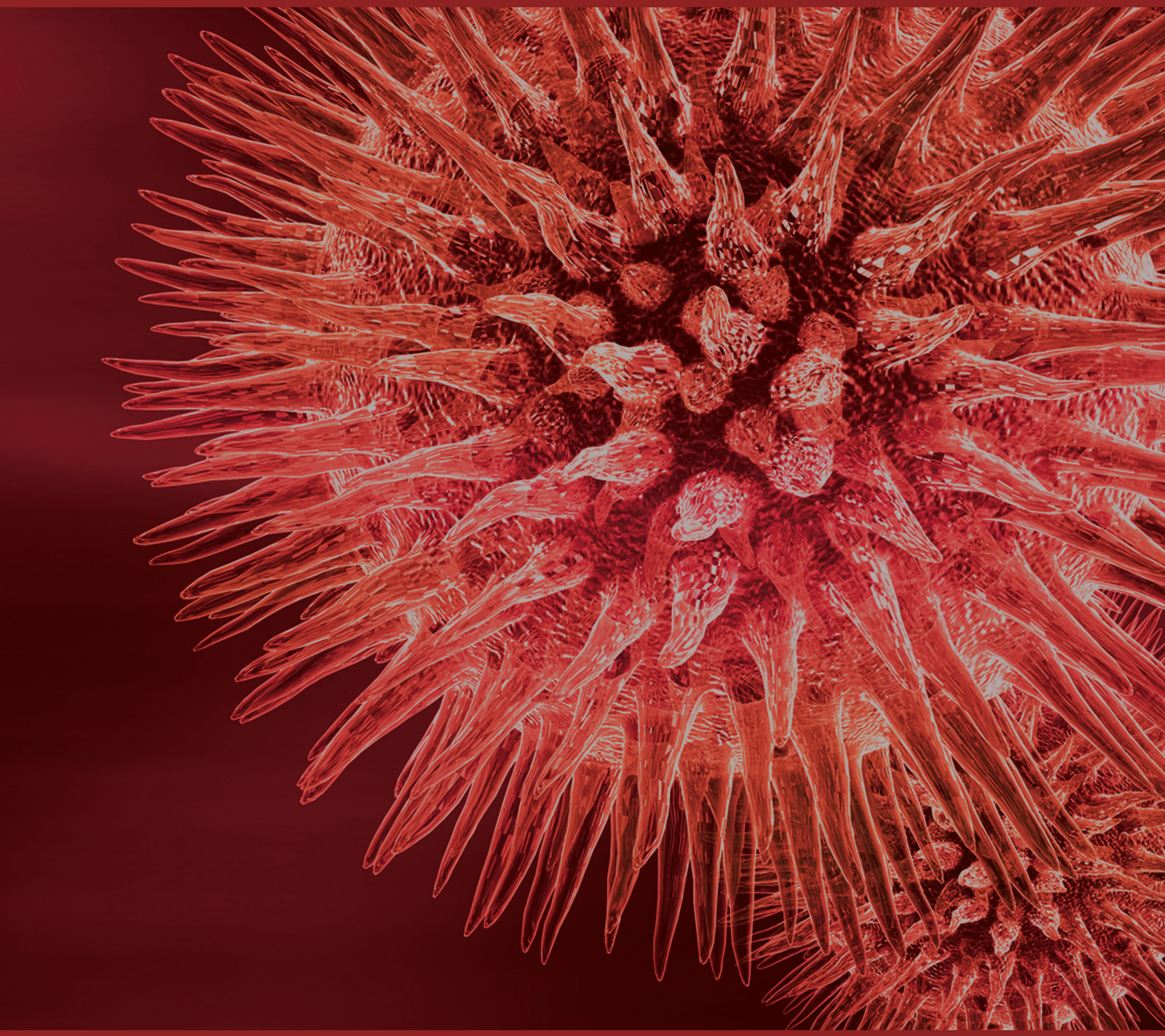
BioMed Research International

Interplay between Tumor Microenvironment and Cancer Cells

Guest Editors: Kallesh D. Jayappa, Ramesh C. Kovi, and Sumanta Chatterjee



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Editorial

Interplay between Tumor Microenvironment and Cancer Cells

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Cancer cell interaction with microenvironment plays a very important role in cancer cell survival, proliferation, and/or metastasis. The cancer cell-microenvironment interaction is a highly complex process and has been one of the leading areas of research in cancer biology. Over the last decade, several components in tumor microenvironment have been identified to have a role in cancer cell survival, proliferation, or metastasis. The underlying mechanisms of tumor cell-microenvironment interactions have been investigated and exploited for cancer therapy. The present issue covers several aspects of cancer cell and tumor microenvironment. This issue contains interesting articles, which were accepted after a rigorous peer review process.

The monocarboxylate transporters (MCTs) are proton-linked plasma membrane transporters that mediate transport of monocarboxylates such as pyruvate and lactate across cellular membranes. Tumor cells depend heavily on anaerobic glycolysis, which generates enormous amount of lactic acid as a byproduct. Lactic acid is toxic to cells and has to be excreted out of the cells through MCTs. An interesting meta-analysis paper by C. D. Bovenzi et al. showed the high expression of MCT4 and CD147 in multiple cancer cells. The elevated MCT4 and CD147 levels were strongly associated with poor overall survival of cancer patients. This work highlighted the importance of MCT4 and CD147 expression in several different cancers and establishes rationale for targeting these molecules for cancer therapy.

As discussed above, as well as by several other reports, mostly the overabundance of ion channels in biological membranes has been implicated in pathogenesis of cancers. Only recently did few studies identify antitumor function of ion channels in some type of cancers. Interestingly, the paper by Z. Xia et al. showed the overexpression of potassium ions in liver cancer cells by the treatment with potassium ions that trigger cancer cell apoptosis, providing convincing evidence in favor of tumor suppression function of potassium ion channels in liver cancer. Given this, the levels of potassium ions in tumor microenvironment can influence the survival of liver cancer cells. Further investigation in this regard is warranted.

Lymphatic vessels are integral part of tumor microenvironment. Although lymphatic vessels were mostly viewed as passive transporters of cancer cells, recent studies demonstrated the active role of these vessels in cancer metastasis. Indeed, these studies collectively established the rationale for targeting lymphangiogenesis for cancer treatment. The paper by X.-L. Ding et al. describes the antilymphangiogenesis effects of Gekko Sulfated Glycopeptide (GSPP) and discusses the potential therapeutic benefits of GSPP in colon carcinoma. The future studies could explore the translational potential of GSPP or related molecules in colon carcinoma or related cancers.

The hormones play a key role in several types of cancers. Gender differences in hormone can influence several aspects of cancer. The paper by S. Caceres et al. developed a male mice xenograft model for mammary tumor and studied the impact of gender differences in hormones on mammary tumor cell proliferation and metastasis. Their preliminary analysis showed the less aggressive nature of mammary tumor cells in male mice compared to female. This work elaborates on the vast literature that describes the importance of extracellular hormone in tumor cell behavior.

The traditional Chinese medicines are being used for cancer treatment for centuries. The review article by J. Xu et al. provides a very nice overview of the selected traditional Chinese medicines that suppress cancer cells by interfering with tumor microenvironment. This article also discusses the synergistic benefits of combining few selected traditional Chinese medicines.

Collectively, this issue will advance our researchers and readers knowledge on various aspects of tumor cell-microenvironment interactions and will provide insight into new research focused in this area and potential therapeutic interventions.

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Research Article

Steroid Tumor Environment in Male and Female Mice Model of Canine and Human Inflammatory Breast Cancer

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Canine inflammatory mammary cancer (IMC) shares clinical and histopathological characteristics with human inflammatory breast cancer (IBC) and has been proposed as a good model for studying the human disease. The aim of this study was to evaluate the capacity of female and male mice to reproduce IMC and IBC tumors and identify the hormonal tumor environment. To perform the study sixty 6–8-week-old male and female mice were inoculated subcutaneously with a suspension of 10^6 IPC-366 and SUM149 cells. Tumors and serum were collected and used for hormonal analysis. Results revealed that IPC-366 reproduced tumors in 90% of males inoculated after 2 weeks compared with 100% of females that reproduced tumor at the same time. SUM149 reproduced tumors in 40% of males instead of 80% of females that reproduced tumors after 4 weeks. Both cell lines produce distant metastasis in lungs being higher than the metastatic rates in females. EIA analysis revealed that male tumors had higher T and SO4E1 concentrations compared to female tumors. Serum steroid levels were lower than those found in tumors. In conclusion, IBC and IMC male mouse model is useful as a tool for IBC research and those circulating estrogens and intratumoral hormonal levels are crucial in the development and progression of tumors.

1. Introduction

Human inflammatory breast cancer (IBC) is the most aggressive mammary neoplasia that affects women [1, 2]. IBC accounts for less than 6% of human breast cancer diagnoses with the poorest survival in women [2, 3]. Canine inflammatory mammary cancer (IMC) has been proposed as the best spontaneous animal model for the study of human IBC [4]. The main histological characteristic of the disease in both species is the massive invasion of dermal lymphatic vessels by neoplastic cells which blocks lymph drainage causing the characteristic edema [5, 6]. In both species, this type of cancer is highly angiogenic and angioinvasive [4, 7–9].

Several human IBC cell lines have been established in order to study the mechanisms of this special type of breast cancer *in vitro* such as SUM149, SUM190, and MDA-IBC-3 [10–12]. Recently IPC-366, an IMC triple negative cell line, has been established [13].

On the other hand, male breast cancer (MBC) is a rare disease that accounts for less than 1% of all breast carcinomas [14] and male inflammatory breast cancer is extremely rare [15]; however, the incidence of MBC is increasing [16]. MBC appears to be biologically similar to female breast cancer [16] and it has been found that clinical and histological features in male and female inflammatory breast cancer are also similar [15]. Hormone imbalance between estrogens and androgens levels is one of the main risk factors for MBC [14], as it is known that androgens exert inhibitory effects in hormone-dependent breast cancer cells [14, 17], but the role of androgens in breast cancer development is still unclear.

Several studies have demonstrated that the tumor tissues have a local steroid synthesis [18]. Biologically active

estrogens are locally produced by estrogen-producing enzymes, such as aromatase that converts circulating androstenedione to estrone or testosterone to estradiol in breast carcinoma [19]. Also intratumoral androgens concentrations were reported to be significantly higher in breast carcinoma [20], and androgen-producing enzymes, such as 17bHSD5 that converts circulating androstenedione to testosterone, and 5a-reductase type 1, which reduces testosterone to DHT, were expressed [21].

The development of xenografts has been a useful tool for improving our understanding of breast cancer progression and metastasis [22–24]. The majority of breast cancer xenografts are performed on female mice. Therefore, the aim of this study was to conduct a male mouse model of IBC and IMC to determine the similarities and differences between female and male tumor progression and tumor hormonal environment.

2. Materials and Methods

2.1. Cell Culture. Canine inflammatory mammary carcinoma cell line, IPC-366, was cultured in Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 Ham (DMEM/F12) containing 10% fetal bovine serum, 1% L-glutamine, and 1% antibiotic-antimycotic (Sigma Aldrich). Its human counterpart, SUM149 cell line, was obtained from Asterand plc (Detroit, MI) and was cultured in Ham's F12 (Fisher Scientific) supplemented with 10% fetal bovine serum, 5 μg/mL insulin, 1 μg/mL hydrocortisone, and antibiotic-antimycotic (Sigma Aldrich).

