Analytical Cellular Pathology

Mesenchymal Changes in Tumorigenesis and Tumor Progression

Lead Guest Editor: Sabina Zurac
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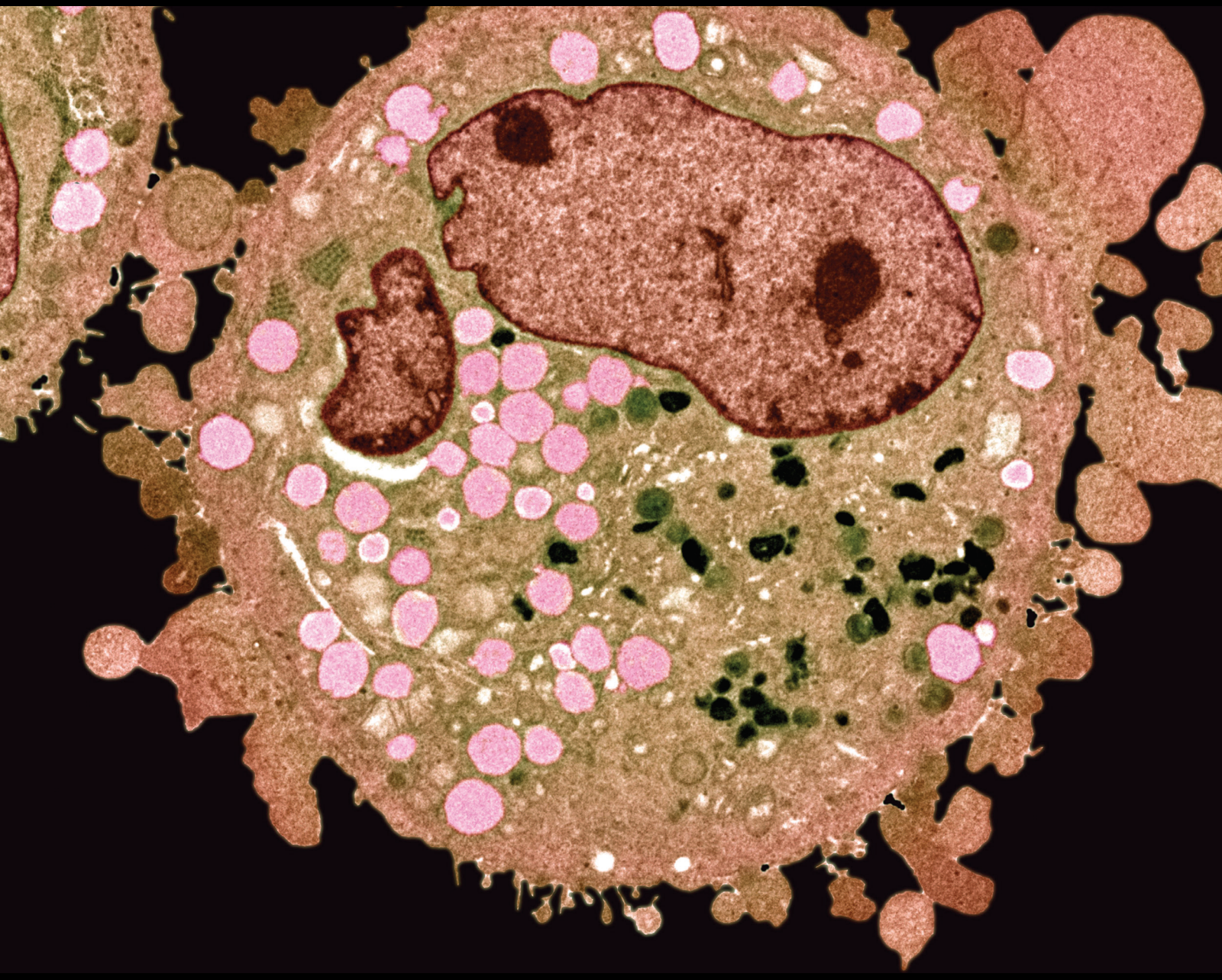

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Contents

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Research Article

Dangerous Liaison: Helicobacter pylori, Ganglionitis, and Myenteric Gastric Neurons: A Histopathological Study

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Chronic inflammation induced by *Helicobacter pylori* (*H. pylori*) infection plays a major role in development of gastric cancer. However, recent findings suggested that progression of inflammation and neoplastic transformation in *H. pylori* infection are more complex than previously believed and could involve different factors that modulate gastric microenvironment and influence host-pathogen interaction. Among these factors, gastric myenteric plexus and its potential adaptive changes in H. pylori infection received little attention. This study is aimed at identifying the impact of *H. pylori*-associated gastritis on number and morphology of nerve cells in the stomach. The distribution of density, inflammation, and programmed cell death in neurons was immunohistochemically assessed in full-thickness archival tissue samples obtained from 40 patients with *H. pylori* infection who underwent surgery for gastric cancer and were compared with findings on samples collected from 40 age- and sex-matched subjects without bacteria. Overall, significant differences were noted between *H. pylori*-positive and *H. pylori*-negative patients. The analysis of tissue specimens obtained from those with infection revealed higher density and larger surface of the myenteric nervous plexus, as well as a significant increase in the number of gastric neuronal cell bodies and glial cells compared to controls. A predominant CD3-immunoreactive T cell infiltrate confined to the myenteric plexus was observed in infected subjects. The presence of mature B lymphocytes, plasma cells, and eosinophils was also noted, but to a lesser extent, within the ganglia. Myenteric ganglionitis was associated with degeneration and neuronal loss. Our results represent the first histopathological evidence supporting the hypothesis that *H. pylori*-induced gastric inflammation may induce morphological changes in myenteric gastric ganglia. These findings could help gain understanding of some still unclear aspects of pathogenesis of *H. pylori* infection, with the possibility of having broader implications for gastric cancer progression.

1. Introduction

H. pylori is one of the most widespread human pathogens and is the strongest known risk factor for malignancies arising within the stomach, mainly due to the persistent inflammatory response induced in the mucosa [1]. However, only a small proportion of colonized individuals develop gastritis and only a small subset of patients with chronic gastritis develop gastric cancer [2]. Furthermore, many of those with gastric inflammation are asymptomatic, while in some patients with overt gastritis, the symptoms persist or recur after eradication treatment [3]. This variability in clinical evolution could be explained by a number of host factors and bacterial virulence factors, but some authors suggested that the pathogenic mechanisms of *H. pylori* infection might be much more complex than generally believed and could involve some less studied individual factors, such as alterations of the gastric enteric nervous system (ENS) [4].

The ENS is by far the largest and most complex part of the autonomic nervous system (ANS), consisting of glial cells and various types of neurons organized in two networks of myenteric ganglia within the gut wall. It was described as the "brain in the gut," since it has the unique ability to control gastrointestinal functions independent of the central nervous system [5]. In the stomach, the ENS is represented mainly by the Auerbach plexus (or the myenteric plexus), which is

situated between the circular and the longitudinal layers of the muscularis propria and provides motor innervation to both muscle layers and secretomotor innervation to the gastric mucosa [6]. Only sparse submucosal ganglia, present mainly in the antrum, form the gastric Meissner plexus [7].

Some studies reported that gastric mucosal colonization by *H. pylori* and subsequent mucosal inflammation may affect central and extragastric peripheral nervous system activity, contributing to intestinal dysfunctions, cardiac arrhythmia, alterations of pancreatic function, and even to some neurological disorders, such as Parkinson's disease and Guillain-Barré syndrome [8–11]. On the other hand, other studies demonstrated that *H. pylori* infection plays a protective role against some esophageal diseases, inflammatory bowel diseases, Alzheimer's disease, and multiple sclerosis, resulting from changes induced in brain-gut axis [4, 11–15]. Based upon these observations, it is likely that *H. pylori* infection may also interact with gastric ENS through different mechanisms: direct neurotoxic effect and microelement deficiency, secondary to functional and morphological changes in the gastric mucosa, activation of neurogenic inflammation, and structural alterations of myenteric ganglia [4]. The last one is best suited for histomorphological and immunohistochemical approach.

Aside from *H. pylori*-associated changes in the neurochemical (neurotransmitter/neuropeptide) content of gastric nerve fibers, too few studies have been done to determine whether the H. pylori-induced gastric inflammation could cause neuroplastic alterations in the myenteric ganglia. Therefore, in this study, we aimed to directly assess the effects of *H. pylori* infection on gastric nervous system morphology, in order to shed light on the potential abnormalities that may result from it. Our hypothesis is that *H. pylori* infection influences the number of myenteric neurons and glial cells and disturbs neuronal homeostasis.

2. Materials and Methods

2.1. Patients and Tissue Samples. This study as designed as an observational retrospective cohort study following the methods previously published by our group [16]. Full-thickness samples of gastric wall were obtained from 40 consecutive patients (31 males, 9 females), mean age 63.43 (SEM = 1.86) undergoing surgery for gastric cancer. *H. pylori* infection was histologically proven in all the enrolled subjects. Archival gastric samples from 40 age- and sex-matched subjects (mean age 63.6, SEM = 1.75) without recent history of *H. pylori* infection, who had been operated for complicated peptic ulcer disease or non-adenocarcinomatous gastric tumors, served as controls and have been selected from same anatomical gastric region as ones of the *H. pylori*-positive group. All samples were harvested from areas at least 5 cm away from any visible lesion. Patients with peritonitis or suffering from different conditions associated with changes in myenteric plexus, as well as patients treated with chemo/radiotherapy, were excluded from the study. Moreover, subjects with morphologic evidence of recently treated *H. pylori* infection (prominent intestinal metaplasia, marked glandular atrophy, or nodular lymphoid aggregates in lamina propria) were not included in the control group.

2.2. Sample Processing and Histological Assessment. Routinely fixed and processed samples were cut in 5 μm thick serial sections with circular layer and myenteric ganglia cut longitudinally. Three gastric cross sections per specimen, cut at a reasonable distance of 200 μm, were mounted on glass slides and then examined. We took this measure to avoid evaluating the same ganglionic area twice in adjacent sections. Before use, slides were deparaffinized, rehydrated, and processed for routine hematoxylin and eosin (H&E) and Giemsa staining and immunohistochemistry. Histopathological findings were assessed on H&E-stained sections, and Giemsa stain technique was used to demonstrate *H. pylori*. The grades of *H. pylori* density, chronic mucosal inflammation, neutrophilic activity, intestinal metaplasia, and glandular atrophy were determined for each specimen and scored as normal, mild, moderate, and marked according to the updated Sydney system [17]. Neuron damage was confirmed when cells with condensed/vacuolated cytoplasm and/or shrunken, pyknotic nuclei were identified and was described as present/absent.

2.3. Immunohistochemical Analysis. Myenteric neurons and glial cells were evaluated by anti-HuC/D and anti-S100 antibodies, respectively. Ganglionic areas were measured by using anti-S100 antibody. Presence and quantification of lymphocytic infiltrate were assessed by using CD3 (T lymphocytes) and CD20 (B lymphocytes) antibodies. Apoptotic activity of myenteric neurons was examined with immunostaining using monoclonal human bcl-2 antibody. Antigen retrieval was performed in citrate buffer (pH 6.0) for HuC/D, whereas Tris-EDTA buffer was used for the rest of antibodies. All slides were microwaved at 500 W for 10 minutes. They were exposed to 3% hydrogen peroxide solution in order to block endogenous peroxidase activity. Sections were incubated with the respective antibodies at 4°C overnight (HuC/D) and for 30-60 minutes at room temperature (other antibodies). The bound antibody was visualized using biotinylated anti-rabbit or anti-mouse secondary antibody, and then streptavidin-peroxidase complex. Diaminobenzidine tetrahydrochloride was used as chromogen substrate. Slides were subsequently counterstained with Mayer's hematoxylin. For each antibody, all slides were simultaneously immunostained in order to rule out differences caused by the staining procedure.

2.4. Quantitative Assessment of Mucosal Inflammation. CD3 and CD20 lymphocytic mucosal inflammation was semiquantitatively graded on a 3-tier scale, according to the percentage of the area in the lamina propria infiltrated by inflammatory cells, as follows: grade 1 (5-30%), grade 2 (30-60%), and grade 3 (>60%). Lymphoid follicles were excluded from analysis, since their random distribution in the mucosa might otherwise generate less consistent results.

2.5. Quantitative Assessment of Myenteric Inflammation and Ganglion Cells. Evaluation of myenteric plexus inflammation was performed as described previously, according to the criteria proposed by Villanacci et al. [16, 18]. Briefly, we

counted only T CD3+ cells the most severely inflamed ganglionic area and grade their density as mild (score 1—four or less lymphocytes observed), moderate (score 2—five to nine cells present), and marked (score 3—ten or more periganglionic lymphocytes identified).

In order to evaluate the immunoreactive ganglionic cells, we used a slightly modified version of a previously described method [19]. For each section, 40 sequential microscopic fields taken along the myenteric plexus were examined at 40x magnification, starting with the first ganglion present on the left side of the section. Examination of the sections and image acquisition were performed using an Olympus BX43 microscope equipped with an Olympus XC30 digital camera (Olympus Corporation, Japan) and ganglionic areas were estimated by an Image Analysis Software (cellSens Dimension, Olympus Corporation, Japan). Each microscopic field corresponded to a 0.36 mm × 0.27 mm rectangle, with an covered area of 0.0972 mm^2. Thereby, the total ganglionic length and tissue area evaluated for each section were 14.4 mm and 3.888 mm^2, respectively.

2.6. Statistical Analysis. For each patient, the results were expressed as mean ± SE. For groups, most data did not follow a parametric distribution, so they are presented using medians and interquartile ranges. The figures are designed as box-whiskers plots. The Wilcoxon test for nonparametric data (two-tailed) was performed to compare groups. The strength of association between variables was evaluated using the γ^2 and Spearman rank correlation tests. A p value < 0.05 was considered statistically significant.

3. Results

On histological examination, there were 20 intestinal, 10 poor cohesive, 7 mixed, and 3 mucinous carcinoma subtypes, according to the 2019 WHO classification of gastric tumors [20]. Most tumors were located in the antrum, along the lesser curvature (27 cases), followed by body (11 cases) and cardia (2 cases). 23 cases were diagnosed as moderately differentiated carcinomas, with the remaining being poorly differentiated.

3.1. Gastric Mucosa. Most cases (22) showed a moderate degree of H. pylori colonization, while 13 cases had a mild bacterial density. In 5 cases, the presence of H. pylori was significant and scored as marked. All patients had chronic gastritis, and neutrophilic activity was observed in 31 (77.5%) of them. Immunohistochemical analysis revealed that the gastric mucosal inflammatory response consisted mainly of CD3+ T cells. Intestinal metaplasia and atrophy were observed in 25 and 21 patients, respectively.

3.2. Gastric Myenteric Plexus. Ganglionic areas were significantly larger (median 0.447 mm^2), and the number of myenteric ganglia was higher (median 29) in *H. pylori*-positive patients, compared to controls (medians 0.231 mm^2 and 20.5, respectively, Figure 1).

An important difference was also found concerning the number of myenteric neurons between patients with *H. pylori*-induced gastritis (median 116.5) and those without infection (median 56.5) (Figure 2(a)), with a significant increment of +171% (individual values varying between 35% and 380%). In addition, more glial cells were identified in myenteric ganglia of infected patients (median 588) compared to controls (median 314) (Figure 2(b)), with a mean increment of +87% (individual values varying between 19% and 172%). Interestingly, in the control group, the number of ganglionic areas (median 20.5) and neuronal density (2-3 neurons per ganglionic area) did not correlate significantly with patients' age or with gastric region. The ratio between glial cells and neurons in myenteric plexus was fairly constant in *H. pylori*-negative patients, (range 5.1-6.8), whereas infected subjects did not display a correlation between glial and neuronal compartments, and the ratio was slightly decreased (range 2.6–6.3, $p = 0.0151$).

Ganglionitis was found in 33 (82.5%) cases with *H. pylori* infection. The inflammatory infiltrate was composed predominantly of CD3-positive T cells, with a minor prevalence of B lymphocytes, plasma cells, and rare eosinophils (Figure 3). T lymphocytic infiltration of myenteric plexus was mild in 17 patients, moderate in 10, and severe in 3 of them, and correlated with T cell density in lamina propria ($p < 0.001$). Occasional inflammatory cells, most of them eosinophils, were present in the vicinity of ganglionic areas in 20 (50%) uninfected patients. Neither CD20-positive B lymphocytes nor plasma cells were observed in the control group.

Degeneration of neuronal cells was obviously more frequently observed in *H. pylori*-infected patients ($p < 0.0001$, Figure 4) but was modestly correlated with T cell ganglionitis ($p = 0.0306$). However, a stronger association ($p = 0.0024$) was found between neurodegenerative changes and the polymorphous inflammatory infiltrate, including T and B lymphocytes and plasma cells.

Myenteric neurons with markedly reduced or lost bcl-2 expression were observed in 23 (57.5%) infected patients, compared to only 3 (5.7%) cases in the control group ($p < 0.0001$, Figure 5). Neuronal apoptosis correlated with the presence of myenteric CD3-positive T cell infiltrate ($p = 0.0056$), but did not correlate with signs of neurodegeneration ($p = 0.627$).

4. Discussion

In the present study, we showed for the first time that the inflammatory process elicited by H. pylori colonization of gastric mucosa can cause inflammation of myenteric plexus and subsequently that myenteric ganglionitis induces structural changes in gastric myenteric ganglia. There is growing evidence that human enteric nervous system can be targeted by the immune response of the host in several chronic inflammatory digestive disorders [21–23]. Moreover, previous reports suggested that impaired neural activity might have a potential role in stomach cancer development [24–26].

4.1. Inflammation of Myenteric Plexus. The presence of periganglionic inflammation, referred to as enteric ganglionitis, or plexitis, reflects imbalanced neuroimmune interactions occurring within the enteric neural microenvironment [27]. In our study, the number of periganglionic inflammatory

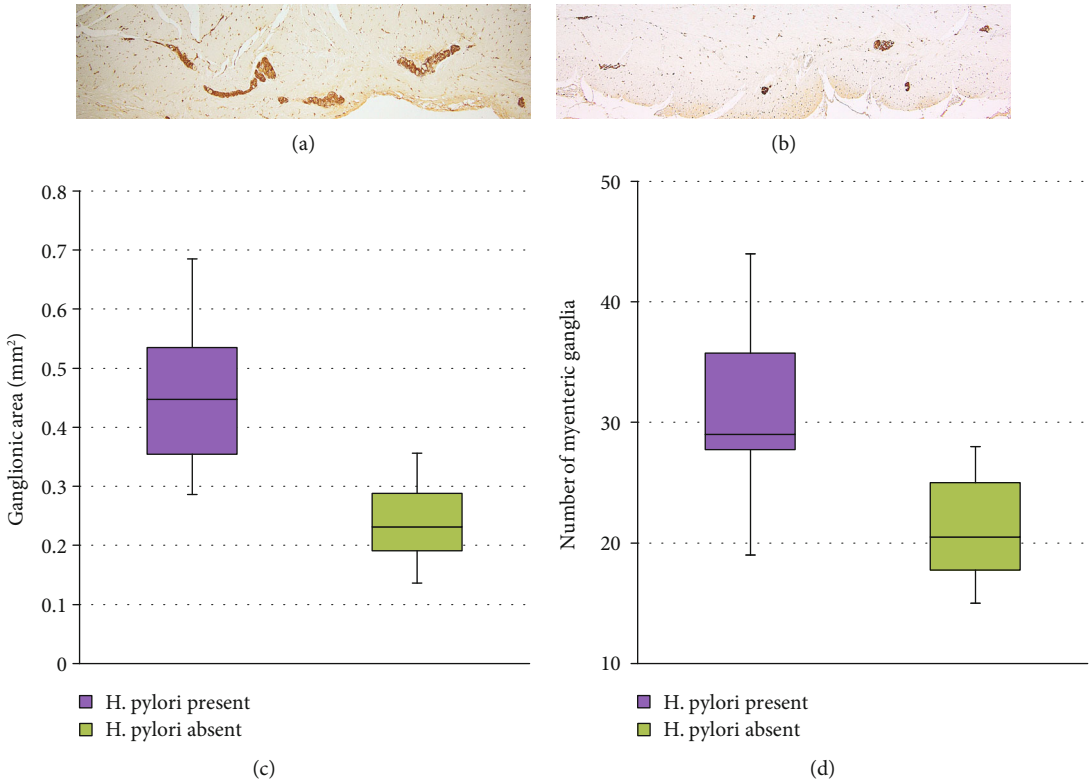

Figure 1: Number and area of myenteric ganglia in the stomach. Representative photomicrographs of S-100 immunostained ganglionic areas in H. pylori-positive patients (a, ×40) and in control patients (b, ×40). Box and whisker plots showing that gastric myenteric ganglia are larger (c, $p < 0.01$) and they are increased in number (d, $p < 0.01$) in H. pylori-infected patients, as compared to controls.

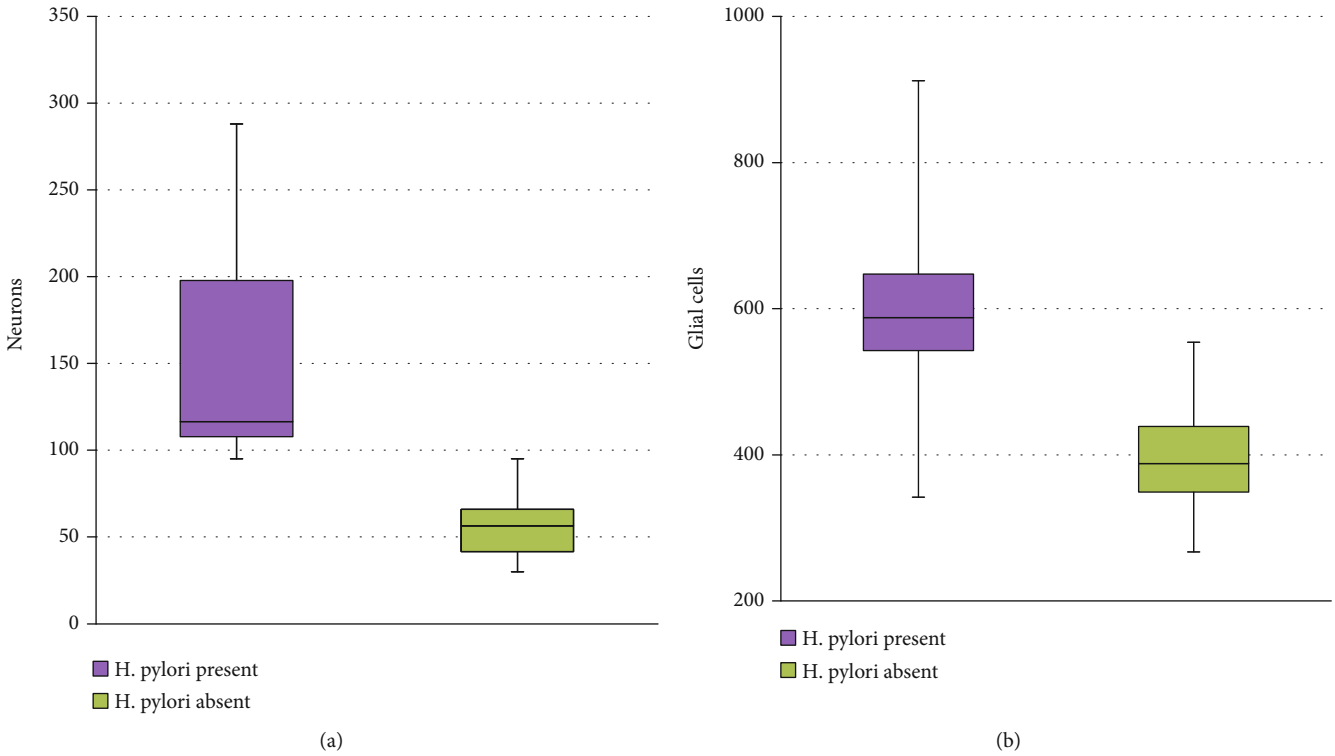

Figure 2: Number of gastric myenteric neurons and glial cells. Graphs showing that significant more myenteric neurons (a, $p < 0.00001$) and glial cells (b, $p < 0.00001$) were detected in the H. pylori-positive group in comparison to the control group.

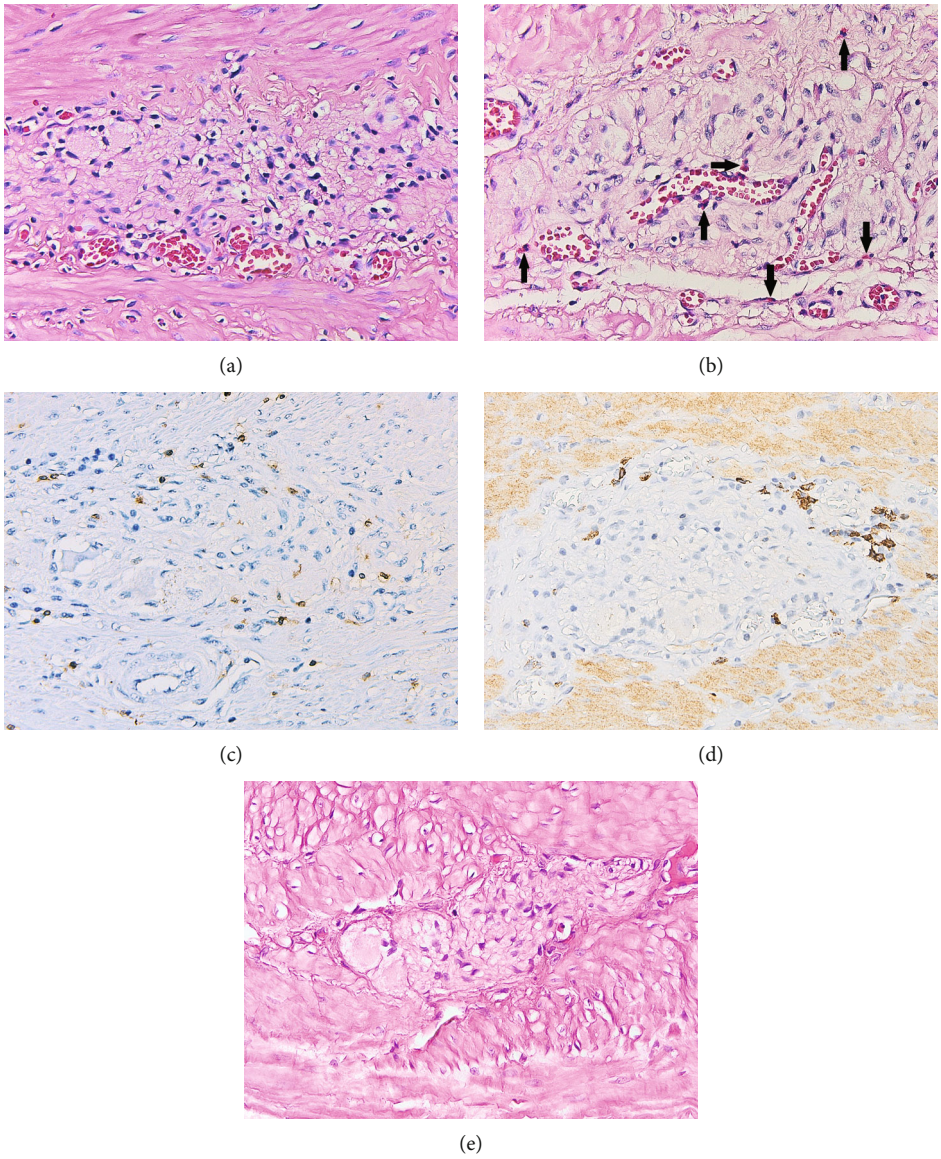

Figure 3: Representative photomicrographs showing different types of inflammatory cells around and within the myenteric plexus in H. pylori-infected patients: lymphocytes (a, H&E stain, 400x); lymphocytes and eosinophils (arrows) (b, H&E stain, 400x); T lymphocytes (c, CD3 stain, 400x); B lymphocytes (d, CD20 stain, 400x). In contrast, no inflammatory cell was noted around myenteric ganglia in control patients (e, H&E stain, 400x).

cells was significantly increased in *H. pylori*-positive patients compared to controls. Although this is an unusual finding, as gastritis is basically a mucosal disease, myenteric plexitis might be hypothesized as responsible for gastric dysmotility frequently described in H. pylori-induced gastritis [4, 28]. The immunohistochemical analysis of the myenteric infiltrate revealed a significant component of CD3-immunoreactive T cells, in agreement with previous reports showing that in inflammatory neuropathies there is a predominant T cytotoxic activity directed against proteins expressed by enteric neurons [18, 22]. However, in the present study, CD20-positive lymphocytes and plasma cells were exclusively identified in patients with *H. pylori* infection, indicating that, in addition to T lymphocyte activation, humoral immune response also participates in myenteric inflammation. Our results confirm previous data documenting the contribution of mature B cells to the immune response by synthesizing and releasing immunoglobulins directed against antigens expressed by myenteric neurons [27].

4.2. Myenteric Neuronal Degeneration and Apoptosis. Neuronal and nerve process degeneration in myenteric plexus has been documented in patients suffering from inflammatory bowel diseases. In our study, signs of neurodegeneration, such as vacuolated or condensed cytoplasm and pyknotic nuclei, were more frequently observed in infected patients, suggesting that *H. pylori* can induce neuronal damage in the myenteric plexus tissue. In addition, we observed a significant relationship between injury of myenteric neurons and periganglionic lymphoplasmacytic inflammatory infiltrate

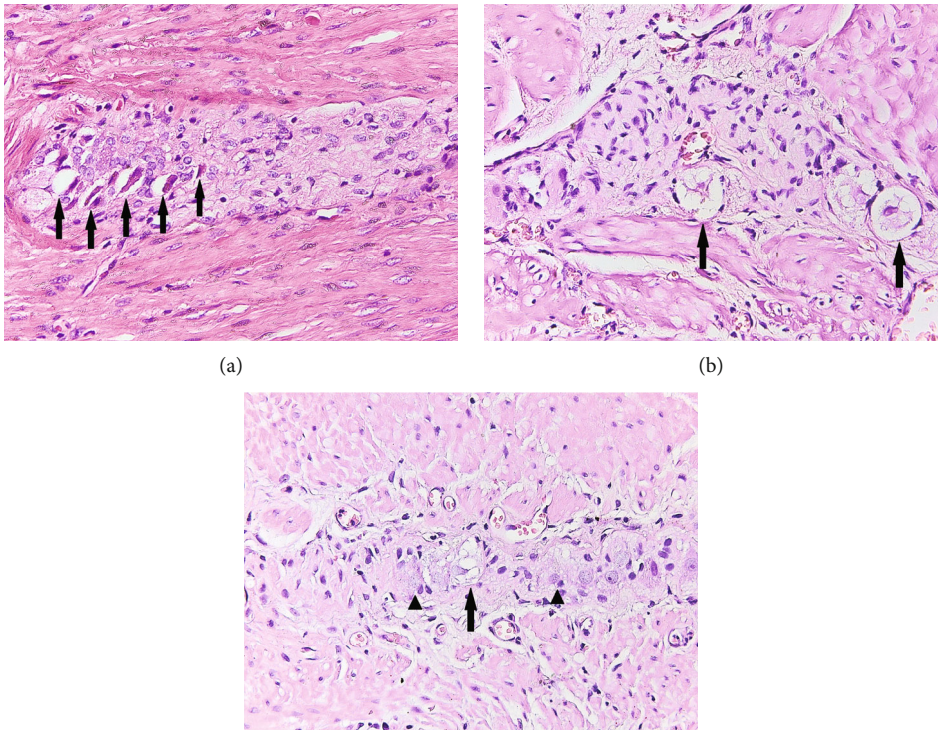

Figure 4: Representative photomicrographs illustrating signs of myenteric neuronal degeneration in H. pylori-positive patients: condensed cytoplasm and pyknotic nuclei (a, H&E stain, 400x) and vacuolated cytoplasm (b and c, H&E stain, 400x). Normal neurons are shown by arrowheads (c).

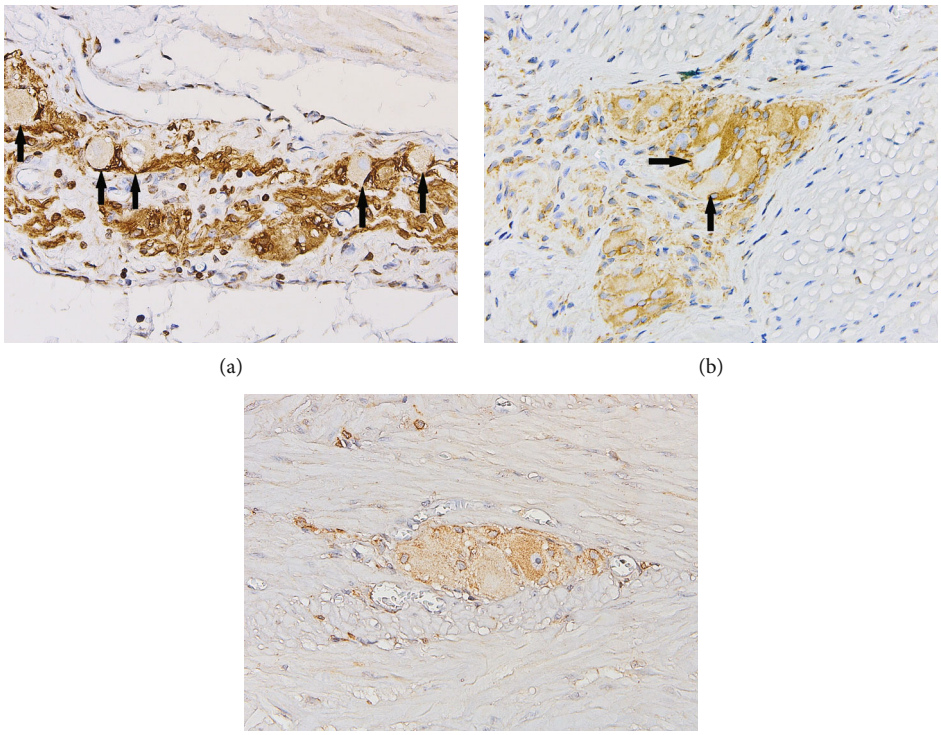

Figure 5: Bcl-2 immunohistochemical labeling of gastric myenteric ganglionic neurons: myenteric neurons with reduced or absent expression of antiapoptotic protein bcl-2 in H. pylori-positive patients (a and b, 400x) and normal bcl-2 expression of neurons in myenteric plexus from a control subject (c, 400x).

($p = 0.0024$). However, a weaker correlation ($p = 0.0306$) with T cell myenteric infiltrate was also noted, indicating that degenerative changes of gastric neurons occur as a result of a concerted action of all the inflammatory cell types (including T cells, B cells, and plasma cells) recruited within myenteric plexus. Our observations confirm previous data showing the degeneration of myenteric neurons under enteric ganglionitis throughout the alimentary tract [29].

Bcl-2 antiapoptotic protein plays an essential role in protecting neurons from programmed cell death, promoting their survival in different types of neural injury. Our results showed, for the first time, that *H. pylori* is able to induce programmed cell death in myenteric gastric neurons. This finding is consistent with previous studies showing that *H. pylori* is able to promote apoptosis in infected gastric epithelial cells [30, 31] and leads to the conclusion that the bacteria might induce apoptosis dysregulation in different cell types of gastric wall. Moreover, we found a significant association between loss of bcl-2 expression in gastric neurons and periganglionic CD3-positive T lymphocytic infiltrate. This finding suggests that T cell-mediated immune response can trigger activation of the apoptotic pathways in myenteric neurons. This hypothesis is supported by similar observations in the central nervous system [32].

4.3. Neuronal and Glial Cell Hyperplasia. A very interesting and surprising finding in this study was the neuronal cell hyperplasia observed in patients with *H. pylori* infection. Variation in the number of enteric neurons was previously described by some authors in inflammatory bowel diseases [33, 34], while other studies failed to demonstrate any significant difference regarding neuron counting [19]. In the context of increased neuronal damage and apoptosis noted in infected patients, we are presently unable to explain the neuronal hyperplasia. In our opinion, the most reasonable hypothesis is that the increased number of gastric myenteric neurons represents a compensatory response to neuronal injury induced by ganglionic inflammation. However, although several possible pathways have been suggested [35–38], the mechanism underlying neuronal hyperplasia remains unknown. Further studies are necessary to elucidate if increased number of neuronal bodies is the result of proliferation and differentiation of neural crest-derived progenitors present in the gut or represents the consequence of transdifferentiation of mature enteric glial cells.

A significant increase in glial compartment was also detected by our analysis. Besides their traditional trophic and supportive functions for enteric neurons, glial cells are involved in enteric neurotransmission [21, 39], neurogenesis [40], and immune signaling [41, 42]; therefore, their number could be influenced by the immune response in the gastrointestinal tract. In our study, the level of neuronal hyperplasia was twice as great as glial cell hyperplasia degree, suggesting that neurons rather than glial cells were more affected in the *H. pylori*-positive patients herein examined. However, it is not clear if the proliferation of gastric glial cells precedes or follows neuronal hyperplasia.

Our study has some limitations. First, the number of patients was relatively small. Our results need to be verified in larger studies to obtain a more reliable estimation. Second, since this was a retrospective study, there may be a bias in the selection of patients, which we tried to minimize by examining 40 consecutive cases. Moreover, some risk factors that might have an impact on gastric myenteric plexus morphology, such as smoking and alcohol consumption, were not considered in this study, which may affect the reliability of the results. In addition, lack of prior research studies on the topic limits the robustness of our results. Future research should address these limitations to validate present findings.

In summary, the data presented provide what we believe is the first evidence that the gastric nervous system can be morphologically altered by host immune response in the setting of *H. pylori* infection. These findings advance our knowledge of the complex mechanisms of interaction between pathogen and host and will hopefully pave the road to a more vast scientific investigation in the area of gastric neural plasticity. Given the recognition of H. pylori as the major cause of gastric cancer, strategies aiming for a better understanding of the mechanisms of carcinogenesis are mandatory for identifying new potential therapeutic targets; therefore, further studies to clarify the involvement of the gastric enteric nervous system in gastric cancer development are needed.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon reasonable request.

Disclosure

Small portion of this study has been briefly presented in abstract form at the 30th European Congress of Pathology, Bilbao, Spain, September 2018 [43].

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

[1] IARC, *Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 100b: A Review of Human Carcinogens: Biological Agents*, Lyon, International Agency for Research on Cancer, 2012.

[2] K. A. Bockerstett and R. J. DiPaolo, "Regulation of gastric carcinogenesis by inflammatory cytokines," *Cellular and Molecular Gastroenterology and Hepatology*, vol. 4, no. 1, pp. 47–53, 2017.

[3] P. Malfertheiner, F. Megraud, C. A. O'Morain et al., "Management of *Helicobacter pylori* infection–the Maastricht IV/Florence Consensus Report," *Gut*, vol. 61, no. 5, pp. 646–664, 2012.

[4] J. Budzyński and M. Kłopocka, "Brain-gut axis in the pathogenesis of *Helicobacter pylori* infection," *World Journal of Gastroenterology*, vol. 20, no. 18, pp. 5212–5225, 2014.

[5] R. K. Goyal and I. Hirano, "The enteric nervous system," *New England Journal of Medicine*, vol. 334, no. 17, pp. 1106–1115, 1996.

[6] J. B. Furness, K. C. Lloyd, C. Sterini, and J. H. Walsh, "Evidence that myenteric neurons of the gastric corpus project to both the mucosa and the external muscle: myectomy operations on the canine stomach," *Cell and Tissue Research*, vol. 266, no. 3, pp. 475–481, 1991.

[7] J. B. Furness, *The enteric nervous system*, Blackwell Publishing, Oxford, 2006.

[8] J. E. Domínguez-Muñoz and P. Malfertheiner, "Effect of *Helicobacter pylori* infection on gastrointestinal motility, pancreatic secretion and hormone release in asymptomatic humans," *Scandinavian Journal of Gastroenterology*, vol. 36, no. 11, pp. 1141–1147, 2001.

[9] J. Budzyński, M. Kłopocka, R. Bujak, M. Swiatkowski, G. Pulkowski, and W. Sinkiewicz, "Autonomic nervous function in *Helicobacter pylori*-infected patients with atypical chest pain studied by analysis of heart rate variability," *European Journal of Gastroenterology and Hepatology*, vol. 16, no. 5, pp. 451–457, 2004.

[10] H. H. Nielsen, J. Qiu, S. Friis, L. Wermuth, and B. Ritz, "Treatment for *Helicobacter pylori* infection and risk of Parkinson's disease in Denmark," *European Journal of Neurology*, vol. 19, no. 6, pp. 864–869, 2012.

[11] J. Kountouras, G. Deretzi, C. Zavos et al., "*Helicobacter pylori* infection may trigger Guillain -Barre syndrome, Fisher syndrome and Bickerstaff brainstem encephalitis," *Journal of the Neurological Sciences*, vol. 305, no. 1-2, pp. 167-168, 2011.

[12] J. H. Rubenstein, J. M. Inadomi, J. Scheiman et al., "Association between *Helicobacter pylori* and Barrett's esophagus, erosive esophagitis, and gastroesophageal reflux symptoms," *Clinical Gastroenterology and Hepatology*, vol. 12, no. 2, pp. 239–245, 2014.

[13] J. Luther, M. Dave, P. D. Higgins, and J. Y. Kao, "Association between *Helicobacter pylori* infection and inflammatory bowel disease: a meta-analysis and systematic review of the literature," *Inflammatory Bowel Diseases*, vol. 16, no. 6, pp. 1077–1084, 2010.

[14] J. Kountouras, M. Boziki, E. Gavalas et al., "Eradication of *Helicobacter pylori* may be beneficial in the management of Alzheimer's disease," *Journal of Neurology*, vol. 256, no. 5, pp. 758–767, 2009.

[15] K. W. Cook, J. Crooks, K. Hussain et al., "*Helicobacter pylori* infection reduces disease severity in an experimental model of multiple sclerosis," *Frontiers in Microbiology*, vol. 6, no. 52, article 25762984, 2015.

[16] L. Sticlaru, F. Stăniceanu, M. Cioplea et al., "Neuroimmune cross-talk inHelicobacter pyloriinfection: analysis of substance P and vasoactive intestinal peptide expression in gastric enteric nervous system," *Journal of Immunoassay and Immunochemistry*, vol. 39, no. 6, pp. 660–671, 2018.

[17] M. F. Dixon, R. M. Genta, J. H. Yardley, and P. Correa, "Classification and grading of gastritis: the updated Sydney system. International workshop on the histopathology of gastritis," *American Journal of Surgical Pathology*, vol. 20, no. 10, pp. 1161–1181, 1994.

[18] V. Villanacci, G. Bassotti, R. Nascimbeni et al., "Enteric nervous system abnormalities in inflammatory bowel diseases," *Neurogastroenterology and Motility*, vol. 20, no. 9, pp. 1009–1016, 2008.

[19] C. Ippolito, C. Segnani, R. de Giorgio et al., "Quantitative evaluation of myenteric ganglion cells in normal human left colon: implications for histopathological analysis," *Cell and Tissue Research*, vol. 336, no. 2, pp. 191–201, 2009.

[20] I. D. Nagtegaal, R. D. Odze, D. Klimstra et al., *WHO Classification of Tumors of the Digestive System*, IARC Press, Lyon, 5th edition, 2019.

[21] V. Vasina, G. Barbara, and L. Talamonti, "Enteric neuroplasticity evoked by inflammation," *Autonomic Neuroscience*, vol. 126-127, pp. 264–272, 2006.

[22] R. De Giorgio, S. Guerrini, G. Barbara et al., "Inflammatory neuropathies of the enteric nervous system," *Gastroenterology*, vol. 126, no. 7, pp. 1872–1883, 2004.

[23] G. Barbara, C. Cremon, R. de Giorgio et al., "Mechanisms underlying visceral hypersensitivity in irritable bowel syndrome," *Current Gastroenterology Reports*, vol. 13, no. 4, pp. 308–315, 2011.

[24] A. C. Polli-Lopes, S. Zucoloto, F. de Queirós Cunha, L. A. da Silva Figueiredo, and S. B. Garcia, "Myenteric denervation reduces the incidence of gastric tumors in rats," *Cancer Letters*, vol. 190, no. 1, pp. 45–50, 2003.

[25] M. Rosso, M. J. Robles-Frías, R. Coveñas, M. V. Salinas-Martín, and M. Muñoz, "The NK-1 receptor is expressed in human primary gastric and colon adenocarcinomas and is involved in the antitumor action of L-733,060 and the mitogenic action of substance P on human gastrointestinal cancer cell lines," *Tumour Biology*, vol. 29, no. 4, pp. 245–254, 2008.

[26] F. Feng, J. Yang, L. Tong et al., "Substance P immunoreactive nerve fibres are related to gastric cancer differentiation status and could promote proliferation and migration of gastric cancer cells," *Cell Biology International*, vol. 35, no. 6, pp. 623–629, 2011.

[27] R. De Giorgio and M. Camilleri, "Human enteric neuropathies: morphology and molecular pathology," *Neurogastroenterology and Motility*, vol. 16, no. 5, pp. 515–531, 2004.

[28] C. L. Zhang, C. H. Geng, Z. W. Yang et al., "Changes in patients' symptoms and gastric emptying after Helicobacter pylori treatment," *World Journal of Gastroenterology*, vol. 22, no. 18, pp. 4585–4593, 2016.

[29] I. E. Demir, K.-H. Schäfer, E. Tieftrunk, H. Friess, and G. O. Ceyhan, "Neural plasticity in the gastrointestinal tract: chronic inflammation, neurotrophic signals, and hypersensitivity," *Acta Neuropathologica*, vol. 125, no. 4, pp. 491–509, 2013.

[30] M. El-Shahat, S. El-Masry, M. Lofty, A.-M. El-Kenawy, and W. A. Nasif, "Relationship of Helicobacter pylori to Bcl-2 family expression, DNA content, and pathological characteristics of gastric cancer," *International Journal of Gastrointestinal Cancer*, vol. 36, no. 2, pp. 61–68, 2005.

[31] S. H. Chu, J. W. Lim, D. G. Kim, E. S. Lee, K. H. Kim, and H. Kim, "Down-regulation of Bcl-2 is mediated by NF-κB activation in helicobacter pylori-induced apoptosis of gastric epithelial cells," *Scandinavian Journal of Gastroenterology*, vol. 46, no. 2, pp. 148–155, 2011.

[32] O. Aktas, O. Ullrich, C. Infante-Duarte, R. Nitsch, and F. Zipp, "Neuronal damage in brain inflammation," *Archives of Neurology*, vol. 64, no. 2, pp. 185–189, 2007.

[33] N. Bernardini, C. Segnani, C. Ippolito et al., "Immunohistochemical analysis of myenteric ganglia and interstitial cells of Cajal in ulcerative colitis," *Journal of Cellular and Molecular Medicine*, vol. 16, no. 2, pp. 318–327, 2012.

[34] K. Geboes and S. Collins, "Structural abnormalities of the nervous system in Crohn's disease and ulcerative colitis," *Neurogastroenterology & Motility*, vol. 10, no. 3, pp. 189–202, 1998.

[35] M. Metzger, P. M. Bareiss, T. Danker et al., "Expansion and differentiation of neural progenitors derived from the human adult enteric nervous system," *Gastroenterology*, vol. 137, no. 6, pp. 2063–2073.e4, 2009.

[36] A. Schuster, M. Klotz, T. Schwab et al., "Maintenance of the enteric stem cell niche by bacterial lipopolysaccharides? Evidence and perspectives," *Journal of Cellular and Molecular Medicine*, vol. 18, no. 7, pp. 1429–1443, 2014.

[37] J. Belkind-Gerson, R. Hotta, N. Nagy et al., "Colitis induces enteric neurogenesis through a 5-HT4-dependent mechanism," *Inflammatory Bowel Diseases*, vol. 21, no. 4, pp. 870–878, 2015.

[38] J. Belkind-Gerson, H. K. Graham, J. Reynolds et al., "Colitis promotes neuronal differentiation of Sox2+ and PLP1+ enteric cells," *Scientific reports*, vol. 7, no. 1, article 2525, 2017.

[39] A. Rühl, "Glial cells in the gut," *Neurogastroenterology and Motility*, vol. 17, no. 6, pp. 777–790, 2005.

[40] C. Laranjeira, K. Sandgren, N. Kessaris et al., "Glial cells in the mouse enteric nervous system can undergo neurogenesis in response to injury," *The Journal of Clinical Investigation*, vol. 121, no. 9, pp. 3412–3424, 2011.

[41] K. Geboes, P. Rutgeerts, N. Ectors et al., "Major histocompatibility class II expression on the small intestinal nervous system in Crohn's disease," *Gastroenterology*, vol. 103, no. 2, pp. 439–447, 1992.

[42] A. Rühl, S. Franzke, S. M. Collins, and W. Stremmel, "Interleukin-6 expression and regulation in rat enteric glial cells," *American Journal of Physiology*, vol. 280, no. 6, pp. G1163–G1171, 2001.

[43] M. Cioplea, L. Sticlaru, C. Popp, G. Micu, and L. Nichita, "Gastric myenteric plexus and Helicobacter pylori infection – is there a relationship?," *Virchows Archiv*, vol. 473, p. S174, 2018.

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Review Article

Epithelial-Mesenchymal Transition in Skin Cancers: A Review

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Epithelial-mesenchymal transition (EMT) is involved in physiologic processes such as embryogenesis and wound healing. A similar mechanism occurs in some tumors where cells leave the epithelial layer and gain mesenchymal particularities in order to easily migrate to other tissues. This process can explain the invasiveness and aggressiveness of these tumors which metastasize, by losing the epithelial phenotype (loss of E-cadherin, desmoplakin, and laminin-1) and acquiring mesenchymal markers (N-cadherin). Complex changes and interactions happen between the tumor cells and the microenvironment involving different pathways, transcription factors, altered expression of adhesion molecules, reorganization of cytoskeletal proteins, production of ECM-degrading enzymes, and changes in specific microRNAs. The purpose of this review is to determine particularities of the EMT process in the most common malignant cutaneous tumors (squamous cell carcinoma, basal cell carcinoma, and melanoma) which still have an increasingly high incidence. More studies are required on this topic in order to establish clear correlations. High costs related to skin cancer therapies in general as well as high impact on patients' quality of life demand finding new, reliable prognostic and therapeutic markers with significant public health impact.

1. Introduction

Epithelial-mesenchymal transition (EMT) is a complex biological process by which epithelial cells acquire special properties that make them more capable of undergoing embryogenesis and promoting normal wound healing. In contrast with these two physiologic aspects, EMT can also take place in the late carcinogenesis, promoting tumor progression and metastasis. During EMT, epithelial tumor cells leave their differentiated properties in order to obtain a mesenchymal-like phenotype, that makes them more invasive and more aggressive, allowing them to migrate into the surrounding tissues [1].

The hallmarks of EMT *in vitro* and *in vivo* include the acquisition of a spindle-like/fibroblastic morphology, the upregulation of mesenchymal markers and extracellular matrix components, the downregulation of epithelial cell surface markers and cytoskeleton components, and the upregulation and/or nuclear translocation of specific transcription factors (i.e., Snail, Slug, Zeb1/2, and Twist1/2) [2] (Figure 1).

EMT implies losing cell-cell junctions and cell polarity. During this process, both gap and adherent junctions are lost. Cadherin-mediated adhesion is a dynamic process that is regulated by several signal transduction pathways. There is also evidence that cadherins are not only targets for

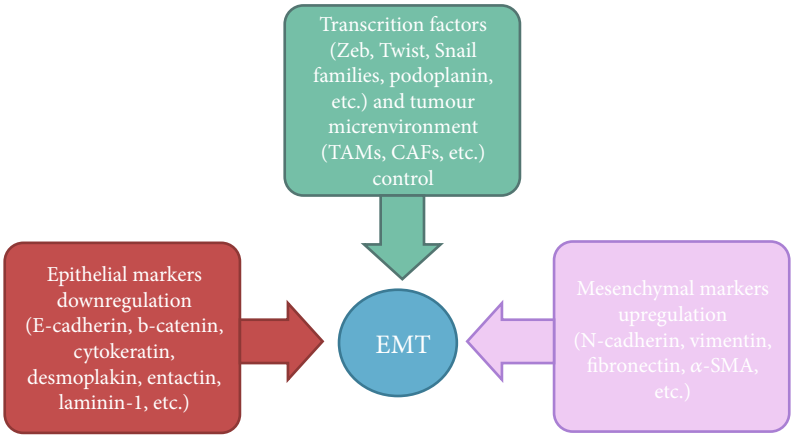

Figure 1: Epithelial-mesenchymal transition hallmarks.

signaling pathways that regulate adhesion but also may themselves send signals that regulate basic cellular processes, such as migration, proliferation, apoptosis, and cell differentiation [3, 4].

All these changes lead to the loss of basal membrane integrity. Moreover, there are cytoskeletal changes regarding the distribution of actin and the replacement of the cytokeratin filaments with vimentin [5, 6].

Single cells can invade lymphatic and hematogenous routes and induce distant metastasis. This phenomenon is facilitated by a decreased expression of E-cadherin, a subtype of cell adhesion molecule expressed by the epithelial cells. This protein is considered a key epithelial marker with tumor suppressor function that inhibits invasion and metastasis. A proof for this is a low transcription of its gene in various malignancies [7]. Moreover, other epithelial markers (cytokeratin, desmoplakin, entactin, and laminin-1) are lost and the cells acquire a mesenchymal phenotype through an increased expression of mesenchymal markers (neural cadherin (N-cadherin), vimentin, fibronectin, and smooth muscle actin alpha (α-SMA)) [1]. It has been shown that these cells with mesenchymal phenotype are often found in the invasion front of primary tumors being involved in invasion and metastasis processes [8].

The EMT process is controlled and enhanced by various transcription factors depending on the skin tumor (such as Zeb, Twist, Snail families, and podoplanin), expressed not only by cancer cells but also within the tumor microenvironment. The microenvironment, also known as the tumor stroma, is composed of tumor-associated macrophages (TAM), cancer-associated fibroblasts (CAFs), lymphocytes, and many other immune cells, that were proved to favor tumor progression and dissemination [9].

Snail and Zeb directly lower E-cadherin expression [10] while other factors, as Twist, have an indirect effect [11]. The maintenance of the epithelial cell polarity is provided by three protein complexes: Par, Crumbs, and Scribble, regulated by the EMT inductors [12]. During EMT, epithelial cell polarity is lost as a result of the Snail 1 suppressor action on Crumbs3 transcription and the loss of Par and Crumbs protein complexes at a junction level [13]. Zeb1 also has suppressive action on gene transcription of cellular polarity by inhibiting Crumbs3 and other genes [14]. TGFβ plays an important role in the loss of cellular polarity in the EMT process, on the one hand, by expressing the Snail and Zeb genes and, on the other hand, by modifying the cytoskeletal architecture [15]. Snail and Zeb transcription factors promote invasion by the expression of matrix metalloproteinases (MMPs) that play a role in destroying the basement membrane. Moreover, MMP3 stimulates the production of reactive oxygen species, thus inducing Snail1 expression and ultimately triggering EMT [16]. Transcription factors confer malignant traits, such as motility, invasiveness, and resistance to apoptosis on neoplastic cells [10, 17–21].

The EMT process can be reversible as the mesenchymal cells become epithelial cells when they reach the secondary sites. This process known as mesenchymal-epithelial transition (MET) facilitates the formation of metastasis [1].

Another process involved in cancer metastasis is collective cancer invasion, in which a group of neoplastic cells, with preserved cell-cell adhesion, move away from the primary tumor. In this case, only a few cells suffer EMT, in order to head the entire group [22–24].

Being so evident of the implication of EMT in cancer progression, in aggressiveness, the aim of this review is to assess different aspects of EMT in the most common malignant skin tumors (squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and melanoma) whose incidence is alarmingly increasing but still with limited therapeutic targets.

2. EMT in Cutaneous SCC

Cutaneous squamous cell carcinoma (cSCC) is easily treated and the cure rate is high, but there are cases where metastasis can occur. An accurate clinical exam correlated with a histological and immunohistochemical investigation can establish the biomarkers involved in the development and evolution of this malignancy and reveal the appropriate treatment for each patient.

EMT in the setting of cSCC is a process far from being completely understood. As within other skin cancers, the phenomenon has been explained through two important

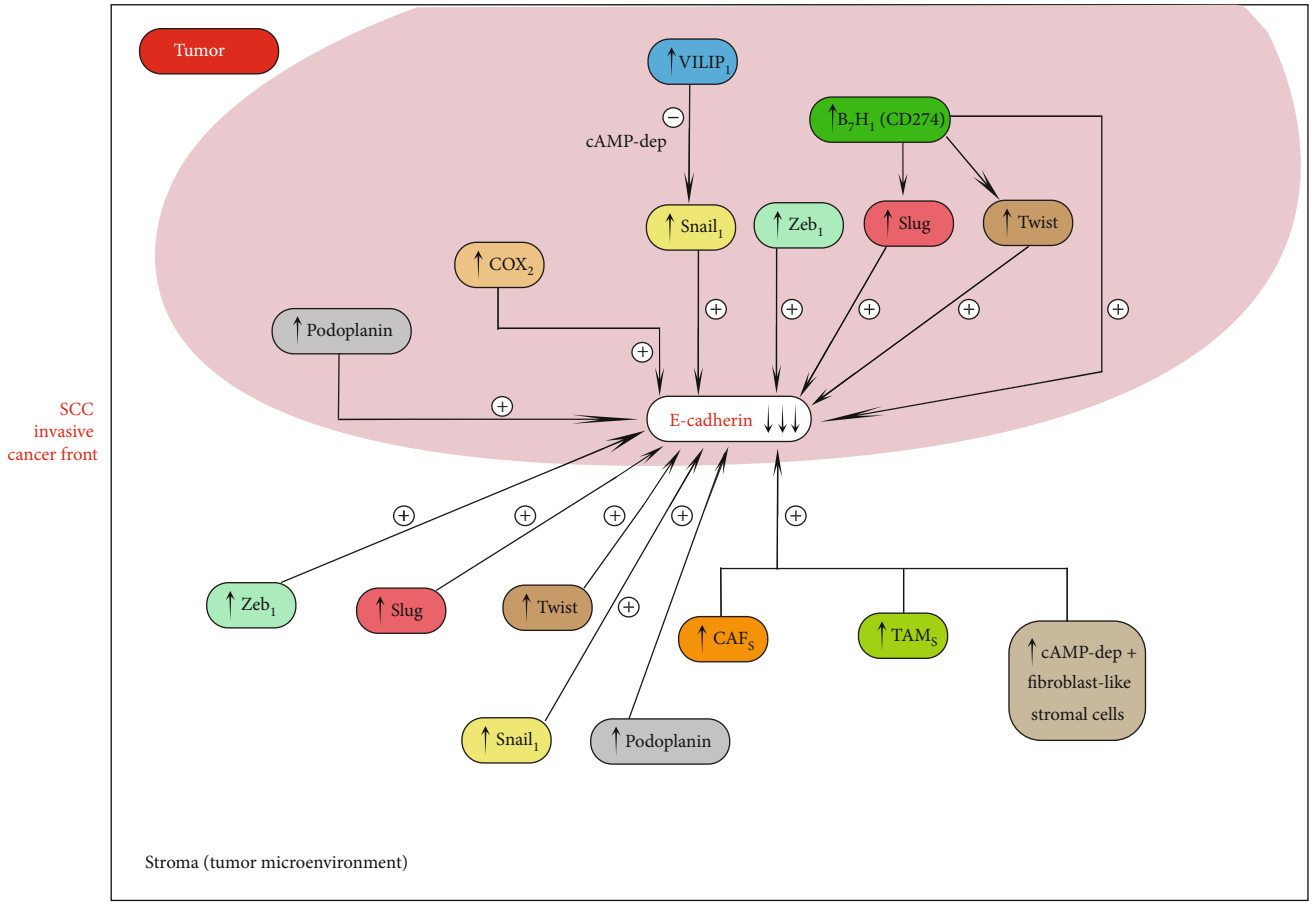

Figure 2: SCC cancer invasive front.

aspects in the cSCC metastasis process: on the one hand, the loss of the expression of epithelial markers in order to invade and disseminate from the primary tumor, on the other hand, the need to revert to an epithelial identity in order to form metastases to distant sites [8]. This hypothesis could have major implications for the management of cSCC, questioning whether therapeutic agents that inhibit EMT [25] or therapeutic agents that inhibit the reversion of EMT [26] would be more appropriate to be used as a treatment. Animal model studies are expected to play major roles in assessing different management strategies designed for skin carcinomas [27].

Often involved in the transformation into a mesenchymal-type phenotype is the acquisition of vimentin and the loss of cell-cell attachment molecules E-cadherin and beta-catenin [26, 28, 29]. Adhesion proteins such as E-cadherin are essential in maintaining cellular integrity. Results from studies demonstrating a reduction in the expression of membranous E-cadherin on cSCC cells, when compared to precursor cSCC lesions and normal skin, indicate EMT as an important process in cSCC progression [30]. Membranous E-cadherin is bound to beta-catenin, which is released when the former is downregulated and can translocate to the nucleus, being able to activate genes involved in proliferation and invasive growth [31]. Membranous E-cadherin expression appears to be correlated with the degree of tumor differentiation, with upregulation in well-differentiated SCCs and attenuated or missing staining in poorly differentiated tumors [24, 32]. Instead, poorly differentiated SCCs seem to have a high cytoplasmic expression of E-cadherin [24]. This translocation from the membrane to the intracytoplasmic region is regarded by many as functional loss of this adhesion molecule, attenuating cellular integrity and thus, promoting malignant transformation and metastasis in the setting of EMT [33–36]. Further evidence that point to EMT as a tumor progression indicator in cSCC is delivered by studies showing a decrease of membranous E-cadherin in corresponding lymph node metastases when compared to primary cSCCs [24, 37]. The membranous downregulation is again accompanied by increased cytoplasmic staining [24, 34].

We will shortly review herein the most important factors reported to influence EMT in cSCC (Figure 2).

Aberrant expression of several transcriptional repressors including Zeb1, Slug, and Twist induces E-cadherin downregulation at the invasive cancer front [38, 39]. These EMT-related proteins can also be expressed by cells from the tumor stroma, such as cancer-associated fibroblasts (CAFs). Activated CAFs are believed to promote tumor progression and decrease patient survival [40–42]. Sasaki et al. analyzed the expression of proteins related to EMT and CAF in different skin cancers, showing that the microenvironment at the tumor invasive front shows different specific

expression patterns in cutaneous BCC, SCC, and MM [9]. High expression levels of podoplanin, PDGFRβ, CD10, S100A4, α-SMA, Zeb1, Slug, and Twist were obtained in the group of cSCCs. The result could represent a useful panel of biomarkers in order to assess skin cancer invasiveness [9].

The contribution of the tumor microenvironment to tumor invasiveness and metastasis is also portrayed by the role of tumor-associated macrophages (TAM). They are shown to induce Snail promoter activity and EMT in MCF-7 breast cancer cells via TNF-α [43] and have been found in higher numbers in cSCC and Bowen's disease (BD) when compared to precancerous lesions [44]. In addition, the cell surface zinc-dependent metalloprotease CD10, expressed in the peritumor fibroblast-like stromal cells of the invasion front of various malignancies [45–48], appears to be increased in cSCC compared to precancerous lesions.

Podoplanin, a mucin-type transmembrane glycoprotein, mediating cellular contractile properties and cytoskeletal reorganization, is upregulated at the leading edge of the tumor in metastatic and poorly differentiated cSCC [24]. Studies comparing primary nonmetastatic cSCC, primary metastatic cSCC, and their corresponding lymphatic metastases demonstrate podoplanin, Twist, Zeb 1, vimentin, and beta-catenin overexpression in metastatic cSCC, with Twist ectopic expression inducing Zeb1, vimentin, and podoplanin expression and also E-cadherin delocalization, resulting in scattered migration pattern *in vitro* [33]. However, EMT marker expression was decreased in metastases compared to the corresponding primary tumors [33]. Overexpression of podoplanin represented a statistically independent prognostic factor for disease-free survival in other studies [24].

PGE2–EP2 signaling pathway is also believed to play an important role in EMT mediation by contributing to E-cadherin downregulation during ultraviolet- (UV-) induced cSCC progression [35]. Cyclooxygenase 2 (COX-2) overexpression in cSCC and precursor lesions was reported in various studies [49–52], and it seems to occur together with inactivation of E-cadherin [53].

B7-H1 (CD274), a T-cell coinhibitory molecule, often expressed in human carcinoma cells, believed to be implicated in the immune escape process also appears to favor EMT. Murine models studied by Cao et al. investigating its expression in a murine methylcholanthrene- (MCA-) induced model of SCC revealed that upregulation of B7-H1 in skin epithelial cells downregulates E-cadherin and upregulates Slug and Twist, promoting EMT [54].

Visinin-like protein 1 (VILIP-1), a neuronal calcium sensor protein, putative tumor migration suppressor gene, modulating cyclic nucleotide levels and inducing cell differentiation, appears to be involved in the process of EMT in cSCC. Studies on SCC mouse model cells by Schönrath et al. found that VILIP-1 suppresses the expression of the EMT-related transcriptional repressor Snail1 in a cAMP-dependent manner [55]. The induction of Snail is inhibited by elevated cAMP levels [55]. Mahloogi et al. used also murine cSCC cells and reported that ectopic expression of VILIP-1 in high-grade SCC lines that did not express VILIP-1 increased cAMP levels, decreased MMP9 and RhoA activity, reducing the invasiveness of the SCC cells [56]. Gonzalez Guerrico et al. suggested that VILIP-1 reduces cell adhesiveness, migration, and invasiveness thorough decreasing fibronectin-specific integrin [57].

In spite of the majority of studies gathering overwhelming proof of EMT implication in cSCC progression, conflicting results by few studies indicate high, predominantly membranous expression of E-cadherin in primary cSCC and cSCC skin metastases. These results dispute the implication of EMT in SCC progression favoring the hypothesis of collective cancer invasion [24]. In this process, adherent cell groups are believed to detach from the primary tumor, favoring malignant transformation and metastasis. Therefore, E-cadherin upregulation is essential to maintain cellular integrity. However, even in this setting, a small number of cells at the leading edge of the adherent cell complex presumably undergo EMT, in order to provide guidance [24].

2.1. Discussions. Different authors have analyzed the role of EMT in cutaneous squamous cell carcinogenesis, using human and animal models, highlighting the expression and activity of epithelial and mesenchymal markers, transcription regulatory factors, and relevant intra- and extracellular pathways.

We identified studies that have investigated EMT contribution to skin SCC mechanisms, current topics of high concern for medical and scientific research. Thus, there is evidence of EMT involvement in actinic keratosis progression into invasive cSCC [58], EMT upregulation in the invasive cSCCs compared to normal skin and with cSCCs *in situ* [30, 59], a particular immunohistochemical pattern of EMT-related protein expression in SCCs [9], and EMT reversion at distant metastasis sites [8, 26].

New insights into the mechanisms of metastasis in SCCs may reveal the distinct contribution of collective cancer invasion and single-cell invasion pathways, in order to optimize the treatment strategy of these patients.

3. EMT in Basal Cell Carcinoma

EMT is also a critical regulator in the progression of cancer metastasis in BCC through SOX2 expression that regulates the EMT processes and proliferation of BCC cells. Some studies showed that overexpression of SOX2 promotes human cancer cell proliferation mainly through promoting migration and invasion via PI3K/AKT by increasing MMP2 expression. In BCC, downregulation of SOX2 leads to low expression of SRPK1 which inhibits the PI3K/AKT signaling pathway decreasing migration and invasion. These data suggest that SRPK1 may be a direct target of SOX2-induced EMT processes in BCC cells as reduced expression of SOX2 may lead to suppression of BCC metastasis (Figure 3). This could be an explanation of why BCCs are usually less aggressive. However, in the very few cases when BCC becomes invasive, it was reported that activation of the PI3K/AKT signaling pathway may abrogate the effects of SOX2 knockdown on BCC cell migration and invasion [60].

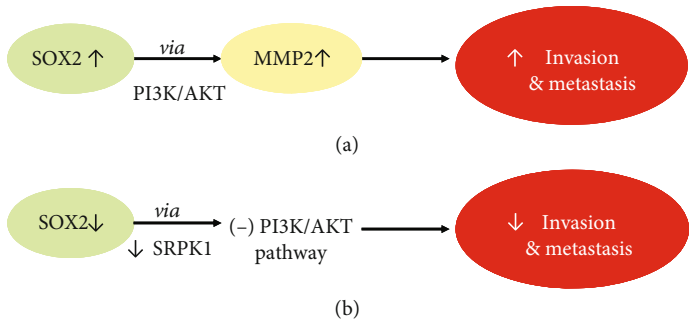

Figure 3: SOX2 pathway in cancer cells (a) vs. BCC cells (b).

Papanikolaou et al. found in all 100 cases of human BCC in their study that ILK (Integrin-Linked Kinase) was overexpressed and it was strongly correlated with tumor invasion and also with EMT features (loss of E-cadherin, Snail, nuclear β-catenin, and α-SMA expression) [61].

Majima et al. showed that tumor cells were positive for Twist1 at the invasive front of the primary tumor, whereas the tumor cells centrally were negative for Twist1.

In nonmetastatic BCC (nodular BCC), tumor cells were Twist1 negative. Double immunofluorescence stains for E-cadherin and N-cadherin showed that E-cadherin was prominently expressed in nodular BCC, whereas this epithelial marker was markedly decreased in the tumor cells of metastatic BCC. For N-cadherin, the tumor cells were negative in nodular BCC and markedly positive in tumor cells at the invasive front of metastatic BCC. Twist1 and N-cadherin were highly expressed in metastatic tumor cells, and E-cadherin expression was markedly decreased in the metastatic tumors. Twist1 is capable of promoting EMT, contributing to aggressive invasion and multiple organ metastases. The expression levels of Snail, a direct transcriptional repressor of E-cadherin, the other transcription factor have been shown to correlate with the depth of tumor invasion in BCC [62].

Tumors of epithelial origin can express transcription factors Snail and Twist1, or the cell adhesion molecule N-cadherin as a mesenchymal marker.

3.1. Discussion. The association of SOX2 expression with the progression of other several human cancer cells has been reported [63–65] but the role of SOX2 in these cancers remains controversial [66] as Yang et al. [67] reported that SOX2 promotes the migration and invasion of laryngeal cancer cells by induction of MMP2 via the PI3K/AKT/mechanistic target of the rapamycin pathway, while Yoon et al. [68] indicated that overexpression of SOX2 is associated with better overall survival in squamous cell lung cancer patients treated with adjuvant radiotherapy.

The SRPK1/PI3K/AKT pathway may be involved in the role of SOX2 in the migration and invasion of BCC cells, and this is why SOX2 may be a novel potential therapeutic target for BCC [60]. SRPK1 is a protein that is dysregulated also in other types of cancer, and this is why SRPK1 inhibition is considered a potential therapeutic target in prostate cancer [69]. A study indicated that SRPK1 has a critical role in the EMT process of human glioblastoma too [70]. It was demonstrated that SRPK1 functions as an oncogene by promoting the activation of PI3K/AKT signaling [71], a pathway involved in the development and progression of human cancer, very well described in lung cancer [72–75].

Meanwhile, knockdown of SOX2 inhibits BCC cell proliferation by upregulating E-cadherin expression and also by lowering vimentin and fibronectin and also by downregulation of the SRPK1-induced EMT signaling pathway [60].

The immunofluorescence assay also confirmed the effects of SOX2 knockdown and overexpression on the epithelial and mesenchymal marker expression levels in BCC cells. SRPK1 overexpression canceled the SOX2 knockdown-inhibited EMT processes of BCC cells. These data suggest that SRPK1 is a direct target of SOX2-induced EMT processes in BCC cells [60].

Aberrant expression of E-cadherin, nuclear beta-catenin, and alpha-SMA correlated with BCC tumor invasion.

In BCC expression, levels of Snail were correlated with the depth of tumor invasion, whereas in cSCC, there is no significant expression of Snail.

4. EMT in Melanoma

Melanocytes are cells derived from neuroectoderm, and during their migration to the epidermis, in the fetal period, they undergo numerous changes similar to EMT-MET ones, thus preserving some particularities. Because of their different origin than the other epidermal cells, melanoma cells were observed to experience a distinct EMT development than other tumor cells derived from the epidermis.

Normally, keratinocytes keep melanocytes from leaving the epidermis through E-cadherin, which is a cell-cell adhesion molecule not present between melanocytes [76, 77]. This molecule is no longer expressed when melanoma cells leave the epidermal layer, suggesting that they lose their epidermal properties, acquiring new specific mesenchymal changes which promote melanoma's invasiveness and progression [78–80]. In a study of Diana et al. which included nevi and dysplastic nevi, the other cell-cell adhesion molecule involved in the EMT, N-cadherin, was present only in the dermal component, being absent in the epidermal or junctional areas, highlighting the idea that its positivity shows a potential malignant transformation of nevi [81].

In melanomas, it has been observed that when switching from radial growth phase (RGP) to vertical growth phase

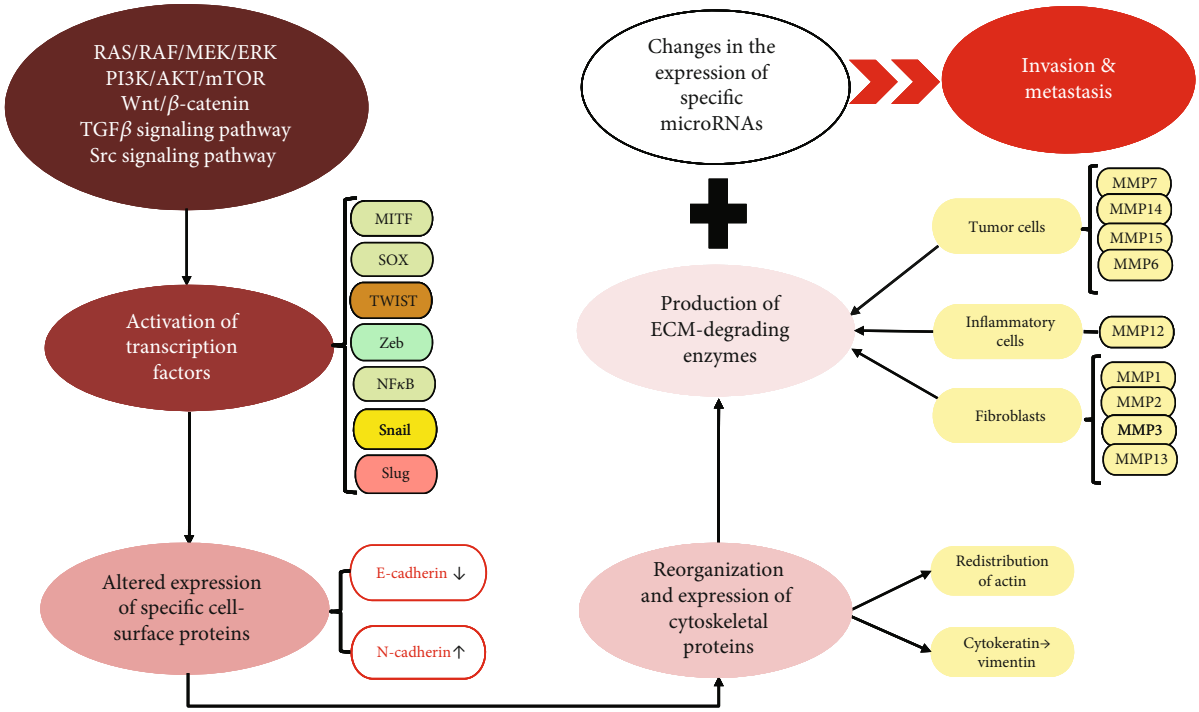

Figure 4: Progression, migration, and invasion pathways in melanoma EMT.

(VGP), there is downregulation of E-cadherin, P-cadherin, and H-cadherin expression, explaining the loss of keratinocyte control over melanoma cells. This is how melanoma cells are gaining properties to evade the epidermis [82–84]. Melanoma is believed to progress characteristically by alternating between proliferative and invasive states, the presence of both types of melanoma cell phenotypes in the same tumor being the main argument [85, 86].

Several pathways are incriminated in the EMT: RAS/-RAF/MEK/ERK, PI3K/AKT/mTOR, Wnt/β-catenin, and Transforming Growth Factor β (TGFβ), Src—and subsequently their effectors, transcription factors—such as microphthalmia-associated transcription factor (MITF), SOX Family, Snail, Slug, Twist, Zeb, and NFκB [2]. An illustrative overview of the progression, migration, and invasion pathways and key points in the EMT of melanoma is described in Figure 4.

Caramel et al. proved in their study that there is a switch in EMT transcription factors between Snail2 and Zeb2 which are found in normal melanocytes and Zeb1 and Twist1 seen in melanoma, changes acquired through the MEK/ERK oncogenic pathway. These modifications were seen to be happening gradually starting from the superficial areas of the melanomas, respectively, from the cortical area of the lymph node metastases (Zeb2/Snail2 positive and Zeb1/-Twist1 negative) until the deeper parts of the tumor and the medullar part of the metastatic lymph nodes (Zeb2/Snail2 negative and Zeb1/Twist1 positive) [87].

MITF, which is a transcription factor involved in melanocyte development and differentiation, was found to be regulated by Zeb2. The switch between Zeb2 and Zeb1 inside the melanoma was observed to be correlated with reduced expression of MITF and consequently with tumor progression [87, 88].