All cell lines were cultured in 25 cm^2 culture flasks and were maintained in a humidified atmosphere of 5% carbon dioxide at 37°C. Cell culture was observed daily by a phase-contrast microscopy.

2.2. Animals. A total of sixty 6–8-week-old female (F) mice and male (M) mice BALB/cJHan®Hsd-Prkdcscid (SCID) (Harlan Laboratories Models, SL, Barcelona, Spain) were used in this study, divided into the following groups: 20 (10 F and 10 M) as serum control group and 40 (F and M) inoculated with IPC-366 ($n = 20$) and SUM149 ($n = 20$) as experimental groups (serum and tumor homogenates). The animals were housed in a flexible-film isolator (Isotec, Harlan Laboratories Models, SL) in cages (1-2 animals per cage), in a room with controlled environmental conditions (20–22°C; 50–55% relative humidity; 10–15 air changes per hour; and a 12 : 12-hour light : dark cycle). Food and water, previously sterilized, were provided ad libitum. Prior to all procedures, animals were anesthetized with isoflurane (IsoVet) at 4% for induction and 1.5% for maintaining sedation, supplied in a fresh gas flow rate of 0.5 L of oxygen/minute, and were observed until fully recovered. Animals were sacrificed by a lethal dose of isoflurane.

Clinical and experimental protocols of this study were approved by the Institutional Animal Care and Use Committee of the University Complutense of Madrid, Spain (number: 115). All procedures were completed in accordance with the Guide for the Care and Use of Laboratory Animals and conformed to the relevant EU Directive.

2.3. Mice Cell Inoculation. A suspension of 10^6 IPC-366 cells and 10^6 SUM149 cells were implanted subcutaneously into the fourth inguinal mammary gland. Mice were inspected twice/week for the development of tumors. If tumors were detected, they were weekly monitored by palpation and measured by calipers. Mice were sacrificed when tumor volume was up to 1500 mm^3. Blood samples were taken from the submandibular venous sinus. Tumors were collected at necropsy for homogenates.

2.4. Steroid Determinations in Serum and Tumor Homogenates. Tumors were homogenized in 4 mL of PBS (pH 7.2) and centrifuged at 1200 g, for 20 min at 4°C. Supernatants were collected and aliquoted individually (−80°C) until hormone assays. Blood samples were centrifuged at 1200 g and 4°C for 20 min and serum was separated and stored frozen at −20°C until assayed. Estrone sulphate (SO4E1: ab R522-2), 17b-estradiol (E2: ab C6E91), androstenedione (A4: ab C9111), testosterone (T: R156), and progesterone (P4: C914) levels of tumor homogenates and serum samples were assayed by enzyme-immunoassay (EIA) previously validated [25]. All antibodies were developed in the Department of Animal Physiology (UCM, Spain).

All hormone concentrations were expressed in ng/g (for tumor homogenates) and ng/mL (for serum samples), except serum E2 concentrations that were expressed in pg/mL.

2.5. Statistics. The statistics software used for data analysis was SAS 9.4 (UCM, Madrid, Spain). The results were expressed as the means ± SD. For tumor progression analysis, to compare both cell lines (IPC-366 and SUM149) in each group the Wilcoxon rank-sum test was performed. For comparisons between groups on each cell line, we used the Kruskal-Wallis test followed by a pairwise nonparametric multiple comparisons test when the overall contrast was significant. Wilcoxon signed ranks test with Bonferroni correction was used for comparisons between weeks on each group and cell line. Differences in hormonal concentrations between group means were analyzed by one-way analysis of variance (ANOVA) followed by appropriate *post hoc* tests for similar variances (Duncan Test) or different ones (Games Howell test). In all statistical comparisons, $p < 0.05$ was accepted as denoting significant differences.

3. Results

3.1. Tumor Growth Progression in Male and Female Mice. IPC-366 and SUM149 cells were injected subcutaneously on female and male SCID mice to observe if there were differences in tumor growth parameters (Table 1). All female mice inoculated with IPC-366 cells reproduced a tumor that was appreciable approximately two weeks after cell injection (16.64 ± 1.72 days). However, 80% of female mice inoculated with SUM149 cells reproduced a tumor with significant

Table 1: Tumor growth parameters of female and male mice inoculated with IPC-366 and SUM149 cells.

Cell line	Gender	% of animals with tumor	Time of palpable tumor (days)	Time of 1.500 mm^3 volume (days)	% of animals with ulceration	% of animals with metastasis
IPC-366 ($n = 20$)	Female	100%	16.64 ± 1.72	42.02 ± 2.35	50%	90%
	Male	90%	15.16 ± 2.60	39.66 ± 3.29	0%*	20%*
SUM149 ($n = 20$)	Female	80%	26.82 ± 2.19^a	53.40 ± 4.86^a	30%	80%
	Male	40%^{*,a}	24.50 ± 3.5^a	51.33 ± 3.66^a	10%	50%^{*,a}

$^*p < 0.05$, significant differences between females and males inoculated with each cell line. ^{a}Significant differences ($p < 0.05$) between cell lines.

Table 2: Serum steroid concentrations in female (F) and male (M) mice in control group, inoculated with IPC-366 and SUM149 cells.

Steroid hormone	Gender	Control	IPC-366	SUM149
SO4E1 (ng/mL)	F	1.30 ± 0.03^a	$0.11 \pm 0.12^{b,1}$	$0.12 \pm 0.01^{b,1}$
	M	$0.29 \pm 0.04^{*,a}$	$0.07 \pm 0.02^{b,1}$	$0.09 \pm 0.03^{b,1}$
E2 (pg/mL)	F	42.79 ± 3.64^a	$6.73 \pm 0.37^{b,1}$	$8.67 \pm 0.71^{b,1}$
	M	$6.00 \pm 0.26^{*,a}$	$2.11 \pm 0.12^{*,b,1}$	$2.91 \pm 0.22^{*,b,1}$
A4 (ng/mL)	F	0.21 ± 0.03^a	$0.64 \pm 0.13^{b,1}$	$0.78 \pm 0.18^{b,1}$
	M	0.14 ± 0.02^a	$0.48 \pm 0.09^{b,1}$	$0.56 \pm 0.11^{b,1}$
T (ng/mL)	F	0.5 ± 0.01^a	$0.31 \pm 0.09^{a,1}$	$0.45 \pm 0.12^{a,1}$
	M	$2.3 \pm 0.6^{*,a}$	$1.66 \pm 0.39^{*,a,1}$	$1.72 \pm 0.28^{*,a,1}$
P4 (ng/mL)	F	3.59 ± 0.04^a	$0.48 \pm 0.22^{b,1}$	$0.57 \pm 0.07^{b,1}$
	M	$1.10 \pm 0.32^{*,a}$	$0.18 \pm 0.03^{*,b,1}$	$0.32 \pm 0.05^{b,1}$

$^*p < 0.05$, significant differences between females and males. Different letters denoted statistical differences ($p < 0.05$) between control and cell lines. Different numbers denoted statistical differences ($p < 0.05$) between cell lines.

difference ($p < 0.05$) in tumor appearance that was in approximately 4 weeks after cell injection (26.82 ± 2.19 days).

Male mice showed similar results on each cell line. Results revealed that 90% and 40% of male mice inoculated with IPC-366 and SUM149, respectively, originated tumors. In SUM149, frequency of tumor appearance in males was halved with respect to females, being a significant difference ($p < 0.05$). IPC-366 and SUM149 males showed no statistically significant differences with respect to females in time of tumor occurrence and time in which tumor volume of 1500 mm^3 was reached.

Results of frequency of mice that developed ulceration and metastasis also differed between males and females inoculated with both cell lines. The percentage of ulcerations and metastasis found in males was reduced in contrast to the females. Frequency of ulceration was significantly higher ($p < 0.05$) in females (IPC-366 50%; SUM149 30%) than in males (IPC-366 10%; SUM149 0%). Also, both models developed spontaneous distant metastasis with significantly higher ($p < 0.05$) frequency in females (IPC-366 90%; SUM149 80%) than males (IPC-366 20%; SUM149 50%).

Tumor growth progression in males and females of IPC-366 and SUM149 cell lines followed a similar pattern (Figure 1). Both models exhibited rapid growth *in vivo* reaching a volume of 1500 mm^3 approximately 6–8 weeks after cell inoculation and no statistical differences were found in tumor progression results.

3.2. Hormonal Tumor Environment. Steroid determinations in tumor homogenates revealed that tumors from males and females differed on estrogen and androgen levels (Figure 2). P4 levels were lower in males compared with female P4 levels but not significantly. However, A4 and T levels were significantly higher ($p < 0.05$) in males than in females. Estrogen levels were higher in female tumors than in males with this difference being significant ($p < 0.05$) in SO4E1 levels, but E2 levels did not show any significant difference.

3.3. Serum Hormonal Concentrations. Results from serum steroid concentrations (Table 2) in control and experimental groups showed that control mice had significantly higher ($p < 0.05$) steroid levels than IPC-366 and SUM149, except A4 concentrations that were significantly higher ($p < 0.05$) in IPC-366 and SUM149 mice than in control mice. Differences between females and males in control and experimental mice were also found. SO4E1, E2, and P4 were significantly higher ($p < 0.05$) in female mice than in males. SUM149 mice did not show any statistical differences in these steroid concentrations. However, IPC-366 mice showed statistical differences ($p < 0.05$) in E2 and P4 levels but not in SO4E1, being higher in females than males. Besides, T levels were significantly higher ($p < 0.05$) in males than females in control, IPC-366, and SUM149 mice, but in A4 concentrations any statistical difference between females and males was found.

4. Discussion

IMC and IBC are considered the most malignant and aggressive subtypes of breast cancer that affect female dogs and humans, respectively, [3–5] and IMC has been suggested as a model to study the human disease [4, 5]. Recently, a triple

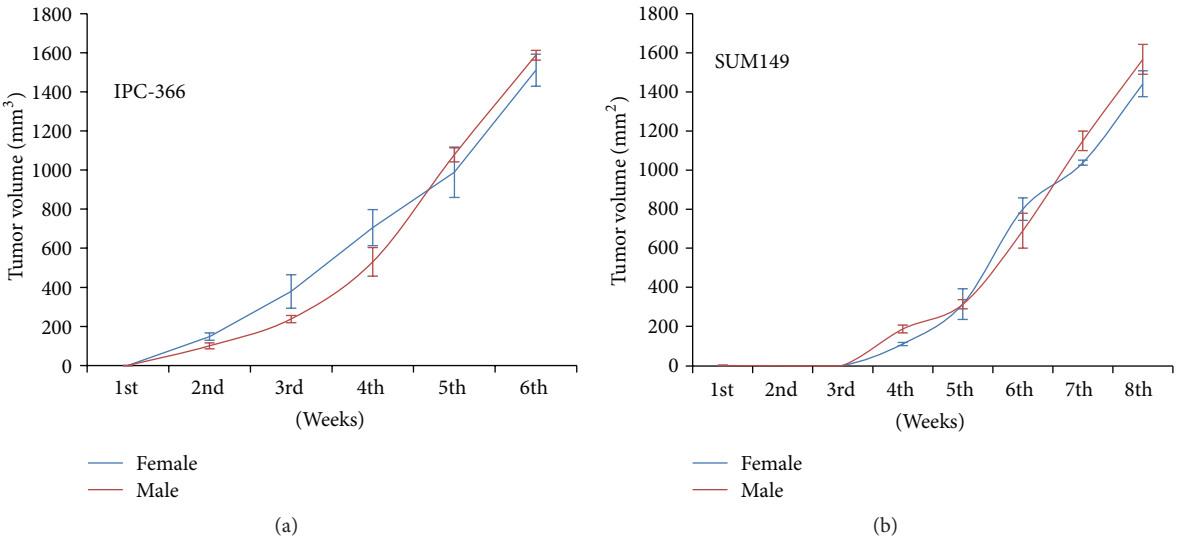

FIGURE 1: *In vivo* tumor growth progression of (a) IPC-366 and (b) SUM149 male and female mice. Tumor growth followed the same pattern in males and females in both cell lines. Lines represent means ± SD. There were no statistical differences between groups.

negative IMC cell line (IPC-366) has been established as a useful tool for breast cancer research [13].