The interaction between beta-catenin, lymphoid enhancer-binding factor 1 (LEF1), and transcription factor 4 (TCF4) was studied in order to see its effects on melanoma. It was observed that the increase in TCF4 and decrease in LEF1 was associated with an invasive transformation of melanoma, in contrast with epithelial tumors where the upregulation of beta-catenin interaction factor LEF1 was seen to promote EMT [89, 90]. The Wnt signaling pathway controls the complex beta-catenin/LEF1 resulting in the regulation of MITF [91].

Another pathway believed to be involved in the EMT of melanoma cells is the oncogenic Notch pathway; however, therapy with Notch inhibitors was not effective on metastatic melanoma until now [92]. The Notch1 signaling pathway increases N-cadherin expression in mesenchymal melanoma cells. As a consequence, the malignant melanocytes acquire a more aggressive phenotype by increasing their invasiveness. Diana et al. observed that while a high presence of Notch1 or N-cadherin alone in the melanoma or the metastases did not bring any significant correlations with overall survival of the patients. The high expression of both Notch1 and N-cadherin in the same lesions correlated with poor prognosis. The authors suggest that this coexpression should be taken into account as a prognostic factor for melanoma patients [81].

In contrast to Notch1, Notch4 was seen to induce opposite changes in melanoma cells, reexpressing epithelial markers (MET-like changes as explained in the introduction). By increasing E-cadherin expression and decreasing Snail2, Twist, vimentin, and MMP2 expression, Notch4

reverts the progressive and invasive pattern of EMT and succeeds in tumor suppression. This is believed to be a reason for the yet unsuccessful therapy with Notch inhibitors [93].

Podoplanin, introduced in the paragraph about SCC, is another researched molecule believed to play different roles in the EMT of some cancers, by losing epithelial-specific markers such as E-cadherin and gaining mesenchymal markers, among which are N-cadherin and fibronectin [94]. It was found to be absent in normal melanocytes and fibroblasts, but present in almost 69% of the melanoma patients studied by Kan et al., yet without any significant correlation with tumor progression or overall survival. However, they observed a worse prognosis and a higher risk for metastases for melanomas with podoplanin positive tumor-associated fibroblasts, suggesting its role as a potential prognostic marker and therapeutic target [95].

As far as immune cells and EMT are concerned, melanoma cells exhibit an interesting particularity. When the change in phenotype is acquired, epithelial to mesenchymal, tumor antigens modify, thus escaping immune surveillance. This is why it is important to target antigens which are common to both epithelial and mesenchymal phenotypes when trying to develop immune therapy [96]. NK cells were seen to promote the change from the proliferative to the invasive state [97]. TAM induce EMT through TGFβ by secreting IL6, IL1, TNFα, and MMPs [98].

Moreover, in order for the EMT to take place, melanoma cells are required to pass through the basement membrane and ECM (extracellular matrix). Specific molecules which have the role of degrading proteins are called matrix metalloproteinases and are released in the tumor environment by tumor cells (MMP7, 14, 15, and 16), inflammatory cells (MMP12), and fibroblasts (MMP1, 2, 3, and 13). When their enzymatic activity surpasses their inhibitors, called tissular inhibitors of matrix metalloproteinase (TIMPs), they favor tumor cells acquire invasiveness and aggressiveness facilitating EMT. As far as TIMPs are concerned, it was observed that an increase in their expression is correlated with a decrease in melanoma's invasiveness [99–102].

60% of melanomas have V600E BRAF mutation. Vemurafenib is a competitive kinase inhibitor with activity against BRAF kinase with V600E that interrupts the B-Raf/MEK step on the B-Raf/MEK/ERK pathway (Figure 4). Patients treated with Vemurafenib had a median overall survival of 14 to 16 months, which significantly improved comparing to the classical treatments, that induced a median survival period of 6 to 10 months [103]. Frequently, after an initial response, the disease progresses due to MEK reactivation [104].

It has been suggested for the melanoma patients who are resistant to Vemurafenib to associate an inhibitor of the EMT. This approach was proposed after noticing cell migration and phenotype switching in these drug-resistant patients while only suppressing the oncogenic signaling pathway BRAF is not sufficient. The targeted molecule was TGFβ, and its inhibition associated with Vemurafenib was seen to defeat the resistance [105]. Other suggestions of future therapies involving EMT were changing the phenotype of melanoma cells to a targetable one or by shifting the cadherin switch [106, 107].

5. Discussion

The differences between melanoma and keratinocyte carcinomas highlight the idea that EMT is a polymorphic and distinct phenomenon with element characteristic to each type of tumor in order to best integrate the cells in the microenvironment [108].

It is assumed that melanoma can metastasize faster than other malignant skin tumors, being more aggressive because normal melanocytes possess from the start elements which contribute to EMT, such as vimentin or some transcription factors (SNAIL2, ZEB2) [109, 110].

Melanoma is a sneaky tumor which has a dynamic character changing back and forth between phenotypes. Due to the unique diversity and plasticity of melanoma cells, it is hard to treat this skin tumor and to foresee its evolution and prognosis.

6. Conclusion

Further studies are needed to assess the onset time of EMT during the process of cutaneous carcinogenesis. Summarizing, the EMT process may influence each stage of skin carcinogenesis, from premalignant changes to distant macrometastatic tumorigenesis. The rationale of inhibiting EMT or inhibiting the reversion of EMT during therapeutic management should also be clarified.

Although the loss of E-cadherin is a critical step in EMT, alone it is not necessarily sufficient to drive EMT.

In this paper, we have focused on the involvement of epithelial-mesenchymal transition in skin cancer mechanisms. We discussed the role of EMT events in cutaneous melanoma, basal cell carcinoma, and squamous cell carcinoma. The results are suggestive rather than conclusive regarding the pathogenic contribution of EMT in different skin neoplasia pathways. The relative paucity of the scientific literature on this topic, the quantitative and qualitative limitations of some studies lead to the need for further insights in order to decipher: EMT contribution to the natural history of cutaneous malignancies, the potential use of EMT markers for an optimized diagnostic staging, and the relevance of the therapeutic modulation of EMT steps.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] R. Kalluri and A. Robert, "The basics of epithelial-mesenchymal transition," *Journal of Clinical Investigation*, vol. 119, no. 6, pp. 1420–1428, 2009.

[2] R. L. Pearlman, M. K. Montes de Oca, H. C. Pal, and F. Afaq, "Potential therapeutic targets of epithelial-mesenchymal transition in melanoma," *Cancer Letters*, no. 391, pp. 125–140, 2017.

[3] A. Hollestelle, J. K. Peeters, M. Smid et al., "Loss of E-cadherin is not a necessity for epithelial to mesenchymal transition in human breast cancer,," *Breast Cancer Research and Treatment*, vol. 138, no. 1, pp. 47–57, 2013.

[4] J. Hülsken, W. Birchmeier, and J. Behrens, "E-cadherin and APC compete for the interaction with beta-catenin and the cytoskeleton," *The Journal of Cell Biology*, vol. 127, no. 6, pp. 2061–2069, 1994.

[5] S. Grünert, M. Jechlinger, and H. Beug, "Diverse cellular and molecular mechanisms contribute to epithelial plasticity and metastasis," *Nature Reviews Molecular Cell Biology*, vol. 4, no. 8, pp. 657–665, 2003.

[6] D. S. Micalizzi, S. M. Farabaugh, and H. L. Ford, "Epithelial-mesenchymal transition in cancer: parallels between normal development and tumor progression," *Journal of Mammary Gland Biology and Neoplasia*, vol. 15, no. 2, pp. 117–134, 2010.

[7] K. Vleminckx, L. Vakaet Jr., M. Mareel, W. Fiers, and F. van Roy, "Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role," *Cell*, vol. 66, no. 1, pp. 107–119, 1991.

[8] J. P. Thiery, "Epithelial-mesenchymal transitions in tumour progression," *Nature Reviews Cancer*, vol. 2, no. 6, pp. 442–454, 2002.

[9] K. Sasaki, T. Sugai, K. Ishida et al., "Analysis of cancer-associated fibroblasts and the epithelial-mesenchymal transition in cutaneous basal cell carcinoma, squamous cell carcinoma, and malignant melanoma," *Human Pathology*, vol. 79, pp. 1–8, 2018.

[10] H. Peinado, D. Olmeda, and A. Cano, "Snail, ZEB and bHLH factors in tumour progression: an alliance against the epithelial phenotype?," *Nature Reviews Cancer*, vol. 7, no. 6, pp. 415–428, 2007.

[11] J. Yang and R. A. Weinberg, "Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis," *Developmental Cell*, vol. 14, no. 6, pp. 818–829, 2008.

[12] G. Moreno-Bueno, F. Portillo, and A. Cano, "Transcriptional regulation of cell polarity in EMT and cancer," *Oncogene*, vol. 27, no. 55, pp. 6958–6969, 2008.

[13] E. L. Whiteman, C. J. Liu, E. R. Fearon, and B. Margolis, "The transcription factor snail represses *Crumbs3* expression and disrupts apico-basal polarity complexes," *Oncogene*, vol. 27, no. 27, pp. 3875–3879, 2008.

[14] S. Spaderna, O. Schmalhofer, M. Wahlbuhl et al., "The transcriptional repressor ZEB1 promotes metastasis and loss of cell polarity in cancer," *Cancer Research*, vol. 68, no. 2, pp. 537–544, 2008.

[15] B. Ozdamar, R. Bose, M. Barrios-Rodiles, H. R. Wang, Y. Zhang, and J. L. Wrana, "Regulation of the polarity protein Par6 by *TGFß Receptors* Controls Epithelial Cell Plasticity," *Science*, vol. 307, no. 5715, pp. 1603–1609, 2005.

[16] D. C. Radisky, D. D. Levy, L. E. Littlepage et al., "Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability," *Nature*, vol. 436, no. 7047, pp. 123–127, 2005.

[17] K. Briegel, "Embryonic transcription factors in human breast cancer," *IUBMB Life (International Union of Biochemistry and Molecular Biology: Life)*, vol. 58, no. 3, pp. 123–132, 2006.

[18] A. Chen, H. Beetham, M. A. Black et al., "E-cadherin loss alters cytoskeletal organization and adhesion in non-malignant breast cells but is insufficient to induce an epithelial-mesenchymal transition," *BMC Cancer*, vol. 14, no. 1, 2014.

[19] M. A. Huber, N. Kraut, and H. Beug, "Molecular requirements for epithelial-mesenchymal transition during tumor progression," *Current Opinion in Cell Biology*, vol. 17, no. 5, pp. 548–558, 2005.

[20] P. Savagner, D. F. Kusewitt, E. A. Carver et al., "Developmental transcription factor slug is required for effective re-epithelialization by adult keratinocytes," *Journal of Cellular Physiology*, vol. 202, no. 3, pp. 858–866, 2005.

[21] J. P. Thiery, H. Acloque, R. Y. J. Huang, and M. A. Nieto, "Epithelial-mesenchymal transitions in development and disease," *Cell*, vol. 139, no. 5, pp. 871–890, 2009.

[22] P. Friedl, J. Locker, E. Sahai, and J. E. Segall, "Classifying collective cancer cell invasion," *Nature Cell Biology*, vol. 14, no. 8, pp. 777–783, 2012.

[23] A. Wicki, F. Lehembre, N. Wick, B. Hantusch, D. Kerjaschki, and G. Christofori, "Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton," *Cancer Cell*, vol. 9, no. 4, pp. 261–272, 2006.

[24] K. Hesse, I. Satzger, V. Schacht et al., "Characterisation of prognosis and invasion of cutaneous squamous cell carcinoma by podoplanin and E-cadherin expression," *Dermatology*, vol. 232, no. 5, pp. 558–565, 2017.

[25] K. Garber, "Epithelial-to-mesenchymal transition is important to metastasis, but questions remain," *Journal of the National Cancer Institute*, vol. 100, no. 4, pp. 232–239, 2008.

[26] J. H. Tsai, J. L. Donaher, D. A. Murphy, S. Chau, and J. Yang, "Spatiotemporal Regulation of Epithelial-Mesenchymal Transition Is Essential for Squamous Cell Carcinoma Metastasis," *Cancer Cell*, vol. 22, no. 6, pp. 725–736, 2012.

[27] M. Neagu, C. Caruntu, C. Constantin et al., "Chemically induced skin carcinogenesis: updates in experimental models (Review)," *Oncology Reports*, vol. 35, no. 5, pp. 2516–2528, 2016.

[28] E. D. Hay, "An overview of epithelio-mesenchymal transformation," *Cells Tissues Organs*, vol. 154, no. 1, pp. 8–20, 1995.

[29] P. Slootweg and S. Zurac, "Prognostic and predictive value of epithelial to mesenchymal transitionassociated markers in oral squamous cell carcinoma," *Current Proteomics*, vol. 10, no. 3, pp. 218–227, 2013.

[30] T. J. Jang, "Epithelial to mesenchymal transition in cutaneous squamous cell carcinoma is correlated with COX-2 expression but not with the presence of stromal macrophages or CD10-expressing cells," *Virchows Archiv*, vol. 460, no. 5, pp. 481–487, 2012.

[31] F. H. Brembeck, M. Rosário, and W. Birchmeier, "Balancing cell adhesion and Wnt signaling, the key role of β-catenin," *Current Opinion in Genetics and Development*, vol. 16, no. 1, pp. 51–59, 2006.

[32] Y. J. Lan, H. Chen, J. Q. Chen, Q. H. Lei, M. Zheng, and Z. R. Shao, "Immunolocalization of vimentin, keratin 17, Ki-67, involucrin, β-catenin and E-cadherin in cutaneous squamous cell carcinoma," *Pathology & Oncology Research*, vol. 20, no. 2, pp. 263–266, 2014.

[33] A. Toll, E. Masferrer, M. E. Hernández-Ruiz et al., "Epithelial to mesenchymal transition markers are associated with an increased metastatic risk in primary cutaneous squamous cell carcinomas but are attenuated in lymph node metastases," *Journal of Dermatological Science*, vol. 72, no. 2, pp. 93–102, 2013.

[34] D. L. V. Vinicius, C. Scapulatempo, N. M. Perpetuo et al., "Prognostic and risk factors in patients with locally advanced cutaneous squamous cell carcinoma of the trunk and extremities," *Journal of Skin Cancer*, vol. 2011, 9 pages, 2011.

[35] S. Brouxhon, S. Kyrkanides, M. K. O'Banion et al., "Sequential down-regulation of E-cadherin with squamous cell carcinoma progression: loss of E-cadherin via a prostaglandin E 2 -EP2-dependent posttranslational mechanism," *Cancer Research*, vol. 67, no. 16, pp. 7654–7664, 2007.

[36] A. Lyakhovitsky, A. Barzilai, M. Fogel, H. Trau, and M. Huszar, "Expression of E-cadherin and beta-catenin in cutaneous squamous cell carcinoma and its precursors," *The American Journal of Dermatopathology*, vol. 26, no. 5, pp. 372–378, 2004.

[37] S. Koseki, T. Aoki, S. Ansai, Y. Hozumi, Y. Mitsuhashi, and S. Kondo, "An immunohistochemical study of E-cadherin expression in human squamous cell carcinoma of the Skin," *The Journal of Dermatology*, vol. 26, no. 7, pp. 416–422, 1999.

[38] M. C. Karlsson, S. F. Gonzalez, J. Welin, and J. Fuxe, "Epithelial-mesenchymal transition in cancer metastasis through the lymphatic system," *Molecular Oncology*, vol. 11, no. 7, pp. 781–791, 2017.

[39] S. J. Serrano-Gomez, M. Maziveyi, and S. K. Alahari, "Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications," *Molecular Cancer*, vol. 15, no. 1, 2016.

[40] H. Li, X. Fan, and J. M. Houghton, "Tumor microenvironment: the role of the tumor stroma in cancer," *Journal of Cellular Biochemistry*, vol. 101, no. 4, pp. 805–815, 2007.

[41] S. H. Omland, E. E. Wettergren, S. Mollerup et al., "Cancer associated fibroblasts (CAFs) are activated in cutaneous basal cell carcinoma and in the peritumoural skin," *BMC Cancer*, vol. 17, no. 1, p. 675, 2017.

[42] B. Zhou, W. L. Chen, Y. Y. Wang et al., "A role for cancer-associated fibroblasts in inducing the epithelial-to-mesenchymal transition in human tongue squamous cell carcinoma," *Journal of Oral Pathology & Medicine*, vol. 43, no. 8, pp. 585–592, 2014.

[43] R. Dong, Q. Wang, X. L. He, Y. K. Chu, J. G. Lu, and Q. J. Ma, "Role of nuclear factor kappa B and reactive oxygen species in the tumor necrosis factor-a-induced epithelial-mesenchymal transition of MCF-7 cells," *Brazilian Journal of Medical and Biological Research*, vol. 40, no. 8, pp. 1071–1078, 2007.

[44] M. Takahara, S. Chen, M. Kido et al., "Stromal CD10 expression, as well as increased dermal macrophages and decreased Langerhans cells, are associated with malignant transformation of keratinocytes," *Journal of Cutaneous Pathology*, vol. 36, no. 6, pp. 668–674, 2009.

[45] M. Albrecht, S. Gillen, B. Wilhelm, J. Doroszewicz, and G. Aumüller, "Expression, localization and activity of neutral endopeptidase in cultured cells of benign prostatic hyperplasia and prostate cancer," *Journal of Urology*, vol. 168, no. 1, pp. 336–342, 2002.

[46] K. Iwaya, H. Ogawa, M. Izumi, M. Kuroda, and K. Mukai, "Stromal expression of CD10 in invasive breast carcinoma: a new predictor of clinical outcome," *Virchows Archiv*, vol. 440, no. 6, pp. 589–593, 2002.

[47] R. Kesse-Adu and S. Shousha, "Myoepithelial markers are expressed in at least 29% of oestrogen receptor negative invasive breast carcinoma," *Modern Pathology*, vol. 17, no. 6, pp. 646–652, 2004.

[48] H. Ogawa, K. Iwaya, M. Izumi et al., "Expression of CD10 by stromal cells during colorectal tumor development," *Human Pathology*, vol. 33, no. 8, pp. 806–811, 2002.

[49] T. Nijsten, C. G. Colpaert, P. B. Vermeulen, A. L. Harris, E. Van Marck, and J. Lambert, "Cyclooxygenase-2 expression and angiogenesis in squamous cell carcinoma of the skin and its precursors: a paired immunohistochemical study of 35 cases," *British Journal of Dermatology*, vol. 151, no. 4, pp. 837–845, 2004.

[50] K. Müller-Decker, G. Reinerth, P. Krieg et al., "Prostaglandin-H-synthase isozyme expression in normal and neoplastic human skin," *International Journal of Cancer*, vol. 82, no. 5, pp. 648–656, 1999.

[51] M. Kagoura, M. Toyoda, C. Matsui, and M. Morohashi, "Immunohistochemical expression of cyclooxygenase-2 in skin cancers," *Journal of Cutaneous Pathology*, vol. 28, no. 6, pp. 298–302, 2001.

[52] C. Denkert, M. Köbel, S. Berger et al., "Expression of cyclooxygenase 2 in human malignant melanoma," *Cancer Research*, vol. 61, no. 1, pp. 303–308, 2001.

[53] M. Tsujii and R. N. DuBois, "Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2," *Cell*, vol. 83, no. 3, pp. 493–501, 1995.

[54] Y. Cao, L. Zhang, Y. Kamimura et al., "B7-H1 overexpression regulates epithelial-mesenchymal transition and accelerates carcinogenesis in skin," *Cancer Research*, vol. 71, no. 4, pp. 1235–1243, 2011.

[55] K. Schönrath, A. J. Klein-Szanto, and K. H. Braunewell, "The putative tumor suppressor VILIP-1 counteracts epidermal growth factor-induced epidermal-mesenchymal transition in squamous carcinoma cells," *PLoS ONE*, vol. 7, no. 3, p. e33116, 2012.

[56] H. Mahloogi, A. M. González-Guerrico, R. Lopez de Cicco et al., "Overexpression of the calcium sensor visinin-like protein-1 leads to a cAMP-mediated decrease of in vivo and in vitro growth and invasiveness of squamous cell carcinoma cells," *Cancer Research*, vol. 63, no. 16, pp. 4997–5004, 2003.

[57] A. M. Gonzalez Guerrico, Z. M. Jaffer, R. E. Page, K. H. Braunewell, J. Chernoff, and A. J. P. Klein-Szanto, "Visinin-like protein-1 is a potent inhibitor of cell adhesion and migration in squamous carcinoma cells," *Oncogene*, vol. 24, no. 14, pp. 2307–2316, 2005.

[58] X. Saenz-Sardà, C. Carrato, L. Pérez-Roca et al., "Epithelial-to-mesenchymal transition contributes to invasion in squamous cell carcinomas originated from actinic keratosis through the differentiated pathway, whereas proliferation plays a more significant role in the classical pathway," *Journal of the European Academy of Dermatology and Venereology*, vol. 32, no. 4, pp. 581–586, 2018.

[59] K. Barrette, S. van Kelst, J. Wouters et al., "Epithelial-mesenchymal transition during invasion of cutaneous squamous cell carcinoma is paralleled by AKT activation," *British Journal of Dermatology*, vol. 171, no. 5, pp. 1014–1021, 2014.

[60] Z.-R. Li, Y. Jiang, J.-Z. Hu, Y. Chen, and Q.-Z. Liu, "SOX2 knockdown inhibits the migration and invasion of basal cell carcinoma cells by targeting the SRPK1-mediated PI3K/AKT

signaling pathway," *Oncology Letters*, vol. 17, no. 2, pp. 1617–1625, 2019.

[61] S. Papanikolaou, V. Bravou, K. Gyftopoulos, D. Nakas, M. Repanti, and H. Papadaki, "ILK expression in human basal cell carcinoma correlates with epithelial-mesenchymal transition markers and tumour invasion," *Histopathology*, vol. 56, no. 6, pp. 799–809, 2010.

[62] Y. Majima, S. Hirakawa, Y. Kito et al., "Twist1 as a possible biomarker for metastatic basal cell carcinoma," *Acta Dermato-Venereologica*, vol. 92, no. 6, pp. 621-622, 2012.

[63] X. F. Liu, W. T. Yang, R. Xu, J. T. Liu, and P. S. Zheng, "Cervical cancer cells with positive Sox2 expression exhibit the properties of cancer stem cells," *PLoS ONE*, vol. 9, no. 1, p. e87092, 2014.

[64] M. Herreros-Villanueva, J. S. Zhang, A. Koenig et al., "SOX2 promotes dedifferentiation and imparts stem cell-like features to pancreatic cancer cells," *Oncogenesis*, vol. 2, no. 8, p. e61, 2013.

[65] S. Chen, Y. Xu, Y. Chen et al., "SOX2 gene regulates the transcriptional network of oncogenes and affects tumorigenesis of human lung cancer cells," *PLoS ONE*, vol. 7, no. 5, 2012.

[66] K. Weina and J. Utikal, "SOX2 and cancer: current research and its implications in the clinic," *Clinical and Translational Medicine*, vol. 3, no. 1, p. 19, 2014.

[67] N. Yang, L. Hui, Y. Wang, H. Yang, and X. Jiang, "SOX2 promotes the migration and invasion of laryngeal cancer cells by induction of MMP-2 via the PI3K/Akt/mTOR pathway," *Oncology Reports*, vol. 31, no. 6, pp. 2651–2659, 2014.

[68] H. I. Yoon, K. H. Park, E. J. Lee et al., "Overexpression of SOX2 is associated with better overall survival in squamous cell lung cancer patients treated with adjuvant radiotherapy," *Cancer Research and Treatment*, vol. 48, no. 2, pp. 473–482, 2016.

[69] A. Mavrou, K. Brakspear, M. Hamdollah-Zadeh et al., "Serine-arginine protein kinase 1 (SRPK1) inhibition as a potential novel targeted therapeutic strategy in prostate cancer," *Oncogene*, vol. 34, no. 33, pp. 4311–4319, 2015.

[70] Z. Liao, J. Wu, M. Wu et al., "Retraction Note to: The Critical Role of SRPK1 in EMT of Human Glioblastoma in the Spinal Cord," *Molecular Neurobiology*, vol. 54, no. 10, p. 8459, 2017.

[71] Y. Chang, Q. Wu, T. Tian et al., "The influence of SRPK1 on glioma apoptosis, metastasis, and angiogenesis through the PI3K/Akt signaling pathway under normoxia," *Tumor Biology*, vol. 36, no. 8, pp. 6083–6093, 2015.

[72] J.-F. Liu, Y.-T. Tsao, and C.-H. Hou, "Fractalkine/CX3CL1 induced intercellular adhesion molecule-1-dependent tumor metastasis through the CX3CR1/PI3K/Akt/NF-κB pathway in human osteosarcoma," *Oncotarget*, vol. 8, no. 33, pp. 54136–54148, 2017.

[73] H. Cui, S. Wu, Y. Shang et al., "*Pleurotus nebrodensis* polysaccharide(PN50G) evokes A549 cell apoptosis by the ROS/AMPK/PI3K/AKT/mTOR pathway to suppress tumor growth," *Food & Function*, vol. 7, no. 3, pp. 1616–1627, 2016.

[74] Y. Sun, Y. Tu, L. He, C. Ji, and B. Cheng, "High mobility group box 1 regulates tumor metastasis in cutaneous squamous cell carcinoma via the PI3K/AKT and MAPK signaling pathways," *Oncology Letters*, vol. 11, no. 1, pp. 59–62, 2016.

[75] J. Meng, X. T. Zhang, X. L. Liu et al., "WSTF promotes proliferation and invasion of lung cancer cells by inducing EMT via PI3K/Akt and IL-6/STAT3 signaling pathways," *Cellular Signalling*, vol. 28, no. 11, pp. 1673–1682, 2016.

[76] J. Pérez-Losada, M. Sánchez-Martín, M. Pérez-Caro, P. A. Pérez-Mancera, and I. Sánchez-García, "The radioresistance biological function of the SCF/kit signaling pathway is mediated by the zinc-finger transcription factor Slug," *Oncogene*, vol. 22, no. 27, pp. 4205–4211, 2003.

[77] P. Pla, R. Moore, O. G. Morali et al., "Cadherins in neural crest cell development and transformation," *Journal of Cellular Physiology*, vol. 189, no. 2, pp. 121–132, 2001.

[78] A. J. Miller and M. C. Mihm Jr., "Melanoma," *New England Journal of Medicine*, vol. 355, no. 1, pp. 51–65, 2006.

[79] M. Y. Hsu, F. E. Meier, M. Nesbit et al., "E-cadherin expression in melanoma cells restores keratinocyte-mediated growth control and down-regulates expression of invasion-related adhesion receptors," *The American Journal of Pathology*, vol. 156, no. 5, pp. 1515–1525, 2000.

[80] J. E. Kim, E. Leung, B. C. Baguley, and G. J. Finlay, "Heterogeneity of expression of epithelial-mesenchymal transition markers in melanocytes and melanoma cell lines," *Frontiers in Genetics*, vol. 4, 2013.

[81] D. Murtas, C. Maxia, A. Diana et al., "Role of epithelial–mesenchymal transition involved molecules in the progression of cutaneous melanoma," *Histochemistry and Cell Biology*, vol. 148, no. 6, pp. 639–649, 2017.

[82] K. Hoek, D. L. Rimm, K. R. Williams et al., "Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas," *Cancer Research*, vol. 64, no. 15, pp. 5270–5282, 2004.

[83] R. Bauer, R. Hein, and A. K. Bosserhoff, "A secreted form of P-cadherin is expressed in malignant melanoma," *Experimental Cell Research*, vol. 305, no. 2, pp. 418–426, 2005.

[84] S. Kuphal, A. C. Martyn, J. Pedley et al., "H-Cadherin expression reduces invasion of malignant melanoma," *Pigment Cell & Melanoma Research*, vol. 22, no. 3, pp. 296–306, 2009.

[85] N. Fenouille, G. Robert, M. Tichet et al., "The p53/p21Cip1/Waf1 pathway mediates the effects of SPARC on melanoma cell cycle progression," *Pigment Cell & Melanoma Research*, vol. 24, no. 1, pp. 219–232, 2011.

[86] M. Rodriguez, E. Aladowicz, L. Lanfrancone, and C. R. Goding, "Tbx3 represses E-cadherin expression and enhances melanoma invasiveness," *Cancer Research*, vol. 68, no. 19, pp. 7872–7881, 2008.

[87] J. Caramel, E. Papadogeorgakis, L. Hill et al., "A Switch in the Expression of Embryonic EMT-Inducers Drives the Development of Malignant Melanoma," *Cancer Cell*, vol. 24, no. 4, pp. 466–480, 2013.

[88] G. Denecker, N. Vandamme, O. Akay et al., "Identification of a ZEB2-MITF-ZEB1 transcriptional network that controls melanogenesis and melanoma progression," *Cell Death and Differentiation*, vol. 21, no. 8, pp. 1250–1261, 2014.

[89] K. Kim, Z. Lu, and E. D. Hay, "Direct evidence for a role of β-catenin/LEF-1 signaling pathway in induction of EMT," *Cell Biology International*, vol. 26, no. 5, pp. 463–476, 2002.

[90] O. M. Eichhoff, A. Weeraratna, M. C. Zipser et al., "Differential LEF1 and TCF4 expression is involved in melanoma cell phenotype switching," *Pigment Cell & Melanoma Research*, vol. 24, no. 4, pp. 631–642, 2011.

[91] L. Larue, "The WNT/Beta-catenin pathway in melanoma," *Frontiers in Bioscience*, vol. 11, no. 1, p. 733, 2006.

[92] S. M. Lee, J. Moon, B. G. Redman et al., "Phase 2 study of RO4929097, a gamma-secretase inhibitor, in metastatic melanoma: SWOG 0933," *Cancer*, vol. 121, no. 3, pp. 432–440, 2015.

[93] E. B. Rad, H. Hammerlindl, C. Wels et al., "Notch4 signaling induces a Mesenchymal–Epithelial–like transition in melanoma cells to suppress malignant behaviors," *Cancer Research*, vol. 76, no. 7, pp. 1690–1697, 2016.

[94] E. Martin-Villar, D. Megias, S. Castel, M. M. Yurrita, S. Vilaro, and M. Quintanilla, "Podoplanin binds ERM proteins to activate RhoA and promote epithelial-mesenchymal transition," *Journal of Cell Science*, vol. 119, no. 21, pp. 4541–4553, 2006.

[95] S. Kan, E. Konishi, T. Arita et al., "Podoplanin expression in cancer-associated fibroblasts predicts aggressive behavior in melanoma," *Journal of Cutaneous Pathology*, vol. 41, no. 7, pp. 561–567, 2014.

[96] K. Woods, A. Pasam, A. Jayachandran, M. C. Andrews, and J. Cebon, "Effects of Epithelial to Mesenchymal Transition on T Cell Targeting of Melanoma Cells," *Frontiers in Oncology*, vol. 4, pp. 1–7, 2014.

[97] L. Huergo-Zapico, M. Parodi, C. Cantoni et al., "NK-cell editing mediates epithelial-to-mesenchymal transition via phenotypic and proteomic changes in melanoma cell lines," *Cancer Research*, vol. 78, no. 14, pp. 3913–3925, 2018.

[98] P. J. Chockley and V. G. Keshamouni, "Immunological consequences of epithelial–mesenchymal transition in tumor progression," *Journal of Immunology*, vol. 197, no. 3, pp. 691–698, 2016.

[99] A. Brînzea, R. I. Nedelcu, G. Turcu, M. Antohe, S. A. Zurac, and D. A. Ion, "TIMPs expression in lentigo maligna/lentigo maligna melanoma versus aged skin–a review of the literature and personal experience," *Romanian Journal of Morphology and Embryology*, vol. 58, no. 3, pp. 717–721, 2017.

[100] S. Zurac, M. Neagu, C. Constantin et al., "Variations in the expression of TIMP1, TIMP2 and TIMP3 in cutaneous melanoma with regression and their possible function as prognostic predictors," *Oncology Letters*, vol. 11, no. 5, pp. 3354–3360, 2016.

[101] V. Thakur and B. Bedogni, "The membrane tethered matrix metalloproteinase MT1-MMP at the forefront of melanoma cell invasion and metastasis," *Pharmacological Research*, vol. 111, pp. 17–22, 2016.

[102] J. Xu, S. Lamouille, and R. Derynck, "TGF-B-induced epithelial to mesenchymal transition," *Cell Research*, vol. 19, no. 2, pp. 156–172, 2009.

[103] A. Ribas, K. B. Kim, L. M. Schuchter et al., "BRIM-2: an open-label, multicenter phase II study of vemurafenib in previously treated patients withBRAFV600E mutation-positive metastatic melanoma," *Journal of Clinical Oncology*, vol. 29, 15_suppl, p. 8509, 2011.

[104] G. A. McArthur, A. Ribas, P. B. Chapman et al., "Molecular analyses from a phase I trial of vemurafenib to study mechanism of action (MOA) and resistance in repeated biopsies from BRAF mutation–positive metastatic melanoma patients (pts)," *Journal of Clinical Oncology*, vol. 29, 15_suppl, p. 8502, 2011.

[105] S. Huang, M. Hölzel, T. Knijnenburg et al., "MED12 controls the response to multiple cancer drugs through regulation of TGF-β receptor signaling," *Cell*, vol. 151, no. 5, pp. 937–950, 2012.

[106] T. Singh and S. K. Katiyar, "Green tea catechins reduce invasive potential of human melanoma cells by targeting COX-2, PGE 2 receptors and epithelial-to-mesenchymal transition," *PLoS ONE*, vol. 6, no. 10, p. e25224, 2011.

[107] M. Sáez-Ayala, M. F. Montenegro, L. Sánchez-del-Campo et al., "Directed phenotype switching as an effective antimelanoma strategy," *Cancer Cell*, vol. 24, no. 1, pp. 105–119, 2013.

[108] J. P. Thiery and J. P. Sleeman, "Complex networks orchestrate epithelial-mesenchymal transitions," *Nature Reviews Molecular Cell Biology*, vol. 7, no. 2, pp. 131–142, 2006.

[109] M. Miettinen, V. P. Lehto, and I. Virtanen, "Presence of fibroblast-type intermediate filaments (vimentin) and absence of neurofilaments in pigmented nevi and malignant melanomas," *Journal of Cutaneous Pathology*, vol. 10, no. 3, pp. 188–192, 1983.

[110] P. B. Gupta, C. Kuperwasser, J. P. Brunet et al., "The melanocyte differentiation program predisposes to metastasis after neoplastic transformation," *Nature Genetics*, vol. 37, no. 10, pp. 1047–1054, 2005.

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Review Article

Tumor Microenvironment in Diffuse Large B-Cell Lymphoma: Role and Prognosis

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Diffuse large B-cell lymphoma (DLBCL) represents 30-40% of all non-Hodgkin lymphomas (NHL) and is a disease with an aggressive behavior. Because about one-third of DLBCL patients will be refractory or resistant to standard therapy, several studies focused on identification of new individual prognostic and risk stratification biomarkers and new potential therapeutic targets. In contrast to other types of cancers like carcinomas, where tumor microenvironment was widely investigated, its role in DLBCL pathogenesis and patient survival is still poorly understood, although few studies had promising results. The composition of TME and its interaction with neoplastic cells may explain the role of several genes (beta2-microglobulin gene, CD58 gene), receptor-like programmed cell death-1 (PD-1) and its ligand (PD-L1), or other cell components (Treg) in tumor evasion of immune surveillance, resulting in tumor progression. Also, it was found that "gene expression profile" of the microenvironmental cells, the phenotype of tumor-associated macrophages (TAM), the expression of matricellular proteins like SPARC and fibronectin, the overexpression of several types of matrix metalloproteinases (MMPs) like MMP-2 and MMP-9, or the tissue inhibitors of matrix metalloproteinases (TIMPs) may lead to a favorable or adverse outcome. With this review, we try to highlight the influence of microenvironment components over lymphoid clone progression and their prognostic impact in DLBCL patients.

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) represents about 30-40% of non-Hodgkin lymphomas (NHL) [1]. Although DLBCL demonstrates an aggressive clinical course, using the established rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone (R-CHOP) standard therapy, this neoplasm is curable in 60-70% of cases [1]. However, about one-third of these patients are refractory to this treatment. It is critical for them to find new therapeutic agents that alone or in addition to R-CHOP therapy may help to improve their survival or to provide an alternative for cases that are not eligible, are refractory, or have relapsed [2]. Recently, new molecular findings in DLBCL genetics have shown that these lymphomas comprise a group of disorders with specific signaling programs [1], and their first target was to identify new potential therapies with greater specificity and with lower toxicity [2].

Current research in this field is focused on identification of new individual prognostic and risk stratification biomarkers in order to predict the outcome and therapy response or that could indicate the patients who may be eligible for more aggressive therapies. Also, they may provide new perspective on current and future possible therapies.

Using gene expression profiling (GEP), Alizadeh et al. [3] found that DLBCL may be divided into two biologically and clinically molecular subgroups, with different prognoses and treatment responses. According to cell-of-origin (COO), these were defined as germinal center B-cell (GCB) (40-50%) or activated B-cell (ABC) (50-60%) subtypes [3]. Also,

there was found a small unclassifiable group (10-15%) [3]. ABC DLBCL cases were found to have a poorer outcome than GCB DLBCL patients when treated with the standard therapy, with a 5-year survival of 44% for the ABC subtype and 87-92% for the GCB subtype [4, 5]. A recent discovery based on a new 20-gene assay permitted also the identification of the ABC vs. the GCB subgroup using formalin-fixed and paraffin-embedded tissue, a method which proved to be accurate and robust [6]. In addition, GCB DLBCLs were found to express genes of germinal center B cells, such as *CD10*, *LMO2*, or *BCL6*, and frequently, they were associated with *REL* amplification, *EZH2* mutation, or t(14;18) translocation [3, 7–13]. The pathogenesis of ABC DLBCLs was believed to be related to activation of the NF-κB signaling pathway via the B-cell receptor (BCR) pathway, but recent studies demonstrated that NF-κB may be expressed in both ABC and GCB DLBCL subgroups and is an adverse prognostic factor [7, 12–20]. *TNFAIP3*, *CARD11*, *CD79B*, *CD79A*, *TRAF2*, *MYD88*, and *REL* are the most commonly altered genes with an adverse impact in the ABC DLBCL subtype [7, 12, 13, 17–21].

Recently, several studies have focused on the potential role of the tumor microenvironment (TME) in DLBCL pathogenesis, but the results remained controversial. It is thought that the role of TME is based on the interactions between tumor cells and stromal elements (fibroblast, blood, and lymphatic vessels), extracellular matrixes, inflammatory, and immune cells (mast cells, macrophages, and T or B lymphocytes). The composition and spatial characteristics of the TME and the interaction between its components and lymphoma cells demonstrate significant heterogeneity depending on the type of lymphoma or the tissue or organ in which lymphoma arises and may have an important impact in the patient's survival, therapy response, and disease progression or relapse.

2. Immune Evasion

Immune evasion is a pathogenetic mechanism used by several types of cancers in their evolution, and avoidance of circulating T-lymphocytes (CTL) or the escape from NK cell recognition are the main processes implied. Challa-Malladi et al. [22] concluded that genetic alterations associated with lack of surface HLA-I and inactivation of the *beta2-microglobulin gene* (*B2M*) are present in 29% of DLBCL cases, leading to escape of tumor cells from CTL. *CD58*, the receptor of the natural killer (NK) cells or T cell $CD2^+$, also has an important role in this process. 21% of DLBCLs, more frequently the ABC subtype, were found to have inactivation of the CD58 gene (*CD58*) that is implied in the loss of recognition of tumor cells by CTL and NK cells [22]. They concluded that both events may be coselected during lymphomagenesis and may be regarded as specific pathogenetic mechanisms [22].

Programmed cell death-1 (*PD-1*) is a surface inhibitory receptor expressed by macrophages, dendritic cells, and T cells [23]. After PD-1 binds to PD-L1 (expressed on an antigen-presenting cell (APC) surface), it plays an important role in regulation of immune response by inhibiting cytokine production and cell-cycle progression in T cells [24].

Many studies tried to find the role of PDL-1 in the mechanism of immune evasion of aggressive B-cell lymphomas [25–28]. In DLBCL, PD-L1 expression was found in both tumor cells and microenvironmental cells, primarily macrophages, and had a controversial role [26, 27, 29, 30]. The DLBCL subgroup with PDL-1^+ in tumor cells was associated with unfavorable prognostic factors like the non-GCB subtype, IPI high-risk group, elevated beta2-microglobulin, resistance to standard therapy, and reduced overall survival (OS) compared with the PD-1 negative subgroup [29–32] (see Table 1). On the other side, a favorable OS was seen in cases with PD-1 expression of a large number of tumor-infiltrating lymphocytes [32–34].

PD-$L1^+$ tumor cells have other various mechanisms to escape T cell immune surveillance, the most important of them being the induction of apoptosis in some of the T cells through the PD-1/PD-L1 pathway [35]. Also, the expression of PD-L1 in myeloid dendritic cells (MDCs) induces T cell immune suppression in the tumor microenvironment [36]. Steidl et al. [25] have found that rearrangements of *CIITA* in B-cell lymphomas determine the overexpression of PD-1 and PD-L1 and may also lead to T cell immune avoidance.

Regulatory T cells (*Treg*) are other cellular components of TME that can contribute to the deprival of neoplastic cells from the effect of several proinflammatory stimuli released by nonneoplastic immune or inflammatory cells. One of the main functions of Tregs is the regulation of antitumor immune responses by inhibiting the cytokine production and suppressing the proliferation of $CD8^+$ T cells, which may lead to an ineffective antitumor response and to the proliferation of cancer cells [37–39]. In DLBCL, the prognostic influence of $FOXP3^+$ Treg is controversial, reported as being associated with a good prognosis in some studies or with an adverse outcome [40–42] or a trend toward a worse prognosis in other studies [43].

3. Stromal Gene Signature

Lenz et al. [4] identified a new gene expression profile of the nontumor cells, determining two different subgroups of DLBCL, predictive of survival and outcome in patients treated with R-CHOP [4].

High expression of "stromal-1 signature" was found in tumors with abundant extracellular matrix elements and a high number of macrophages. This subtype encodes elements of extracellular matrix, like osteonectin, various types of collagen and laminin, fibronectin, antiangiogenic factor thrombospondin, connective-tissue growth factor (CTGF), and remodeling proteins (MMP2, MMP9, MMP14, PLAU, and TIMP2) [4]. The "stromal-1" response was associated with a better prognosis [4].

"Stromal-2 signature" encodes markers of endothelial cells (CD31, von Willebrand factor), regulators of angiogenesis (vascular endothelial growth factor (VEGF) receptor, endothelial tyrosine kinase (TEK), and components of caveolae), and genes usually expressed in adipocytes, like *RBP4*, *ADIPOQ*, *PLIN*, and *FABP4* [4]. High expression of this

Table 1: Characteristics of PDL-1$^+$ and mPDL-1$^+$ cases in various retrospective studies.

	Number of patients (n)	Treatment regimens	PDL-1$^+$ tumor cells/mPDL-1$^+$	OS/PFS	Specific results
Kiyasu et al. [29]	1253	R-CHOP/R-CHOP-like, CHOP	(i) *PDL-1$^+$ tumor cells*: 10.5% (threshold ≥ 30%) (ii) *mPDL-1$^+$*: 15.3% (threshold ≥ 20%)	(i) *OS HR*: 1.809 (CI: 1.051-3.112)	(i) *PDL-1$^+$* is a poor prognostic factor; was significantly associated with the presence of B symptoms, IPI high-risk group, elevated serum soluble IL-2 receptor levels, EBV infection, and non-GCB subtype (ii) *mPDL-1$^+$*: was found to be associated with IPI high-risk group, EBV infection, and non-GCB subtype
Hu et al. [30]	204	R-CHOP/R-CHOP-like, CHOP	(i) *PDL-1$^+$ tumor cells*: 49% (threshold ≥ 5%) (ii) *mPDL-1$^+$*: 21.6% (threshold ≥ 20%)	(i) *5-year OS* and *PFS*: 50% vs. 67.3% and 39.6% vs. 59.6%	(i) *PDL-1$^+$* was found to be an independent risk factor for OS; it was associated with elevated beta2-microglobulin, resistance to first-line chemotherapy, and non-GCB subtype (ii) *mPDL-1$^+$*: was associated with first-line chemotherapy resistance
Xing et al. [31]	86	R-CHOP (85%)	(i) *PDL-1$^+$ tumor cells*: 16% (threshold ≥ 30%) (ii) *mPDL-1$^+$*: 27% (threshold ≥ 5%)	(i) *Median OS and PFS*: 21 months and 18.5 months	(i) *PDL-1$^+$* is a statistically significant factor for OS; it was also associated with higher initial staging, greater extralymphatic organ involvement and non-GCB subtype
Fang et al. [32]	76	R-CHOP/R-CHOP-like, surgery, and surgery$^+$ chemotherapy	(i) *PDL-1$^+$ tumor cells*: 26.3%	(i) *OS HR*: 2.547 (CI: 0.964-6.730)	(i) *PDL-1$^+$* cases had a worse clinical outcome; it is not an independent prognostic marker for patients' OS

subtype was correlated with poorer outcome and increased tumor blood vessel density [4].

Several studies tried to develop a new biologic prognostic model (BPM) and modified BPM (mBPM) based on COO and stromal-1 and stromal-2 responses, in order to determine DLBCL progression and response to therapy [44, 45]. The system uses three adverse prognostic markers—expression of SPARC, non-GCB subgroup, and high microvascular density [44, 45]. Cases with low score of BPM and mBPM showed a better survival rate [45] and higher rate of complete response to therapy [44] but without any impact on the patient's OS [44, 45].

Recently, Ciavarella et al. [46] analyzed GEP datasets from 175 cases of DLBCL using the computational method CIBERSORT to identify microenvironmental prognostic genes. Furthermore, they used the NanoString technology on FFPE to asses both TME genes and COO, in order to develop a reproducible assay [46]. They found that cases with higher proportions of myofibroblasts, dendritic cells (DCs), and $CD4^+$ T cells had longer OS, independently of the COO [46]. In contrast, cases with a higher number of activated NK and plasma cells correlated with poorer outcome [46]. When they had integrated the two prognosticators, TME and COO, the survival prediction was improved [46].

4. Tumor-Associated Macrophages

The role of tumor-associated macrophages (TAMs) has been widely studied in the pathogenesis of various cancers, especially because of their controversial role. On the one side, they can kill tumor cells, but on the other side, they may favor tumor growth, invasion, and progression by inducing immunosuppression and synthesis of higher levels of angiogenic factors such as VEGF, interleukin 8 (IL-8), TNF-alfa, metalloproteases, and fibroblast growth factor 1 (FGF-1) [47].

In lymphomas, tumor cells release several soluble mediators, leading to continuous B-cell-receptor (BCR) stimulation and T cell and $CD14^+$ monocyte recruitment and through them, to B-cell abnormal proliferation and rescue from apoptosis [48–51]. Khalifa et al. [52] found that lymphomas with an increased number of $CD14^+$ monocytes and with loss of human leukocyte antigen-DR (HLA-DR) expression were more aggressive and more frequently associated with refractory disease or relapse to treatment.

There are also some discrepancies in the prognosis of TAM in DLBCL outcome, depending on the macrophage phenotype M1 (CD68/HLA-DR) or M2 (CD68/CD163). Riihijärvi et al. [53] found in their study that both $CD68^+$ TAM and CD68 mRNA levels were associated with adverse prognostic factors for OS in patients treated with CHOP, but among patients that were treated with R-CHOP, the prognostic of $CD68^+$ was favorable and the patients had improved OS. Marchesi et al. [54] ($n = 61$), Nam et al. [55] ($n = 165$), and Wada et al. [56] ($n = 101$) also concluded that M2 TAM is a significant factor for poor prognosis, being an independent predictor for shorter OS and PFS. On the other side, in several studies, no significant correlation was found between TAM and patient survival [57–59].

Marinaccio et al. [60] demonstrated opposing roles of inhibition and promotion of angiogenesis based on the M1 and M2 phenotypes of TAM, M1 macrophage having antitumor and antiangiogenic roles, and M2 macrophage acting as immunosuppressive and proangiogenic. Therefore, they concluded that since the expression of CD68 or CD163 is associated with an adverse outcome in patients treated with R-CHOP, double staining for CD68 and CD163 may be a better method of predicting outcomes of DLBCL [60].

Legumain is a cysteine protease, secreted by tumor cells undergoing hypoxia and also by TAM and is thought to have several roles in cancer pathogenesis. In tumors, the overexpression of legumain was found in correlation with angiogenesis, expansion of the tumor, and cleavage of the ECM [61, 62]. *In vitro* experiments of Shen et al. [62] showed first that M2 TAMs induced cleavage of ECM and formation of several vessel tubes demonstrating their proangiogenic role, but subsequent administration of legumain's inhibitors demonstrated that these effects were actually mediated by legumain.

5. Extracellular Matrix

Extracellular matrix (ECM) is composed by a mixture of several proteins, mineral deposits, and proteoglycans, synthesized by stromal cells, and has roles in supporting the cells and regulating intercellular interactions [49, 63, 64]. ECM composition is constantly changing by interactions between its components and different enzymes, contributing to progression of several types of malignancies [4, 49, 65]. Genes coding several ECM components, like collagens, laminin, metalloproteases, and matricellular proteins, were related to "stromal-1 signature" which have been associated with a favorable prognosis in DLBCL [4].

Among matricellular proteins, *SPARC* (secreted protein acidic rich in cysteine), also called osteonectin, is a marker expressed by a subset of macrophages and has ambiguous roles in tumor pathogenesis. It is considered in some studies as a tumor suppressor and in others a tumor promoter, favoring epithelial-to-mesenchymal transition (EMT), tissue invasion, or metastasis, depending on the tissue and cell type [66, 67].

In types of leukemia like myelogenous leukemia with MLL abnormalities that do not express SPARC, in pancreatic carcinoma, or in ovarian carcinoma, SPARC was associated with tumor suppression [68–70]. On the other side, lymphomas and types of leukemia with SPARC overexpression presented increased tumor growth [71]. In DLBCL, SPARC positivity of stromal cells was associated with longer OS and EFS than negative cases [4, 58]. Abdou et al. [44] reported high levels of SPARC in patients with adverse prognostic factors such as splenic involvement, but without any effect on patient overall survival, and concluded that DLBCL TEM could modulate tumor progression behavior.

In DLBCL, Brandt et al. [72] evaluated the expression of *fibronectin* (*Fn1*), another "stromal-1 gene," and SPARC and concluded that combined immunohistochemical assessment of both of them is an important predictor of survival. They reported that "patients with double positive DLBCL had a

significantly longer OS than the negative group and a better association with OS data than the expression of SPARC or Fn1 taken separately" [72].

The expression and synthesis of various types of *matrix metalloproteinases* (*MMPs*) in some aggressive B-cell lymphomas could be determined by neoplastic cell type and by the surrounding environment [72]. *Osteopontin* (*OPN*) is a TME matricellular protein, usually expressed by both normal and cancer cells that was demonstrated to have an important role in tumor invasion and metastasis through its function as a regulator of the enzymatic activity of MMPs [73–77]. Several studies suggested that overexpression of MMP-2 and MMP-9, upon their role in OPN-induced tumor invasion, is associated with an aggressive phenotype of cancers [78–81]. Their role in the dissemination and progression of aggressive NHL was also highlighted in the special literature [75, 82–84].

IL-6 is another promoter of tumorigenesis [85–88], and in addition to OPN, it activates MMP-2 and MMP-9 and stimulates the expression of the tissue inhibitor of metalloproteinase (TIMP) by neoplastic and stromal cells [89]. IL-6 levels were reported to be associated with a poor prognosis in DLBCL [90]. *In vitro* studies conducted by Malaponte et al. [75] demonstrated that OPN, but not IL-6, stimulation was associated with increased MMP-9 and MMP-2 secretion and activation, suggesting that higher levels of IL-6 found in NHL and their role in proliferation, invasion, and migration of lymphoma cells may probably be attributed to the activation of other molecular pathways.

Usually, *tissue inhibitors of metalloproteinases* (*TIMPs*) have roles in maintaining the balance between ECM components through the inhibition of the MMP's activity, but several studies reported contradictory actions, especially in lymphomas, suggesting that they may actually contribute to tumor progression [49, 91, 92].

TIMP-1 is produced by both neoplastic lymphocytes and TME elements [49, 92, 93], and by activating different signaling pathways, it is an inhibitor of germinal center B-cell apoptosis and a promoter of neoplastic cell survival [92, 94]. The antiapoptotic role of TIMP-1, which may contribute to the poor prognosis of aggressive B-cell neoplasms, is determined by the binding of TIMP-1 to a putative cell-surface receptor, independent of its MMP inhibitory function [92].

TIMP-1 expression in DLBCL was assessed by Choi et al. [92], and they reported that it is an independent prognostic marker of poor prognosis and highlighted its possible role in the tumor progression but without any correlation with histogenetic origin or the presence of EBV infection.

6. Vasculogenesis

The angiogenic mechanism in DLBCL could be explained by different interactions of neoplastic cells and TME elements. The role of TAMs and mast cells in tumor progression and angiogenesis was demonstrated by their capacity of releasing several proangiogenic cytokines such as VEGF, IL-8, fibroblast growth factor 2 (FGF-2), and TNF-alfa [60, 95, 96]. Also, mast cells, by their role as regulators of MMPs and plasminogen activator (PA) activity, stimulate the proliferation of endothelial cells and the release of proangiogenic factors [60, 95–98].

Another angiogenic mechanism is related to a "stromal-2 signature" component—CXC chemokine ligand 12 (CXCL12) or stromal-cell-derived factor 1 (SDF-1)—a chemokine that can recruit $CXCR4^+$ endothelial cells from the bone marrow [4, 99, 100]. The angiogenic role of the adipocyte-associated gene expression is related to adipocyte precursors that have the potential to differentiate into endothelial cells [101]. Finally, neoplastic B lymphocytes can also receive directly proliferation and/or survival signals through the overexpression of VEGF receptors [49, 102].

Upon these mechanisms, the relationship between MVD and DLBCL behavior was the object of many studies. High expression of the "stromal-2 gene signature" was found in cases with increased MVD, being correlated with an adverse outcome and with a shorter OS rate [4, 45, 60, 103]. In a cohort of 74 patients with DLBCL, Gomez-Gelvez et al. [43] reported contradictory results, high MVD being associated with better PFS and EFS. In the experiment conducted by Abdou et al. [44], MVD was found to be associated with poor prognostic parameters such as splenic involvement, high mitotic rate, and capsular invasion.

7. Conclusion

Our review of recent literature demonstrates once again that DLBCL is a disease with complex pathogenesis and behavior not only from the perspective of genetic alterations of lymphoid cells but also from the perspective of TME composition and its elements' interactions with neoplastic cells. These findings are important steps in understanding DLBCL pathogenesis and its unpredictable evolution. In addition to current COO classification and other prognostic markers, microenvironment assessment will discriminate better the subsets of patients with worse prognosis leading to the beginning of a new therapeutic era that will allow the administration of personalized therapy.

Patients with aggressive diseases that have relapsed or are refractory to current standard therapy may benefit from novel treatment strategies like antiangiogenic treatments, inhibition of legumain, administration of monoclonal antibodies targeting antigens of the myeloid-lineage cells, or immunotherapy targeting the PD-1/PD-L1 pathway.

Although the understanding of DLBCL biology has improved, the molecular mechanism by which several elements of TME confer aggressiveness is still poorly understood and further studies with larger cohorts and longer follow-up are recommended.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

[1] S. H. Swerdlow, E. Campo, N. L. Harris et al., *World Health Organisation Classification of Tumors of Hematopoietic and Lymphoid Tissues*, IARC Press, Lyon, France, 2008.

[2] L. H. Sehn and R. D. Gascoyne, "Diffuse large B-cell lymphoma: optimizing outcome in the context of clinical and biologic heterogeneity," *Blood*, vol. 125, no. 1, pp. 22–32, 2015.

[3] A. A. Alizadeh, M. B. Eisen, R. E. Davis et al., "Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling," *Nature*, vol. 403, no. 6769, pp. 503–511, 2000.

[4] G. Lenz, G. Wright, S. S. Dave et al., "Stromal gene signatures in large-B-cell lymphomas," *New England Journal of Medicine*, vol. 359, no. 22, pp. 2313–2323, 2008.

[5] W. W. L. Choi, D. D. Weisenburger, T. C. Greiner et al., "A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy," *Clinical Cancer Research*, vol. 15, no. 17, pp. 5494–5502, 2009.

[6] D. W. Scott, G. W. Wright, P. M. Williams et al., "Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue," *Blood*, vol. 123, no. 8, pp. 1214–1217, 2014.

[7] K. Karube, A. Enjuanes, I. Dlouhy et al., "Integrating genomic alterations in diffuse large B-cell lymphoma identifies new relevant pathways and potential therapeutic targets," *Leukemia*, vol. 32, no. 3, pp. 675–684, 2018.

[8] S. L. Barrans, P. A. Evans, S. J. O'Connor et al., "The t(14;18) is associated with germinal center-derived diffuse large B-cell lymphoma and is a strong predictor of outcome," *Clinical Cancer Research*, vol. 9, no. 6, pp. 2133–2139, 2003.

[9] G. Lenz, G. W. Wright, N. C. T. Emre et al., "Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways," *Proceedings of the National Academy of Sciences*, vol. 105, no. 36, pp. 13520–13525, 2008.

[10] R. D. Morin, N. A. Johnson, T. M. Severson et al., "Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin," *Nature Genetics*, vol. 42, no. 2, pp. 181–185, 2010.

[11] J. G. Lohr, P. Stojanov, M. S. Lawrence et al., "Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 10, pp. 3879–3884, 2012.

[12] L. Li, Z. Y. Xu-Monette, C. Y. Ok et al., "Prognostic impact of c-Rel nuclear expression and REL amplification and crosstalk between c-Rel and the p53 pathway in diffuse large B-cell lymphoma," *Oncotarget*, vol. 6, no. 27, pp. 23157–23180, 2015.

[13] L. Pasqualucci, V. Trifonov, G. Fabbri et al., "Analysis of the coding genome of diffuse large B-cell lymphoma," *Nature Genetics*, vol. 43, no. 9, pp. 830–837, 2011.

[14] Q. Zhao, W. Fu, H. Jiang et al., "Clinicopathological implications of nuclear factor κB signal pathway activation in diffuse large B-cell lymphoma," *Human Pathology*, vol. 46, no. 4, pp. 524–531, 2015.

[15] R. E. Davis, K. D. Brown, U. Siebenlist, and L. M. Staudt, "Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells," *Journal of Experimental Medicine*, vol. 194, no. 12, pp. 1861–1874, 2001.

[16] J. Wang, M. Zhou, Q. G. Zhang et al., "Prognostic value of expression of nuclear factor kappa-B/p65 in non-GCB DLBCL patients," *Oncotarget*, vol. 8, no. 6, pp. 9708–9716, 2017.

[17] M. Zhang, Z. Y. Xu-Monette, L. Li et al., "RelA NF-κB subunit activation as a therapeutic target in diffuse large B-cell lymphoma," *Aging*, vol. 8, no. 12, pp. 3321–3340, 2016.

[18] N. Knies, B. Alankus, A. Weilemann et al., "Lymphomagenic CARD11/BCL10/MALT1 signaling drives malignant B-cell proliferation via cooperative NF-κB and JNK activation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 112, no. 52, pp. E7230–E7238, 2015.

[19] M. Compagno, W. K. Lim, A. Grunn et al., "Mutations of multiple genes cause deregulation of NF-κB in diffuse large B-cell lymphoma," *Nature*, vol. 459, no. 7247, pp. 717–721, 2009.

[20] R. E. Davis, V. N. Ngo, G. Lenz et al., "Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma," *Nature*, vol. 463, no. 7277, pp. 88–92, 2010.

[21] A. Rosenwald, G. Wright, W. C. Chan et al., "The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma," *New England Journal of Medicine*, vol. 346, no. 25, pp. 1937–1947, 2002.

[22] M. Challa-Malladi, Y. K. Lieu, O. Califano et al., "Combined genetic inactivation of β2-microglobulin and CD58 reveals frequent escape from immune recognition in diffuse large B cell lymphoma," *Cancer Cell*, vol. 20, no. 6, pp. 728–740, 2011.

[23] G. J. Freeman, A. J. Long, Y. Iwai et al., "Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation," *Journal of Experimental Medicine*, vol. 192, no. 7, pp. 1027–1034, 2000.

[24] M. E. Keir, M. J. Butte, G. J. Freeman, and A. H. Sharpe, "PD-1 and its ligands in tolerance and immunity," *Annual Review of Immunology*, vol. 26, pp. 677–704, 2008.

[25] C. Steidl, S. P. Shah, B. W. Woolcock et al., "MHC class II transactivator *CIITA* is a recurrent gene fusion partner in lymphoid cancers," *Nature*, vol. 471, no. 7338, pp. 377–381, 2011.

[26] D. J. Andorsky, R. E. Yamada, J. Said, G. S. Pinkus, D. J. Betting, and J. M. Timmerman, "Programmed death ligand 1 is expressed by non–Hodgkin lymphomas and inhibits the activity of tumor-associated T cells," *Clinical Cancer Research*, vol. 17, no. 13, pp. 4232–4244, 2011.

[27] B. J. Chen, B. Chapuy, J. Ouyang et al., "PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies," *Clinical Cancer Research*, vol. 19, no. 13, pp. 3462–3473, 2013.

[28] D. D. W. Twa, F. C. Chan, S. Ben-Neriah et al., "Genomic rearrangements involving programmed death ligands are recurrent in primary mediastinal large B-cell lymphoma," *Blood*, vol. 123, no. 13, pp. 2062–2065, 2014.

[29] J. Kiyasu, H. Miyoshi, A. Hirata et al., "Expression of programmed cell death ligand 1 is associated with poor overall survival in patients with diffuse large B-cell lymphoma," *Blood*, vol. 126, no. 19, pp. 2193–2201, 2015.

[30] L. Y. Hu, X. L. Xu, H. L. Rao et al., "Expression and clinical value of programmed cell death-ligand 1 (PD-L1) in diffuse large B cell lymphoma: a retrospective study," *Chinese Journal of Cancer*, vol. 36, no. 1, p. 94, 2017.

[31] W. Xing, K. Dresser, R. Zhang et al., "PD-L1 expression in EBV-negative diffuse large B-cell lymphoma: clinicopathologic features and prognostic implications," *Oncotarget*, vol. 7, no. 37, article 11045, pp. 59976–59986, 2016.

[32] X. Fang, B. Xiu, Z. Yang et al., "The expression and clinical relevance of PD-1, PD-L1, and TP63 in patients with diffuse large B-cell lymphoma," *Medicine*, vol. 96, no. 15, p. e6398, 2017.

[33] S. Muenst, S. Hoeller, N. Willi, S. Dirnhofer, and A. Tzankov, "Diagnostic and prognostic utility of PD-1 in B cell lymphomas," *Disease Markers*, vol. 29, no. 1, 53 pages, 2010.

[34] M. J. Ahearne, K. Bhuller, R. Hew, H. Ibrahim, K. Naresh, and S. D. Wagner, "Expression of PD-1 (CD279) and FoxP3 in diffuse large B-cell lymphoma," *Virchows Archiv*, vol. 465, no. 3, pp. 351–358, 2014.

[35] H. Dong and L. Chen, "B7-H1 pathway and its role in the evasion of tumor immunity," *Journal of Molecular Medicine*, vol. 81, no. 5, pp. 281–287, 2003.

[36] T. J. Curiel, S. Wei, H. Dong et al., "Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity," *Nature Medicine*, vol. 9, no. 5, pp. 562–567, 2003.

[37] S. Hori, T. Nomura, and S. Sakaguchi, "Control of regulatory T cell development by the transcription factor Foxp3," *Science*, vol. 299, no. 5609, pp. 1057–1061, 2003.

[38] J. D. Fontenot, J. P. Rasmussen, L. M. Williams, J. L. Dooley, A. G. Farr, and A. Y. Rudensky, "Regulatory T cell lineage specification by the forkhead transcription factor foxp3," *Immunity*, vol. 22, no. 3, pp. 329–341, 2005.

[39] G. Roncador, P. . J. Brown, L. Maestre et al., "Analysis of FOXP3 protein expression in human $CD4^{+}CD25^{+}$ regulatory T cells at the single-cell level," *European Journal of Immunology*, vol. 35, no. 6, pp. 1681–1691, 2005.

[40] A. Tzankov, C. Meier, P. Hirschmann, P. Went, S. A. Pileri, and S. Dirnhofer, "Correlation of high numbers of intratumoral FOXP3+ regulatory T cells with improved survival in germinal center-like diffuse large B-cell lymphoma, follicular lymphoma and classical Hodgkin's lymphoma," *Haematologica*, vol. 93, no. 2, pp. 193–200, 2008.

[41] N. R. Lee, E. K. Song, K. Y. Jang et al., "Prognostic impact of tumor infiltrating FOXP3 positive regulatory T cells in diffuse large B-cell lymphoma at diagnosis," *Leukemia & Lymphoma*, vol. 49, no. 2, pp. 247–256, 2008.

[42] S. Hasselblom, M. Sigurdadottir, U. Hansson, H. Nilsson-Ehle, B. Ridell, and P. O. Andersson, "The number of tumour-infiltrating TIA-1+ cytotoxic T cells but not FOXP3 + regulatory T cells predicts outcome in diffuse large B-cell lymphoma," *British Journal of Haematology*, vol. 137, no. 4, pp. 364–373, 2007.

[43] J. C. Gomez-Gelvez, M. E. Salama, S. L. Perkins, M. Leavitt, and K. V. Inamdar, "Prognostic impact of tumor microenvironment in diffuse large B-cell lymphoma uniformly treated with R-CHOP chemotherapy," *American Journal of Clinical Pathology*, vol. 145, no. 4, pp. 514–523, 2016.

[44] A. G. Abdou, N. Asaad, M. Kandil, M. Shabaan, and A. Shams, "Significance of stromal-1 and stromal-2 signatures and biologic prognostic model in diffuse large B-cell lymphoma," *Cancer Biology & Medicine*, vol. 14, no. 2, pp. 151–161, 2017.

[45] A. M. Perry, T. M. Cardesa-Salzmann, P. N. Meyer et al., "A new biologic prognostic model based on immunohistochemistry predicts survival in patients with diffuse large B-cell lymphoma," *Blood*, vol. 120, no. 11, pp. 2290–2296, 2012.

[46] S. Ciavarella, M. C. Vegliante, M. Fabbri et al., "Dissection of DLBCL microenvironment provides a gene expression-based predictor of survival applicable to formalin-fixed paraffin-embedded tissue," *Annals of Oncology*, vol. 29, no. 12, pp. 2363–2370, 2018.

[47] C. Brigati, D. M. Noonan, A. Albini, and R. Benelli, "Tumors and inflammatory infiltrates: friends or foes?," *Clinical & Experimental Metastasis*, vol. 19, no. 3, pp. 247–258, 2002.

[48] C. G. Mueller, C. Boix, W. H. Kwan et al., "Critical role of monocytes to support normal B cell and diffuse large B cell lymphoma survival and proliferation," *Journal of Leukocyte Biology*, vol. 82, no. 3, pp. 567–575, 2007.

[49] M. Cacciatore, C. Guarnotta, M. Calvaruso et al., "Microenvironment-centred dynamics in aggressive B-cell lymphomas," *Advances in Hematology*, vol. 2012, Article ID 138079, 12 pages, 2012.

[50] F. Guilloton, G. Caron, C. Ménard et al., "Mesenchymal stromal cells orchestrate follicular lymphoma cell niche through the CCL2-dependent recruitment and polarization of monocytes," *Blood*, vol. 119, no. 11, pp. 2556–2567, 2012.

[51] R. Küppers, "Mechanisms of B-cell lymphoma pathogenesis," *Nature Reviews Cancer*, vol. 5, no. 4, pp. 251–262, 2005.

[52] K. A. Khalifa, H. M. Badawy, W. M. Radwan, M. A. Shehata, and M. A. Bassuoni, "$CD14^{+}$HLA-DR low/$^{-}$ monocytes as indicator of disease aggressiveness in B-cell non-Hodgkin lymphoma," *International Journal of Laboratory Hematology*, vol. 36, no. 6, pp. 650–655, 2014.

[53] S. Riihijärvi, I. Fiskvik, M. Taskinen et al., "Prognostic influence of macrophages in patients with diffuse large B-cell lymphoma: a correlative study from a Nordic phase II trial," *Haematologica*, vol. 100, no. 2, pp. 238–245, 2015.

[54] F. Marchesi, M. Cirillo, A. Bianchi et al., "High density of CD68+/CD163+ tumour-associated macrophages (M2-TAM) at diagnosis is significantly correlated to unfavorable prognostic factors and to poor clinical outcomes in patients with diffuse large B-cell lymphoma," *Hematological Oncology*, vol. 33, no. 2, pp. 110–112, 2015.

[55] S. J. Nam, H. Go, J. H. Paik et al., "An increase of M2 macrophages predicts poor prognosis in patients with diffuse large B-cell lymphoma treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone," *Leukemia & Lymphoma*, vol. 55, no. 11, pp. 2466–2476, 2014.

[56] N. Wada, M. A. Zaki, Y. Hori et al., "Tumour-associated macrophages in diffuse large B-cell lymphoma: a study of the Osaka Lymphoma Study Group," *Histopathology*, vol. 60, no. 2, pp. 313–319, 2012.

[57] Q. C. Cai, H. Liao, S. X. Lin et al., "High expression of tumor-infiltrating macrophages correlates with poor prognosis in patients with diffuse large B-cell lymphoma," *Medical Oncology*, vol. 29, no. 4, pp. 2317–2322, 2012.

[58] S. Hasselblom, U. Hansson, M. Sigurdardottir, H. Nilsson-Ehle, B. Ridell, and P. O. Andersson, "Expression of CD68+ tumor-associated macrophages in patients with diffuse large B-cell lymphoma and its relation to prognosis," *Pathology International*, vol. 58, no. 8, pp. 529–532, 2008.

[59] P. N. Meyer, K. Fu, T. Greiner et al., "The stromal cell marker SPARC predicts for survival in patients with diffuse large B-

cell lymphoma treated with rituximab," *American Journal of Clinical Pathology*, vol. 135, no. 1, pp. 54–61, 2011.

[60] C. Marinaccio, G. Ingravallo, F. Gaudio et al., "Microvascular density, CD68 and tryptase expression in human diffuse large B-cell lymphoma," *Leukemia Research*, vol. 38, no. 11, pp. 1374–1377, 2014.

[61] Z. Liu, M. Xiong, J. Gong et al., "Legumain protease-activated TAT-liposome cargo for targeting tumours and their microenvironment," *Nature Communications*, vol. 5, no. 1, 2014.

[62] L. Shen, H. Li, Y. Shi et al., "M2 tumour-associated macrophages contribute to tumour progression via legumain remodelling the extracellular matrix in diffuse large B cell lymphoma," *Scientific Reports*, vol. 6, article 30347, 2016.

[63] B. Geiger, A. Bershadsky, R. Pankov, and K. M. Yamada, "Transmembrane crosstalk between the extracellular matrix and the cytoskeleton," *Nature Reviews Molecular Cell Biology*, vol. 2, no. 11, pp. 793–805, 2001.

[64] T. R. Kyriakides and P. Bornstein, "Matricellular proteins as modulators of wound healing and the foreign body response," *Thrombosis and Haemostasis*, vol. 90, no. 12, pp. 986–992, 2003.

[65] M. R. Ng and J. S. Brugge, "A stiff blow from the stroma: collagen crosslinking drives tumor progression," *Cancer Cell*, vol. 16, no. 6, pp. 455–457, 2009.

[66] S. Sangaletti, C. Tripodo, P. Portararo et al., "Stromal niche communalities underscore the contribution of the matricellular protein SPARC to B-cell development and lymphoid malignancies," *OncoImmunology*, vol. 3, no. 6, article e28989, 2014.

[67] A. Chlenski and S. L. Cohn, "Modulation of matrix remodeling by SPARC in neoplastic progression," *Seminars in Cell & Developmental Biology*, vol. 21, no. 1, pp. 55–65, 2010.

[68] J. F. DiMartino, N. J. Lacayo, M. Varadi et al., "Low or absent *SPARC* expression in acute myeloid leukemia with *MLL* rearrangements is associated with sensitivity to growth inhibition by exogenous SPARC protein," *Leukemia*, vol. 20, no. 3, pp. 426–432, 2006.

[69] N. Sato, N. Fukushima, N. Maehara et al., "SPARC/osteonectin is a frequent target for aberrant methylation in pancreatic adenocarcinoma and a mediator of tumor-stromal interactions," *Oncogene*, vol. 22, no. 32, pp. 5021–5030, 2003.

[70] M. J. Socha, N. Said, Y. Dai et al., "Aberrant promoter methylation of SPARC in ovarian cancer," *Neoplasia*, vol. 11, no. 2, pp. 126–135, 2009.

[71] N. Martínez, F. I. Camacho, P. Algara et al., "The molecular signature of mantle cell lymphoma reveals multiple signals favoring cell survival," *Cancer Research*, vol. 63, no. 23, pp. 8226–8232, 2003.

[72] S. Brandt, C. Montagna, A. Georgis et al., "The combined expression of the stromal markers fibronectin and SPARC improves the prediction of survival in diffuse large B-cell lymphoma," *Experimental Hematology & Oncology*, vol. 2, no. 1, p. 27, 2013.

[73] S. R. Rittling and A. F. Chambers, "Role of osteopontin in tumour progression," *British Journal of Cancer*, vol. 90, no. 10, pp. 1877–1881, 2004.

[74] P. Y. Wai and P. C. Kuo, "Osteopontin: regulation in tumor metastasis," *Cancer and Metastasis Reviews*, vol. 27, no. 1, pp. 103–118, 2008.

[75] G. Malaponte, S. Hafsi, J. Polesel et al., "Tumor microenvironment in diffuse large B-cell lymphoma: matrixmetalloproteinases activation is mediated by osteopontin overexpression," *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, vol. 1863, no. 3, pp. 483–489, 2016.

[76] S. Curran and G. I. Murray, "Matrix metalloproteinases: molecular aspects of their roles in tumour invasion and metastasis," *European Journal of Cancer*, vol. 36, no. 13, pp. 1621–1630, 2000.

[77] A. E. Kossakowska, S. A. Huchcroft, S. J. Urbanski, and D. R. Edwards, "Comparative analysis of the expression patterns of metalloproteinases and their inhibitors in breast neoplasia, sporadic colorectal neoplasia, pulmonary carcinomas and malignant non-Hodgkin's lymphomas in humans," *British Journal of Cancer*, vol. 73, no. 11, pp. 1401–1408, 1996.

[78] B. V. Kallakury, S. Karikehalli, A. Haholu, C. E. Sheehan, N. Azumi, and J. S. Ross, "Increased expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinases 1 and 2 correlate with poor prognostic variables in renal cell carcinoma," *Clinical Cancer Research*, vol. 7, no. 10, pp. 3113–3119, 2001.

[79] H. C. Yeh, S. M. Lin, M. F. Chen, T. L. Pan, P. W. Wang, and C. T. Yeh, "Evaluation of serum matrix metalloproteinase (MMP)-9 to MMP-2 ratio as a biomarker in hepatocellular carcinoma," *Hepato-Gastroenterology*, vol. 57, no. 97, pp. 98–102, 2010.

[80] K. W. Min, D. H. Kim, S. I. Do et al., "Expression patterns of stromal MMP-2 and tumoural MMP-2 and -9 are significant prognostic factors in invasive ductal carcinoma of the breast," *APMIS*, vol. 122, no. 12, pp. 1196–1206, 2014.

[81] B. Yang, F. Tang, B. Zhang, Y. Zhao, J. Feng, and Z. Rao, "Matrix metalloproteinase-9 overexpression is closely related to poor prognosis in patients with colon cancer," *World Journal of Surgical Oncology*, vol. 12, no. 1, p. 24, 2014.

[82] A. E. Kossakowska, S. J. Urbanski, and A. Janowska-Wieczorek, "Matrix metalloproteinases and their tissue inhibitors-expression, role and regulation in human malignant non-Hodgkin's lymphomas," *Leukemia & Lymphoma*, vol. 39, no. 5-6, pp. 485–493, 2000.

[83] B. Hazar, G. Polat, E. Seyrek, Ö. Bağdatoğlğlu, A. Kanik, and N. Tiftik, "Prognostic value of matrix metalloproteinases (MMP-2 and MMP-9) in Hodgkin's and non-Hodgkin's lymphoma," *International Journal of Clinical Practice*, vol. 58, no. 2, pp. 139–143, 2004.

[84] K. I. Sakata, M. Satoh, M. Someya et al., "Expression of matrix metalloproteinase 9 is a prognostic factor in patients with non-Hodgkin lymphoma," *Cancer*, vol. 100, no. 2, pp. 356–365, 2004.

[85] Y. Wang, L. Li, X. Guo et al., "Interleukin-6 signaling regulates anchorage-independent growth, proliferation, adhesion and invasion in human ovarian cancer cells," *Cytokine*, vol. 59, no. 2, pp. 228–236, 2012.

[86] D. M. Hilbert, M. Kopf, B. A. Mock, G. Köhler, and S. Rudikoff, "Interleukin 6 is essential for in vivo development of B lineage neoplasms," *Journal of Experimental Medicine*, vol. 182, no. 1, pp. 243–248, 1995.

[87] J. F. Seymour, M. Talpaz, F. Cabanillas, M. Wetzler, and R. Kurzrock, "Serum interleukin-6 levels correlate with prognosis in diffuse large-cell lymphoma," *Journal of Clinical Oncology*, vol. 13, no. 3, pp. 575–582, 1995.

[88] T. Ara and Y. A. DeClerck, "Interleukin-6 in bone metastasis and cancer progression," *European Journal of Cancer*, vol. 46, no. 7, pp. 1223–1231, 2010.