Animal models are of great value in cancer research. However, the microenvironment around the tumor is crucial for tumor development [26] and hormonal secretion plays an important role.

This study was intended to develop a male animal model for elucidating which endocrine factors may be involved in breast carcinogenesis, comparing tumor growth and intratumoral steroids levels in female and male mice inoculated with IBC and IMC cell lines (SUM149 and IPC-366 cell lines, resp.).

Our results revealed that both cell lines were capable of reproducing tumors in male mice at the same time compared to female mice, but the frequency of tumor appearance was lower than in female mice. The reason of the lower frequency rates found in male mice could be due to the androgen environment that male mice provide as it is known that androgens exert inhibitory effects in hormone-dependent breast cancer cells [17]. To our knowledge, this is the first male animal model for breast cancer research, as male breast cancer development is still unclear. We also found that metastases and ulceration rates were also lower in males. Tumor metastasis comprises different processes that lead tumor cells move away from the tumor to a distant location [27] and some authors suggested that stromal cells regulate the production of various factors implicated in metastasis process such as COX2, TNF-α, IL-6, and IL-11 [28]. However, hormone levels could also exert an influence on metastatic process. In point of fact, several authors' associate levels of expression of SO4E1 with lymph node metastases [29] that is also in agreement with our results in female mice, where we found higher amount levels of intratumoral SO4E1 and metastatic rates.

In ER positive breast carcinomas, androgens are well known to suppress cell proliferation but there is poor knowledge of the roles of androgens in triple negative carcinomas [30]. It is known that ER/PR negative carcinomas are associated with decreased hormone levels of androgens and estrogens when compared to ER/PR positive cancers [18]; this hypothesis was supported by Blankenstein et al. that observed significant estradiol levels in ER-negative tumors [31].

Wiebe suggested that P4 metabolites produced within breast tissues might function as cancer promoting or inhibiting agents, since P4 serves as the precursor for the major steroid hormones (androgens and estrogens). Tumor progression could be related to changes in local P4 levels [32]. Our results reveled that intratumoral P4 levels were decreased in male mice compared to those found in females. The decrease of intratumoral levels of P4 in males compared to females might be due to the low frequency of metastasis found in males, as it is proposed that P4 metabolites might play a role in the acquisition of metastatic potential [32].

Estrogens are known to be responsible for development and progression of breast cancer by stimulating cell proliferation [33]. E2 is the most potent estrogen whose effects are mediated by binding to the estrogen receptor (ER) [27, 34]. Likewise, it is known that androgens suppress cell proliferation in breast cancer cells [17]; however, their role in carcinogenesis on breast tissue is still unclear and there is some controversy on their effects on breast cancer [35]. Some studies revealed that androgens mediated cell growth via aromatization in epithelial breast cancer cells [36].

In situ production of steroids plays an important role on steroid signaling in hormone-dependent carcinomas. These tumors do not depend on circulating steroid levels but produce steroid hormones locally from circulating precursors [37]. Additionally, the local synthesis of steroids has been proposed in the canine mammary gland [38] and the ability of

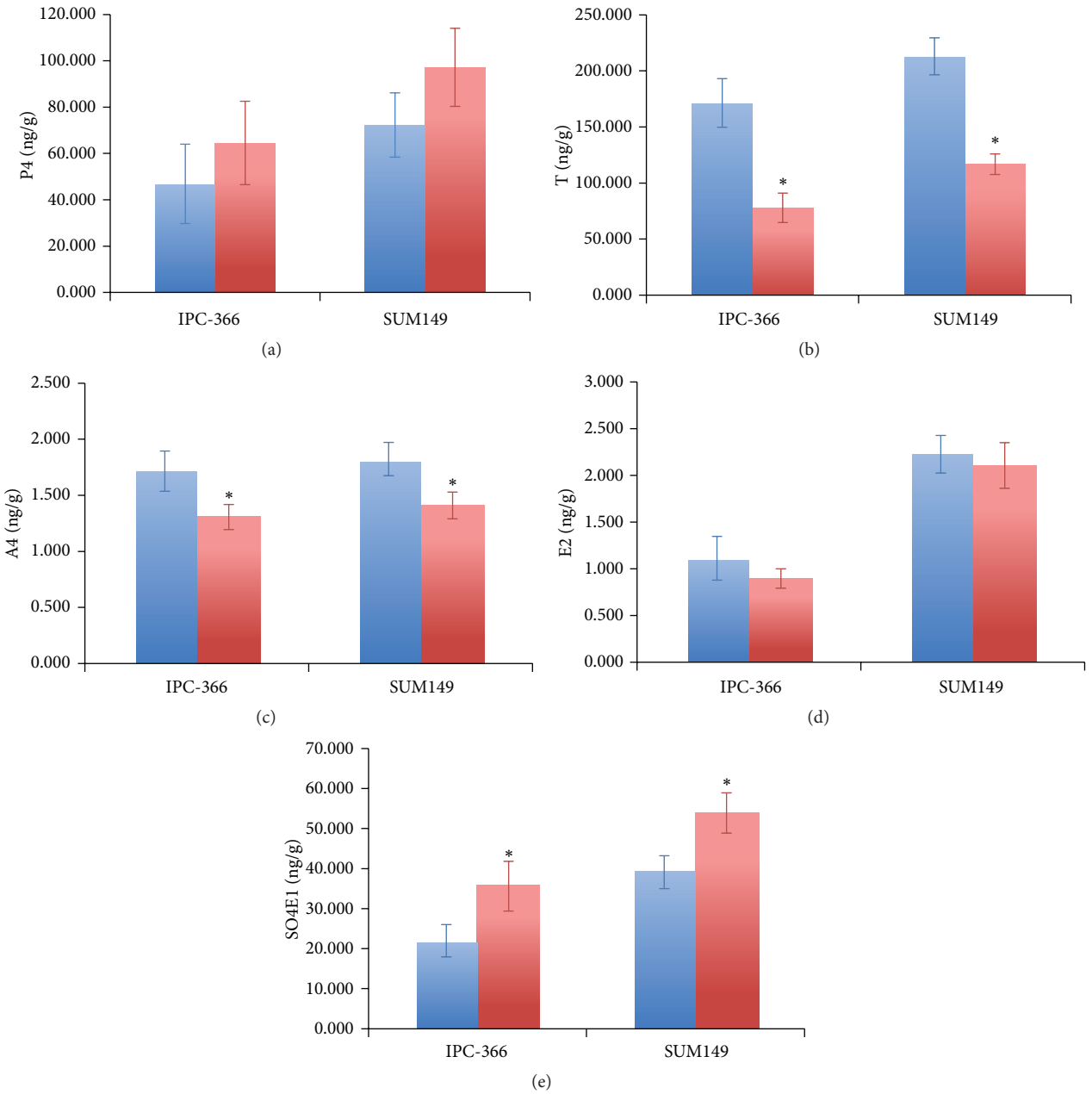

Figure 2: Hormonal levels of (a) P4, (b) T, (c) A4, (d) E2, and (e) SO4E1 of male and female IPC-366 and SUM149 tumors homogenates. Estrogens were higher in female tumors than in males instead of androgens that were higher in males tumors than females tumors. Bar represents means ± SD. $^{*}p < 0.05$ denoted significant differences between females and males.

canine and human breast cancer cell lines to produce steroid hormones in *in vitro* conditions [39].

Serum hormone levels were lower than those found in control group, suggesting that estrogens and androgens locally produced in tissues act without being released into the bloodstream [20]. These results provide evidence that tumoral tissue uptake plasma steroid from circulation and also produce the biosynthesis of them [31, 38]. Estrogens tissue levels will be the result of biosynthesis and degradation of the estrogenic enzymes. Apart from the enzyme activity, estrogen biosynthesis will also depend on the availability of substrate. The breast does not have the precursor steroids such as androstenediol or testosterone, which directly convert to E2, DHEA, and A4, and can contribute to E2 biosynthesis via estrone [31].

In this study we found several differences in intratumoral estrogen and androgen levels between females and males. Males had higher levels of intratumoral androgens instead of females that presented higher levels of estrogens. Our study confirms the hypothesis of estrogen local production in IMC and IBC [38, 40, 41] because higher SO4E1 and E2 concentrations were found in tumor than in serum. As

androgens suppress cell proliferation, the high intratumoral androgen levels found in males could be associated with the low frequency of tumors in males inoculated with SUM149 and also the low metastases rates in both cell lines. Probably, intratumoral androgens exert an effect on the stromal cells by blocking the metastatic process in male mice. Planas-Silva and Waltz found that E2 promotes reversible epithelial-to-mesenchymal transition in ERα-positive cells [42] and these changes in the cells could lead to metastasis [27]. Thus, the high estrogen intratumoral levels found in female mice could also be implicated on the metastatic process and the malignancy of the cells. We have found that in females SO4E1 intratumoral levels were significantly higher than in males, and E2 levels were similar in both models. Probably, the amounts of SO4E1 found in females could act as a reservoir of estrogens [43]. In the case of males, to counteract high T intratumoral levels and promote tumor progression, tumor cells use SO4E1 reservoirs to produce biologically active estrogens (E2) and thus promote cell proliferation.

5. Conclusions

In this study we determined an IBC and IMC male mouse model useful for male and women inflammatory breast cancer. We also have found that hormonal tumor environment is crucial for tumor development and progression; high amounts of intratumoral androgens could be associated with a low risk of metastatic capacity.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

References

[1] K. W. Hance, W. F. Anderson, S. S. Devesa, H. A. Young, and P. H. Levine, "Trends in inflammatory breast carcinoma incidence and survival: the surveillance, epidemiology, and end results program at the National Cancer Institute," *Journal of the National Cancer Institute*, vol. 97, no. 13, pp. 966–975, 2005.

[2] H. Yamauchi, W. A. Woodward, V. Valero et al., "Inflammatory breast cancer: what we know and what we need to learn," *Oncologist*, vol. 17, no. 7, pp. 891–899, 2012.

[3] F. A. Tavassoli, "Inflammatory carcinoma. Infiltrating carcinomas: special types," in *Pathology of the Breast*, pp. 538–541, McGraw-Hill, New York, NY, USA, 2nd edition, 1999.