[89] A. E. Kossakowska, D. R. Edwards, C. Prusinkiewicz et al., "Interleukin-6 regulation of matrix metalloproteinase (MMP-2 and MMP-9) and tissue inhibitor of metalloproteinase (TIMP-1) expression in malignant non-Hodgkin's lymphomas," *Blood*, vol. 94, no. 6, pp. 2080–2089, 1999.

[90] H. Ü. Teke, E. Gündüz, O. M. Akay, C. Bal, and Z. Gülbaş, "Are the high serum interleukin-6 and vascular endothelial growth factor levels useful prognostic markers in aggressive non-Hodgkin lymphoma patients?," *Turkish Journal of Hematology*, vol. 32, no. 1, pp. 21–28, 2015.

[91] T. Sato, A. Ito, and Y. Mori, "Interleukin 6 enhances the production of tissue inhibitor of metalloproteinases (TIMP) but not that of matrix metalloproteinases by human fibroblats," *Biochemical and Biophysical Research Communications*, vol. 170, no. 2, pp. 824–829, 1990.

[92] J. W. Choi, J. S. An, J. H. Lee, E. S. Lee, K. H. Kim, and Y. S. Kim, "Clinicopathologic implications of tissue inhibitor of metalloproteinase-1-positive diffuse large B-cell lymphoma," *Modern Pathology*, vol. 19, no. 7, pp. 963–973, 2006.

[93] A. E. Kossakowska, S. J. Urbanski, and D. R. Edwards, "Tissue inhibitor of metalloproteinases-1 (TIMP-1) RNA is expressed at elevated levels in malignant non-Hodgkin's lymphomas," *Blood*, vol. 77, no. 11, pp. 2475–2481, 1991.

[94] L. Guedez, L. Courtemanch, and M. Stetler-Stevenson, "Tissue inhibitor of metalloproteinase (TIMP)-1 induces differentiation and an antiapoptotic phenotype in germinal center B cells," *Blood*, vol. 92, no. 4, pp. 1342–1349, 1998.

[95] B. Barleon, S. Sozzani, D. Zhou, H. A. Weich, A. Mantovani, and D. Marme, "Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1," *Blood*, vol. 87, no. 8, pp. 3336–3343, 1996.

[96] B. L. Gruber, M. J. Marchese, and R. Kew, "Angiogenic factors stimulate mast-cell migration," *Blood*, vol. 86, no. 7, pp. 2488–2493, 1995.

[97] D. Ribatti, A. Vacca, A. Marzullo et al., "Angiogenesis and mast cell density with tryptase activity increase simultaneously with pathological progression in B-cell non-Hodgkin's lymphomas," *International Journal of Cancer*, vol. 85, no. 2, pp. 171–175, 2000.

[98] R. J. Blair, H. Meng, M. J. Marchese et al., "Human mast cells stimulate vascular tube formation. Tryptase is a novel, potent angiogenic factor," *The Journal of Clinical Investigation*, vol. 99, no. 11, pp. 2691–2700, 1997.

[99] A. Orimo, P. B. Gupta, D. C. Sgroi et al., "Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion," *Cell*, vol. 121, no. 3, pp. 335–348, 2005.

[100] S. Rafii, D. Lyden, R. Benezra, K. Hattori, and B. Heissig, "Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy?," *Nature Reviews Cancer*, vol. 2, no. 11, pp. 826–835, 2002.

[101] V. Planat-Benard, J.-S. Silvestre, B. Cousin et al., "Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives," *Circulation*, vol. 109, no. 5, pp. 656–663, 2004.

[102] D. Gratzinger, S. Zhao, R. J. Tibshirani et al., "Prognostic significance of VEGF, VEGF receptors, and microvessel density in diffuse large B cell lymphoma treated with anthracycline-based chemotherapy," *Laboratory Investigation*, vol. 88, no. 1, pp. 38–47, 2008.

[103] T. M. Cardesa-Salzmann, L. Colomo, G. Gutierrez et al., "High microvessel density determines a poor outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus chemotherapy," *Haematologica*, vol. 96, no. 7, pp. 996–1001, 2011.

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Review Article

The Role of Matrix Metalloproteinases in the Epithelial-Mesenchymal Transition of Hepatocellular Carcinoma

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The epithelial-mesenchymal transition (EMT) is a transformation process mandatory for the local and distant progression of many malignant tumors, including hepatocellular carcinoma (HCC). Matrix metalloproteinases (MMPs) play significant roles in cellular regeneration, programmed death, angiogenesis, and many other essential tissular functions, involved in the normal development and also in pathological processes, such as the EMT. This paper reviews the roles of MMPs in the EMT involved in HCC invasion, as well as the ancillary roles that MMP cross-activation and tissue inhibitors play in modulating this process. While gelatinases MMP-2 and MMP-9 are the MMPs commonly cited in the EMT of HCC, MMPs belonging to other classes have been proven to be involved in this process, favoring not only invasion and metastasis (MMP-1, MMP-3, MMP-7, MMP-10, MMP-11, MMP-13, MMP-14, MMP-16, MMP-26, and MMP-28) but also angiogenesis (MMP-8 and MMP-10). There is also data suggesting that other MMPs with a suspected or demonstrated role in the EMT of other cancers may also have some degree of involvement in HCC. The auto- and cross-activation of MMPs may complicate this issue, as pinpointing the extent of implication of each MMP may be extremely difficult. The homeostasis between MMPs and their tissue inhibitors is essential in preventing tumor progression, and the disturbance of this stability is another entailed factor in the EMT of HCC, which is addressed herein.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the leading causes of death worldwide and develops in a context of long-term liver injury, inflammation, and regeneration [1]. With a mortality of 9.1% worldwide, HCC is the fifth most common cancer and is considered a significant global health burden, by itself and through its potential unnoticeable or overt complications [2–4]. With recent progress, diagnosis hindrances of rare or atypical forms of HCC have been surmounted, and novel therapies appear promising in complementing the available resources for managing this disease [5–7].

However, a better understanding of the underlying pathological mechanisms in the development of HCC may uncover more efficient ways to limit tumor growth and dissemination.

Epithelial-mesenchymal transition (EMT) is defined as a transformation process, in which epithelial cell features are lost in favor of adopting mesenchymal traits; the process usually implies loss of the apicobasal cell polarity, through intracellular adhesion alteration. EMT is considered essential for oncogenesis, enabling tumors to acquire aggressive features such as invasiveness and the ability to metastasize [8].

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endoproteases responsible for degrading the extracellular matrix (ECM) by breaking down various proteins in its structure. MMPs promote a wide spectrum of processes, including cell proliferation and migration, and could play a role in cell apoptosis, angiogenesis, tissue regeneration, and immune response [9]. In malignancies, such as HCC, MMPs function within the tumor microenvironment to induce changes during EMT and help to facilitate EMT via invasion and metastasis behaviors [10].

MMPs seem to play important roles, as the members of this family have various implications in the complex pathogenesis of EMT in HCC. This paper is aimed at thoroughly presenting their functions in this process.

2. Matrix Metalloproteinases

2.1. General Description. MMPs belong to metzincins, a family of Zn^{2+}-dependent, Ca^{2+}-containing endoproteases comprising of 24 members in mammals (23 in humans). MMPs are produced as zymogens (pro-MMPs) that are activated by other enzymes or free radicals through the cysteine switch mechanism [11].

Metalloproteinases are named incrementally, starting with MMP-1 and ending with MMP-28, but not including MMP-4, MMP-5, MMP-6, and MMP-22, since these enzymes were discovered simultaneously by different research teams. MMP-18 was identified in Xenopus with no known human orthologue [12]. Based on the target substrate and chemical structure, MMPs are split into several groups: collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, and other nonclassified MMPs [13].

MMPs promote the degradation of various ECM proteins, leading to architectural changes in the cell and tissue environments. Different MMPs have different efficacies in breaking down various proteins. Some of the substrates targeted by MMPs include collagen, gelatin, aggrecan, entactin, fibronectin, laminin, tenascin, and vitronectin. MMPs can also degrade myelin basic protein and casein [14, 15].

Cytokines, chemokines, and various receptors may also be targeted by various MMPs. As such, MMPs not only break down ECM components but also are involved in regulating intra- and intercellular signaling pathways by proteolysis [16].

MMP-1, MMP-2, MMP-3, MMP-11, and MMP-13 are among the MMPs constitutively expressed in normal livers, while the others may appear in various pathological processes, such as acute or chronic liver injury [17].

There are several levels of regulation for MMPs: gene transcription, secretion compartmentalization, proenzyme activation, endocytosis, and inhibition of enzyme activity [15, 16, 18].

The inhibition of MMPs is performed by either endogenous or exogenous inhibitors. Tissue inhibitors of metalloproteinases (TIMPs) may inhibit more than one metalloproteinase and are widely distributed in the human body [19]. Either absolute or relative changes in MMP or TIMP levels can trigger important changes in specific MMP activity [15]. TIMP-1 and TIMP-2 can block MMP effects in promoting tumor cell proliferation and migration and can also inhibit angiogenesis and apoptosis. Conversely, apoptosis is induced by TIMP-3 through TNF-α receptor stabilization and by TIMP-4 via overexpression and through different mechanisms, depending on the region involved [20].

2.2. MMP Interaction. Once activated, MMPs subsequently activate other MMPs that are in an inactive zymogen form (pro-MMPs). This may lead to a cascade of activation, and this network of interaction between MMPs may potentiate their effects in the EMT.

MMP-3 and MMP-10 activate MMP-1, MMP-7, MMP-8, and MMP-9, enhancing ECM degradation [21]. MMP-14 activates both MMP-2 and MMP-13, in the presence of TIMP-2, with a demonstrated effect in tumor invasion and metastasis, by promoting cell migration [22].

MMP-15, MMP-16, and MMP-24 also activate MMP-2 on the cell surface, affecting the ECM integrity [23]. Other interactions between MMPs, such as activation of MMP-13 by MMP-15, MMP-2, and MMP-3, as well as activation of MMP-9 by MMP-2 and MMP-13 and activation of MMP-2 by MMP-1, MMP-17, MMP-7, MMP-13, and MMP-25, were also described [24].

MMP-1 was also shown to be activated by MMP-7, and MMP-13 may also be activated by MMP-10, while MMP-14 and MMP-26 are capable of autoactivation [22, 25]. Activated MMP-7 can also determine the activation of MMP-9 and MMP-13, while MMP-12 could activate MMP-2 and MMP-3 [26].

This intricate web of MMP cross-activation is able to completely cleave the ECM if the strict and multilevel regulation of MMPs is overwhelmed [26]. Also, the multiple interactions make it difficult to accurately determine the individual role of each MMP in an *in vivo* setting of HCC cells going through EMT.

3. EMT and HCC

EMT is a biological process defined as a rigorously programmed shift from epithelial to mesenchymal cell features that plays a substantial role in embryogenesis and organ development and also tissue repair and regeneration, as well as tumor invasion and metastasis [27]. EMT is triggered and sustained by multiple molecular processes, which, in some cases, may be used as biomarkers. Some of the genomic processes include activation of transcription factors and alterations of microRNAs, while nongenomic processes involve release of MMPs, cytoskeletal reconfiguration, and expression of specific proteins on the cell surface [28].

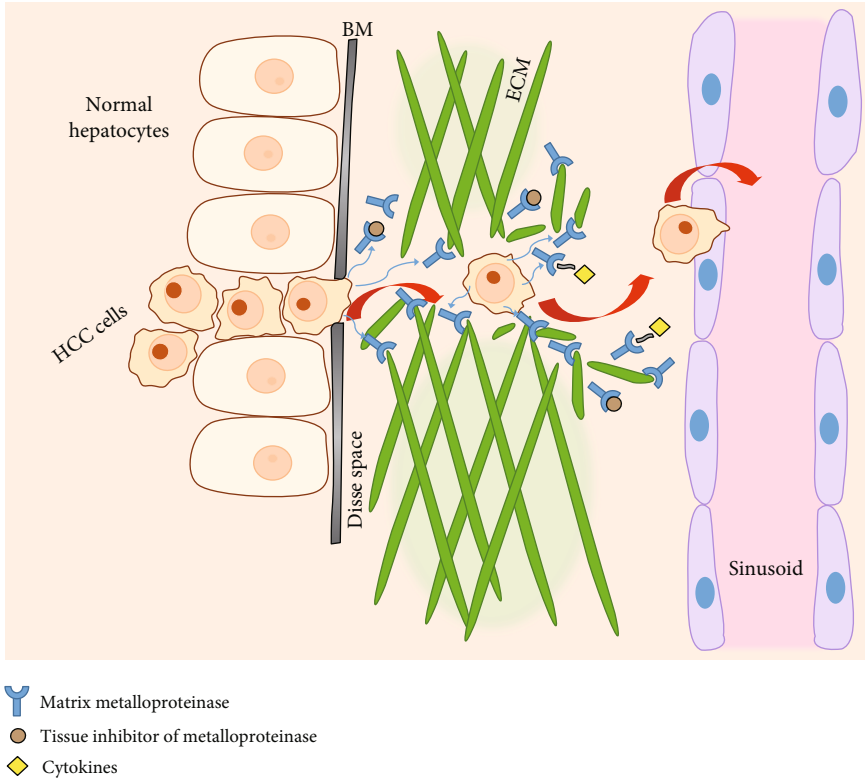

Figure 1: Schematic representation of the epithelial-mesenchymal transition of hepatocellular carcinoma (HCC) cells. Matrix metalloproteinases (MMPs) break down the extracellular matrix (ECM) and activate various cytokines facilitating the local invasion of the HCC cells through the basement membrane (BM) and Disse space, favoring their advance towards the liver sinusoids. Tissue inhibitors of metalloproteinases block the activity of some MMPs, balancing the process.

The main features of the EMT include the downregulation of epithelial markers, such as E-cadherin, beta-catenin, tight junction protein-1, laminin, and cytokeratin, and the upregulation of mesenchymal markers such as N-cadherin, vimentin, and α-smooth muscle actin (α-SMA). A number of transcription factors are involved in the EMT of HCC, including Snail, Twist, and zinc finger E-box binding protein 1 (ZEB1), and their presence is associated with a poor prognosis [29–31]. These EMT transcription factors activate multiple cellular signaling pathways and molecules, such as Akt, MAPK, STAT3, transforming growth factor beta (TGF-β), β-catenin, Wnt, Ras, Notch, nuclear factor κB (NF-κB), tumor necrosis factor alpha (TNF-α), and hypoxia-inducible factor-1 alpha (HIF-1α) [31].

The involvement of several MMPs in tumor progression through EMT was demonstrated in various digestive (gastric, pancreatic, and colorectal) and nondigestive (lung, ovarian, mammary, and prostate) cancers, with similarities to HCC, but entailing tissue-specific pathogeneses [32–38].

Some effects of the EMT in HCC, such as tumor invasion, were demonstrated on HepG2 and Huh-7 cell lines; for instance, Snail was reported to present a strong effect in increasing invasion, correlated with cell dedifferentiation. The MMP gene family is upregulated by Snail expression in HepG2 cells in candidate genes relating to tumor migration; therefore, MMPs may play an important part in the EMT of HCC [39].

4. MMPs with a Demonstrated Role in the EMT of Invasive HCC

Degradation of the basement membrane and subsequently of the ECM is critical for invasion and metastasis, and these complications are major factors of poor prognosis in HCC patients [40]. MMPs are key factors in providing the invasive and metastatic traits of malignant tumor cells by enabling their infiltration and migration in the process of EMT. Tumor cell migration depends on the increased release and activation of MMPs, as well as their cell membrane expression, leading to a breakdown of the ECM and favoring infiltration [41] (see Figure 1).

MMP-1 is also named collagenase-1, due to the fact that it promotes degradation of the interstitial collagens and is normally regulated by TIMP-1. Overexpression of both MMP-1 and TIMP-1 is associated with an elevated invasive and migratory capacity of the HCC cells, most likely by ECM degradation in the process of EMT [42]. Overexpression of miR-526b is associated with increased cell proliferation, venous infiltration, and a poor prognosis, and MMP-1 is recognized as a direct target of miR-526b [43].

MMP-2 is a collagenase that represents the main proteolytic enzyme among MMPs and is a major promoter of tumor cell invasion and metastasis through breaking down of the basement membrane and favoring the local and distant infiltration of tumor cells [44]. MMP-2 is not normally found

in liver cells but is expressed in HCC cells, especially in the fibrolamellar variant [45]. In the EMT of HCC, MMP-2 seems to be linked to HIF-1α, a known enhancer of tumor invasion and metastasis, which downregulates E-cadherin and upregulates MMP-2 [46].

MMP-3, or stromelysin-1, can degrade a variety of ECM substrates, including collagens, laminins, fibronectin, osteopontin, and proteoglycans, while also demonstrating a proteolytic activity on cell surface protein ectodomains. Hepatocyte growth factor (HGF) stimulates MMP-3 to initiate and maintain the EMT of HCC, favoring the invasion of ECM by liver cancer cells [47]. The role of MMP-3 in the invasiveness of HCC can be demonstrated by its *in vivo* expression, while HGF-induced invasion can be demonstrated by using an antibody to MMP-3, which blocks the invasion [48]. Features of invasion and migration in HCC can be stimulated by some cytokines, such as IL-1β, TNF-α, and interferon gamma, which can induce a significant MMP-3 mRNA production that in normal circumstances is at low levels [49].

MMP-7 is also known as matrilysin, and it cleaves many protein components of the ECM, including collagen, entactin, osteopontin, fibronectin, laminin, elastin, and proteoglycans, and also pro-MMP-2 and pro-MMP-9, as well as other proteins [50]. MMP-7 was found to function as a prometastatic factor by promoting the migratory and invasive ability of cancer cells, and overexpression of MMP-7 was found in HCC specimens and cells, favoring EMT. MMP-7 is a direct target of miR-489 in HCC, and miR-489 inhibits the migration and invasion of HCC. The underexpression of miR-489 facilitates tumor migration that plays a role in HCC progression, via targeting MMP-7 [51].

MMP-8, or collagenase-2, plays a role in cell proliferation and migration, as well as in angiogenesis, through the development of capillary-like network structures [52]. Upregulation of MMP-8 and TGF-β1 activates the PI3K/Akt/Rac1 pathway, altering the EMT phenotype, inducing HCC invasion and migration [53]. The use of apigenin in HCC Huh-7 cells inhibits the migration capabilities of tumor cells through downregulation of vimentin, type I collagen, VEGF, and MMP-8, thus regulating angiogenesis and migration and promoting EMT [54].

Alongside MMP-2, *MMP-9*, also known as gelatinase B, is one of the most studied MMPs in the pathogenesis of EMT in HCC. MMP-9 degrades the ECM, activates IL-1β, and cleaves several chemokines [55]. MMP-9 seems to play a major role in tumor angiogenesis, through its critical intervention in the regulation of growth plate angiogenesis and recruitment of endothelial stem cells [56]. Overexpression of MMP-9 in HCC leads to a higher TNM stage through an increase of lymph node invasion as well as promoting metastasis and also to poor differentiation and an overall poor prognosis [57]. MMP-9 is considered a consistent progression marker alongside extracellular matrix protein 2, related to invasion and metastasis, and they represent targets to as many as 285 consistently downregulated and 149 upregulated genes appearing in the EMT of HCC [58].

MMP-10, or stromelysin-2, is mainly found in epithelial cells and is involved in tumor cell invasion and metastasis by targeting several pro-MMPs, as well as breaking down ECM components such as collagen, gelatin, elastin, fibronectin, proteoglycans, and laminin [59]. MMP-10 contributes to HCC development, participating in tumor angiogenesis, growth, and lung dissemination, induced by hypoxia, an increased CXCR4 expression, stromal-derived factor-1, and increased C-Jun transcriptional activity, resulting in the EMT of HCC cells [60–62].

MMP-11, also known as stromelysin-3, has a relatively limited substrate, by only cleaving the insulin-like growth factor-binding protein-1, the laminin receptor, and the native alpha3 chain of collagen VI. Nevertheless, MMP-11 overexpression is a factor of poor prognosis in various human carcinomas. Interestingly, this proteinase is not expressed in malignant cells themselves but is secreted by adjacent mesenchymal cells that do not present specific malignant features [63]. One of the novel biomarkers of tumor aggressiveness and potential targets for HCC treatment is miR-125a, which decreases the EMT activity by downregulating MMP-11 and VEGF, *in vitro* and *in vivo*, resulting in an inhibition of HCC invasion and migration [64].

MMP-13 (collagenase-3) is activated by TGF-β and is important in HCC invasion and metastasis. TGF-β seems to only be involved in invasive HCC types, and the stimulating effects on MMP-13 expression are correlated with a feedback repression of miR-127 [65]. High levels of MMP-13 and of gelatinases are responsible for the degradation of the basement membrane, favoring EMT [66].

MMP-14 is a membrane-type MMP that plays an important role in cancer metastasis by degrading the ECM, increasing the secretion of pro-MMP-2 and pro-MMP-9, and interacting with TIMP-2. The increased expression of MMP-14 seems to be correlated with high rates of portal vein invasion, intrahepatic metastasis, and recurrence in HCC [67]. Pravastatin reduces the rates of local invasion and distant metastasis in HCC by decreasing the expression of MMP-14 required for MMP-2 activation [68].

MMP-16 is a membrane-type MMP localized on the surface of fibroblasts, capable of degrading various ECM components, including collagen, and is an activator of MMP-2 [69]. MMP-16 induces EMT in HCC, promoting cancer cell invasion and metastases; silencing MMP-16 expression hinders the EMT process by increasing the expression of epithelial cell marker E-cadherin while repressing mesenchymal markers vimentin and N-cadherin [40].

MMP-26, or matrilysin-2, breaks down several EMC components and activates MMP-9 through cleavage. MMP-26 may be activated in HCC Huh7 cells when stimulated by fibroblast growth factors that increase tumor proliferation and migration, with the involvement of the extracellular signal-regulated kinase (ERK) and NF-κB pathways [70]. Also, tumor formation in distal organs was detected in mice that received MMP-26+CXCR4+HepG2 HCC cells, suggesting that MMP-26 plays a role in the EMT of HCC [71].

MMP-28, also known as epilysin, is the most recently identified MMP and degrades casein. MMP-28 promotes and maintains EMT through activation of TGF-β signaling and upregulating Snail transcription factor [72]. Elevated

Table 1: Summary of the roles of MMPs involved in the EMT of HCC and their interactions.

MMP	Category	Role in the EMT of HCC	Cross-activated by MMP	References
MMP-1	Collagenase	Invasion and metastasis	MMP-3, MMP-7, and MMP-10	[21, 25, 43, 44]
MMP-2	Gelatinase	Invasion and metastasis	MMP-1, MMP-7, MMP-12, MMP-13, MMP-14, MMP-15, MMP-16, MMP-17, MMP-24, and MMP-25	[22–26, 44–46]
MMP-3	Stromelysin	Invasion and metastasis	MMP-12	[47–60, 74]
MMP-7	Matrilysin	Invasion and metastasis	MMP-3, MMP-10	[21, 50, 51]
MMP-8	Collagenase	Angiogenesis and migration	MMP-3, MMP-10	[21, 52–54]
MMP-9	Gelatinase	Angiogenesis, invasion, and metastasis	MMP-2, MMP-3, MMP-7, MMP-10, and MMP-13	[21, 24, 55–58, 74]
MMP-10	Stromelysin	Angiogenesis, invasion, and metastasis	N/A	[59–62]
MMP-11	Stromelysin	Invasion and metastasis	N/A	[63, 64]
MMP-13	Collagenase	Invasion and metastasis	MMP-2, MMP-3, MMP-7, MMP-10, MMP-14, and MMP-15	[22, 25, 26, 65, 66]
MMP-14	Membrane-type	Invasion and metastasis	MMP-14	[22, 67, 68]
MMP-16	Membrane-type	Invasion and metastasis	N/A	[40, 69]
MMP-26	Matrilysin	Invasion and metastasis	MMP-26	[25, 70, 71]
MMP-28	Other	Invasion and metastasis	N/A	[72, 73]

levels of MMP-28 in HCC are correlated with a poor prognosis, due to higher TNM stage and increased rates of portal vein invasion and metastasis, the latter apparently depending on Notch3 signals [73].

The MMPs with demonstrated involvement in the EMT of HCC are presented in Table 1, alongside their MMP activators.

5. Other MMPs with Possible Involvement in Tumor Pathogenesis

MMP-12 degrades elastin, thus earning its alternate name: macrophage elastase. MMP-12 expression affects overall survival time of patients with HCC who underwent curative resection but does not seem to be involved in HCC invasiveness or metastasis [75].

MMP-15 is classified into the membrane-type MMPs that are important for pericellular proteolysis, and the expression level of MMP-15 is associated with tumor growth of human fibrosarcoma and gastric cancer cells as well as tumor progression and intratumoral angiogenesis in non-small-cell lung cancer [74, 76, 77]. However, in regard to MMP-15 involvement in HCC, only indirect and nondefinitive data is available to date [77].

MMP-17 is a relatively newly discovered membrane-type MMP that is glycosylphosphatidylinositol- (GPI-) anchored, but little information is available in regard to its physiological roles [78]. No involvement of MMP-17 in the EMT of HCC was found in the literature, but it was implicated in breast cancer progression, apparently by facilitating *in vivo* and *in vitro* breast cancer cell proliferation through outside-in EGFR signaling, but without acting as a protease [79].

MMP-19, also known as stromelysin-4, could be involved in processes such as neovascularization and angiogenesis or lymphocyte extravasation, but its role in cancer evolution is unclear [80].

MMP-20, or enamelysin, is a tooth-specific MMP, which under normal conditions is only associated with ameloblasts and odontoblasts but was recently identified in colon, breast, and lung cancers [81]. HCC cells with increased serine protease inhibitor Kazal-type- (SPINK-) 6 expression associate a significant downregulation of MMP-20, as well as MMP-9, suggesting that MMP-20 may also play a role in ECM degradation and tumor cell invasion and migration [82].

MMP-21 is involved in establishing left-right asymmetry by cleaving specific targets at the embryonic node and possibly activating latent TGF-β factors [83]. The involvement of MMP-21 in cell adhesion as well as in cell migration is made possible by the vitronectin-like domain in the catalytic site [84]. The increased expression of MMP-21 is correlated with a poor prognosis due to the higher TNM stage, tumor invasion, and metastasis in other types of malignancies, such as gastric cancer and colorectal cancer [85]. However, implication in HCC is undetermined as of yet.

MMP-23 may possess a novel mechanism for cellular localization, due to a lack of C-terminal transmembrane domain or GPI anchor found in the membrane-type MMPs [86]. MMP-23 is upregulated in a Mdr2-knockout model of chronic inflammation-mediated HCC, possibly playing a still unclear role in the hepatocarcinogenesis process occurring in long-term liver inflammation [87].

MMP-24 is a membrane-type MMP that activates MMP-2 by cleavage and was identified as a biomarker of lung and gastric adenocarcinoma progression and metastasis [88]. MMP-24 is expressed after partial hepatectomy, but no studies were found citing its role in the EMT of HCC [17].

MMP-25 is another GPI-anchored membrane-type MMP that is expressed in several human cancers, including brain, colon, urothelial, and prostate cancers [89]. It was suggested that MMP-25 may be important for tumor cell invasion because elevated levels are identified in the tumor

progression process of invasive colon cancer [90]. Also, MMP-25 reduces the levels of alpha-1 proteinase inhibitor, stimulating the ECM degradation and the subsequent tumor invasion and migration [91].

MMP-27 has a unique C-terminal extension which does not lead to stable membrane insertion, favoring its retention in the endoplasmic reticulum [92]. MMP-27 was initially speculated as a marker of poor prognosis in breast cancer patients, but later studies did not confirm these findings [93]. Enhanced melanoma progression is often observed in patients that present mutations in the genes coding MMP-8 and MMP-27 [94]. The effect of MMP-27 on HCC invasiveness is yet to be determined.

6. TIMP Contribution

The balance between matrix metalloproteinases and TIMPs seems to be a key factor in maintaining a normal configuration of the ECM and of the basement membrane, as well as preventing tumor cell invasion and migration [95]. Each human TIMP may inhibit several MMPs with various affinities, but noninhibitory interactions may also occur between a series of MMP-TIMP couples, such as coactivation of pro-MMP-2 by TIMP-2 and activation protection of pro-MMP-9 by TIMP-1 [96].

TIMP-1 overexpression facilitates the EMT of HCC cells, through functions that are independent of MMPs, such as modulating apoptosis, mitogenic activity, and cellular proliferation and morphology [97]. TIMP-1 can be used as a marker of lung metastasis in HCC, due to the fact that transcripts for TIMPs were clearly demonstrated in the metastatic HCC nodules in the lung [98]. Also, TIMP-1 initiates the transformation from liver fibroblasts to carcinoma-associated fibroblasts in the tumor milieu of HCC in progression [99].

TIMP-2 serum and tissue concentrations are lower in HCC patients with metastasis and are higher in those without; furthermore, patients with high levels of TIMP-2 have higher survival rates than those with low levels [100]. Besides the aforementioned decreases in MMP-14 and, subsequently, MMP-2 levels, statins also trigger a decline in the expression of TIMP-2 and also TIMP-1, restoring the MMP-TIMP balance and reducing the progression and metastatic rates of HCC in a preclinical model [68].

TIMP-3 may have a role in decreasing the aggressiveness of HCC, by inhibiting portal vein invasion and lymph node metastasis, probably by suppressing tumorigenesis and angiogenesis by interacting with integrin $\alpha 7$ and angiotensin II type 2 receptor [101]. However, TIMP-3 was found to be associated with tumor progression and negative clinical outcome in squamous cell carcinomas of the head and neck, so its definitive role in EMT remains to be established [102].

Exogenous TIMPs, such as fucoidan, maintain the ECM homeostasis by increasing TIMP-1 and decreasing MMP-2 and MMP-9 through downregulation of TGF-β signaling, implicitly decreasing the aggressiveness of HCC by preventing the EMT [103].

TIMPs play complex roles in balancing the activated MMPs and their roles in various processes, such as ECM degradation, angiogenesis, and tumor invasion and migration, but some of the effects of TIMPs are MMP-independent and have been demonstrated to favor HCC progression in some cases [104]. Serum concentrations of TIMPs, as well as some MMP/TIMP ratios, seem to correlate with the prognosis and overall survival of patients with HCC, suggesting their potential use as biomarkers for HCC [105]. The interactions between MMPs and TIMPs have been modeled according to evolutionary game theory, providing a better understanding of their dynamics in the presence or absence of cancer cells, while also offering alternate courses in cancer progression control; restoring the MMP-TIMP balance may represent an important adjuvant therapy, limiting cancer invasion and modulating the metabolisms and interactions between cancer cells and their opponents [106].

7. Conclusions

The microenvironment of HCC cells determines the invasiveness and metastasis of tumor cells. A key factor in limiting the aggressiveness of HCC, the ECM integrity, is maintained, among other factors, by normal ratios of MMPs and TIMPs. Overexpression of various MMPs can lead to extreme ECM breakdown and significantly increased EMT. Moreover, MMPs can cross-activate, and the imbalance between MMPs and TIMPs seems to play a major role in cell migration.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

[1] H. Gupta, G. S. Youn, M. J. Shin, and K. T. Suk, "Role of gut microbiota in hepatocarcinogenesis," *Microorganisms*, vol. 7, no. 5, p. 121, 2019.

[2] M. C. S. Wong, J. Y. Jiang, W. B. Goggins et al., "International incidence and mortality trends of liver cancer: a global profile," *Scientific Reports*, vol. 7, no. 1, article 45846, 2017.

[3] C. Scheau, A. I. Badarau, A. E. Ghergus, G. A. Popa, and I. G. Lupescu, "Minimal hepatic encephalopathy diagnosis by magnetic resonance spectroscopy. A case report," *Journal of Gastrointestinal and Liver Diseases*, vol. 22, no. 4, pp. 455–459, 2013.

[4] M. Colombo, "Natural history of hepatocellular carcinoma," *Cancer Imaging*, vol. 5, no. 1, pp. 85–88, 2005.

[5] A. E. Scheau, C. Scheau, and I. G. Lupescu, "Nodule-in-nodule imaging pattern in hepatocellular carcinoma treated by transarterial chemoembolization - a multiparametric magnetic resonance imaging study," *Journal of Gastrointestinal and Liver Diseases*, vol. 26, no. 4, pp. 387–393, 2017.

[6] M. Yarchoan, P. Agarwal, A. Villanueva et al., "Recent developments and therapeutic strategies against hepatocellular carcinoma," *Cancer Research*, vol. 79, no. 17, pp. 4326–4330, 2019.

[7] C. Scheau, I. A. Badarau, C. Caruntu et al., "Capsaicin: effects on the pathogenesis of hepatocellular carcinoma," *Molecules*, vol. 24, no. 13, p. 2350, 2019.

[8] A. Brinzea, R. I. Nedelcu, G. Turcu, M. Antohe, S. A. Zurac, and D. A. Ion, "TIMPs expression in lentigo maligna/lentigo maligna melanoma versus aged skin - a review of the literature and personal experience," *Romanian Journal of Morphology and Embryology*, vol. 58, no. 3, pp. 717–721, 2017.

[9] N. Cui, M. Hu, and R. A. Khalil, "Biochemical and biological attributes of matrix metalloproteinases," *Progress in Molecular Biology and Translational Science*, vol. 147, pp. 1–73, 2017.

[10] B. N. Smith and N. A. Bhowmick, "Role of EMT in metastasis and therapy resistance," *Journal of Clinical Medicine*, vol. 5, no. 2, p. 17, 2016.

[11] M. Brkic, S. Balusu, C. Libert, and R. E. Vandenbroucke, "Friends or foes: matrix metalloproteinases and their multifaceted roles in neurodegenerative diseases," *Mediators of Inflammation*, vol. 2015, Article ID 620581, 27 pages, 2015.

[12] M. A. Stolow, D. D. Bauzon, J. Li et al., "Identification and characterization of a novel collagenase in Xenopus laevis: possible roles during frog development," *Molecular Biology of the Cell*, vol. 7, no. 10, pp. 1471–1483, 1996.

[13] A. Jabłońska-Trypuć, M. Matejczyk, and S. Rosochacki, "Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 31, pp. 177–183, 2016.

[14] A. Kucukguven and R. A. Khalil, "Matrix metalloproteinases as potential targets in the venous dilation associated with varicose veins," *Current Drug Targets*, vol. 14, no. 3, pp. 287–324, 2013.

[15] J. Liu and R. A. Khalil, "Matrix metalloproteinase inhibitors as investigational and therapeutic tools in unrestrained tissue remodeling and pathological disorders," *Progress in Molecular Biology and Translational Science*, vol. 148, pp. 355–420, 2017.

[16] H.-J. Ra and W. C. Parks, "Control of matrix metalloproteinase catalytic activity," *Matrix Biology*, vol. 26, no. 8, pp. 587–596, 2007.

[17] S. Duarte, J. Baber, T. Fujii, and A. J. Coito, "Matrix metalloproteinases in liver injury, repair and fibrosis," *Matrix Biology*, vol. 44-46, pp. 147–156, 2015.

[18] L. Yadav, N. Puri, V. Rastogi, P. Satpute, R. Ahmad, and G. Kaur, "Matrix metalloproteinases and cancer - roles in threat and therapy," *Asian Pacific Journal of Cancer Prevention*, vol. 15, no. 3, pp. 1085–1091, 2014.

[19] S. Zurac, M. Neagu, C. Constantin et al., "Variations in the expression of TIMP1, TIMP2 and TIMP3 in cutaneous melanoma with regression and their possible function as prognostic predictors," *Oncology Letters*, vol. 11, no. 5, pp. 3354–3360, 2016.

[20] F. Mannello and G. Gazzanelli, "Tissue inhibitors of metalloproteinases and programmed cell death: conundrums, controversies and potential implications," *Apoptosis*, vol. 6, no. 6, pp. 479–482, 2001.

[21] J. Batra, J. Robinson, A. S. Soares, A. P. Fields, D. C. Radisky, and E. S. Radisky, "Matrix metalloproteinase-10 (MMP-10) interaction with tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2: binding studies and crystal structure," *The Journal of Biological Chemistry*, vol. 287, no. 19, pp. 15935–15946, 2012.

[22] K. J. Davies, "The complex interaction of matrix metalloproteinases in the migration of cancer cells through breast tissue stroma," *International Journal of Breast Cancer*, vol. 2014, Article ID 839094, 5 pages, 2014.

[23] Y. Itoh, "Membrane-type matrix metalloproteinases: their functions and regulations," *Matrix Biology*, vol. 44-46, pp. 207–223, 2015.

[24] S. Chakraborti, M. Mandal, S. Das, A. Mandal, and T. Chakraborti, "Regulation of matrix metalloproteinases: an overview," *Molecular and Cellular Biochemistry*, vol. 253, no. 1-2, pp. 269–285, 2003.

[25] E. Hijová, "Matrix metalloproteinases: their biological functions and clinical implications," *Bratisl Lek Listy*, vol. 106, no. 3, pp. 127–132, 2005.

[26] T. Djuric and M. Živković, "Overview of MMP biology and gene associations in human diseases," in *The Role of Matrix Metalloproteinase in Human Body Pathologies*, IntechOpen, 2017.

[27] H. Lin, B. Yang, and M. Teng, "T-cell immunoglobulin mucin-3 as a potential inducer of the epithelial-mesenchymal transition in hepatocellular carcinoma," *Oncology Letters*, vol. 14, no. 5, pp. 5899–5905, 2017.

[28] R. Kalluri and R. A. Weinberg, "The basics of epithelial-mesenchymal transition," *The Journal of Clinical Investigation*, vol. 119, no. 6, pp. 1420–1428, 2009.

[29] K. Mima, H. Hayashi, H. Kuroki et al., "Epithelial-mesenchymal transition expression profiles as a prognostic factor for disease-free survival in hepatocellular carcinoma: clinical significance of transforming growth factor-β signaling," *Oncology Letters*, vol. 5, no. 1, pp. 149–154, 2013.

[30] M. H. Yang, C. L. Chen, G. Y. Chau et al., "Comprehensive analysis of the independent effect of twist and snail in promoting metastasis of hepatocellular carcinoma," *Hepatology*, vol. 50, no. 5, pp. 1464–1474, 2009.

[31] T. Wan, T. Zhang, X. Si, and Y. Zhou, "Overexpression of EMT-inducing transcription factors as a potential poor prognostic factor for hepatocellular carcinoma in Asian populations: a meta-analysis," *Oncotarget*, vol. 8, no. 35, pp. 59500–59508, 2017.

[32] W. Li, S. Li, L. Deng et al., "Decreased MT1-MMP in gastric cancer suppressed cell migration and invasion via regulating MMPs and EMT," *Tumour Biology*, vol. 36, no. 9, pp. 6883–6889, 2015.

[33] W. Jiang, Y. Zhang, K. T. Kane et al., "CD44 regulates pancreatic cancer invasion through MT1-MMP," *Molecular Cancer Research*, vol. 13, no. 1, pp. 9–15, 2015.

[34] C. Ding, J. Luo, L. Li et al., "Gab2 facilitates epithelial-to-mesenchymal transition via the MEK/ERK/MMP signaling

in colorectal cancer," *Journal of Experimental & Clinical Cancer Research*, vol. 35, no. 1, p. 5, 2016.

[35] Y. Liu, X. Sun, J. Feng et al., "MT2-MMP induces proteolysis and leads to EMT in carcinomas," *Oncotarget*, vol. 7, no. 30, pp. 48193–48205, 2016.

[36] M. C. Vos, E. Hollemans, N. Ezendam et al., "MMP-14 and CD44 in epithelial-to-mesenchymal transition (EMT) in ovarian cancer," *Journal of Ovarian Research*, vol. 9, no. 1, p. 53, 2016.

[37] A. Lochter, S. Galosy, J. Muschler, N. Freedman, Z. Werb, and M. J. Bissell, "Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells," *The Journal of Cell Biology*, vol. 139, no. 7, pp. 1861–1872, 1997.

[38] C. M. Chen, C. L. Lin, H. L. Chiou et al., "Loss of endothelial cell-specific molecule 1 promotes the tumorigenicity and metastasis of prostate cancer cells through regulation of the TIMP-1/MMP-9 expression," *Oncotarget*, vol. 8, no. 8, pp. 13886–13897, 2017.

[39] A. Miyoshi, Y. Kitajima, K. Sumi et al., "Snail and SIP1 increase cancer invasion by upregulating MMP family in hepatocellular carcinoma cells," *British Journal of Cancer*, vol. 90, no. 6, article BF6601685, pp. 1265–1273, 2004.

[40] Z. Shen, X. Wang, X. Yu, Y. Zhang, and L. Qin, "MMP16 promotes tumor metastasis and indicates poor prognosis in hepatocellular carcinoma," *Oncotarget*, vol. 8, no. 42, pp. 72197–72204, 2017.

[41] K. Nabeshima, T. Inoue, Y. Shimao, and T. Sameshima, "Matrix metalloproteinases in tumor invasion: role for cell migration," *Pathology International*, vol. 52, no. 4, pp. 255–264, 2002.

[42] Y.-L. Lai, C.-L. Gong, C.-K. Fu et al., "The contribution of matrix metalloproteinase-1 genotypes to hepatocellular carcinoma susceptibility in Taiwan," *Cancer Genomics & Proteomics*, vol. 14, no. 2, pp. 119–125, 2017.

[43] X. Liu, L. Yang, J. Tu et al., "MicroRNA-526b servers as a prognostic factor and exhibits tumor suppressive property by targeting Sirtuin 7 in hepatocellular carcinoma," *Oncotarget*, vol. 8, no. 50, pp. 87737–87749, 2017.

[44] B. Wang, Y.-M. Ding, P. Fan, B. Wang, J.-H. Xu, and W.-X. Wang, "Expression and significance of MMP2 and HIF-1α in hepatocellular carcinoma," *Oncology Letters*, vol. 8, no. 2, pp. 539–546, 2014.

[45] K. E. Schoedel, V. Z. Tyner, T. H. Kim, G. K. Michalopoulos, and W. M. Mars, "HGF, MET, and matrix-related proteases in hepatocellular carcinoma, fibrolamellar variant, cirrhotic and normal liver," *Modern Pathology*, vol. 16, no. 1, pp. 14–21, 2003.

[46] S. W. Jing, Y. D. Wang, M. Kuroda et al., "HIF-1α contributes to hypoxia-induced invasion and metastasis of esophageal carcinoma via inhibiting E-cadherin and promoting MMP-2 expression," *Acta Medica Okayama*, vol. 66, no. 5, pp. 399–407, 2012.

[47] K. Si-Tayeb, A. Monvoisin, C. Mazzocco et al., "Matrix metalloproteinase 3 is present in the cell nucleus and is involved in apoptosis," *The American Journal of Pathology*, vol. 169, no. 4, pp. 1390–1401, 2006.

[48] A. Monvoisin, C. Bisson, K. Si-Tayeb, C. Balabaud, A. Desmouliere, and J. Rosenbaum, "Involvement of matrix metalloproteinase type-3 in hepatocyte growth factor-induced invasion of human hepatocellular carcinoma cells," *International Journal of Cancer*, vol. 97, no. 2, pp. 157–162, 2002.

[49] K. Okamoto, C. Ishida, Y. Ikebuchi et al., "The genotypes of IL-1 beta and MMP-3 are associated with the prognosis of HCV-related hepatocellular carcinoma," *Internal Medicine*, vol. 49, no. 10, pp. 887–895, 2010.

[50] Y. Yokoyama, F. Grünebach, S. M. Schmidt et al., "Matrilysin (MMP-7) is a novel broadly expressed tumor antigen recognized by antigen-specific T cells," *Clinical Cancer Research*, vol. 14, no. 17, pp. 5503–5511, 2008.

[51] Y. Lin, J. Liu, Y. Huang, D. Liu, G. Zhang, and H. Kan, "MicroRNA-489 plays an anti-metastatic role in human hepatocellular carcinoma by targeting matrix metalloproteinase-7," *Translational Oncology*, vol. 10, no. 2, pp. 211–220, 2017.

[52] C. Fang, G. Wen, L. Zhang et al., "An important role of matrix metalloproteinase-8 in angiogenesis in vitro and in vivo," *Cardiovascular Research*, vol. 99, no. 1, pp. 146–155, 2013.

[53] G. Qin, M. Luo, J. Chen et al., "Reciprocal activation between MMP-8 and TGF-β1 stimulates EMT and malignant progression of hepatocellular carcinoma," *Cancer Letters*, vol. 374, no. 1, pp. 85–95, 2016.

[54] B. R. Kim, Y. K. Jeon, and M. J. Nam, "A mechanism of apigenin-induced apoptosis is potentially related to anti-angiogenesis and anti-migration in human hepatocellular carcinoma cells," *Food and Chemical Toxicology*, vol. 49, no. 7, pp. 1626–1632, 2011.

[55] G. Opdenakker, P. van den Steen, B. Dubois et al., "Gelatinase B functions as regulator and effector in leukocyte biology," *Journal of Leukocyte Biology*, vol. 69, no. 6, pp. 851–859, 2001.

[56] B. Heissig, K. Hattori, S. Dias et al., "Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand," *Cell*, vol. 109, no. 5, pp. 625–637, 2002.

[57] S. J. Sun, N. Wang, Z. W. Sun, J. Chen, and H. W. Cui, "miR-5692a promotes the invasion and metastasis of hepatocellular carcinoma via MMP9," *European Review for Medical and Pharmacological Sciences*, vol. 22, no. 15, pp. 4869–4878, 2018.

[58] Z. C. Nwosu, D. A. Megger, S. Hammad et al., "Identification of the consistently altered metabolic targets in human hepatocellular carcinoma," *Cellular and Molecular Gastroenterology and Hepatology*, vol. 4, no. 2, pp. 303–323.e1, 2017.

[59] G. Zhang, M. Miyake, A. Lawton, S. Goodison, and C. J. Rosser, "Matrix metalloproteinase-10 promotes tumor progression through regulation of angiogenic and apoptotic pathways in cervical tumors," *BMC Cancer*, vol. 14, no. 1, p. 310, 2014.

[60] O. García-Irigoyen, M. U. Latasa, S. Carotti et al., "Matrix metalloproteinase 10 contributes to hepatocarcinogenesis in a novel crosstalk with the stromal derived factor 1/C-X-C chemokine receptor 4 axis," *Hepatology*, vol. 62, no. 1, pp. 166–178, 2015.

[61] K. M. F. Sze, G. K. Y. Chu, J. M. F. Lee, and I. O. L. Ng, "C-terminal truncated hepatitis B virus x protein is associated with metastasis and enhances invasiveness by c-jun/matrix metalloproteinase protein 10 activation in hepatocellular carcinoma," *Hepatology*, vol. 57, no. 1, pp. 131–139, 2013.

[62] P. T. Gao, G. Y. Ding, X. Yang et al., "Invasive potential of hepatocellular carcinoma is enhanced by loss of selenium-binding protein 1 and subsequent upregulation of CXCR4," *American Journal of Cancer Research*, vol. 8, no. 6, pp. 1040–1049, 2018.

[63] N. Dali-Youcef, K. Hnia, S. Blaise et al., "Matrix metalloproteinase 11 protects from diabesity and promotes metabolic switch," *Scientific Reports*, vol. 6, no. 1, article 25140, 2016.

[64] Q. Bi, S. Tang, L. Xia et al., "Ectopic expression of miR-125a inhibits the proliferation and metastasis of hepatocellular carcinoma by targeting MMP11 and VEGF," *PLoS One*, vol. 7, no. 6, article e40169, 2012.

[65] Z. Yang, Y. Zhang, and L. Wang, "A feedback inhibition between miRNA-127 and TGFβ/c-Jun cascade in HCC cell migration via MMP13," *PloS one*, vol. 8, no. 6, article e65256, 2013.

[66] S. S. Sume, A. Kantarci, A. Lee, H. Hasturk, and P. C. Trackman, "Epithelial to mesenchymal transition in gingival overgrowth," *The American Journal of Pathology*, vol. 177, no. 1, pp. 208–218, 2010.

[67] T. Li, J. Xie, C. Shen et al., "miR-150-5p inhibits hepatoma cell migration and invasion by targeting MMP14," *PLoS One*, vol. 9, no. 12, article e115577, 2014.

[68] D. Taras, J. F. Blanc, A. Rullier et al., "Pravastatin reduces lung metastasis of rat hepatocellular carcinoma via a coordinated decrease of MMP expression and activity," *Journal of Hepatology*, vol. 46, no. 1, pp. 69–76, 2007.

[69] J.-C. Jung, P. X. Wang, G. Zhang, Y. Ezura, M. E. Fini, and D. E. Birk, "Collagen fibril growth during chicken tendon development: matrix metalloproteinase-2 and its activation," *Cell and Tissue Research*, vol. 336, no. 1, pp. 79–89, 2009.

[70] S. Wang, H. Lin, T. Zhao et al., "Expression and purification of an FGF9 fusion protein in E. coli, and the effects of the FGF9 subfamily on human hepatocellular carcinoma cell proliferation and migration," *Applied Microbiology and Biotechnology*, vol. 101, no. 21, pp. 7823–7835, 2017.

[71] C. Yu, Z. Wang, X. Xu, W. Xiang, and X. Huang, "Circulating hepatocellular carcinoma cells are characterized by CXCR4 and MMP26," *Cellular Physiology and Biochemistry*, vol. 36, no. 6, pp. 2393–2402, 2015.

[72] S. A. Illman, K. Lehti, J. Keski-Oja, and J. Lohi, "Epilysin (MMP-28) induces TGF-β mediated epithelial to mesenchymal transition in lung carcinoma cells," *Journal of Cell Science*, vol. 119, no. 18, pp. 3856–3865, 2006.

[73] J. Zhou, X. Zheng, M. Feng et al., "Upregulated MMP28 in hepatocellular carcinoma promotes metastasis via Notch3 signaling and predicts unfavorable prognosis," *International Journal of Biological Sciences*, vol. 15, no. 4, pp. 812–825, 2019.

[74] L. Chen, Q. Zhou, B. Xu et al., "MT2-MMP expression associates with tumor progression and angiogenesis in human lung cancer," *International Journal of Clinical and Experimental Pathology*, vol. 7, no. 6, pp. 3469–3477, 2014.

[75] M.-K. He, Y. Le, Y.-F. Zhang et al., "Matrix metalloproteinase 12 expression is associated with tumor FOXP3$^+$ regulatory T cell infiltration and poor prognosis in hepatocellular carcinoma," *Oncology Letters*, vol. 16, no. 1, pp. 475–482, 2018.

[76] E. Ito, I. Yana, C. Fujita et al., "The role of MT2-MMP in cancer progression," *Biochemical and Biophysical Research Communications*, vol. 393, no. 2, pp. 222–227, 2010.

[77] Y. Inagaki, K. Shiraki, K. Sugimoto et al., "Epigenetic regulation of proliferation and invasion in hepatocellular carcinoma cells by CBP/p300 histone acetyltransferase activity," *International Journal of Oncology*, vol. 48, no. 2, pp. 533–540, 2016.

[78] M. José Blanco, I. Rodriguez-Martin, A. Rodriguez Learte et al., "Developmental expression of membrane type 4-matrix metalloproteinase (Mt4-mmp/Mmp17) in the mouse embryo," *PLoS One*, vol. 12, no. 9, article e0184767, 2017.

[79] A. Paye, A. Truong, C. Yip et al., "EGFR activation and signaling in cancer cells are enhanced by the membrane-bound metalloprotease MT4-MMP," *Cancer Research*, vol. 74, no. 23, pp. 6758–6770, 2014.

[80] C. Kolb, S. Mauch, H. H. Peter, U. Krawinkel, and R. Sedlacek, "The matrix metalloproteinase RASI-1 is expressed in synovial blood vessels of a rheumatoid arthritis patient," *Immunology Letters*, vol. 57, no. 1-3, pp. 83–88, 1997.

[81] D. Kraus, J. Reckenbeil, S. Perner, J. Winter, and R. Probstmeier, "Expression pattern of matrix metalloproteinase 20 (MMP20) in human tumors," *Anticancer Research*, vol. 36, no. 6, pp. 2713–2718, 2016.

[82] K. Ge, J. Huang, W. Wang et al., "Serine protease inhibitor kazal-type 6 inhibits tumorigenesis of human hepatocellular carcinoma cells via its extracellular action," *Oncotarget*, vol. 8, no. 4, pp. 5965–5975, 2017.

[83] A. Guimier, G. C. Gabriel, F. Bajolle et al., "*MMP21* is mutated in human heterotaxy and is required for normal left-right asymmetry in vertebrates," *Nature Genetics*, vol. 47, no. 11, pp. 1260–1263, 2015.

[84] Y. Xie, A. Mustafa, A. Yerzhan et al., "Nuclear matrix metalloproteinases: functions resemble the evolution from the intracellular to the extracellular compartment," *Cell Death Discovery*, vol. 3, no. 1, article 17036, 2017.

[85] Y.-C. Yeh and B. Sheu, "Matrix metalloproteinases and their inhibitors in the gastrointestinal cancers: current knowledge and clinical potential," *Metalloproteinases In Medicine*, vol. 1, pp. 3–13, 2014.

[86] D. Pei, T. Kang, and H. Qi, "Cysteine array matrix metalloproteinase (CA-MMP)/MMP-23 is a type II transmembrane matrix metalloproteinase regulated by a single cleavage for both secretion and activation," *Journal of Biological Chemistry*, vol. 275, no. 43, pp. 33988–33997, 2000.

[87] E. Stoyanov, G. Ludwig, L. Mizrahi et al., "Chronic liver inflammation modifies DNA methylation at the precancerous stage of murine hepatocarcinogenesis," *Oncotarget*, vol. 6, no. 13, pp. 11047–11060, 2015.

[88] R. A. Okimoto, F. Breitenbuecher, V. R. Olivas et al., "Inactivation of Capicua drives cancer metastasis," *Nature Genetics*, vol. 49, no. 1, pp. 87–96, 2017.

[89] A. Sohail, Q. Sun, H. Zhao, M. M. Bernardo, J.-A. Cho, and R. Fridman, "MT4-(MMP17) and MT6-MMP (MMP25), a unique set of membrane-anchored matrix metalloproteinases: properties and expression in cancer," *Cancer Metastasis Reviews*, vol. 27, no. 2, pp. 289–302, 2008.

[90] Q. Sun, C. R. Weber, A. Sohail et al., "MMP25 (MT6-MMP) is highly expressed in human colon cancer, promotes tumor growth, and exhibits unique biochemical properties," *The Journal of Biological Chemistry*, vol. 282, no. 30, pp. 21998–22010, 2007.

[91] A. Sircana, E. Paschetta, F. Saba, F. Molinaro, and G. Musso, "Recent insight into the role of fibrosis in nonalcoholic steatohepatitis-related hepatocellular carcinoma," *International Journal of Molecular Sciences*, vol. 20, no. 7, article 1745, 2019.

[92] A. Cominelli, M. Halbout, F. N'Kuli et al., "A Unique C-terminal Domain Allows Retention of Matrix Metalloproteinase-27 in the Endoplasmic Reticulum," *Traffic*, vol. 15, no. 4, pp. 401–417, 2014.

[93] E. Zografos, A. K. Anagnostopoulos, A. Papadopoulou et al., "Serum proteomic signatures of male breast cancer," *Cancer Genomics & Proteomics*, vol. 16, no. 2, pp. 129–137, 2019.

[94] A. Noël, A. Gutiérrez-Fernández, N. E. Sounni et al., "New and paradoxical roles of matrix metalloproteinases in the tumor microenvironment," *Frontiers in Pharmacology*, vol. 3, p. 140, 2012.

[95] E. Roeb, R. Winograd, B. Breuer, H. Nguyen, and S. Matern, "Increased TIMP-1 activity results in increased expression of gelatinases and altered cell motility," *Journal of Cellular Biochemistry*, vol. 75, no. 2, pp. 346–355, 1999.

[96] E. S. Radisky, M. Raeeszadeh-Sarmazdeh, and D. C. Radisky, "Therapeutic potential of matrix metalloproteinase inhibition in breast cancer," *Journal of Cellular Biochemistry*, vol. 118, no. 11, pp. 3531–3548, 2017.

[97] E. Roeb, A.-K. Bosserhoff, S. Hamacher et al., "Enhanced migration of tissue inhibitor of metalloproteinase overexpressing hepatoma cells is attributed to gelatinases: relevance to intracellular signaling pathways," *World Journal of Gastroenterology*, vol. 11, no. 8, pp. 1096–1104, 2005.

[98] H. Nakatsukasa, K. Ashida, T. Higashi et al., "Cellular distribution of transcripts for tissue inhibitor of metalloproteinases 1 and 2 in human hepatocellular carcinomas," *Hepatology*, vol. 24, no. 1, pp. 82–88, 1996.

[99] T. Song, C. Dou, Y. Jia, K. Tu, and X. Zheng, "TIMP-1 activated carcinoma-associated fibroblasts inhibit tumor apoptosis by activating SDF1/CXCR4 signaling in hepatocellular carcinoma," *Oncotarget*, vol. 6, no. 14, pp. 12061–12079, 2015.

[100] G. Giannelli, C. Bergamini, F. Marinosci et al., "Clinical role of MMP-2/TIMP-2 imbalance in hepatocellular carcinoma," *International Journal of Cancer*, vol. 97, no. 4, pp. 425–431, 2002.

[101] X. Gu, M. Fu, Y. Ding et al., "TIMP-3 expression associates with malignant behaviors and predicts favorable survival in HCC," *PLoS One*, vol. 9, no. 8, article e106161, 2014.

[102] J. W. Kornfeld, S. Meder, M. Wohlberg et al., "Overexpression of TACE and TIMP3 mRNA in head and neck cancer: association with tumour development and progression," *British Journal of Cancer*, vol. 104, no. 1, pp. 138–145, 2011.

[103] M.-D. Yan, C.-J. Yao, J.-M. Chow et al., "Fucoidan elevates microRNA-29b to regulate DNMT3B-MTSS1 axis and inhibit EMT in human hepatocellular carcinoma cells," *Marine Drugs*, vol. 13, no. 10, pp. 6099–6116, 2015.

[104] J. D. Yang, I. Nakamura, and L. R. Roberts, "The tumor microenvironment in hepatocellular carcinoma: current status and therapeutic targets," *Seminars in Cancer Biology*, vol. 21, no. 1, pp. 35–43, 2011.

[105] A. Daniele, R. Divella, M. Quaranta et al., "Clinical and prognostic role of circulating MMP-2 and its inhibitor TIMP-2 in HCC patients prior to and after trans-hepatic arterial chemo-embolization," *Clinical Biochemistry*, vol. 47, no. 3, pp. 184–190, 2014.

[106] J. Salimi Sartakhti, M. H. Manshaei, and M. Sadeghi, "MMP-TIMP interactions in cancer invasion: an evolutionary game-theoretical framework," *Journal of Theoretical Biology*, vol. 412, pp. 17–26, 2017.

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Review Article

Proteomic Technology "Lens" for Epithelial-Mesenchymal Transition Process Identification in Oncology

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The epithelial-mesenchymal transition (EMT) is a complex transformation process that induces local and distant progression of many malignant tumours. Due to its complex array of proteins that are dynamically over-/underexpressed during this process, proteomic technologies gained their place in the EMT research in the last years. Proteomics has identified new molecular pathways of this process and brought important insights to develop new therapy targets. Various proteomic tools and multiple combinations were developed in this area. Out of the proteomic technology armentarium, mass spectrometry and array technologies are the most used approaches. The main characteristics of the proteomic technology used in this domain are high throughput and detection of minute concentration in small samples. We present herein, using various proteomic technologies, the identification in cancer cell lines and in tumour tissue EMT-related proteins, proteins that are involved in the activation of different cellular pathways. Proteomics has brought besides standard EMT markers (e.g., cell-cell adhesion proteins and transcription factors) other future potential markers for improving diagnosis, monitoring evolution, and developing new therapy targets. Future will increase the proteomic role in clinical investigation and validation of EMT-related biomarkers.

1. Introduction

The epithelial-to-mesenchymal transition (EMT) process allows the cellular differentiation from polarized epithelial phenotype to mesenchymal characteristics. Also an active process in embryogenesis and wound healing, EMT plays a decisive role in cancer development being highly coordinated at genetic, epigenetic, and proteome levels by different regulators [1].

The EMT process is marked by loss of epithelial marker E-cadherin, induced by the upregulation of certain transcriptional repressors (e.g., SNAIL1/2, TWIST, and ZEB1/2). As the EMT process occurs, the epithelial cells acquire multiple cell-surface and cytoskeletal markers, as well as expression of certain extracellular proteins and transcription factors [2, 3] (Figure 1). Thus, gaining a set of mesenchymal markers supports and stabilizes the newly acquired cellular phenotype. One of the main changes in the expression of cell-surface markers is the cadherin switch, from E-cadherin high expression in epithelial cells to its low profile correlated with an increased expression of N-cadherin in mesenchymal cells [4]. EMT also alters cell-extracellular matrix (ECM) interactions, and as a result, integrins such as $\beta 6$ integrin, $\alpha 5$ integrin, and syndecan-1 could also be used as biomarkers for EMT, depending on the type of cancer [5]. In terms of cytoskeletal

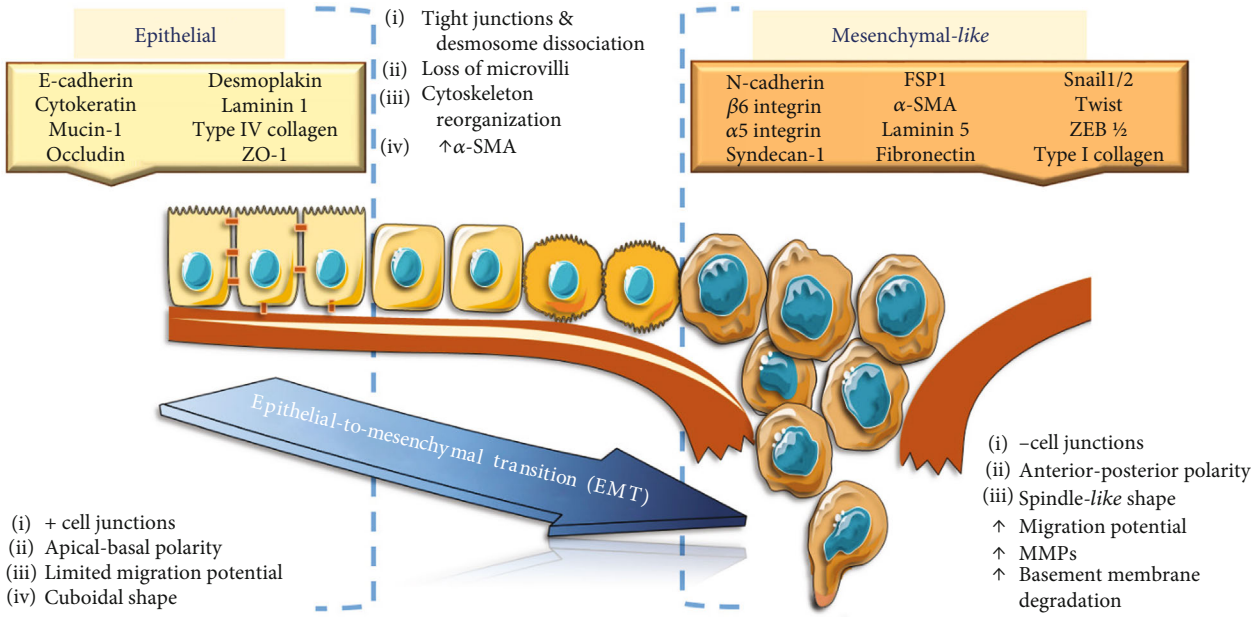

Figure 1: Markers and main molecular changes during epithelial-to-mesenchymal transition.

markers, the expression of fibroblast-specific protein 1 (FSP1), vimentin, α-smooth muscle actin (α-SMA) correlated with low expression or loss of cytokeratins, mucin-1, occludin, and desmoplakin is an indicator of cells undergoing EMT [6]. Another cytoskeletal marker, β-catenin, can be a marker of either epithelial normal cells or mesenchymal cells, depending on its cell localization [5].

In addition, FOXO proteins modulate EMT process during tumour development events contributing to the design of additional studies for future development of FOXO proteins as therapeutic targets [7].

Regarding EMT initiation, there are still debates whether metabolic changes are contributory or just an effect of EMT triggering. Catabolic and anabolic pathways that characterize glucids, amino acids, and lipid metabolism are equally involved. Depending on the tumour cell type, the EMT is based on an aerobic glycolytic program and unveils a lipogenic metabolism depending on the EMT inducer (e.g., TNF-α versus TGF-β-derived Snail1). However, all these metabolic events lead to membrane fluidity increase and destabilization of lipid rafts. This would upregulate membrane cholesterol content making inhibitors of cholesterol synthesis to act for stabilizing lipid rafts, reducing membrane fluidity, and therefore opposing EMT phenomena [8]. An overview of the protein profiling of the EMT process is depicted in Figure 1.

In the very last years, proteomic strategies were enrolled for analysing different milestones in ETM course, real powerful tools for catching and quantifying ETM marks starting from the cellular surface to intracellular events. The proteomic domain comprises a large array of technologies, from traditional ELISA to newer ones, but all of them gaining their place in oncology investigations. MS, with its various variants, like matrix-assisted laser desorption/ionization (MALDI) and electrospray, was subject for Nobel prizes [9]. Soon enough, this proteomic technology was assisted by increased automation and computational analytics software. These last MS's acquired traits have pushed the technology in clinical proteomics [10]. More recently, multiple reaction monitoring was introduced using triple quad MS technology and/or several tribrid high-resolution MS. The evolution of these new types of MS was a consequence of the clinical proteomic needs seeking to measure detailed proteomic panels further to be used in early detection, disease and/or therapy monitoring, patient stratification, and so on [11]. Another proteomic technology that is highly used in clinical approaches is protein microarray (PM) technology with all its functional versions like multiplexed, tissue arrays, direct, and reverse phase [12]. Combinations of various array technologies can now not only show that the protein is deregulated in terms of concentration and structure but also show where this protein is located in a diseased tissue [13]. Hence, from identifying and validating myriads of biomarkers to identifying complex proteomic networks involved in tumourigenesis [14], PM has accelerated clinical proteomics, offering new insights in disease and therapy monitoring.

Tumourigenesis is such a complex process involving a myriad of more subtle events like chronic inflammation and viral infection [15–20]; thus, the proteins that are up-/downregulated in this complex process can be in minute concentrations at one time point. Thus, besides evaluating extremely small amounts of a biological sample for a huge panel of proteins, their actual quantities are in minute concentrations, some of them probably just a few hundred per cell. In clinical proteomics, samples contain small amounts of cells, and if the sample is just a biopsy and not an excised tumour, the sample is even smaller with actually a few hundred to a thousand cells. Invented more than 15 years ago, the reverse phase protein array (RPPA) has been used broadly in medicine [13]. Therefore, RPPA was developed to measure proteins and their phosphorylated forms that exist in low levels in small biopsy samples [12].

Through a time-resolved multiomic strategy, a correlation between proteome changes, phosphoproteome signalling, and histone alterations during TGF-β-induced EMT was recently highlighted. Thus, Erk signalling activation and histone H3K27me3 posttranslational modification were the most significantly upregulated marks in TGF-β-induced EMT that could be turned in therapeutic strategies for EMT-related diseases [21].

Using antibody-based protein profiling with matching transcriptomic data, it was defined an EMT signature consisting of 239 genes in an analysis comprising 736 cancer cell lines. Thus, histone deacetylase inhibitors as inducers of EMT and kinase inhibitors as mesenchymal-to-epithelial transition promoters were the most prevailing markers uncovered [22]. The EMT is linked with and promotes cancer stem cell (CSC) formation and mediates drug resistance, making tumour microenvironment a promising source of clinical markers and/or therapeutic targets in cancer [23].

EMT candidate markers that may help to diagnose diseases or monitor treatment efficiently could be detected *via* proteomic tools. One of the most engaged technologies is mass spectrometry- (MS-) based platforms that allow analysing multiple analytes and samples and moreover allow differentiation between epithelial and mesenchymal tumours and in addition could depict how these can be assessed for future target discovery and validation [24], contributing for better understanding of the physiological and pathological bases in EMT process [25].

Even more, an intricate process like ETM would beneficiate from the latest proteomic approaches to study the dynamics of this complex process.

2. EMT Proteomics

2.1. Cellular Models in EMT Proteomics. Although many studies regarding EMT rely on the significant role that endothelial cells (EC) play in this crucial process of tumour invasiveness and migration [2], the EMT connection with tumourigenesis and metastatic course was initially unveiled in various epithelial cancer cell line models [26]. Furthermore, EMT in tumourigenesis is a demanding task as certain molecular similarities are shared by human tumour cells with mesenchymal phenotype and stromal fibroblasts [27]. Therefore, such studies using different cancer cell line models provided data regarding the relevance of EMT to the metastatic process [28]. Moreover, this challenge is nowadays transposed in studies involving human tumour samples to highlight the clinical role of the EMT process [26, 29].

The interference of EMT with tumour cell fate is studied in various *in vitro* models of cancers focusing mainly on cellular morphology in a definite *omic* context generated by tumour cell transformation. The myriad of factors released in tumour milieu are seized nowadays with high-throughput proteomics (MS, 2D electrophoresis, arrays platforms, etc.) that further decipher those specific alterations imprinted in the tumour milieu.

2.1.1. Breast and Genital Cancer. Factors released by EC generate alterations specific for EMT and moreover could tip the balance toward EMT or toward MET. Thus, in a model with human breast cancer SK-BR-3 cells exposed to EC supernatants, it was revealed by quantitative MS proteomics that *nidogen-1* inhibits SK-BR-3 cell migration. This nidogen-1 is exclusively derived from confluent EC and reveals a novel pathway of cancer progression controlled by EC [2].

Proteomic analysis is able to decode fine molecular mechanisms of EMT triggering. For example, SNAIL is one of the transcription factors overexpressed in EMT and is directly correlated with cancer invasiveness [30]. Overexpression of SNAIL in breast adenocarcinoma cell line MCF7 was validated by molecular and functional proteomic tests. Comprehensive proteomic subcellular fractionation corroborated with GEL-LC-MS/MS revealed 4,289 intracellular proteins involved in cell cycle and epigenetic control. This complex protein network analysis show that SNAIL overexpression led to cell cycle arrest in G0/G1. Moreover, SNAIL upregulation is favoured by HDAC1 inhibition suggesting an interrelation between these two proteins in EMT initiation. These molecular mechanisms underlying EMT might also represent effective strategies for cancer metastasis management [31]. Another modulator of EMT, TWIST, is a highly conserved transcription factor that also acts as a major regulator of EMT; however, little is known about signalling regulation by TWIST in cancer cells. By iTRAQ labelling combined with 2D LC-MS/MS, 194 proteins mainly involved in MAPK, PI3K/AKT, and WNT signalling were identified with a highly modified expression in Twist-overexpressing MCF10A mammary epithelial cells. Ingenuity Pathway Analysis showed that ITGB1 integrin interconnects ILK kinase and FAK kinase as well as MAPK, PI3K/AKT, and WNT pathways. Amplified TWIST and ITGB1 expressions are associated with tumour progression and EMT in MCF10A cells. Inactivating ILK, FAK, MAPK, or PI3K/AKT signalling would also suppress EMT, so the ITGB1-FAK/ILK axis revealed by proteomic inquires conducts the TWIST-induced EMT in human breast cancer cells [32].

More recently, breast cancer metastasis was associated with alterations in genes encoding for complex I components of mitochondria. A quantitative proteomic analysis in a model with highly metastatic MDA-MB-231 cells has assessed certain proteins encoded by these genes and pointed out that it could have significant value in EMT. Using the iTRAQ labelling, it was found that NDUFB9, one of the respiratory complex I subunits, was downregulated; moreover, NDUFB9 knockdown cells MDA-MB-231 exhibit decreased expression of E-cadherin and an increased expression of vimentin and fibronectin-1, as assessed by immunoblotting analysis. Downregulation of NDUFB9 activates Akt/mTOR signalling pathway, which led to EMT endowing MDA-MB-231 cells with a metastatic phenotype. Thus, complex I mitochondrial deficiency could be a potential biomarker for assessing EMT process with possible clinical utility [33]. Another study model with MDA-MB-231 cells was used to assess the transition from a nontumourigenic epithelial-like phenotype to an aggressive mesenchymal-like one. HMGA1, a nonhistone chromatin protein, is a key regulator of EMT process. The proteins isolated from HMGA1-silenced MDA-MB-231 cells were analysed using label-free shotgun MS. Data obtained

generated an HMGA1 protein signature of 21 members endorsed with prognostic value in breast cancer. Further, qRT-PCR, Western blot, and immunohistochemistry validated the association of three proteins (KIFC1, LRRC59, and TRIP13) with HMGA1 expression levels and with tumour cell dissemination. Besides their potential as a druggable target, these factors aid in elucidating EMT in a triple-negative breast tumour [34].

EMT process is a very appropriate barograph in assessing therapeutic effects of certain drug agents in breast cancer. Thus, the effect of two retinoic acid isomers was examined in MDA-MB-231 human breast cancer cell line. Bottom-up proteomic strategies (2D SDS-PAGE, MALDI-TOF/TOF) were applied for identifying more than 50 proteins affected by retinoic acid isomers, from which 9 proteins are associated with the tumour process. Exposure to retinoic acid isoforms led to a decreasing of the protein level related to cellular metabolism, apoptosis, and regulation of the transcription process or EMT, namely, annexins, nucleoside diphosphate kinase B, and vimentin [35]. EMT process could guide the therapy options in cancer cells expressing oncogenic Ras mutants. An MS-based method using KRasG12V-transfected MCF10A (MCF10A-KRasG12V) cells were used for molecular profiling of the cell membrane proteins. This combined proteomic scrutiny identified over 500 cell-surface proteins deregulated in MCF10A-KRasG12V cells that depict as a real proteomic map the phenotypic changes consistent with EMT process and further uncovers potential therapeutic targets [36]. Intercellular communication in EMT is another topic of interest. Recent data involving proteomic assessment demonstrated that the protein cargo of exosomes reflects the *epithelial/mesenchymal phenotype* of secreting breast cancer cells. Thus, breast cancer phenotypes may be differentiated based on their protein content carried by exosomes [37].

In endometrioid carcinoma (ENC), it was reported previously that aldehyde dehydrogenase 1 (ALDH1), a potential marker of normal and malignant stem cells, is related to the tumourigenic potential. Using shotgun proteomics, the levels of several proteins were compared in human ENC cells with high and low ALDH1 expressions; it was noticed that *serum deprivation-response protein* (SDPR) was particularly expressed in cells with high ALDH1 expression. SDPR is a protein required for the formation of caveolae. By means of SDPR-knockout ENC cells generated with CRISPR/Cas9 tool, it was shown that SDPR was correlated with invasion, migration, and EMT [38].

At distant sites, the interaction of circulating tumour cells (CTCs) with the microenvironment is crucial for metastatic colonization, with the participation of the extracellular vesicles (EVs). Tumour EVs replicate the *epithelial* phenotype predominant in the primary carcinoma, whereas CTCs are regarded as *EMT* phenotype. The epithelial-like EVs were characterized using *SILAC proteome analysis* in Hec1A endometrial cell line model. There was an *in vitro* indication of improved adhesion of CTC to a functionalized endothelium, suggesting a contribution of the epithelial-like EVs in the homing of CTCs at metastatic sites and indirectly in EMT commencement [39].

Approaches with proteins map in EMT were extended to ovarian cancer where three cell lines—the cell line-derived ovarian cancer stem cells (OCSCs), 3AO, and Caov3—were subjected to proteome pattern surveying using *liquid chromatography- (LC-) MS/MS label-free quantitative proteomics*. Over 70 proteins were found most differentially expressed, and among them, stonin 2 (STON2) was suggested to downregulate the stemness of the ovarian cancer cell which is characterized by EMT-related markers. The identified protein network revealed that STON2 modulate stemness in ovarian cancer cells *via* epigenetic effectors such as DNMT1, and therefore, STON2 has a role in ovarian cancer biology and could represent a therapeutic target [40].

Oct4A is a well-known biomarker for cancer stem cells, and recently, its key roles were revealed in ovarian tumour cell survival, metastasis, and drug resistance. An MS-based proteomic analysis performed in ovarian cancer shRNA Oct4A knockdown cell line uncovers important alterations in protein networks related to cytoskeleton, ECM, cell proliferation, drug resistance, and EMT, sustaining Oct4A role in modulating EMT related to ovarian tumours [41].

2.1.2. Renal Carcinoma. Multiomic platforms were enrolled also in exploring EMT in renal carcinoma through modulators of transcription factors such as SNAIL, well known to be involved in EMT initiation [2]. Thus, BRCA1-associated protein 1 (BAP1) is an enzyme from the ubiquitinase family whose encoding gene BAP1 is mutated in almost 10% of clear cell renal cell carcinomas (ccRCC). Lower BAP1 expression is related to prolonged overall survival; quantitative proteomics of BAP1 knockout ccRCC cell lines revealed a decreased expression of transcriptional repressor SNAIL and reduced activity of Rho-GTPase events that uphold EMT process [42].

2.1.3. Melanoma. In melanoma, EMT process is related to some transcription factors that affect cellular physiology. For instance, a higher expression of the osteogenic master gene RUNX2 has been reported in melanoma cells associated to tumour progression and EMT. In a melanoma cell model with RUNT-deleted cells by CRISPR/Cas9 technique, a reduced cell proliferation, increased apoptosis, and reduced EMT traits, indicating RUNX2 as a likely therapeutic target [43], were reported.