[4] L. Peña, M. D. Perez-Alenza, A. Rodriguez-Bertos, and A. Nieto, "Canine inflammatory mammary carcinoma: histopathology, immunohistochemistry and clinical implications of 21 cases," *Breast Cancer Research and Treatment*, vol. 78, no. 2, pp. 141–148, 2003.

[5] M. D. Pérez-Alenza, E. Tabanera, and L. Peña, "Inflammatory mammary carcinoma in dogs: 33 cases (1995–1999)," *Journal of the American Veterinary Medical Association*, vol. 219, no. 8, pp. 1110–1114, 2001.

[6] S. H. Giordano and G. N. Hortobagyi, "Inflammatory breast cancer: clinical progress and the main problems that must be addressed," *Breast Cancer Research*, vol. 5, no. 6, pp. 284–288, 2003.

[7] C. G. Kleer, K. L. van Golen, and S. D. Merajver, "Molecular biology of breast cancer metastasis. Inflammatory breast cancer: clinical syndrome and molecular determinants," *Breast Cancer Research*, vol. 2, no. 6, pp. 423–429, 2000.

[8] I. Van der Auwera, S. J. Van Laere, G. G. Van den Eynden et al., "Increased angiogenesis and lymphangiogenesis in inflammatory versus noninflammatory breast cancer by real-time reverse transcriptase-PCR gene expression quantification," *Clinical Cancer Research*, vol. 10, no. 23, pp. 7965–7971, 2004.

[9] M. Clemente, M. D. Pérez-Alenza, and L. Peña, "Metastasis of canine inflammatory versus non-inflammatory mammary tumours," *Journal of Comparative Pathology*, vol. 143, no. 2-3, pp. 157–163, 2010.

[10] A. H. Klopp, L. Lacerda, A. Gupta et al., "Mesenchymal stem cells promote mammosphere formation and decrease E-Cadherin in normal and malignant breast cells," *PLoS ONE*, vol. 5, no. 8, Article ID e12180, 2010.

[11] D. L. Holliday and V. Speirs, "Choosing the right cell line for breast cancer research," *Breast Cancer Research*, vol. 13, no. 4, article 215, 2011.

[12] S. V. Fernandez, F. M. Robertson, J. Pei et al., "Inflammatory breast cancer (IBC): clues for targeted therapies," *Breast Cancer Research and Treatment*, vol. 140, no. 1, pp. 23–33, 2013.

[13] S. Caceres, L. Peña, P. J. DeAndres et al., "Establishment and characterization of a new cell line of canine inflammatory mammary cancer: IPC-366," *PLoS ONE*, vol. 10, no. 3, Article ID e0122277, 2015.

[14] C. Gómez-Raposo, F. Zambrana Tévar, M. Sereno Moyano, M. López Gómez, and E. Casado, "Male breast cancer," *Cancer Treatment Reviews*, vol. 36, no. 6, pp. 451–457, 2010.

[15] T. Yamamoto, K. Iriyama, and T. Araki, "Male inflammatory breast cancer," *Surgery Today*, vol. 27, no. 7, pp. 669–671, 1997.

[16] A. Agrawal, A. A. Ayantunde, R. Rampaul, and J. F. R. Robertson, "Male breast cancer: a review of clinical management," *Breast Cancer Research and Treatment*, vol. 103, no. 1, pp. 11–21, 2007.

[17] T. Hanamura, T. Niwa, S. Nishikawa et al., "Androgen metabolite-dependent growth of hormone receptor-positive breast cancer as a possible aromatase inhibitor-resistance mechanism," *Breast Cancer Research and Treatment*, vol. 139, no. 3, pp. 731–740, 2013.

[18] K. M. McNamara, T. Yoda, K. Takagi, Y. Miki, T. Suzuki, and H. Sasano, "Androgen receptor in triple negative breast cancer," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 133, no. 1, pp. 66–76, 2013.

[19] K. Takagi, Y. Miki, S. Nagasaki et al., "Increased intratumoral androgens in human breast carcinoma following aromatase inhibitor exemestane treatment," *Endocrine-Related Cancer*, vol. 17, no. 2, pp. 415–430, 2010.

[20] T. Suzuki, Y. Miki, T. Moriya et al., "In situ production of sex steroids in human breast carcinoma," *Medical Molecular Morphology*, vol. 40, no. 3, pp. 121–127, 2007.

[21] T. Suzuki, A. D. Darnel, J.-I. Akahira et al., "5α-reductases in human breast carcinoma: possible modulator of in situ androgenic actions," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 5, pp. 2250–2257, 2001.

[22] M. Lelekakis, J. M. Moseley, T. J. Martin et al., "A novel orthotopic model of breast cancer metastasis to bone," *Clinical and Experimental Metastasis*, vol. 17, no. 2, pp. 163–170, 1999.

[23] A. Fantozzi and G. Christofori, "Mouse models of breast cancer metastasis," *Breast Cancer Research*, vol. 8, article 212, 2006.

[24] B. A. Ruggeri, F. Camp, and S. Miknyoczki, "Animal models of disease: pre-clinical animal models of cancer and their applications and utility in drug discovery," *Biochemical Pharmacology*, vol. 87, no. 1, pp. 150–161, 2014.

[25] F. L. Queiroga, M. D. Pérez-Alenza, G. Silvan, L. Peña, C. Lopes, and J. C. Illera, "Role of steroid hormones and prolactin in canine mammary cancer," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 94, no. 1–3, pp. 181–187, 2005.

[26] M. De Jong and T. Maina, "Of mice and humans: are they the same?—implications in cancer translational research," *Journal of Nuclear Medicine*, vol. 51, no. 4, pp. 501–504, 2010.

[27] S. S. Roy and R. K. Vadlamudi, "Role of estrogen receptor signaling in breast cancer metastasis," *International Journal of Breast Cancer*, vol. 2012, Article ID 654698, 8 pages, 2012.

[28] E. R. Simpson and S. R. Davis, "Minireview: aromatase and the regulation of estrogen biosynthesis—some new perspectives," *Endocrinology*, vol. 142, no. 11, pp. 4589–4594, 2001.

[29] V. N. Kristensen and A. L. Børresen-Dale, "Molecular epidemiology of breast cancer: genetic variation in steroid hormone metabolism," *Mutation Research/Reviews in Mutation Research*, vol. 462, no. 2-3, pp. 323–333, 2000.

[30] R. Shibuya, T. Suzuki, Y. Miki et al., "Intratumoral concentration of sex steroids and expression of sex steroid-producing enzymes in ductal carcinoma in situ of human breast," *Endocrine-Related Cancer*, vol. 15, no. 1, pp. 113–124, 2008.

[31] M. A. Blankenstein, J. Van de Ven, I. Maitimu-Smeele et al., "Intratumoral levels of estrogens in breast cancer," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 69, no. 1–6, pp. 293–297, 1999.

[32] J. P. Wiebe, "Progesterone metabolites in breast cancer," *Endocrine-Related Cancer*, vol. 13, no. 3, pp. 717–738, 2006.

[33] B. Xie, S. W. Tsao, and Y. C. Wong, "Sex hormone-induced mammary carcinogenesis in female Noble rats: the role of androgens," *Carcinogenesis*, vol. 20, no. 8, pp. 1597–1606, 1999.

[34] G. S. Chetrite, J. Cortes-Prieto, J. C. Philippe, F. Wright, and J. R. Pasqualini, "Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 72, no. 1-2, pp. 23–27, 2000.

[35] J. P. Garay and B. H. Park, "Androgen receptor as a targeted therapy for breast cancer," *American Journal of Cancer Research*, vol. 2, no. 4, pp. 434–445, 2012.

[36] J. Jensen, J. W. Kitlen, P. Briand, F. Labrie, and A. E. Lykkesfeldt, "Effect of antiestrogens and aromatase inhibitor on basal growth of the human breast cancer cell line MCF-7 in serum-free medium," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 84, no. 4, pp. 469–478, 2003.

[37] K. M. McNamara and H. Sasano, "The intracrinology of breast cancer," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 145, pp. 172–178, 2015.

[38] L. Peña, G. Silván, M. D. Pérez-Alenza, A. Nieto, and J. C. Illera, "Steroid hormone profile of canine inflammatory mammary carcinoma: a preliminary study," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 84, no. 2-3, pp. 211–216, 2003.

[39] J. C. Illera, S. Caceres, L. Peña et al., "Steroid hormone secretion in inflammatory breast cancer cell lines," *Hormone Molecular Biology and Clinical Investigation*, vol. 24, no. 3, pp. 137–145, 2015.

[40] J. C. Illera, M. D. Pérez-Alenza, A. Nieto et al., "Steroids and receptors in canine mammary cancer," *Steroids*, vol. 71, no. 7, pp. 541–548, 2006.

[41] A. R. Sánchez-Archidona, M. A. Jiménez, D. Pérez-Alenza et al., "Steroid pathway and oestrone sulphate production in canine inflammatory mammary carcinoma," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 104, no. 3-5, pp. 93–99, 2007.

[42] M. D. Planas-Silva and P. K. Waltz, "Estrogen promotes reversible epithelial-to-mesenchymal-like transition and collective motility in MCF-7 breast cancer cells," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 104, no. 1-2, pp. 11–21, 2007.

[43] S. F. Wong, K. Reimann, and L. C. Lai, "Effect of transforming growth factor-β1, insulin-like growth factor-I and insulin-like growth factor-II on cell growth and oestrogen metabolism in human breast cancer cell lines," *Pathology*, vol. 33, no. 4, pp. 454–459, 2001.

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Research Article

The Antitumor Effect of Gekko Sulfated Glycopeptide by Inhibiting bFGF-Induced Lymphangiogenesis

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Objective. To study the antilymphangiogenesis effect of Gekko Sulfated Glycopeptide (GSPP) on human lymphatic endothelial cells (hLECs). *Methods*. MTS was conducted to confirm the antiproliferation effect of GSPP on hLECs; flow cytometry was employed to detect hLECs cycle distribution; the antimigration effect of GSPP on hLECs was investigated by wound healing experiment and transwell experiment; tube formation assay was used to examine its inhibitory effect on the lymphangiogenesis; western blotting was conducted to detect the expression of extracellular signal-regulated kinase1/2 (Erk1/2) and p-Erk1/2 after GSPP and basic fibroblast growth factor (bFGF) treatment. Nude mice models were established to investigate the antitumor effect of GSPP in vivo. Decreased lymphangiogenesis caused by GSPP in vivo was verified by immunohistochemical staining. *Results*. In vitro, GSPP (10 μg/mL, 100 μg/mL) significantly inhibited bFGF-induced hLECs proliferation, migration, and tube-like structure formation ($P < 0.05$) and antagonized the phosphorylation activation of Erk1/2 induced by bFGF. In vivo, GSPP treatment (200 mg/kg/d) not only inhibited the growth of colon carcinoma, but also inhibited the tumor lymphangiogenesis. *Conclusion*. GSPP possesses the antitumor ability by inhibiting bFGF-inducing lymphangiogenesis in vitro and in vivo, which may further inhibit tumor lymphatic metastasis.

1. Introduction

There are two vital ways for cancer cells to disseminate: the blood pathway, leading to the invasion of distant organs such as liver, brain, bone, or lung, and the lymphatic pathway, leading to the invasion of the lymph nodes draining the organs where the tumor evolves [1]. For a long time, the study on tumor metastasis has been centered on tumor angiogenesis and hematogenous metastasis [2]. In the recent years, tumor lymphangiogenesis catches the eyes of researchers [3]. One of the most popular views on cancerous lymphangiogenesis is that tumor forms new lymphatic vessels on the basis of the existing ones. Studies have demonstrated that tumor-induced lymphangiogenesis plays a significant role in tumor cells traffic and lymph node metastasis [4–6]. Further, lymphatic metastasis is a major and early step during tumor progression. The presence of lymphatic metastasis is a key determinant of cancer staging, treatment, and prognosis [7, 8]. Thus, antilymphangiogenesis is a new target for cancer therapy. However, there are no effective antilymphangiogenesis drugs in clinic until now.

It was reported that vascular endothelial growth factor-C3 (VEGF-C3) and VEGF-D were identified as the stimulators of the proliferation of lymphatic endothelial cells. Recent studies showed that basic fibroblast growth factor (bFGF) was another effective stimulator for lymphangiogenesis and could regulate lymphatic endothelial cell proliferation, migration, and tube formation as well [9–11]. bFGF is a heparin dependent growth factor and can activate the intracellular signal transduction pathways only in the form of bFGF-heparin-FGFR terpolymers structure [12]. bFGF is bound to heparan sulfate (HS) in the extracellular matrix (ECM) and is released

in an active form when the ECM-HS is degraded by heparanase expressed in normal and malignant cells [13, 14].

We previously isolated a novel polysaccharide, Gekko Sulfated Glycopeptide (GSPP) from *Gekko swinhonis* Günther, and confirmed it as a homogeneous sulfated polysaccharide-protein complex with O-glycopeptide linkages. The molecular weight of GSPP was estimated to be over 2000 kDa [15]. Its direct effects on the proliferation, differentiation, and migration of hepatoma cells have been studied [15–17]. Our further study showed that GSPP had a similar structure with heparin and competed with heparin to disturb the bFGF-heparin-FGFR terpolymers forming, further blocking bFGF's biological effect. GSPP could inhibit tumor angiogenesis by reducing bFGF production, inhibiting the release of bFGF from the extracellular matrix, and disturbing the binding of bFGF to its low affinity receptor. By inhibiting bFGF-induced angiogenesis, GSPP significantly inhibited the growth of nude mice xenografted tumors [12].

In this study, whether GSPP could inhibit the lymphangiogenesis goaded our interests. Here, we investigated the potent antilymphangiogenesis ability of GSPP in vitro and in vivo and found that GSPP significantly inhibited bFGF-induced cell proliferation, migration, and tube formation in hLECs. And GSPP demonstrated an excellent antitumor effect through inhibiting lymphangiogenesis in vivo.

2. Materials and Methods

2.1. Cell Lines. hLECs were purchased from CHI Scientific Inc. (Jiangsu, China). The certificate analysis sheet supplied by CHI Scientific Inc. for each vial of cells indicated that more than 95% of the cells were hLECs (CD31 and podoplanin double positive). This was determined by Fluorescence Activating Cell Sorter (FACS). Cells were cultured in EGM-2 media according to the supplier's instructions (CHI Scientific Inc., Jiangsu, China). Cells before 6 generations were used in this study.

2.2. Antibodies and Reagents. GSPP used in this study was prepared in advance, which is the same batch with that in previous study [15]. The dried powder is stored in −80°C. MTS test kit was purchased from Promega Corporation (Madison, Wisconsin, USA). Fibronectin from human plasma was purchased from Sigma (St. Louis, MO, USA). Recombinant human FGF-basic (154 a.a.) was purchased from PeproTech Corporation (Rocky Hill, NJ, USA). Antibodies against phospho-Erk1/2 (p44/42 MAPK, lot: 9101S) and total-Erk1/2 (p44/42 MAPK, 137F5, lot: 4695) were obtained from Cell Signaling Technology (Danvers, MA, USA). Antibody against LYVE-1 (lot: 33504-1) was bought from Abcam Corporation (Cambridge, MA, USA). Antibody against β-actin (lot: A2228) was bought from Sigma Corporation (St. Louis, MO, USA).

2.3. hLECs Proliferation Assay. 96-well plate was precoated with fibronectin for 20 minutes at 37°C in 5% humidified CO_2. 100 μL hLECs suspension (1×10^5 cells/mL) was seeded into each well of a 96-well plate. After 24 h, the medium was discarded and replaced with drug-containing medium. 6 groups were set up as GSPP 10 μg/mL, GSPP 100 μg/mL, bFGF 10 ng/mL, GSPP 10 μg/mL with bFGF 10 ng/mL, GSPP 100 μg/mL with bFGF 10 ng/mL, and negative control group. After cells were exposed to the drugs for indicated times (0, 1, 2, 3, 4, 5, and 6 d), 20 μL MTS solution reagent was added into each well and incubated at 37°C for 1–4 h and then the OD value was measured with a Microplate reader (iMark, Bio-Rad) at 490 nm. The media were not changed during the treatment period.

2.4. Cell Cycle Detection. A flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA) was used to evaluate cell cycle distribution. hLECs (5×10^5 cells/2 mL) were seeded into 6-well plates and treated with GSPP (10 μg/mL, 100 μg/mL) alone or combined with bFGF 10 ng/mL for 48 h. Cells were collected and washed twice in cold PBS, fixed in 70% methanol (−20°C) overnight. Then, cells were washed with PBS twice again and incubated with RNase (20 μg/mL) in 37°C for 1 h. Propidium iodide (50 μg/mL) was added before being detected by flow cytometry system.

2.5. Wound Healing Experiment. hLECs were seeded in 24-well plates at the density of 1×10^5 cells/mL. After cell attachment, hLECs were starved with serum-free EBM-2 for 24 h. A linear wound about 1 mm in width was made by scratching the monolayer cell culture with a pipette tip after cell confluency. Then, EBM-2 with different concentrations of GSPP (10 μg/mL, GSPP 100 μg/mL) and/or bFGF (10 ng/mL) with 15% FBS were added. After 0 and 6 h, the photographs of wound healing width of hLECs were observed and taken under an invert microscope. The migration width was measured by the Photoshop software. The migration ratio was calculated as the migration width of experiment group/the migration width of control group.

2.6. Transwell Experiment. After being starved with serum-free medium for 24 h, hLECs (5×10^4 cells) in EBM-2 media with different concentrations of GSPP (10 μg/mL, GSPP 100 μg/mL) were added to the upper chambers of the transwell insert (BD Biosciences, Bedford, MA). EBM-2 containing bFGF was added to the lower chamber to induce cell migration. After being incubated for 12 h at 37°C, cells on the top surface of the membranes were wiped off with cotton balls, and the cells that migrated on the underside of inserts were fixed with methanol and stained with crystal violet. Five different digital images were taken per well, and the numbers of migrated cells were counted and calculated.

2.7. Tube Formation Assay. Matrigel was thawed at 4°C overnight. 96-well plate and 100 μL pipette tips were also kept at 4°C overnight and both the plate and tips were placed on ice during the entire experiment process. 30 uL Matrigel was loaded in each well of the 96-well plates and the plate was incubated at 37°C in a tissue culture incubator for 30 min to allow the matrix to polymerize. Trypsinized LECs were adjusted to the appropriate cell density (1.5×10^4 cells/well) with different concentration of GSPP and bFGF as described in the proliferation assay. 100 μL hLECs suspension was added on top of the gel in the 96-well plate. The plate was then

incubated at 37°C in a tissue culture incubator and the formation of the capillary-like tubes was observed after 4 h. Then, hLECs were observed under inverted microscope and 9 photographs (×40) were taken per hole. The numbers of matrix form of closed irregular polygon were recorded and calculated.

2.8. Western Blot Experiment. For western blot analysis of Erk and p-Erk protein expression, confluent cultures of hLECs in 6-hole pate were homogenized in lysis buffer. The protein concentrations were determined using the BCA Protein Quantitation Kit. Equal amounts of lysate protein were subjected to 10% SDS-polyacrylamide gel electrophoresis. The proteins were transferred onto PVDF membranes for immunoblot analysis. Blocking was performed with 5% nonfat dry milk in 0.1% Tween 20 in TBS, followed by immunoblotting with a polyclonal goat anti-human Erk antibody, p-Erk antibody, and β-actin antibody. Specific binding was detected by the ECL plus Western Blotting Detection System.

2.9. In Vivo Nude Mice Model. This study was approved by the Tianjin Cancer Institutional Animal ethics Committee (number 2014044). Animal care and experimental procedures followed the Tianjin Medical University guidelines for the care and use of laboratory animals. 4–6-week-old male BALB/c mice were purchased from LianHe LiHua cooperation. To establish a heterotopic colon carcinoma nude mice model, 2.5×10^6 HT-29 cells in 200 μL PBS were injected into the right flank of BALB/c mice. When tumors grew to 10 mm diameter size, tumors were cut off and cut into 1 mm × 1 mm chips and then transplanted to other 24 BALB/c mice, which were divided into four groups of 8 mice each group. Mice were treated daily with an intraperitoneal injection of either 0.1 mL GSPP (20, 200 mg/kg/day in PBS) or PBS (control) for 21 days. The length and width of the tumors were measured with a caliper every 2-3 days. All mice were sacrificed 24 days after tumor inoculation and the tumors were excised and weighted and the tumor volumes were calculated using the standard formula $V = ab^2/2$ (a, lengths of the tumors; b, widths of the tumors).

2.10. Immunohistochemistry Staining. Tumor specimens were immediately removed from sacrificed mice and prepared for immunohistological examination. Tumors were fixed in 10% (v/v) neutral buffered formalin overnight, embedded in paraffin and sectioned to a 5 μm thickness. Tumor sections were deparaffinized via immersion in xylene, dehydrated in a graded series of ethanol, and washed with distilled water. Thereafter, tumor sections were boiled in 10 mM sodium citrate buffer (pH = 6.0) for 10 min and cooled at room temperature. To inhibit endogenous peroxidase activity, tumor sections were incubated with methanol containing 1% (v/v) hydrogen peroxide for 10 min. Tumor sections were then blocked with 1% BSA and then incubated overnight with anti-LYVE-1 antibody; tumor sections were probed with peroxidase-conjugated secondary antibodies and incubated with DAB until the desired stain intensity developed. After counterstaining with Harris hematoxylin, tumor sections were examined under an inverted microscope (E100; Nikon, Japan). To analyze immunohistochemical signals within the specimens, all tumor sections were digitized under a ×40 magnification and images were captured.

2.11. Statistical Analysis. The data were expressed as the mean ± SD from triplicate experiments and were analyzed using SPSS software (Version 20.0; Chicago, USA). One-way ANOVA and two-way ANOVA were applied to analyze the significance of groups with one factor and two factors, respectively. P value <0.05 was considered statistically significant. All of the experiments were repeated at least three times.

3. Results

3.1. Morphological Characteristics of Lymphatic Endothelial Cells In Vitro Culture. After 3–5 h of primary cell culture, cells were able to grow adhering to the wall. After 1 d, endothelial cells spread forming groups and, after 1 week, cells grew densely and formed a single layer with the characteristic of "pebbles." Inverted microscopy observation: lymphatic endothelial cells were irregular ovoid with big nucleus and there were many small vacuoles in the cytoplasm (Figure 1(a)).