To another scale, phenotype switching toward more aggressive forms is related to EMT. The NK cells may increase the malignancy of melanoma cells by inducing changes relevant to EMT that are reliant on NKp30 or NKG2D receptors and a concomitant IFNγ and TNFα release. Melanoma cells suffering EMT either increase their HLA-I surface expression or inhibit tumour-recognizing activating receptors thus avoiding the NK cell attack. In several different melanoma cell lines derived from metastatic melanoma resections, MS analysis revealed proteomic profiles induced by coculture with NK cells or by EMT cytokines; it was observed a partial overlapping in proteomic pattern depending on the milieu exposure (NK cells or EMT factors) that could be exploited in innovative antitumour therapies NK cell-based [44].

2.1.4. Lung Cancer. In lung cancer cells, functional proteomic screening was enrolled to assess EMT via Raf-MEK-ERK pathway modulation by KAP1. In A549 lung cancer cells, it was found that knockdown KAP1 arrested cells in the G0/G1 phase and decreased growth, metastasis, and EMT process; thus, Raf-MEK-ERK pathway represents a source of therapeutic inquires and regulate lung cancer development [45].

2.1.5. Digestive Tumours. A key step in hepatocellular carcinoma (HCC) development is the migration of malignant hepatocytes into blood vessels that would sustain the spreading of HCC tumour cells. This transendothelial migration depends on TGF-β which promotes EMT that would favour HCC cell spreading. In a model of hepato-transendothelial migration using EMT-transformed hepatocytes (MIM-RT) and liver sinusoidal endothelial cells (mLSECs) [46, 47], specific molecular changes in both migrating hepatocytes and endothelial cells were detected by MS. There was reported a proteome pattern comprising 36 and 559 regulated proteins in hepatocytes and endothelial cells, respectively, suggesting that transendothelial migration also depends on intercellular interactions and not on TGF-β only. There were identified alterations on peroxiredoxin-3, epoxide hydrolase, transgelin-2, and collectin 12, markers associated clinically with patient's survival. This hepatocellular plasticity sustained by an EMT phenotype induced by TGF-β provides valuable clues in HCC invasion mechanisms [48].

EMT regulation is also ensued at the epigenetic level, so UHRF1 maintains the optimum level of DNMT1-mediated DNA methylation, being involved in various tumour processes. Recently, it was shown that UHRF2 has a role in EMT process regulation by acting as a coregulator of the EMT-transcription factors (TFs). The following human gastric cancer cell lines—SGC7901, MKN74, N87, and MKN45—with ectopically expressed UHRF2 were subjected to proteome profiling that has revealed upregulation of many EMT-TFs in UHRF2-overexpressing cells. Moreover, by ChIP-seq, it was identified that UHRF2 and EMT-TFs share the same genomic binding motifs, and the interactome analysis highlights that UHRF2 interact with TFs (e.g., TCF7L2), proteins involved in chromatin remodelling and histone alterations, data confirmed by immunoprecipitation combined with MS. Giving this evidence from multidimensional proteomic analysis, a role of UHRF2 in transcription coregulator for EMT and metastasis mechanisms was proposed [49].

2.1.6. Other Cancers. The differential action of TGFβ, known as EMT inducer on cancer-associated fibroblasts (CAFs) and on epithelial tumour cells (ETCs), has been recently investigated at the proteomic level. In a coculture system comprising fluorescently labelled CAFs and ETCs stimulated with TGFβ, cells were separated using FACS and subjected to quantitative MS. It was shown that TGFβ treatment upregulates extracellular matrix proteins and increased N-cadherin levels in CAFs, whereas ETCs were found low responders to TGFβ. The authors conclude that TGFβ treatment could change proteome pattern in fibroblasts and epithelial tumour cells and thus modulate EMT phenotype [50].

A similar approach involving quantitative MS combined with gene arrays was used in NCI-H226 mesothelioma cells to assess EMT pathway in the frame of BAP1 tumour suppressor activity influence. Analysed proteome and gene expression revealed enrichment in proteins related to cytoskeleton as well as enhancement in markers related to EMT. Further functional evaluation in BAP1 wild-type, BAP1 knocked down, and BAP1 noncatalytically expressing NCI-H226 mesothelioma cells indicate that BAP1 enzymatic activity was a requisite to maintain these proteomic and genomic phenotypes [51].

A complex proteomic approach corroborated with qPCR target validation was used to identify differentially expressed proteins in a coculture of human glioma U251 cells treated with human bone marrow mesenchymal stem cells (hBMSCs). It was registered that hBMSCs could inhibit cell proliferation and could induce apoptosis of U251 cells. By means of proteomics, there were identified 11 differentially expressed proteins involved in biological processes mostly related to the PI3K/AKT pathway. In addition, hBMSC treatment led to inhibition of EMT-like and PI3K/AKT pathways. These proteomic data highlight the antitumour properties and EMT inhibitors of hBMSCs with the potential to be explored in glioma therapy [52].

2.2. EMT Markers in Tumour Tissue—Proteomic Approaches

2.2.1. Digestive Tract Tumours. Colorectal cancer (*CRC*) actually represents the most studied solid cancer for EMT proteomic analysis, and various proteomic technologies applied in patient's samples were employed to study the complexity of EMT process underlying CRC. The worldwide high incidence of CRC and its metastatic capacity leading to disabilities and unfortunately death streamline the proteomic assessment that has revealed a high array of deregulated proteins in tumour samples that were found linked to EMT. Thus, recently, using laser capture microdissection, CRC tissue samples were collected and further subjected to iTRAQ-based quantitative proteomic analysis to evaluate global proteomic profiling of CRC tumour microenvironment. Comparing samples of tumour microenvironment vascular endothelial cells (VECs) from patients subjected to antiangiogenic therapy, a large array of differentially expressed proteins was identified in VEC, comprising over 200 different protein types. The majority of these proteins were proven to be involved in EMT process, ECM-receptors, focal adhesion, PI3K-Akt signalling pathway, angiogenesis, and HIF-1 signalling pathways. Owing to this large proteomic profiling, future targets related to EMT could be developed in CRC [53].

The cellular heterogeneity of tumour microenvironment with various clones of cancer cells, stroma cells, cancer stem cells, and immune cells represents a rich reservoir for clinical meanings in EMT process. In over 400 CRC samples, Xu et al. investigated various EMT markers along with immune cell markers investigated. Protein expression of certain stemness markers (e.g., Nanog, Lgr5, and CD44v6) and infiltrating immune cells were highly correlated; furthermore, all these markers positively correlated with E-cadherin or Snail.

Authors even denominated a protein cluster (SIE) that could predict 5-year survival of CRC patients. This cluster is comprised of proteins that are related to cancer stemness, immune status, and EMT process [54].

Using high-resolution proteomic technologies, namely, nano-LC-MS/MS coupled to Orbitrap mass spectrometry, liver metastases of CRC revealing that the ECM is deregulated by cancer cell-derived peptidyl-arginine deiminase 4 (PAD4) were evaluated. Citrullination of collagen type I as a key component of ECM is involved in the EMT promotion and liver metastasis, showing PAD4 involvement in the progression of CRC to metastasis [55].

The study of functional proteome in over 250 CRC tumour samples that were further compared to over 450 samples from The Cancer Genome Atlas (TCGA) has identified various protein patterns. The 163 validated proteins were accomplished with RPPA. This large study pointed out that proteins that append to the EMT pattern characterized a subtype A while high Akt/TSC/mTOR pathway characterized subtype B. Prognostic relevance of these patterns was analysed, and the authors concluded that eight proteins could predict tumour recurrence, and these proteins were collagen VI, FOXO3a, INPP4B, LcK, phospho-PEA15, phospho-PRAS40, Rad51, and phospho-S6 [56].

After developing genomic studies, proteomic confirmations have established that high-mobility gene group A2 (HMGA2) has as direct downstream target IL11 that modulates cell migration through pSTAT3-dependent signalling pathway. Therefore, in over 120 CRC sample tissues, a strong positive correlation was found between HMGA2 and IL11 expression and further was associated with poor prognosis and with other clinical parameters like tumour size and lymph node tumour invasion. Thus, Wu et al. pointed out that HMGA2 and IL11 can be new therapy targets in CRC [57].

In a recent report, Zhang et al. have studied in CRC samples that prostate transmembrane protein androgen induced 1 (PMEPA1) is linked to EMT. Gene expression microarray and further immunoproteomics indicate that PMEPA1 is highly expressed in tumours compared to normal tissue and is associated with a poor prognosis. EMT process is promoted by PMEPA1 through the activation of bone morphogenetic protein (BMP) signalling through TGF-β action [58].

Integrin-linked kinase (ILK) was also studied in relation to CRC progression and chemoresistance. In around 150 tumour samples, it was shown through immunohistochemical analysis that ILK expression correlates with EMT and CSC markers. Moreover, this overexpression is associated with metastasis and chemoresistance [59]. In a larger cohort comprising over 400 CRC samples, immunohistochemistry showed a particular S100A8+ cell type harboured in the tumoural stroma that was found associated with EMT markers, like E-cadherin and SNAIL, and moreover, this association could predict CRC prognosis [60].

Using proteomic and genomic technologies aided by bioanalysis, several EMT biomarkers were identified in CRC samples (e.g., BGN, MMP1, LGALS1, SERPINB5, and TM4SF4) and appended to the pathway of TGFβ/Snail triggered with TNFα/NFκB. The authors point out that poor prognosis is associated with these biomarkers that are reported as being involved in EMT process [61].

Lipocalin2 (LCN2) expression in 400 CRC samples was studied with various proteomic technologies involving immunohistochemistry and Western blot and linked to EMT process. LCN2 was found highly expressed in over 60% of the tumour samples, and it was found significantly correlated with the presence of E-cadherin in the membrane and with the absence of nuclear β-catenin. The authors point out that LCN2 can be a negative regulator of EMT in CRC, acting upstream of NF-κB/snail signalling network. Thus, therapeutical manipulation of LCN2 and NF-κB/snail pathway can be a future approach in CRC [62].

Interleukin-13 (IL-13) can be another candidate molecule triggering EMT. Thus, by Western blot and immunoblot analysis, it was reported in CRC samples a positive correlation between IL-13Rα1 and ZEB1 probably demonstrating that IL-13/IL-13Rα1/STAT6/ZEB1 axes promote EMT and aggressiveness of this cancer [63].

Growth differentiation factor 15 (GDF15) can enhance EMT process *via* TGF-β receptor that further activates Smad2 and Smad3 pathways. Indeed, in CRC tissues, GDF15 is overexpressed and was correlated with its increased serum levels in patients diagnosed with CRC. A high level of GDF15 prognosticated reduced overall survival in CRC. GDF15 activates EMT and thus can be considered a new prognostic marker [64].

In a study involving highly invasive colon cancer cell lines and CRC samples, correlations between Cdc42BPA overexpression and clinic-pathological patient parameters were found. Using tissue microarray, it was found that Cdc42BPA expression is higher in CRC samples when compared to adjacent normal tissues. Cdc42BPA expression correlated with metastasis and worse prognosis [65].

Gastric cancer (*GC*) is a very heterogeneous disease characterized by a high rate of dissemination; thus, studies that focus on the EMT process seek to evaluate pathways that can prognosticate the disease outcome and new therapy approaches. In GC, the FGFR1 expression was reported positively correlated with SNAI1, VIM, and ZEB1 expression but negatively correlated with CDH1. Furthermore, FGFR1 expression was associated with peritoneal dissemination of the tumour and with EMT that was reflected in poor prognosis for GC patients [66]. Recent proteomic reports have shown that GC comprises two molecular subtypes, the mesenchymal (MP) and epithelial (EP) phenotypes. While MP subtype is associated with poor prognosis and high resistance to chemotherapy, EP subtype induces better survival rates and sensitivity to chemotherapy. Integrative proteomic analysis has revealed proteins involved in the EMT-related pathways and insulin-like growth factor 1 (IGF1)/IGF1 receptor (IGF1R) pathway. With this newly acquired knowledge regarding these pathways, novel therapeutic targets can become eligible and developed in GC [67].

In HCC, the metastasis process is correlated with subtle processes like GnT-V-mediated N-glycosylation of marker CD147/basigin. This is actually a tumour-associated glycoprotein upregulated when EMT process is triggered *via* TGF-β1 activation. Moreover, GnT-V expression is

controlled by PI3K/Akt pathway so that this recent study provided new evidence for developing specific drugs that can impede metastasis [68]. In HCC tissues, proteomic studies were performed to investigate the differences between early recurrence and late recurrence cases. 2D fluorescence gel electrophoresis was used as the main proteomic technology, and after investigating over 1,600 proteins, 19 proteins were selected based on their capacity to differentiate between these two groups of patients. Transglutaminase 2 (TGM2) was found upregulated in the early recurrence patients, and TGM2's mRNA level was correlated with EMT-related genes. This study has shown, once more, that proteomic evaluations in HCCs can be further developed for identifying new therapeutic targets in metastatic HCC [69]. Another study has enrolled quantitative proteomics and Ingenuity Pathway Analysis to prove that TGM2 overexpression in HCC is correlated with inflammatory signalling pathways. Moreover, the promotion of EMT process in HCC was shown to be mediated by pseudohypoxia triggered by TGM2/VHL/HIF-1a pathway [70].

In primary liver carcinoma, a proteomic retrospective study has shown that a novel peptide containing the RGD (Arg-Gly-Asp)-sequence derived from the C-terminal portion of fibrinogen was detected in the sera of metastatic patients. This peptide was associated to neoangiogenesis and EMT process [71].

2.2.2. Breast and Genital Cancers. In terms of applying proteomic technologies to depict EMT process, the second most studied cancer is breast cancer (BC). Like in CRC, breast cancer has a high incidence and, at the molecular level, is characterized by various subtypes. In the search to evaluate complex proteomic biomarkers in BC, various proteomic technologies were recently applied. Liquid chromatography-selected reaction monitoring MS (LC-SRM) was reported to aid the oncology domain because it can quantify multiple biomarkers. In BC, the American Society of Clinical Oncology (ASCO) has validated some tissue markers for evaluating prognosis and guiding therapy, like estrogen receptor, progesterone receptor, and HER2/Neu receptor tyrosine kinase. LC-SRM technology assessed these proteins and their phosphorylation status, and furthermore, these proteins correlated with Ki-67 (proliferation marker) and vimentin (tumour aggressiveness markers) as related to EMT process. Within this study, Chen et al. reported the design of a three-tier multiplexed assay platform, for evaluating the complex biology of BC tissues [72].

Like in other solid cancers, tumour ECM has an important role in BC tumourigenesis. Using an array of proteomic approaches (2D differential gel electrophoresis, MALDI-MS, and immunoblotting), the deregulated expression of ECM proteins was studied. There is a specific ECM pattern characterized by a series of dysregulated proteins, e.g., fibrinogen-β chain, collagen α-1(VI) chain, and α-1B-glycoprotein. In triple-negative BC that displays the mentioned ECM pattern, there is an increase of FGG and $\alpha5\beta1/\alpha v\beta3$ integrins accompanied by a decrease of detyrosinated α-tubulin and mimecan. These deregulated expressions induce integrin disorganization involving focal adhesion kinase and activation of EMT-related Rho GTPases. This proteomic profiling in BC could be developed in prognosis biomarkers [73]. Inside triple-negative BC, there is a subset of claudin-low (CLOW) type. The Clinical Proteomic Tumour Analysis Consortium (CPTAC) has recently reported a proteogenomic evaluation of this BC CLOW type. Dihydropyrimidinase-like-3 (DPYSL3) protein was found specific for CLOW subset, and it was suggested that DPYSL3 constitutes a key protein in the negative feedback for EMT. Thus, through this protein, CLOW tumours can be identified as being sensitive to PAK signalling inhibitors during EMT process [74].

In BC, fibroblasts trigger the activation of breast cancer stem cells (BCSCs), while CAFs induce EMT and therefore favour a stem cell profile. Cells isolated from BC samples, namely, normal fibroblasts (NFs) and CAFs, subjected to proteomic analyses have revealed some interesting findings. Proteomics has shown that these cells are heterogeneous and can trigger BCSC generation, with an emphasis on CAF potency. Thus, CAFs induced aldehyde dehydrogenase-1-positive (ALDH1+) BCSCs, while NFs generated mostly CD44+CD24-type BCSCs [75].

Using a newly established flow cytometry surface proteomics combined with cellular functional analysis, several characteristics of metastatic breast cancer (MBrCa) explants were studied. On these cells, several markers were found upregulated: CD200, CD51/CD61 (from the integrin $\alpha5/\beta3$ family), CD26 (dipeptidyl peptidase-4), CD165 (c-Cbl), and CD54 (ICAM-1). When EMT process evolves and it is accompanied by invasion, a series of proteins are once more upregulated (e.g., CD26, CD63 (LAMP3), CD105 (Endoglin), CD107a (LAMP1), CD108 (Semaphorin 7A), CD109 (Integrin $\beta4$), CD151 (Raph blood group), and disialoganglioside G2). When comparing these data to standard breast cancer cell lines (MDA-MB-231, MCF7, and BT-474), the authors have shown that MBrCa have a clear mesenchymal pattern and that surface proteome is different when compared to standard BC cell lines [76].

Basal-like breast cancer (BLBC) is a type of BC associated with poor prognosis. Using several genomic and proteomic technologies, aldo-keto reductase 1 member B1 (AKR1B1) was found overexpressed in BLBC. The authors show a positive feedback loop where Twist2 induces the transcription of AKR1B1 activating nuclear factor κB (NF-κB) that further upregulates Twist2. Thus, this positive loop will activate EMT complex process. Moreover, epalrestat, AKR1B1 inhibitor, could suppress CSC characteristics in BLBC tumours [77].

Invasive lobular carcinoma (ILC) is a subtype of histological BC, having the second occurrence after invasive ductal carcinoma (IDC). In a broad genomic, proteomic (e.g., RPPA), transcriptomic, and clinical data performed on ILC, Michaut et al. reported subtyping ILCs. Thus, ILC has an "immune-related subtype" characterized by upregulation of PD-L1, PD-1, and CTLA-4 and higher sensitivity to DNA-damaging cytostatics and a "hormone-related subtype", which is related to EMT and several genomic traits (e.g., gain of chromosomes 1q and 8q and loss of chromosome 11q) [78]. Previously reported by other groups [79], the study of

Michaut et al. confirmed that ILCs can beneficiate from new therapeutical agents as PI3K pathway inhibitors [78].

In the class of genital cancers, high-grade serous ovarian cancer (HGSOC) has a high recurrence rate, mainly due to the high rate of drug resistance. In an established human ovarian carcinoma cell line, isolated from a patient with chemorefractory HGSOC, complex characterization was performed using genomic, transcriptomic, and proteomic technologies like MS. High levels of alpha-enolase and vimentin were found characterizing an EMT profile of this cell line [80]. In ovarian cancer, EGFR family of protein overexpression depicts an aggressive behaviour. In an ovarian adenocarcinoma cell line (Caov-3), the inducement of EMT was studied using subcellular proteome enrichment, GEL-LC-MS/MS, and SILAC platform. Signalling pathways like PI3K/Akt/mTOR and Ras/Erk MAPK were found activated when EMT was induced with EGF in this Caov-3. The study shows that EGF-induced EMT in ovarian cancer cells induces also deregulations in protein synthesis, cell cycle control, CSC generation, and the clinical poor prognosis of the patients [81].

In cervical cancer cells, another protein with enzyme function, O-GlcNAc transferase (OGT), was proven to be involved in EMT regulation. Gao et al. described in 2018 for the first time OGT-interacting proteins, like PRMT5/WDR77 complex, PRC2 complex, the ten-eleven translocation (TET) family, CRL4B complex, and nucleosome remodelling and deacetylase (NuRD) complex. OGT upregulation in cervical cancer was shown to be related to worse prognosis. The study pointed out that OGT is related to EMT and can be both prognosis biomarker and future target therapy [82].

Using immunoprecipitation and MS-based quantitative proteomic approaches in a long established cervical cancer cell line (HeLa), ADAM12 was reported to interact with various proteins. Among these proteins, myoferlin was reported as regulating ADAM12 expression, reducing its specific substrate, E-cadherin. All these mechanistic links regulate cell adhesion and metastasis [83].

2.2.3. Other Cancers. There are several sparse studies that have used proteomic analysis to evaluate EMT process in the metastatic outcome of cancers.

(1) Head and Neck Cancers. In head and neck squamous cell carcinoma (HNSCC), recurrence has a high incidence, most probably due to minimal residual disease (MRD). In a complex research flow involving also membrane proteomic methodology [84], several interesting protein expressions were found. Thus, EGFR was found highly expressed and moreover found constitutively phosphorylated; CD10, a marker for CSC, was as well found overexpressed. Therefore, Roh et al. show that in HNSCC, markers appending to EMT process can be hallmarks of recurrence and they can be further developed for testing MRD in these patients [85]. In human oral squamous cell carcinomas (OSCCs), nucleosome remodelling and deacetylase (NuRD) complex regulates tumourigenesis processes, and the loss of the subunit Deleted in Oral Cancer 1 (DOC1) associates with protumourigenesis and EMT processes. When restoring DOC1 in OSCC cells, EMT process is reversed through a regulatory process in which SWI/SNF and NURD have antagonistic functions that control chromatin and transcription [86]. Using RPPA profiling in nasopharyngeal carcinoma (NPC), total protein expression and protein functions were analysed in correlation to metastasis. In NPC, metastasis was associated with proteins regulating signalling pathways that control the cell cycle, apoptosis, and EMT [87].

(2) Renal Cancers. Renal cell carcinoma (RCC) is characterized by a high degree of metastasis. Zhou et al., using a proteomic approach, have shown that in tumour tissues, MYSM-1 is downregulated. Tissue microarray has shown that the low expression is associated with poor clinical prognosis. Inducing overexpression of MYSM-1 suppressed cell proliferation, migration, and invasion, inhibiting EMT process [88]. In clear cell renal cell carcinoma (ccRCC), an integrated proteomic and transcriptomic evaluation has shown that in all metastasis stages of ccRCC, the most differentially expressed molecules were TGF-β and proteins related to EMT. From this complex process, serpin peptidase inhibitor clade H member 1 (SERPINH1) was found strongly associated with poor clinical prognosis. These proteins related to EMT could stratify ccRCC patients that need a more aggressive therapeutical approach [89]. In urothelial carcinoma (UC), MS and quantitative proteomics were used for investigating in a patient's urine biomarkers associated to EMT. Shotgun proteomics identified over 200 candidate proteins in which signalling pathways of SH3 domain binding glutamic acid-rich protein like 3 (SH3BGRL3) were the most prominent one. SH3BGRL3 expression associates with risk of progression for UC patients. SH3BGRL3 is involved in EMT process promotion and cell migration. Thus, evaluation of urinary SH3BGRL3 can identify a subset of patients that need a more aggressive treatment in order to impede the disease progression [90].

3. EMT Highlights in Therapy

3.1. New Therapy Targets. As already stated prior, there are several types of solid cancers, e.g., BC and digestive cancers, which are taking advantages on proteomic approaches seeking for new therapy targets.

3.1.1. Breast Cancer. In BC, proteomic studies have been performed using quantitative multiplexed proteomic tandem mass tags (TMTs) to address new therapy targets. Hence, in a recent study, TACC3 (transforming acidic coiled-coil protein 3) inhibitor, KHS101, suppresses cancer cell stemness and EMT processes and induces apoptosis. Upon applying this proteomic analysis, multiple protumoural processes were shown to be hindered by this inhibitor; thus, KHS101 can be foreseen as a multitargeting inhibitor in BC [91]. Among the genes that characterize BC cells that develop EMT or its reverse process MET, those that encode for proteins involved in DNA replication and repair pathways, ABC transporter, Hedgehog, Notch, and several metabolic pathways were found deregulated, and these can be also future therapy targets [92].

In a BC cell line model using several proteomic methods, new drugs were screened to abolish EMT processes. Thus, a combination of resveratrol (RSVL) with salinomycin (SAL) showed good efficacy in these cell lines. Western blots, colony formation, and flow cytometry for cellular apoptosis were used to show that EMT-associated proteins (e.g., fibronectin, vimentin, N-cadherin, and slug), inflammation-related proteins (e.g., Cox2, NF-κB, and p53), and apoptotic molecules (Bax, Bcl-2) were found to drive cells through the inverse process, namely, MET upon therapy. Hence, in triple-negative BC, RSVL can positively potentiate SAL [93].

3.1.2. Digestive Tract Cancers

(1) HCC. In HCC, quantitative proteomic analysis was employed to study EMT and associated molecular events. When activating HCC cell lines (HepG2 and Huh7), an increased EMT profile was observed. But when introducing also metformin, EMT and metastasis were inhibited, and moreover, metformin could inhibit AKT/GSK-3β signalling induced by bFGF-mediated activation [94]. Another process studied in HCC was inflammation, knowing that an inflammatory status promotes tumourigenesis [95]. In a mouse model, label-free quantitative (LFQ) proteomics was performed for protein identification during the transformation of hepatic cells subjected to an inflammatory milieu to a precancerous cellular pattern. In this process, several proteins were identified as being deregulated from integrin, Rho family GTPases, IL-8, and ILK signalling pathways. Deregulations in the processes that regulate focal adhesion and actin cytoskeleton were as well found. Western blot has shown that proteins in EMT were upregulated (e.g., p-STAT3, TWIST, SNAIL, vimentin, and MMP-9). This study demonstrated some new therapy targets in HCC from the inflammatory-related pathways activating EMT [96]. MMPs as proteins related to EMT process were reported in several other cancers, like melanoma [97], nonmelanoma [98, 99], and other skin-related tumours [100, 101], and modulated through therapy induction.

(2) CRC. In CRC cell lines, it was shown that cyclic AMP (cAMP) response element binding protein 1 (CREB1) can be a new therapy target. Luteolin was tested in cell lines, and it inhibited CREB1 expression blocking therefore EMT. Moreover, luteolin induced MET process and reduced EMT-protein expressions inhibiting tumour cell migration. When comparing proteomic profile of CRC cell lines, HCT-116, treated or not with luteolin, a large panel of proteins, over 360 proteins, with different expressions were identified. Immunoblot evaluation has shown that CREB1 protein is decreased upon luteolin treatment and that this expression is inhibited at the transcriptional level. Besides the knowledge presented in this study, the authors point out that "proteomics is a powerful platform" to be used in deciphering the mechanism of action for drugs that can target EMT [102].

3.1.3. Lung Cancer. In the inflammatory environment of tumours, macrophages have an M2-phenotype that is protumourigenic. Zhu et al. showed that in lung cancer, M2 macrophages secrete factors that induce tumour cell migration, invasion, and EMT. In various cancers, β-elemene was shown to inhibit tumour proliferation [103]. In lung cancers, it was shown that β-elemene switched the polarization of protumourigenic M2-macrophages to M1. Moreover, this natural-related compound inhibited EMT characteristics of lung cancer cells and increased their radiosensitivity [104].

3.1.4. Urinary Tract Cancers. In prostate cancer (PC), the effects of plectranthoic acid (PA), extracted from natural sources, were investigated on EMT, migration, and invasion in PC cell lines. The study showed that PA reversed EMT and the proteomic analysis has pinpointed Rac1 as significantly inhibited upon treatment, indicating thus PA as a new adjuvant drug in PC [105]. Another natural product, caffeic acid phenethyl ester (CAPE), was studied as a future advent drug in PC. In PC-3 and DU-145 prostate cancer cell lines using several proteomic technologies (gelatin zymography, Western blotting), EMT-related proteins were investigated upon CAPE treatment, and it was shown that MMP-9 and MMP-2 have reduced activities. Micro-Western Array, a proteomic platform, performed on this treated cell lines has shown that the compound reduced β-catenin abundance, NF-κB activity, PI3K-Akt signalling, and EMT. In a mouse model of induced tumours, the immunohistochemical analysis has shown that the treatment increased ROR2 and Wnt5a expression and decreased Ki67, Frizzle 4, NF-κB p65, MMP-9, Snail, β-catenin, and phosphorylation of IκBα protein expression. In this study, proteomic technologies could pinpoint a new drug in PC that hinders aggressiveness and EMT processes [106].

In RCC, slightly different results were reported. Thus, using proteomic-based analyses, it was shown that HDACi do not induce EMT, contrarily inducing reduced proliferation and apoptosis. HDACi reduced E-cadherin and platelet-derived growth factor receptor-β (PDGFRβ), reducing RCC metastatic capacity. Moreover, in animal models, lung metastasis was reduced by HDACi [107].

3.1.5. Neurodegenerative Disorders. Histone deacetylase (HDAC) inhibitors have a long history of drugs, generally in neurodegenerative disorders [108], but recently, these drugs were tested also in oncology. In UC cell lines (RT112, VM-Cub-1, SW1710, and UM-UC-3), proteome and transcriptome investigations showed that HDAC5 overexpression in one of the investigated cell lines induced a decrease in cell proliferation but a switch to an EMT. This study signals proteomic mechanisms that are more subtle and more individualized in various tumour cell types as prior acknowledged [109].

In the neuroblastoma mouse model, the proteomic profile of murine anchorage-dependent (AD) cells and anchorage-independent (AI) tumour spheres was reported. Through complex proteomics, it was shown that there are upregulated proteins from the tumourigenic pathways in AI tumour spheres compared to AD cells. These proteins were regulating metastatic and EMT-related pathways. Thus, more specifically, survivin, CDC2, and the enzyme poly (ADP-ribose) polymerase 1 were found upregulated. The approved drug, sunitinib, inhibited PDGFRβ, MYCN, and

SOX2, and survivin in AI reduced tumour sphere self-renewal. Moreover, the radiosensitivity of AI was enhanced upon treatment. In neuroblastoma, there is a high heterogeneity of tumour cells, so all cell types should be targeted to have an overall efficient therapy [110].

3.2. Therapy Resistance with EMT Traits. Resistance to chemotherapy is a widely spread process along cancer. In this resistance process, EMT can induce resistance to cancer therapies, as proven throughout multiple types of cancers [111].

3.2.1. Breast and Digestive Cancers. In CRC patients, recurrence after chemotherapy is probably due to CSCs. Using a yeast-2-hybrid system and 2D gel-based proteomics, recently, it was shown that there is a molecular link between CSC and EMT. Thus, E3-ubiquitin ligase FBXW7 binds to EMT-inducing transcription factor ZEB2 and further induces its degradation. The FBXW7-ZEB2 tandem regulates many cancer cell features like stemness, chemoresistance, and metastasis. An increased expression of ZEB2 in tumours is correlated with reduced ZEB2 expression in cancer-associated stroma sustaining a tumour-stromal crosstalk and EMT activation [112]. In CRC, the chemoresistance is associated with TP53 gene mutation. Investigating the mechanism of EMT associated to chemoresistance, EFNB2 was found as mutant p53 target responsible for chemoresistance. The acetylated version of mutant p53 protein is enlisted on the EFNB2 promoter and upregulates its expression in conjunction with coactivator p300. Silencing EFNB2 induces chemosensitivity in tumours that display mutant p53. Patients that had tumours with high expression of EFNB2 had low clinical response to neoadjuvant therapy. Targeting the ephrin-B2 axes can increase the therapeutic capacity of cytostatics in tumours that display mutant p53 [113]. In HCC, quantitative proteomics and phosphoproteomics were performed on metastatic tissues resistant to sorafenib treatment. Analysis has shown that there are overexpressed pathways involved in tumour progression and resistance, (e.g., EMT and cell adhesion). Using this proteomic protocol, mechanisms of resistance based on EMT processes can be elucidated and further can be developed through precision medicine [114].

In HER2-positive breast and gastric cancer, resistance to trastuzumab installs during disease evolution. Liu et al. established a set of cell lines (trastuzumab-resistant MKN45, NCI N87 gastric cancer sublines) in order to study the underlining resistance mechanisms. Using label-free quantitative proteomics, altered pathways related to EMT process were found in MKN45/R cells, the key altered pathway being Wnt/β-catenin. Using Western blot, proteins like Wnt3A, FZD6, and CTNNB1 were confirmed as increased, while GSK-3β was found decreased. Using a specific Wnt/&-catenin inhibitor in these cells, proliferation was reduced and EMT was reversed. The authors conclude that Wnt/β-catenin pathway sustains trastuzumab resistance, and using Wnt/β-catenin inhibitors can overcome the resistance in this case [115]. For investigating resistance in BC HER2-positive breast cancer, cell lines were generated. This cell line was resistant to either lapatinib or AZD8931, and resistance was associated to their EMT phenotype. A global proteomic methodology was applied to these cells, and a novel set of EMT-related proteins was identified. Targeting EMT-associated kinases (e.g., Src and Axl) inhibited cell proliferation thus providing an additional therapy option [116].

EMT association with chemoresistance in BC focused on PD-L1 expression was also studied. In a complex transcriptomic and proteomic endeavour, expression profiling for over 500 patients was performed. A positive correlation between PD-L1 expression and stemness score of the cancer tissue was found. Global proteomic analysis showed that AKT has a central role in PD-L1 expression. Downregulation of PD-L1 expression reduced the cancer cell stemness and EMT characteristics and brings new backgrounds for anti-PD-L1 therapy in BC [117].

Breast cancer cells (cell line MDA-MB-231) treated with metapristone reduce metastasis and their interference with endothelial cells in the process of migration. Using iTRAQ technology to assess the effect of metapristone on MDA-MB-231 cells, over 5,000 proteins were identified, out of which over 300 proteins were differently expressed in treated cells. These proteins were involved in seminal cellular processes, like translation, transcription, replication, and signal transduction. Proteins like E-cadherin, vimentin, TGF-β receptor I/II, smad2/3, β-catenin, caveolin, and dystroglycan are associated with signalling pathways (e.g., TGF-β and Wnt) linked to EMT process. Using immunoblot and immunofluorescence, the validation of EMT-related proteins (E-cadherin, vimentin) was done [118].

3.2.2. Melanoma. In BRAFV600E-mutated melanoma tumours, the acquired resistance to MEK1/2 inhibitors (MEKi) reinstalls ERK1/2 signalling. In contrast, resistance installed by KRASG13D amplification drives ZEB1-dependent EMT and chemoresistance [119]. In melanoma patients, resistance to new therapy occurs due to the high plasticity and heterogeneity of this skin cancer. Several proteomic studies were focused on MAPK inhibitor (MAPKi) resistance. These studies have pointed out that BRAFi resistance is associated with lysosomal compartment, cell adhesion, and EMT process. When switching to the invasive state, melanoma cells gain EMT characteristics and become resistant to MAPKi. The authors point out that proteomic studies can further reveal other subtle mechanisms underlying MAPKi resistance mechanisms and develop biomarkers for identifying early resistance pattern [120, 121].

3.2.3. Non-Small-Cell Lung Cancer. Drug resistance in non-small-cell lung cancer (NSCLC) was studied by evaluating proteomic profiling in a resistant A549 CDDP-resistant (CPr-A549) cell line. A panel of 15 proteins was found differentially expressed in CPr-A549. These proteins are involved in misfolding of proteins, endoplasmic reticulum stress, positively correlated with EMT and CSC markers. These proteins could be further developed in NSCLC patient prognosis and survival markers [122].

In NSCLC, the resistance to tyrosine kinase inhibitors (TKIs) is also related to the EMT process and CAFs. In experimental models with cell lines like HCC827 and PC9 cells, the interaction with CAFs induced EMT phenotype

Table 1: Proteomic platforms for EMT process evaluation in various types of cancer tissues.

Proteomic platform(s) in EMT assessing	Cancer type/sample type	Evaluated EMT-related process	Comments regarding EMT-related proteins	Ref
iTRAQ-based quantitative proteomics	Colorectal cancer/tumour tissue	Tumourigenesis	TME proteins—ECM-receptors, focal adhesion, PI3K-Akt signalling pathway, angiogenesis, HIF-1 signalling pathways	[53, 54]
Reverse phase protein array	Colorectal cancer/tumour tissue	Recurrence	Collagen VI, FOXO3a, INPP4B, LcK, phospho-PEA15, phospho-PRAS40, Rad51, phospho-S6	[56]
Tissue microarray	Colorectal cancer/tumour tissue	Metastasis	Cdc42BPA highly correlated with metastasis	[65]
Yeast-2-hybrid system and 2D gel-based proteomics	Colorectal cancer	Therapy resistance	FBXW7-ZEB2 tandem involved in stemness, chemoresistance, metastasis	[112]
Two-dimensional fluorescence gel electrophoresis	Hepatocellular carcinoma	Metastasis	GnT-V-mediated N-glycosylation of marker CD147/basigin. Upregulated and controlled by PI3K/Akt pathway	[68]
Label-free quantitative proteomics	Hepatocellular carcinoma	Inflammation	Inflammatory milieu has integrin, Rho family GTPases, IL-8, and ILK signalling pathway deregulations	[96]
Quantitative proteomic analysis	Hepatocellular carcinoma	Therapy resistance	Metformin inhibits AKT/GSK-3β signalling	[94]
Quantitative (phospho)proteomics	Hepatocellular carcinoma resistance	Therapy resistance	Proteins related to EMT and cell adhesion were associated with sorafenib treatment resistance	[114]
Label-free quantitative proteomics	Gastric cancer	Therapy resistance	Wnt/β-catenin pathway sustains trastuzumab resistance	[115]
Micro-Western Array	Prostate cancer	Therapy resistance	Caffeic acid phenethyl ester reduced β-catenin, NF-κB, and PI3K-Akt signalling	[106]
Liquid chromatography tandem-mass spectrometry	Prostate cancer	Therapy resistance	Signalling pathways in therapy-resistant tumours	[126]
Liquid chromatography-selected reaction monitoring mass spectrometry	Breast cancer	Aggressiveness	Estrogen, progesterone receptors, HER2/Neu receptor correlated with Ki-67 and vimentin	[72]
2D-differential gel electrophoresis, MALDI-MS, immunoblotting	Breast cancer	Aggressiveness	ECM pattern—fibrinogen-β chain, collagen α-1(VI) chain, and α-1B-glycoprotein	[73]
Flow cytometric surface proteomics	Breast cancer	Aggressiveness	Upregulated proteins CD200, CD51/CD61, CD26, CD165, and CD54	[76]
Reverse phase protein arrays	Breast cancer	Subtyping	Immune-related subtype and a hormone-related subtype	[78]
Quantitative multiplexed proteomic tandem mass tags	Breast cancer	Therapy resistance	TACC3 inhibitor, suppresses cancer cell stemness	[91]
Western blot, colony formation, flow cytometry for cellular apoptosis	Breast cancer	Therapy resistance	Resveratrol and salinomycin reverse EMT	[93]
iTRAQ technology	Breast cancer	Therapy resistance	E-cadherin, vimentin, TGF-β receptor I/II, smad2/3, β-catenin, caveolin, dystroglycan overexpression	[118]
Mass spectrometry	High-grade serous ovarian cancer	Tumourigenesis	Alpha-enolase and vimentin overexpression	[80]
GEL-LC-MS/MS and SILAC	Ovarian adenocarcinoma	Tumourigenesis	Activated signalling pathways PI3K/Akt/mTOR and Ras/Erk MAPK	[81]

Table 1: Continued.