3.2. GSPP Inhibited bFGF-Induced Proliferation of hLECs. MTS assay was performed to investigate the antiproliferation effect of GSPP. After hLECs were exposed to GSPP (0, 10, and 100 μg/mL) and/or bFGF (10 ng/mL) for 0, 1, 2, 3, 4, 5, or 6 d, OD values were detected. The results demonstrated that bFGF promoted the proliferation of hLECs significantly ($P < 0.05$), while GSPP alone did not inhibit proliferation of hLECs ($P > 0.05$). Trypan Blue staining showed that the viability of hLECs was not affected by GSPP (results were not shown). But GSPP could abrogate bFGF-induced proliferation of hLECs significantly in a dose- and time-dependent manner ($P < 0.05$, Figure 1(b)). In the cell cycle assay, bFGF significantly promoted hLECs into proliferation cycle with a high proportion of S and G2 phase cells, while GSPP alone has no effect on the cell cycle distribution of hLECs. When combined with 100 μg/mL GSPP, cell proliferative activity was blocked distinctly with an increasing proportion of G1 phase cells compared with bFGF alone group (Figure 1(d)).

3.3. bFGF-Induced p-Erk Was Downregulated by GSPP in hLECs. Erk1/2 signal pathway is reported to be involved in cell growth, migration, and angiogenesis. The promoting effect of bFGF on vessel cell proliferation and migration may be partly associated with an increased level of Erk phosphorylation [18, 19]. To explore the possible mechanism of GSPP in inhibiting bFGF-induced lymphangiogenesis, Erk and p-Erk protein expressions were detected by western blotting. Results showed that bFGF significantly increased the expression of p-Erk in hLECs and GSPP decreased the bFGF-induced p-Erk significantly. No significant difference of the expression of total Erk was observed among each group (Figure 1(c)).

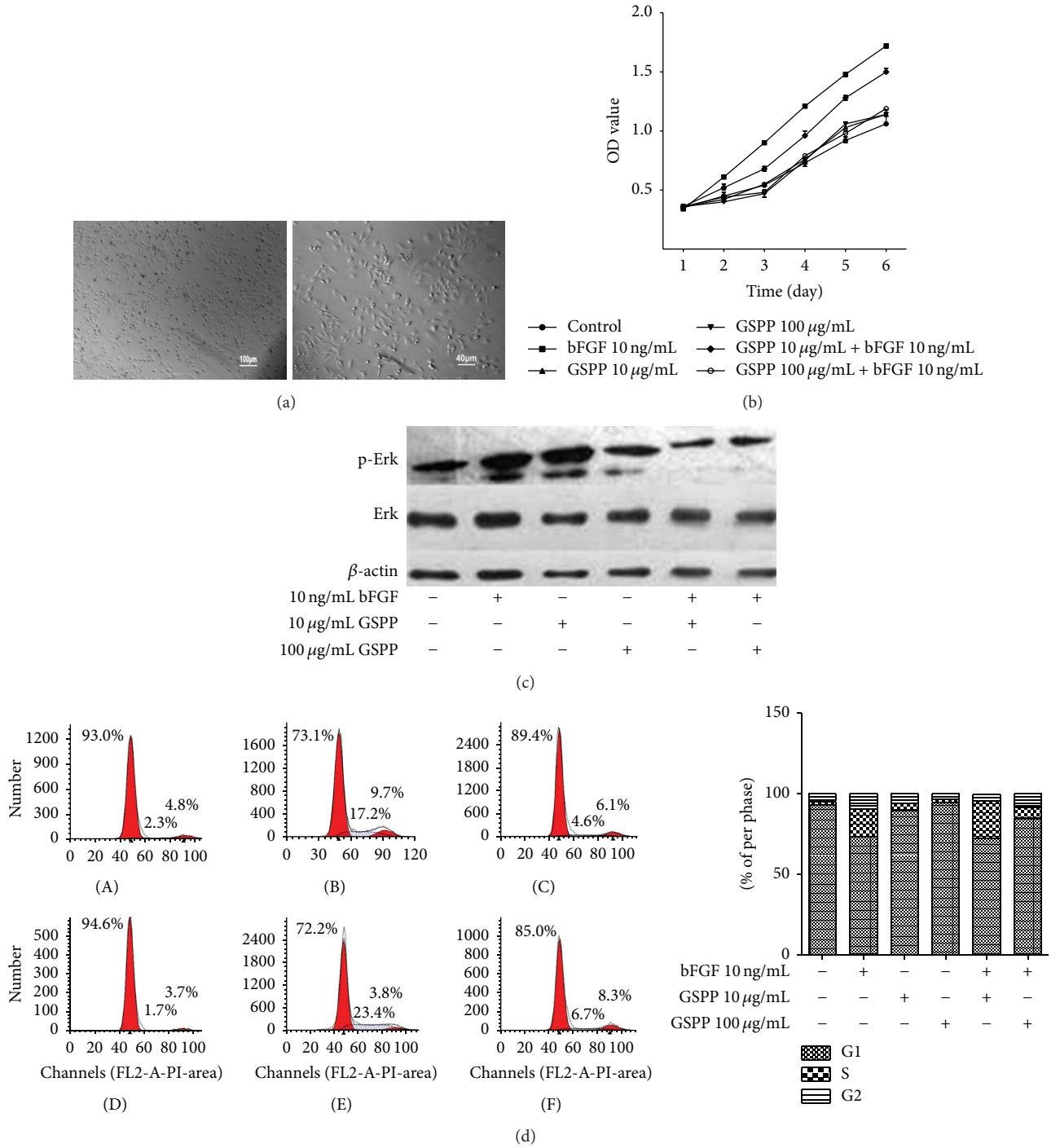

Figure 1: GSPP inhibited bFGF-induced proliferation of hLECs and Erk phosphorylation. (a) Morphological characteristics of hLECs in vitro culture hLECs were compressed ovoid, short fusiform, or polygon and formed a single layer with the characteristic of "pebbles." (b) hLECs growth curves (1×10^5 cells/mL) were incubated with different concentration of GSPP (0, 10, and 100 μg/mL) and/or bFGF (10 ng/mL) for 0, 1, 2, 3, 4, 5, and 6 d, then cell proliferation was quantified by MTS assay, and cell growth curve was made. (c) The changes of Erk and p-Erk protein expression level of hLECs cultured in 6-well plate were incubated with different concentration of GSPP (0, 10, and 100 μg/mL) and/or bFGF (10 ng/mL); Erk and p-Erk protein levels were monitored by western blot analysis of whole-cell lysates. (d) Cell cycle analysis. Left, histogram of cell cycle distribution. Right, statistical analysis of cell cycle percentage. After exposure to GSPP (0, 10, and 100 μg/mL) and/or bFGF (10 ng/mL) for 48 h, cell cycle distribution was determined by propidium iodide labeling. (A) Control, (B) bFGF 10 ng/mL, (C) GSPP 10 μg/mL, (D) GSPP 100 μg/mL, (E) GSPP 10 μg/mL + bFGF 10 ng/mL, and (F) GSPP 100 μg/mL + bFGF 10 ng/mL. Data were presented as mean ± SD of three independent experiments. hLECs, human lymphatic endothelial cells; bFGF, basic fibroblast growth factor; GSPP, Gekko Sulfated Glycopeptide.

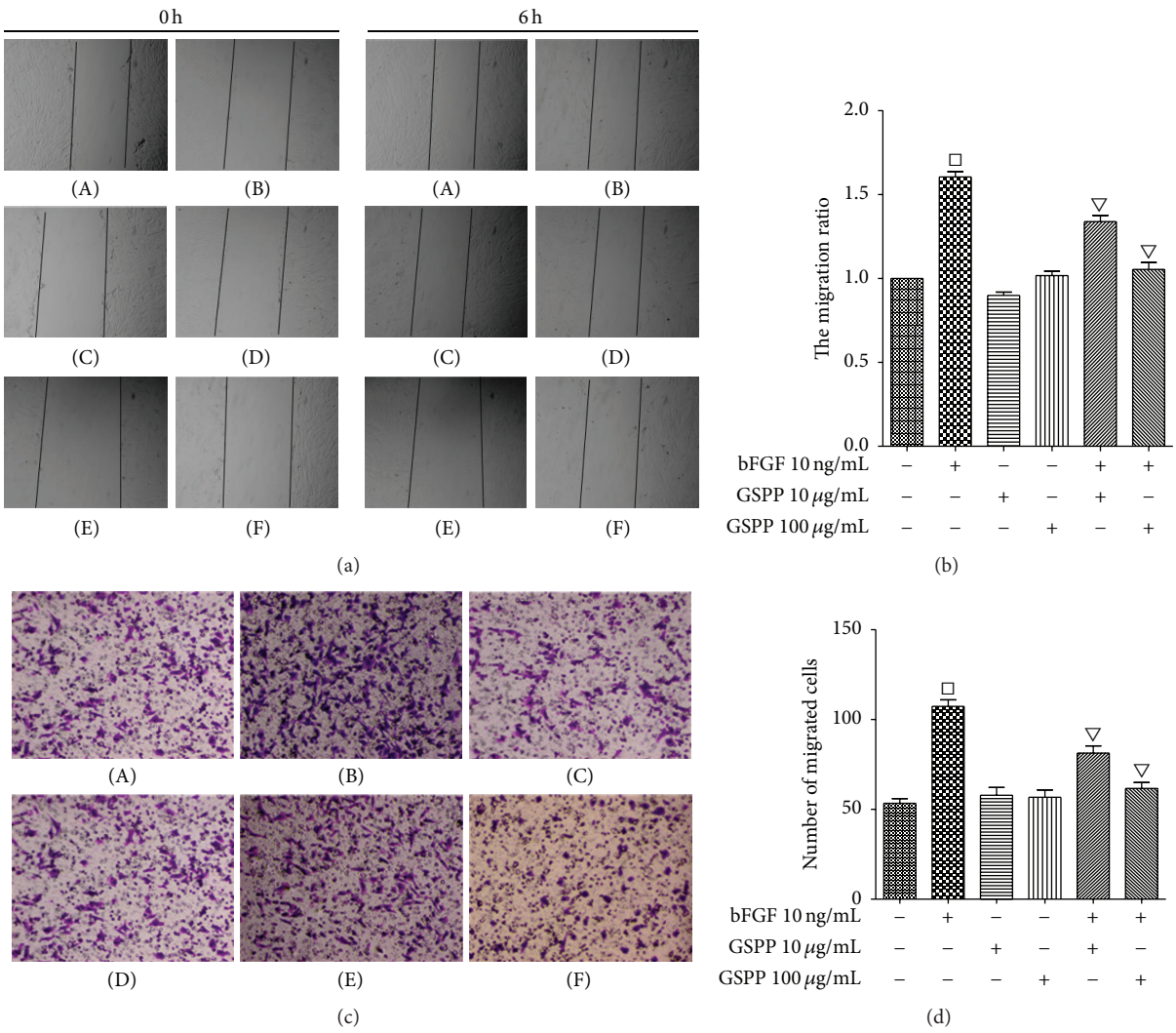

Figure 2: GSPP inhibits bFGF-induced migration of hLECs. (a) Wound healing assay. Left, representative images of injury width in wound healing assay (×40). Right, quantification of the migration ratio of hLECs compared to control. hLECs (1×10^5 cells/well) were seeded in 24-well plates and wounds were generated after cell confluence. After hLECs were treated with different concentrations of GSPP (0, 10, and 100 μg/mL) and/or bFGF (10 ng/mL) for 0 h and 6 h, the photos were taken and the injury width was measured. The migration ratio was calculated as the migration width of experiment group/the migration width of control group. (b) Transwell assays. Left, representative images of migrated LECs in transwell assay (×40). Right, quantification of migrated LECs compared to control. hLECs (5×10^4 cells/well) in EBM-2 with different concentrations of GSPP (0, 10, and 100 μg/mL) were added to the upper chamber of the transwell insert. EBM-2 containing bFGF (10 ng/mL) or not was added to the lower chamber to induce cell migration. After 12 h at 37°C, cells on the top surface of the membranes were wiped off with cotton balls, and the cells that migrated on the underside of inserts were fixed with methanol and stained with crystal violet. Five different digital images were taken per well, and the number of migrated cells was counted. (A) Control, (B) bFGF 10 ng/mL, (C) GSPP 10 μg/mL, (D) GSPP 100 μg/mL, (E) GSPP 10 μg/mL + bFGF 10 ng/mL, and (F) GSPP 100 μg/mL + bFGF 10 ng/mL. Data were presented as mean ± SD of three independent experiments; $^{\square}P < 0.05$ versus control group and $^{\triangledown}P < 0.05$ versus bFGF-single use group.

3.4. GSPP Inhibited bFGF-Induced Migration of hLECs. Wound healing experiment and transwell experiment were conducted to investigate the antimigration effect of GSPP. It was showed that bFGF significantly upregulated the migration distance of hLECs ($P < 0.05$), which was antagonized by GSPP significantly ($P < 0.05$) at 6 h (Figure 2(a)). The same results were obtained in transwell experiment. The number of migrated cells in bFGF-treated group increased significantly compared to the negative control cells ($P < 0.05$). No significant difference of the cell numbers was observed between the GSPP-treated group and negative control ($P > 0.05$). However, concomitant treatment with 10 μg/mL, 100 μg/mL GSPP, and bFGF inhibited the migration of hLECs compared with the bFGF-single use group ($P < 0.05$) (Figure 2(b)).

3.5. GSPP Abrogated bFGF-Induced hLECs Lymphangiogenesis In Vitro. Tube-like formation assay was conducted to examine the inhibitory effect of GSPP on lymphangiogenesis. hLECs were added on top of the gel in the 96-well plate, incubated at 37°C in a tissue culture incubator, and the formation

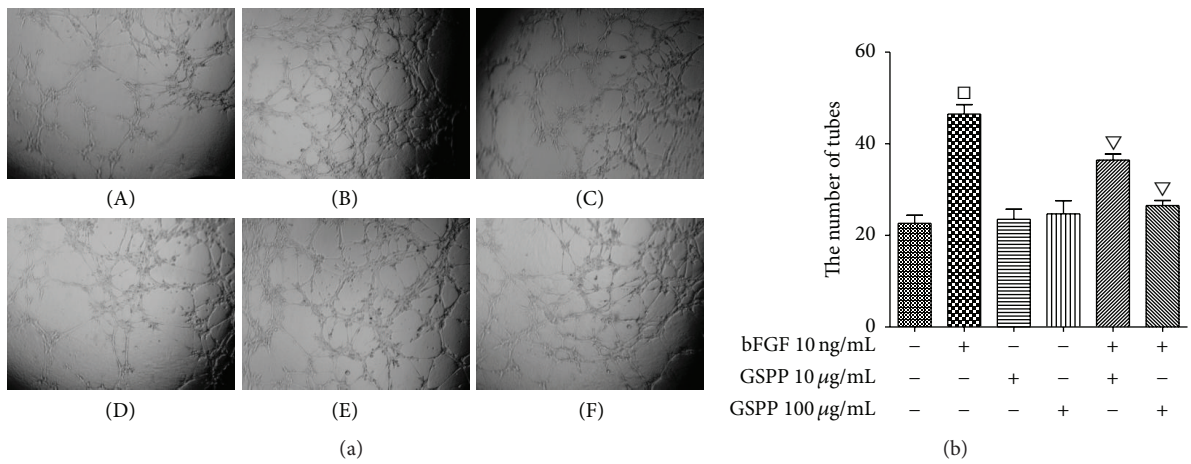

Figure 3: GSPP inhibits bFGF-induced lymphangiogenesis in vitro. In vitro tube formation assay. hLECs (1.5×10^4 cells/well) were seeded in Matrigel-coated 96-well plates and treated with different concentration of GSPP (0, 10, and 100 μg/mL) and/or bFGF (10 ng/mL) for 4 h and the tube-like structure formation was observed. (a) Representative images of tube formation (×40). (b) Quantification of inhibitory ratios of tube branches. (A) Control, (B) bFGF 10 ng/mL, (C) GSPP 10 μg/mL, (D) GSPP 100 μg/mL, (E) GSPP 10 μg/mL + bFGF 10 ng/mL, and (F) GSPP 100 μg/mL + bFGF 10 ng/mL. Data were presented as mean ± SD of three independent experiments; $^{\square}P < 0.05$ versus control group and $^{\triangledown}P < 0.05$ versus bFGF-single use group.

of the capillary-like tubes was observed after 4 h. There was no difference in the number of tube-like structures formation between GSPP treatment group and the negative control group in hLECs ($P > 0.05$). The number of tube-like structures treated with bFGF group was much more than the negative control group ($P < 0.05$). However, GSPP significantly attenuated bFGF-induced tube formation in a dose-dependent manner with simultaneous incubation with GSPP and bFGF (Figures 3(a) and 3(b)).

3.6. GSPP Inhibited Tumor Growth and Lymphangiogenesis in HT-29 Colon Carcinoma Nude Mice Model. The antitumor activity of GSPP was investigated in HT-29 colon carcinoma xenograft model using BALB/c nude mice. Growth of the tumors was significantly inhibited in the mice treated with GSPP compared with the growth of tumors in control mice (Figure 4(a)). In control group (intraperitoneal injection with PBS), tumors grew rapidly and reached an average volume of 459.03 ± 28.92 mm^3 (mean ± SD) by day 24 after being transplanted with HT-29 tumor blocks, while the sizes of tumors in 20 and 200 mg/kg/day GSPP-treated groups were only 348.25 ± 62.2 mm^3 and 255.18 ± 60.72 mm^3, respectively (75.9% and 55.9% decrease) ($P < 0.01$, Figure 4(b)). The tumor weight of the control group was 0.28 ± 0.03 g, whereas the weights of GSPP-treated groups decreased to 0.22 ± 0.04 g and 0.14 ± 0.03 g, respectively ($P < 0.01$, Figure 4(c)). To evaluate the adverse effects of GSPP, we measured the weights and visceral index of the mice and found that there was no significant difference between the control and GSPP-treated groups (Figure 4(d)). Tumor lymphangiogenesis was analyzed using immunohistochemical staining with LYVE-1 antibody. Results showed that 200 mg/kg/day GSPP markedly reduced tumor microvessel density in the tissue sections compared with the control group (Figure 4(e)). These results indicated that GSPP efficiently inhibits tumor growth in carcinoma animal model. Suppression of tumor growth due to GSPP could be caused by inhibition of lymphangiogenesis.

4. Discussion

Metastasis is a key cause for the failure of tumor treatment and patient death. The lymph node metastasis is the first step of the tumor dissemination and is also the main sign of poor prognosis of tumor [20–22]. More and more studies showed that the tumor-induced lymphangiogenesis played an extremely important role in cancer cells spreading to some local lymph nodes and distant metastasis [23–25]. Studies on antitumor lymphangiogenesis have gradually become a research hotspot, and bFGF as an important factor to promote lymphangiogenesis has been researched intensively.

Gekko swinhonis Günther was a traditional Chinese medicine, which has been used as an anticancer drug in traditional Chinese medicine for hundreds of years, especially in hepatoma [26]. Soaking in alcohol is very useful for pharmaceutical ingredients dissolving out and is also the most common method of extraction. Oral administration and external use were the main methods of administration. In our previous study, we isolated GSPP from *Gekko swinhonis* Günther and confirmed that GSPP could induce hepatoma cell differentiation, inhibit cell proliferation and migration in vitro, and inhibit hepatic carcinoma growth in vivo [12, 15, 17]. In this study, we verified another mechanism of GSPP's antitumor effects by inhibiting tumor lymphangiogenesis. Results showed that GSPP significantly inhibited bFGF-induced human hLECs proliferation, migration, and tube-like structure formation in vitro. Moreover, GSPP treatment (200 mg/kg/d) not only inhibited the growth of breast carcinoma, but also inhibited the lymphangiogenesis in vivo. The dosage was determined by previous studies, which is a safe dose with an appropriate tumor inhibition rate [12]. Through