Proteomic platform(s) in EMT assessing	Cancer type/sample type	Evaluated EMT-related process	Comments regarding EMT-related proteins	Ref
Mass spectrometry-based quantitative proteomic approaches	Cervical cancer	Tumourigenesis	Myoferlin regulates ADAM12 expression	[83]
Membrane proteomic methodology	Head and neck squamous cell carcinoma	Tumourigenesis	EGFR constitutively phosphorylated	[85]

and overexpression of specific EMT markers. All these recent data were obtained using proteomic-based methods. Annexin 2, a protein influencing mainly the cellular motility [123], was found significantly increased on NSCLC cells by CAFs along with an increased secretion of growth factors HGF and IGF-1. Knocking down ANXA2 induced an inverse EMT phenotype. Thus, if a new therapeutical approach is to be designed in the future, inhibition of tandem HGF/c-met and IGF-1/IGF-1R networks could reduce EMT and gefitinib resistance [124].

3.2.4. Radioresistance in Solid Tumours. Tumour resistance to radiotherapy is another challenge in tumour therapies. In PC, metastasis and recurrence postradiotherapy can occur. In an animal model, using a radioresistant xenograft mouse, pathways that can induce this resistance were investigated. Xenografts that were resistant in comparison to nonresistant cells (PC-3RR compared to PC-3) were analysed using liquid chromatography tandem-MS. There were reported almost 380 proteins and over 50 pathways that were significantly differently expressed in PC-3RR compared to PC-3 xenograft and that the deregulated glycolysis pathway links to PC radioresistance. Within the glycolysis pathway, lactate dehydrogenase A enzyme (LDHA) is a crucial node, and if knocked down (e.g., with siRNA or with a LDHA specific inhibitor like FX-11), PC-3RR cells would develop radiosensitivity, reduced EMT phenotype, hypoxia, and apoptosis [125, 126].

3.2.5. Other Therapy Resistance Inducers. Associated with various cancer types, Nogo-B receptor (NgBR), was found highly expressed in BC, lung cancer, HCC, and so on. It seems that in all these types of cancer NgBR promotes EMT. Dong et al. have shown that in Bel7402/5FU cells, increased expression of NgBR is associated with chemoresistance. If this receptor is knocked down in this cell line, proteins p53 and p21 are reduced. NgBR expression in HCC is correlated with a poor prognosis. Thus, targeting NgBR and combining with chemotherapy (e.g., 5-FU) an increased therapeutic efficacy can be obtained [125]. In lung cancer, NgBR was studied as EMT inducer. Indeed, the NgBR knockdown of NSCLC cells inhibited EMT process, while NgBR overexpression induced EMT, through EMT-related proteins, mainly Snail1, the transcription factor repressing E-cadherin expression. NgBR overexpression favoured Ras membrane localization and the activation of MEK/ERK signalling. These results can aid to developing new therapeutic strategy in NSCLC [127].

In several tumour cell types, high EMT state and therapy resistance depend on the lipid-peroxidase pathway. This pathway activation protects tumour cell from ferroptosis. Using proteomic technologies, the enzymes that govern this process were identified. The lipid metabolism specific for tumour cells is dependent on phospholipid glutathione peroxidase (GPX4), an enzyme that prevents ferroptotic cell death. Therapy-resistant tumour cells have high EMT pattern and high expression of ZEB1. This configuration was proven in carcinomas, in TGFβ-resistance in melanoma, and in treatment-induced transdifferentiation in PC and sarcomas. If this abnormal lipid peroxidase pathway can be targeted and reversed, then EMT-driven therapy resistance can be eluded [111].

An outline of proteomic technologies used for identification of EMT-related molecules in various cancers is presented in Table 1.

Nevertheless, it should be highlighted that proteomic approaches emerge in the EMT field by preparing the clinical setting ground because there are already plentiful preclinical approaches in this domain. There are *omic* platforms such as protein microarrays and/or XMAP arrays that provide besides multiplexing qualitative and quantitative detection for discovery and validation of EMT-related markers. Subtle molecular modifications are detected with multidimensional-electrophoresis and MS platforms that could discover and identify specific biomolecules of EMT trait. With the constant evolution of proteomic analysis, deficiencies associated with these techniques can hardly be stated. However, an important shortage related to omic platforms to be implemented in clinics could be linked to high equipment costs and way above medium personnel expertise. Nevertheless, protein microarrays open the clinical setting by designing special formats for diagnosis/early diagnosis or therapy monitoring [128]. A small volume of analysed samples, simultaneous detection and short operating time, and increased specificity and sensibility of the tested analytes sustain the importance of proteomic technologies in any biomedical attempt EMT process included [128, 129].

4. Conclusion

EMT is involved in key steps of tumour progression, because it mainly facilitates metastasis. Recently, EMT research has taken on board proteomic technologies to identify new molecular insights of the process and to further evaluate the possibilities to develop new therapy targets. Various proteomic tools and multiple combinations were developed in this

area. Out of the proteomic technology armentarium, mass spectrometry and array technologies are the most used approaches [129]. The main characteristics of the proteomic technology used in this domain are high throughput and detection of minute concentration in small samples. Therefore, in various cancer cell lines and tumour tissues, EMT-related proteins were newly detected proteins that are involved in the activation of different cellular pathways. Proteomics has brought besides standard EMT markers (e.g., cell-cell adhesion proteins and transcription factors) other future potential markers for improving diagnosis, monitoring evolution, and developing new therapy targets. Thus, proteomics will steadily increase its role in clinical investigation and validation of EMT-related biomarkers [24].

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

[1] S. Lamouille, J. Xu, and R. Derynck, "Molecular mechanisms of epithelial-mesenchymal transition," *Nature Reviews Molecular Cell Biology*, vol. 15, no. 3, pp. 178–196, 2014.

[2] R. Kalluri and R. A. Weinberg, "The basics of epithelial-mesenchymal transition," *Journal of Clinical Investigation*, vol. 119, no. 6, pp. 1420–1428, 2009.

[3] P. G. Santamaria, G. Moreno-Bueno, F. Portillo, and A. Cano, "EMT: present and future in clinical oncology," *Molecular Oncology*, vol. 11, no. 7, pp. 718–738, 2017.

[4] Z. Peng, C. X. Wang, E. H. Fang, G. B. Wang, and Q. Tong, "Role of epithelial-mesenchymal transition in gastric cancer initiation and progression," *World Journal of Gastroenterology*, vol. 20, no. 18, pp. 5403–5410, 2014.

[5] M. Zeisberg and E. G. Neilson, "Biomarkers for epithelial-mesenchymal transitions," *Journal of Clinical Investigation*, vol. 119, no. 6, pp. 1429–1437, 2009.

[6] E. Prieto-García, C. V. Díaz-García, I. García-Ruiz, and M. T. Agulló-Ortuño, "Epithelial-to-mesenchymal transition in tumour progression," *Medical Oncology*, vol. 34, no. 7, p. 122, 2017.

[7] Z. Ma, Z. Xin, W. Hu et al., "Forkhead box O proteins: crucial regulators of cancer EMT," *Seminars in Cancer Biology*, vol. 50, pp. 21–31, 2018.

[8] A. Morandi, M. L. Taddei, P. Chiarugi, and E. Giannoni, "Targeting the metabolic reprogramming that controls epithelial-to-mesenchymal transition in aggressive tumours," *Frontiers in Oncology*, vol. 7, p. 40, 2017.

[9] K. TanakaBiographical. Nobel Prize.org. Nobel Media AB 2019. Sat. 1 Jun 2019. https://www.nobelprize.org/prizes/chemistry/2002/tanaka/biographical.

[10] C. Constantin, A. Bulman, D. McCarthy, and M. Neagu, "Surface-enhanced laser desorption/ionization mass spectrometry for biomarker discovery in cutaneous melanoma," *Current Proteomics*, vol. 14, no. 2, pp. 100–111, 2017.

[11] M.-L. Popa, R. Albulescu, M. Neagu, M. E. Hinescu, and C. Tănase, "Multiplex assay for multiomics advances in personalized-precision medicine," *Journal of Immunoassay and Immunochemistry*, vol. 40, no. 1, pp. 3–25, 2019.

[12] M. Neagu, M. Bostan, and C. Constantin, "Protein microarray technology - assisting personalized medicine in oncology," *World Academy of Sciences Journal*, vol. 1, pp. 113–124, 2019.

[13] L. Charboneau, H. Scott, T. Chen et al., "Utility of reverse phase protein arrays: applications to signalling pathways and human body arrays," *Briefings in Functional Genomics and Proteomics*, vol. 1, no. 3, pp. 305–315, 2002.

[14] C. Matei, M. Tampa, C. Caruntu et al., "Protein microarray for complex apoptosis monitoring of dysplastic oral keratinocytes in experimental photodynamic therapy," *Biological Research*, vol. 47, no. 1, p. 33, 2014.

[15] M. Neagu, C. Constantin, C. Caruntu, C. Dumitru, M. Surcel, and S. Zurac, "Inflammation: a key process in skin tumourigenesis," *Oncology Letters*, vol. 17, no. 5, pp. 4068–4084, 2019.

[16] T. Tampa, M. I. Mitran, C. I. Mitran et al., "Mediators of inflammation – a potential source of biomarkers in oral squamous cell carcinoma," *Journal of Immunology Research*, vol. 2018, Article ID 1061780, 12 pages, 2018.

[17] C. Vajaitu, C. C. Draghici, I. Solomon et al., "The central role of inflammation associated with checkpoint inhibitor treatments," *Journal of Immunology Research*, vol. 2018, Article ID 4625472, 10 pages, 2018.

[18] S. R. Georgescu, C. I. Mitran, M. I. Mitran et al., "New insights in the pathogenesis of HPV infection and the associated carcinogenic processes: the role of chronic inflammation and oxidative stress," *Journal of Immunology Research*, vol. 2018, Article ID 5315816, 10 pages, 2018.

[19] D. Boda, A. O. Docea, D. Calina et al., "Human papilloma virus: apprehending the link with carcinogenesis and unveiling new research avenues," *International Journal of Oncology*, vol. 52, no. 3, pp. 637–655, 2018.

[20] S. R. Georgescu, M. Tampa, M. I. Mitran et al., "Potential pathogenic mechanisms involved in the association between lichen planus and hepatitis C virus infection," *Experimental and Therapeutic Medicine*, vol. 17, no. 2, pp. 1045–1051, 2019.

[21] C. Lu, S. Sidoli, K. Kulej, K. Ross, C. H. Wu, and B. A. Garcia, "Coordination between TGF-β cellular signalling and epigenetic regulation during epithelial to mesenchymal transition," *Epigenetics & Chromatin*, vol. 12, no. 1, p. 11, 2019.

[22] S. Koplev, K. Lin, A. B. Dohlman, and A. Ma'ayan, "Integration of pan-cancer transcriptomics with RPPA proteomics reveals mechanisms of epithelial-mesenchymal transition," *PLoS Computational Biology*, vol. 14, no. 1, article e1005911, 2018.

[23] H. Li, F. Xu, S. Li, A. Zhong, X. Meng, and M. Lai, "The tumour microenvironment: an irreplaceable element of tumour budding and epithelial-mesenchymal transition-mediated cancer metastasis," *Cell Adhesion & Migration*, vol. 10, no. 4, pp. 434–446, 2016.

[24] D. Vergara, P. Simeone, J. Franck et al., "Translating epithelial mesenchymal transition markers into the clinic: novel insights from proteomics," *EuPA Open Proteomics*, vol. 10, pp. 31–41, 2016.

[25] P. Bottoni, M. A. Isgrò, and R. Scatena, "The epithelial-mesenchymal transition in cancer: a potential critical topic for translational proteomic research," *Expert Review of Proteomics*, vol. 13, no. 1, pp. 115–133, 2016.

[26] K. T. Yeung and J. Yang, "Epithelial–mesenchymal transition in tumor metastasis," *Molecular Oncology*, vol. 11, pp. 28–39, 2017.

[27] J. H. Tsai and J. Yang, "Epithelial-mesenchymal plasticity in carcinoma metastasis," *Genes & Development*, vol. 27, no. 20, pp. 2192–2206, 2013.

[28] H. Škovierová, T. Okajčeková, J. Strnádel, E. Vidomanová, and E. Halašová, "Molecular regulation of epithelial-to-mesenchymal transition in tumorigenesis," *International Journal of Molecular Medicine*, vol. 41, no. 3, pp. 1187–1200, 2018.

[29] F. Marcucci, G. Stassi, and R. De Maria, "Epithelial–mesenchymal transition: a new target in anticancer drug discovery," *Nature Reviews Drug Discovery*, vol. 15, no. 5, pp. 311–325, 2016.

[30] D. A. Ferraro, F. Patella, S. Zanivan et al., "Endothelial cell-derived nidogen-1 inhibits migration of SK-BR-3 breast cancer cells," *BMC Cancer*, vol. 19, no. 1, p. 312, 2019.

[31] C. de Souza Palma, M. L. Grassi, C. H. Thomé et al., "Proteomic analysis of epithelial to mesenchymal transition (EMT) reveals cross-talk between SNAIL and HDAC1 proteins in breast cancer cells," *Molecular & Cellular Proteomics*, vol. 15, no. 3, pp. 906–917, 2016.

[32] J. Yang, Y. Hou, M. Zhou et al., "Twist induces epithelial-mesenchymal transition and cell motility in breast cancer via ITGB1-FAK/ILK signalling axis and its associated downstream network," *The International Journal of Biochemistry & Cell Biology*, vol. 71, pp. 62–71, 2016.

[33] L.-D. Li, H.-F. Sun, X.-X. Liu et al., "Down-regulation of NDUFB9 promotes breast cancer cell proliferation, metastasis by mediating mitochondrial metabolism," *PLoS One*, vol. 10, no. 12, article e0144441, 2015.

[34] E. Maurizio, J. R. Wiśniewski, Y. Ciani et al., "Translating proteomic into functional data: an high mobility group A1 (HMGA1) proteomic signature has prognostic value in breast cancer," *Molecular & Cellular Proteomics*, vol. 15, no. 1, pp. 109–123, 2016.

[35] D. Flodrova, L. Toporova, M. Lastovickova et al., "Consequences of the natural retinoid/retinoid X receptor ligands action in human breast cancer MDA-MB-231 cell line: focus on functional proteomics," *Toxicology Letters*, vol. 281, pp. 26–34, 2017.

[36] X. Ye, K. C. Chan, A. M. Waters et al., "Comparative proteomics of a model MCF10A-KRasG12V cell line reveals a distinct molecular signature of the $KRas^{G12V}$ cell surface," *Oncotarget*, vol. 7, no. 52, pp. 86948–86971, 2016.

[37] S. W. Wen, L. G. Lima, R. J. Lobb et al., "Breast cancer-derived exosomes reflect the cell-of-origin phenotype," *Proteomics*, vol. 19, no. 8, article e1800180, 2019.

[38] S. Tahara, S. Nojima, K. Ohshima et al., "Serum deprivation-response protein regulates aldehyde dehydrogenase 1 through integrin-linked kinase signalling in endometrioid carcinoma cells," *Cancer Science*, vol. 110, no. 5, pp. 1804–1813, 2019.

[39] J. Mariscal, P. Fernandez-Puente, V. Calamia et al., "Proteomic characterization of epithelial-like extracellular vesicles in advanced endometrial cancer," *Journal of Proteome Research*, vol. 18, no. 3, pp. 1043–1053, 2019.

[40] S. Xu, Y. Yue, S. Zhang et al., "STON2 negatively modulates stem-like properties in ovarian cancer cells via DNMT1/MUC1 pathway," *Journal of Experimental & Clinical Cancer Research*, vol. 37, no. 1, p. 305, 2018.

[41] C. Samardzija, D. W. Greening, R. Escalona et al., "Knockdown of stem cell regulator Oct4A in ovarian cancer reveals cellular reprogramming associated with key regulators of cytoskeleton-extracellular matrix remodelling," *Scientific Reports*, vol. 7, article 46312, 2017.

[42] P. Chen, H. Wang, W. Zhang et al., "Loss of BAP1 results in growth inhibition and enhances mesenchymal-epithelial transition in kidney tumour cells," *Molecular & Cellular Proteomics*, vol. 18, no. 7, pp. 1320–1329, 2019.

[43] M. Deiana, L. Dalle Carbonare, M. Serena et al., "New insights into the Runt domain of RUNX2 in melanoma cell proliferation and migration," *Cell*, vol. 7, no. 11, p. 220, 2018.

[44] L. Huergo-Zapico, M. Parodi, C. Cantoni et al., "NK-cell editing mediates epithelial-to-mesenchymal transition via phenotypic and proteomic changes in melanoma cell lines," *Cancer Research*, vol. 78, no. 14, pp. 3913–3925, 2018.

[45] G. J. Wu, J. Pen, Y. Huang et al., "KAP1 inhibits the Raf-MEK-ERK pathway to promote tumourigenesis in A549 lung cancer cells," *Molecular Carcinogenesis*, vol. 57, no. 10, pp. 1396–1407, 2018.

[46] A. N. Fischer, B. Herrera, M. Mikula et al., "Integration of Ras subeffector signalling in TGF-β mediated late stage hepatocarcinogenesis," *Carcinogenesis*, vol. 26, pp. 931–942, 2005.

[47] P. Koudelkova, G. Webe, and W. Mikulits, "Liver sinusoidal endothelial cells escape senescence by loss of p19ARF," *PLoS One*, vol. 10, no. 11, article e0142134, 2015.

[48] P. Koudelkova, V. Costina, G. Weber et al., "Transforming growth factor-β drives the transendothelial migration of hepatocellular carcinoma cells," *International Journal of Molecular Sciences*, vol. 18, no. 10, article 2119, 2017.

[49] M. Lai, L. Liang, J. Chen et al., "Multidimensional proteomics reveals a role of UHRF2 in the regulation of epithelial-mesenchymal transition (EMT)," *Molecular & Cellular Proteomics*, vol. 15, no. 7, pp. 2263–2278, 2016.

[50] M. M. Koczorowska, C. Friedemann, K. Geiger, M. Follo, M. L. Biniossek, and O. Schilling, "Differential effect of TGFβ on the proteome of cancer associated fibroblasts and cancer epithelial cells in a co-culture approach - a short report," *Cellular Oncology*, vol. 40, no. 6, pp. 639–650, 2017.

[51] L. Hebert, D. Bellanger, C. Guillas et al., "Modulating BAP1 expression affects ROS homeostasis, cell motility and mitochondrial function," *Oncotarget*, vol. 8, no. 42, pp. 72513–72527, 2017.

[52] L. Lu, G. Chen, J. Yang et al., "Bone marrow mesenchymal stem cells suppress growth and promote the apoptosis of glioma U251 cells through downregulation of the PI3K/AKT signalling pathway," *Biomedicine & Pharmacotherapy*, vol. 112, article 108625, 2019.

[53] G. Wang, Q. Yang, M. Li et al., "Quantitative proteomic profiling of tumour-associated vascular endothelial cells in colorectal cancer," *Biology Open*, vol. 8, no. 5, article bio042838, 2019.

[54] F. Xu, S. Li, J. Zhang et al., "Cancer stemness, immune cells, and epithelial-mesenchymal transition cooperatively predict

prognosis in colorectal carcinoma," *Clinical Colorectal Cancer*, vol. 17, no. 3, pp. e579–e592, 2018.

[55] A. E. Yuzhalin, A. N. Gordon-Weeks, M. L. Tognoli et al., "Colorectal cancer liver metastatic growth depends on PAD4-driven citrullination of the extracellular matrix," *Nature Communications*, vol. 9, no. 1, p. 4783, 2018.

[56] C. N. Clarke, M. S. Lee, W. Wei et al., "Proteomic features of colorectal cancer identify tumour subtypes independent of oncogenic mutations and independently predict relapse-free survival," *Annals of Surgical Oncology*, vol. 24, no. 13, pp. 4051–4058, 2017.

[57] J. Wu, Y. Wang, X. Xu et al., "Transcriptional activation of FN1 and IL11 by HMGA2 promotes the malignant behavior of colorectal cancer," *Carcinogenesis*, vol. 37, no. 5, pp. 511–521, 2016.

[58] L. Zhang, X. Wang, C. Lai, H. Zhang, and M. Lai, "PMEPA1 induces EMT via a non-canonical TGF-β signalling in colorectal cancer," *Journal of Cellular and Molecular Medicine*, vol. 23, no. 5, pp. 3603–3615, 2019.

[59] D. Tsoumas, S. Nikou, E. Giannopoulou et al., "ILK expression in colorectal cancer is associated with EMT, cancer stem cell markers and chemoresistance," *Cancer Genomics & Proteomics*, vol. 15, no. 2, pp. 127–141, 2018.

[60] S. Li, F. Xu, H. Li et al., "S100A8$^+$ stroma cells predict a good prognosis and inhibit aggressiveness in colorectal carcinoma," *Oncoimmunology*, vol. 6, no. 1, article e1260213, 2016.

[61] H. Li, A. Zhong, S. Li et al., "The integrated pathway of TGFβ/Snail with TNFα/NFκB may facilitate the tumour-stroma interaction in the EMT process and colorectal cancer prognosis," *Scientific Reports*, vol. 7, no. 1, article 4915, 2017.

[62] M. Feng, J. Feng, W. Chen et al., "Lipocalin2 suppresses metastasis of colorectal cancer by attenuating NF-κB-dependent activation of snail and epithelial mesenchymal transition," *Molecular Cancer*, vol. 15, no. 1, p. 77, 2016.

[63] H. Cao, J. Zhang, H. Liu et al., "IL-13/STAT6 signalling plays a critical role in the epithelial-mesenchymal transition of colorectal cancer cells," *Oncotarget*, vol. 7, no. 38, pp. 61183–61198, 2016.

[64] C. Li, J. Wang, J. Kong et al., "GDF15 promotes EMT and metastasis in colorectal cancer," *Oncotarget*, vol. 7, no. 1, pp. 860–872, 2016.

[65] H. F. Hu, W. W. Xu, Y. Wang et al., "Comparative proteomics analysis identifies Cdc42-Cdc42BPA signaling as prognostic biomarker and therapeutic target for colon cancer invasion," *Journal of Proteome Research*, vol. 17, no. 1, pp. 265–275, 2018.

[66] D. Shimizu, T. Saito, S. Ito et al., "Overexpression of FGFR1 promotes peritoneal dissemination via epithelial-to-mesenchymal transition in gastric cancer," *Cancer Genomics - Proteomics*, vol. 15, no. 4, pp. 313–320, 2018.

[67] S. C. Oh, B. H. Sohn, J. H. Cheong et al., "Clinical and genomic landscape of gastric cancer with a mesenchymal phenotype," *Nature Communications*, vol. 9, no. 1, article 1777, 2018.

[68] J. Cui, W. Huang, B. Wu et al., "*N*-Glycosylation by *N*-acetylglucosaminyltransferase V enhances the interaction of CD147/basigin with integrin β1 and promotes HCC metastasis," *The Journal of Pathology*, vol. 245, no. 1, pp. 41–52, 2018.

[69] H. Yamaguchi, K. Kuroda, M. Sugitani, T. Takayama, K. Hasegawa, and M. Esumi, "Transglutaminase 2 is upregulated in primary hepatocellular carcinoma with early recurrence as determined by proteomic profiles," *International Journal of Oncology*, vol. 50, no. 5, pp. 1749–1759, 2017.

[70] H. Ma, L. Xie, L. Zhang et al., "Activated hepatic stellate cells promote epithelial-to-mesenchymal transition in hepatocellular carcinoma through transglutaminase 2-induced pseudohypoxia," *Communications Biology*, vol. 1, p. 168, 2018.

[71] M. Tsuru, M. Sata, M. Tanaka et al., "Retrospective proteomic analysis of a novel, cancer metastasis-promoting RGD-containing peptide," *Translational Oncology*, vol. 10, no. 6, pp. 998–1007, 2017.

[72] Y. Chen, D. Britton, E. R. Wood et al., "Quantitative proteomics of breast tumours: tissue quality assessment to clinical biomarkers," *Proteomics*, vol. 17, no. 6, article 1600335, 2017.

[73] M. Moriggi, M. Giussani, E. Torretta et al., "ECM remodeling in breast cancer with different grade: contribution of 2D-DIGE proteomics," *Proteomics*, vol. 18, no. 24, article 1800278, 2018.

[74] R. Matsunuma, D. W. Chan, B.-J. Kim et al., "DPYSL3 modulates mitosis, migration, and epithelial-to-mesenchymal transition in claudin-low breast cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 115, no. 51, pp. E11978–E11987, 2018.

[75] B. Wang, C. Xi, M. Liu et al., "Breast fibroblasts in both cancer and normal tissues induce phenotypic transformation of breast cancer stem cells: a preliminary study," *PeerJ*, vol. 6, article e4805, 2018.

[76] V. S. Donnenberg, J. J. Zhang, E. Moravcikova et al., "Antibody-based cell-surface proteome profiling of metastatic breast cancer primary explants and cell lines," *Cytometry Part A*, vol. 93, no. 4, pp. 448–457, 2018.

[77] X. Wu, X. Li, Q. Fu et al., "AKR1B1 promotes basal-like breast cancer progression by a positive feedback loop that activates the EMT program," *The Journal of Experimental Medicine*, vol. 214, no. 4, pp. 1065–1079, 2017.

[78] M. Michaut, S. F. Chin, I. Majewski et al., "Integration of genomic, transcriptomic and proteomic data identifies two biologically distinct subtypes of invasive lobular breast cancer," *Scientific Reports*, vol. 6, no. 1, article 18517, 2016.

[79] G. Ciriello, M. L. Gatza, A. H. Beck et al., "Comprehensive molecular portraits of invasive lobular breast cancer," *Cell*, vol. 163, no. 2, pp. 506–519, 2015.

[80] P. N. Teng, N. W. Bateman, G. Wang et al., "Establishment and characterization of a platinum- and paclitaxel-resistant high grade serous ovarian carcinoma cell line," *Human Cell*, vol. 30, no. 3, pp. 226–236, 2017.

[81] M. L. Grassi, C. S. Palma, C. H. Thomé, G. P. Lanfredi, A. Poersch, and V. M. Faça, "Proteomic analysis of ovarian cancer cells during epithelial-mesenchymal transition (EMT) induced by epidermal growth factor (EGF) reveals mechanisms of cell cycle control," *Journal of Proteomics*, vol. 151, pp. 2–11, 2017.

[82] J. Gao, Y. Yang, R. Qiu et al., "Proteomic analysis of the OGT interactome: novel links to epithelial-mesenchymal transition and metastasis of cervical cancer," *Carcinogenesis*, vol. 39, no. 10, pp. 1222–1234, 2018.

[83] Y. Zhou, L. Xiong, Y. Zhang, R. Yu, X. Jiang, and G. Xu, "Quantitative proteomics identifies myoferlin as a novel regulator of a disintegrin and metalloproteinase 12 in HeLa cells," *Journal of Proteomics*, vol. 148, pp. 94–104, 2016.

[84] A. Zougman, P. J. Selby, and R. E. Banks, "Suspension trapping (STrap) sample preparation method for bottom-up

proteomics analysis," *Proteomics*, vol. 14, no. 9, pp. 1000–1006, 2014.

[85] V. Roh, P. Abramowski, A. Hiou-Feige et al., "Cellular barcoding identifies clonal substitution as a hallmark of local recurrence in a surgical model of head and neck squamous cell carcinoma," *Cell Reports*, vol. 25, no. 8, pp. 2208–2222.e7, 2018.

[86] A. Mohd-Sarip, M. Teeuwssen, A. G. Bot et al., "DOC1-dependent recruitment of NURD reveals antagonism with SWI/SNF during epithelial-mesenchymal transition in oral cancer cells," *Cell Reports*, vol. 20, no. 1, pp. 61–75, 2017.

[87] T. Xu, B. Su, P. Huang et al., "Novel biomarkers of nasopharyngeal carcinoma metastasis risk identified by reverse phase protein array based tumour profiling with consideration of plasma Epstein-Barr virus DNA load," *Proteomics - Clinical Applications*, vol. 11, no. 5-6, article 1600090, 2017.

[88] L. Zhou, L. Shi, H. Guo, and X. Yao, "MYSM-1 suppresses migration and invasion in renal carcinoma through inhibiting epithelial-mesenchymal transition," *Tumour Biology*, vol. 37, no. 12, pp. 15583–15591, 2016.

[89] Y. Qi, Y. Zhang, Z. Peng et al., "SERPINH1 overexpression in clear cell renal cell carcinoma: association with poor clinical outcome and its potential as a novel prognostic marker," *Journal of Cellular and Molecular Medicine*, vol. 22, no. 2, pp. 1224–1235, 2018.

[90] C.-Y. Chiang, C.-C. Pan, H.-Y. Chang et al., "SH3BGRL3 protein as a potential prognostic biomarker for urothelial carcinoma: a novel binding partner of epidermal growth factor receptor," *Clinical Cancer Research*, vol. 21, no. 24, pp. 5601–5611, 2015.

[91] L. Campo and E. K. Breuer, "Inhibition of TACC3 by a small molecule inhibitor in breast cancer," *Biochemical and Biophysical Research Communications*, vol. 498, no. 4, pp. 1085–1092, 2018.

[92] E. Bigagli, L. Cinci, M. D'Ambrosio, and C. Luceri, "Transcriptomic characterization, chemosensitivity and regulatory effects of exosomes in spontaneous EMT/MET transitions of breast cancer cells," *Cancer Genomics Proteomics*, vol. 16, no. 3, pp. 163–173, 2019.

[93] G. Rai, S. Suman, S. Mishra, and Y. Shukla, "Evaluation of growth inhibitory response of resveratrol and salinomycin combinations against triple negative breast cancer cells," *Biomedicine & Pharmacotherapy*, vol. 89, pp. 1142–1151, 2017.

[94] W. Chengye, T. Yu, S. Ping et al., "Metformin reverses bFGF-induced epithelial-mesenchymal transition in HCC cells," *Oncotarget*, vol. 8, no. 61, pp. 104247–104257, 2017.

[95] M. Neagu, C. Constantin, I. D. Popescu et al., "Inflammation and metabolism in cancer cell—mitochondria key player," *Frontiers in Oncology*, vol. 9, p. 348, 2019.

[96] K. Lu, G. Liu, L. Yang et al., "Sustainable inflammation transforms hepatic cells by causing oxidative stress injury and potential epithelial-mesenchymal transition," *International Journal of Oncology*, vol. 49, no. 3, pp. 971–980, 2016.

[97] S. Zurac, M. Neagu, C. Constantin et al., "Variations in the expression of TIMP1, TIMP2 and TIMP3 in cutaneous melanoma with regression and their possible function as prognostic predictors," *Oncology Letters*, vol. 11, no. 5, pp. 3354–3360, 2016.

[98] V. Voiculescu, B. Calenic, M. Ghita et al., "From normal skin to squamous cell carcinoma: a quest for novel biomarkers," *Disease Markers*, vol. 2016, Article ID 4517492, 14 pages, 2016.

[99] M. Lupu, C. Caruntu, M. A. Ghita et al., "Gene expression and proteome analysis as sources of biomarkers in basal cell carcinoma," *Disease Markers*, vol. 2016, Article ID 9831237, 9 pages, 2016.

[100] M. Tampa, C. Caruntu, M. Mitran et al., "Markers of oral lichen planus malignant transformation," *Disease Markers*, vol. 2018, Article ID 1959506, 13 pages, 2018.

[101] A. Ion, I. M. Popa, L. M. L. Papagheorghe et al., "Proteomic approaches to biomarker discovery in cutaneous T-cell lymphoma," *Disease Markers*, vol. 2016, Article ID 9602472, 8 pages, 2016.

[102] Y. Liu, T. Lang, B. Jin et al., "Luteolin inhibits colorectal cancer cell epithelial-to-mesenchymal transition by suppressing CREB1 expression revealed by comparative proteomics study," *Journal of Proteomics*, vol. 161, pp. 1–10, 2017.

[103] T. Zhu, Y. Xu, B. Dong et al., "β-Elemene inhibits proliferation of human glioblastoma cells through the activation of glia maturation factor β and induces sensitization to cisplatin," *Oncology Reports*, vol. 26, no. 2, pp. 405–413, 2011.

[104] X. Yu, M. Xu, N. Li et al., "β-Elemene inhibits tumour-promoting effect of M2 macrophages in lung cancer," *Biochemical and Biophysical Research Communications*, vol. 490, no. 2, pp. 514–520, 2017.

[105] N. Akhtar, D. N. Syed, R. K. Lall, B. Mirza, and H. Mukhtar, "Targeting epithelial to mesenchymal transition in prostate cancer by a novel compound, plectranthoic acid, isolated from *Ficus microcarpa*," *Molecular Carcinogenesis*, vol. 57, no. 5, pp. 653–663, 2018.

[106] J. C. Tseng, C. Y. Lin, L. C. Su, H. H. Fu, S. D. Yang, and C. P. Chuu, "CAPE suppresses migration and invasion of prostate cancer cells via activation of non-canonical Wnt signaling," *Oncotarget*, vol. 7, no. 25, pp. 38010–38024, 2016.

[107] N. Kiweler, B. Brill, M. Wirth et al., "The histone deacetylases HDAC1 and HDAC2 are required for the growth and survival of renal carcinoma cells," *Archives of Toxicology*, vol. 92, no. 7, pp. 2227–2243, 2018.

[108] E. Hahnen, J. Hauke, C. Tränkle, I. Y. Eyüpoglu, B. Wirth, and I. Blümcke, "Histone deacetylase inhibitors: possible implications for neurodegenerative disorders," *Expert Opinion on Investigational Drugs*, vol. 17, no. 2, pp. 169–184, 2008.

[109] A. A. Jaguva Vasudevan, M. J. Hoffmann, M. L. C. Beck et al., "HDAC5 expression in urothelial carcinoma cell lines inhibits long-term proliferation but can promote epithelial-to-mesenchymal transition," *International Journal of Molecular Sciences*, vol. 20, no. 9, article 2135, 2019.

[110] T. J. Abou-Antoun, J. Nazarian, A. Ghanem, S. Vukmanovic, and A. D. Sandler, "Molecular and functional analysis of anchorage independent, treatment-evasive neuroblastoma tumourspheres with enhanced malignant properties: a possible explanation for radio-therapy resistance," *PLoS One*, vol. 13, no. 1, article e0189711, 2018.

[111] V. S. Viswanathan, M. J. Ryan, H. D. Dhruv et al., "Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway," *Nature*, vol. 547, no. 7664, pp. 453–457, 2017.

[112] N. Li, R. Babaei-Jadidi, F. Lorenzi et al., "An FBXW7-ZEB2 axis links EMT and tumour microenvironment to promote colorectal cancer stem cells and chemoresistance," *Oncogene*, vol. 8, no. 3, p. 13, 2019.

[113] S. K. Alam, V. K. Yadav, S. Bajaj et al., "DNA damage-induced ephrin-B2 reverse signalling promotes chemoresistance and

drives EMT in colorectal carcinoma harboring mutant p53," *Cell Death & Differentiation*, vol. 23, no. 4, pp. 707–722, 2016.

[114] E. Dazert, M. Colombi, T. Boldanova et al., "Quantitative proteomics and phosphoproteomics on serial tumour biopsies from a sorafenib-treated HCC patient," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 5, pp. 1381–1386, 2016.

[115] W. Liu, J. Yuan, Z. Liu, J. Zhang, and J. Chang, "Label-free quantitative proteomics combined with biological validation reveals activation of Wnt/β-catenin pathway contributing to trastuzumab resistance in gastric cancer," *International Journal of Molecular Sciences*, vol. 19, no. 7, article 1981, 2018.

[116] H. Creedon, L. Gómez-Cuadrado, Ž. Tarnauskaitė et al., "Identification of novel pathways linking epithelial-to-mesenchymal transition with resistance to HER2-targeted therapy," *Oncotarget*, vol. 7, no. 10, pp. 11539–11552, 2016.

[117] S. Almozyan, D. Colak, F. Mansour et al., "PD-L1 promotes OCT4 and Nanog expression in breast cancer stem cells by sustaining PI3K/AKT pathway activation," *International Journal of Cancer*, vol. 141, no. 7, pp. 1402–1412, 2017.

[118] S. Yu, C. Yan, X. Yang et al., "Pharmacoproteomic analysis reveals that metapristone (RU486 metabolite)intervenes E-cadherin and vimentin to realize cancer metastasis chemoprevention," *Scientific Reports*, vol. 6, article 22388, 2016.

[119] M. J. Sale, K. Balmanno, J. Saxena et al., "MEK1/2 inhibitor withdrawal reverses acquired resistance driven by BRAFV600E amplification whereas KRASG13D amplification promotes EMT-chemoresistance," *Nature Communications*, vol. 10, no. 1, article 2030, 2019.

[120] V. Paulitschke, O. Eichhoff, P. F. Cheng, M. P. Levesque, and C. Höller, "Proteomics approaches to understanding mitogen-activated protein kinase inhibitor resistance in melanoma," *Current Opinion in Oncology*, vol. 28, no. 2, pp. 172–179, 2016.

[121] V. M. Voiculescu, C. V. Lisievici, M. Lupu et al., "Mediators of inflammation in topical therapy of skin cancers," *Mediators of Inflammation*, vol. 2019, Article ID 8369690, 15 pages, 2019.

[122] M. R. Milone, R. Lombardi, M. S. Roca et al., "Novel pathways involved in cisplatin resistance identified by a proteomics approach in non-small-cell lung cancer cells," *Journal of Cellular Physiology*, vol. 234, no. 6, pp. 9077–9092, 2019.

[123] T. Kling, R. Ferrarese, D. Ó hAilín et al., "Integrative modeling reveals annexin A2-mediated epigenetic control of mesenchymal glioblastoma," *eBioMedicine*, vol. 12, pp. 72–85, 2016.

[124] Y. Yi, S. Zeng, Z. Wang et al., "Cancer-associated fibroblasts promote epithelial-mesenchymal transition and EGFR-TKI resistance of non-small cell lung cancers via HGF/IGF-1/ANXA2 signalling," *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, vol. 1864, no. 3, pp. 793–803, 2018.

[125] C. Dong, B. Zhao, F. Long et al., "Nogo-B receptor promotes the chemoresistance of human hepatocellular carcinoma via the ubiquitination of p53 protein," *Oncotarget*, vol. 7, no. 8, pp. 8850–8865, 2016.

[126] J. Hao, P. Graham, L. Chang et al., "Proteomic identification of the lactate dehydrogenase A in a radioresistant prostate cancer xenograft mouse model for improving radiotherapy," *Oncotarget*, vol. 7, no. 45, pp. 74269–74285, 2016.

[127] D. Wu, B. Zhao, X. Qi et al., "Nogo-B receptor promotes epithelial–mesenchymal transition in non-small cell lung cancer cells through the Ras/ERK/Snail1 pathway," *Cancer Letters*, vol. 418, pp. 135–146, 2018.

[128] J.-y. Li, X.-y. Yang, X.-f. Wang et al., "Siglec-5 is a novel marker of critical limb ischemia in patients with diabetes," *Scientific Reports*, vol. 7, no. 1, article 11272, 2017.

[129] R. A. Mathias and R. J. Simpson, "Towards understanding epithelial-mesenchymal transition: a proteomics perspective," *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, vol. 1794, no. 9, pp. 1325–1331, 2009.