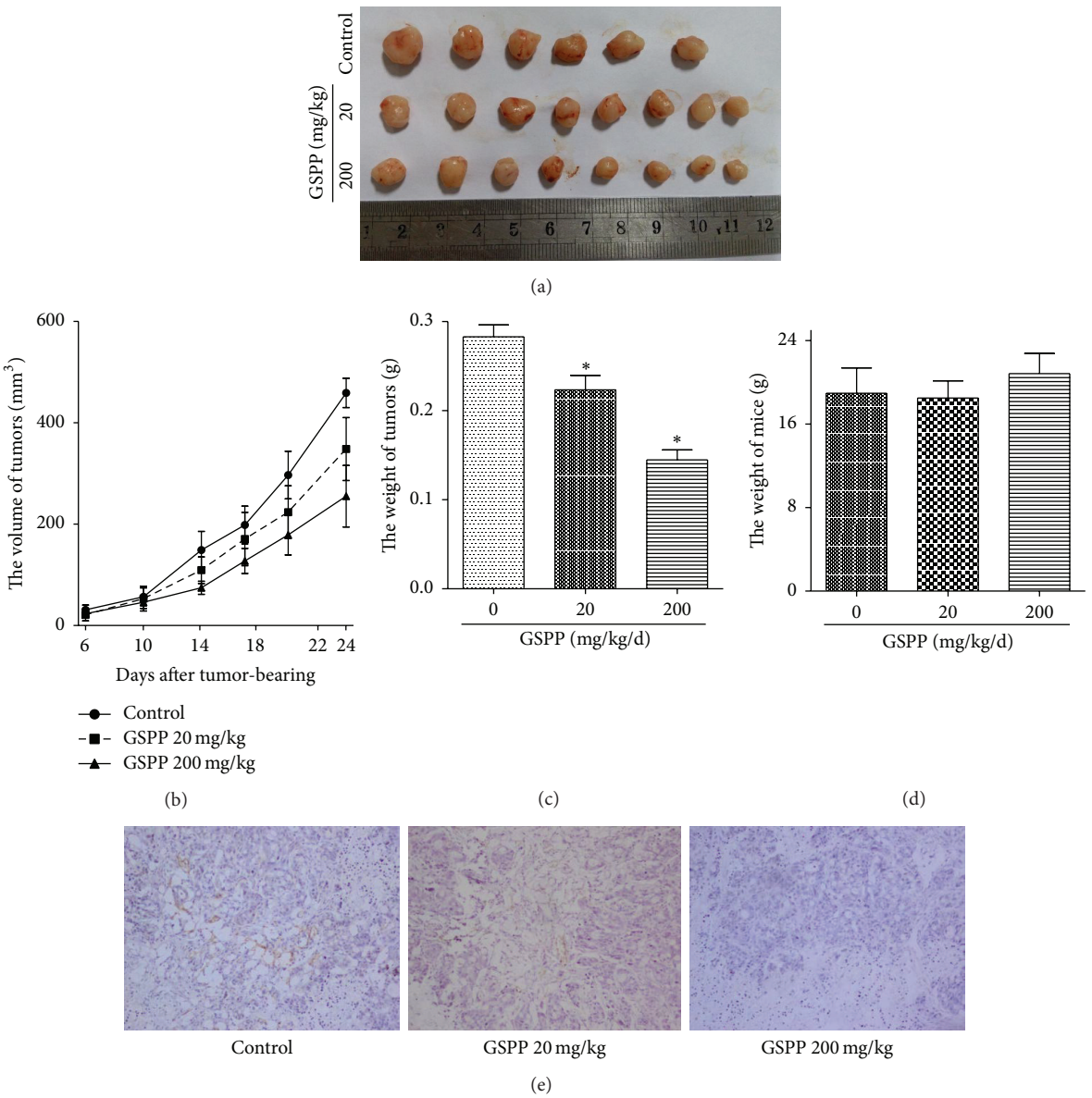

Figure 4: GSPP inhibited colon carcinoma HT-29 xenograft growth and lymphangiogenesis in vivo. Male nu/nu nude mice were inoculated subcutaneously with colon carcinoma HT-29 cells. Three days after inoculation, mice were treated with GSPP (20 or 200 mg/kg) or PBS every day for 21 days via intraperitoneal injection. The lengths and widths of tumors were measured individually every 3 days. At the end of the experiment, the implanted tumors were sectioned. (a) Effect of GSPP on tumor volume. Left, image of excised tumors. Right, tumor growth curves. (b) Effect of GSPP on tumor weight. (c) Effect of GSPP on mouse weight. Mice were weighed at the end of the experiment. (d) Tumor lymphatic microvessel density. The implanted tumors were sectioned and stained against LYNE-1 antibody. Tumor lymphatic vessels are shown as LYNE-1 positive (yellow color). Data were presented as mean ± SD of three independent experiments; $^{*}P < 0.05$ versus control group.

this study, we further uncovered the mechanisms of GSPP's antitumor effects besides inhibiting cancer cells proliferation and migration, inducing tumor cell differentiation, and inhibiting cancer angiogenesis and cancer-associated fibroblast growth (unpublished), showing that GSPP is a promising antitumor drug in future cancer treatment.

bFGF, as an important prolymphangiogenesis factor generated by tumor cells, can significantly promote lymphatic vessel endothelial cell proliferation and migration and promote tumor lymphangiogenesis by a variety of ways [27]. The most classic way on which bFGF promotes tumor lymphangiogenesis is through VEGF-A, VEGF-C, and VEGF-D, which are known prolymphangiogenesis factors to promote tumor lymphatic vessel grow [23, 28]. bFGF is a heparin dependent growth factor, which means that it can have effect only in the form of bFGF-heparin-FGFR terpolymers structure to further activate the intracellular signal transduction pathways. GSPP is a kind of polysaccharide sulfate, which

is similar to the structure of heparin active site, both of which contain a sulfuric acid base. Previous study showed that GSPP works by three distinct mechanisms: (a) blocking the bFGF production, (b) inhibiting the release of bFGF from the extracellular matrix, and (c) directly binding to bFGF and competitively inhibiting the binding of bFGF to its low affinity receptor heparin/HS [12]. In this study, we found that GSPP alone has no effect on the viability, growth of hLECs. And it does not inhibit the migration and tube formation of hLECs, which may be due to the absence of bFGF. bFGF plays an important role in the growth, migration, and lymphangiogenesis of hLECs. With the addition of exogenous bFGF, which simulates the environment in the body, GSPP significantly inhibited bFGF-induced cell proliferation, migration, and tube formation. This means that GSPP works through directly binding to bFGF and competitively inhibiting the binding of bFGF to its low affinity receptor heparin/HS.

Extracellular signal-regulated kinase1/2 (Erk1/2) is a protein kinase, separated and identified in the early 1990s, and its signal transduction is involved in cell growth, development, and differentiation [19]. Studies found that the promoting effect of bFGF on endothelial cell proliferation and migration of part is associated with an increased level of Erk phosphorylation [18, 29]. To explore the possible mechanism of GSPP's inhibiting effect of bFGF-induced lymphangiogenesis, western blot was performed. We examined the Erk and p-Erk expression level changes in lymphatic endothelial cells after being exposed to GSPP for a certain time. Our results showed that bFGF significantly increased the expression of p-Erk in hLECs. However, cotreatment with GSPP and bFGF decreased the expression of p-Erk in hLECs compared with the bFGF-single use group. This further proved that GSPP could bind to bFGF and competitively inhibit the binding of bFGF to its low affinity receptor, thus blocking the bFGF-induced phosphorylation of Erk1/2 in hLECs.

5. Conclusions

Our study showed that inhibiting bFGF-induced lymphangiogenesis was one of GSPP's anticancer mechanisms. GSPP, as an effective bFGF-targeted inhibitor, can be a notable antilymphatic metastasis drug in future cancer treatment.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Xiu-Li Ding and Ya-Nan Man contributed equally to this work.

Acknowledgments

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References

[1] J. Robert, "Biology of cancer metastasis," *Bulletin du Cancer*, vol. 100, no. 4, pp. 333–342, 2013.

[2] R. Bicknell, "Vascular targeting and the inhibition of angiogenesis," *Annals of Oncology*, vol. 5, supplement 4, pp. S45–S50, 1994.

[3] D. Ribatti, "History of research on angiogenesis," *Chemical Immunology and Allergy*, vol. 99, pp. 1–14, 2014.

[4] R. Shayan, M. G. Achen, and S. A. Stacker, "Lymphatic vessels in cancer metastasis: bridging the gaps," *Carcinogenesis*, vol. 27, no. 9, pp. 1729–1738, 2006.

[5] J. P. Sleeman and W. Thiele, "Tumor metastasis and the lymphatic vasculature," *International Journal of Cancer*, vol. 125, no. 12, pp. 2747–2756, 2009.

[6] R. L. Ferris, M. T. Lotze, S. P. L. Leong, D. S. B. Hoon, and D. L. Morton, "Lymphatics, lymph nodes and the immune system: barriers and gateways for cancer spread," *Clinical and Experimental Metastasis*, vol. 29, no. 7, pp. 729–736, 2012.

[7] S. S. Sundar and T. S. Ganesan, "Role of lymphangiogenesis in cancer," *Journal of Clinical Oncology*, vol. 25, no. 27, pp. 4298–4307, 2007.

[8] M. G. Achen, B. K. McColl, and S. A. Stacker, "Focus on lymphangiogenesis in tumor metastasis," *Cancer Cell*, vol. 7, no. 2, pp. 121–127, 2005.

[9] R. H. Adams and K. Alitalo, "Molecular regulation of angiogenesis and lymphangiogenesis," *Nature Reviews Molecular Cell Biology*, vol. 8, no. 6, pp. 464–478, 2007.

[10] P. M. Linares and J. P. Gisbert, "Role of growth factors in the development of lymphangiogenesis driven by inflammatory bowel disease: a review," *Inflammatory Bowel Diseases*, vol. 17, no. 8, pp. 1814–1821, 2011.

[11] L. K. Chang, G. Garcia-Cardeña, F. Farnebo et al., "Dose-dependent response of FGF-2 for lymphangiogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 32, pp. 11658–11663, 2004.

[12] S.-X. Zhang, C. Zhu, Y. Ba et al., "Gekko-sulfated glycopeptide inhibits tumor angiogenesis by targeting basic fibroblast growth factor," *The Journal of Biological Chemistry*, vol. 287, no. 16, pp. 13206–13215, 2012.

[13] M. Benezra, R. Ishai-Michaeli, S. A. Ben-Sasson, and I. Vlodavsky, "Structure-activity relationships of heparin-mimicking compounds in induction of bFGF release from extracellular matrix and inhibition of smooth muscle cell proliferation and heparanase activity," *Journal of Cellular Physiology*, vol. 192, no. 3, pp. 276–285, 2002.

[14] M. Fannon, K. E. Forsten, and M. A. Nugent, "Potentiation and inhibition of bFGF binding by heparin: a model for regulation of cellular response," *Biochemistry*, vol. 39, no. 6, pp. 1434–1445, 2000.

[15] D. Chen, W.-J. Yao, X.-L. Zhang et al., "Effects of Gekko sulfated polysaccharide-protein complex on human hepatoma SMMC-7721 cells: inhibition of proliferation and migration," *Journal of Ethnopharmacology*, vol. 127, no. 3, pp. 702–708, 2010.

[16] X. Wu, D. Chen, and G.-R. Xie, "Effects of Gekko sulfated polysaccharide on the proliferation and differentiation of hepatic cancer cell line," *Cell Biology International*, vol. 30, no. 8, pp. 659–664, 2006.

[17] X.-Z. Wu, D. Chen, and X.-Q. Han, "Anti-migration effects of Gekko sulfated glycopeptide on human hepatoma SMMC-7721 cells," *Molecules*, vol. 16, no. 6, pp. 4958–4970, 2011.

[18] L. Yang, J. Zhang, C. Wang et al., "Interaction between 8-hydroxyquinoline ruthenium(II) complexes and basic fibroblast growth factors (bFGF): inhibiting angiogenesis and tumor growth through ERK and AKT signaling pathways," *Metallomics*, vol. 6, no. 3, pp. 518–531, 2014.

[19] K. Balmanno and S. J. Cook, "Tumour cell survival signalling by the ERK1/2 pathway," *Cell Death and Differentiation*, vol. 16, no. 3, pp. 368–377, 2009.

[20] N. E. Tobler and M. Detmar, "Tumor and lymph node lymphangiogenesis—impact on cancer metastasis," *Journal of Leukocyte Biology*, vol. 80, no. 4, pp. 691–696, 2006.

[21] Y. Cao, "Opinion: emerging mechanisms of tumour lymphangiogenesis and lymphatic metastasis," *Nature Reviews Cancer*, vol. 5, no. 9, pp. 735–743, 2005.

[22] K. Alitalo, T. Tammela, and T. V. Petrova, "Lymphangiogenesis in development and human disease," *Nature*, vol. 438, no. 7070, pp. 946–953, 2005.

[23] M. Skobe, T. Hawighorst, D. G. Jackson et al., "Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis," *Nature Medicine*, vol. 7, no. 2, pp. 192–198, 2001.

[24] S. A. Stacker, C. Caesar, M. E. Baldwin et al., "VEGF-D promotes the metastatic spread of tumor cells via the lymphatics," *Nature Medicine*, vol. 7, no. 2, pp. 186–191, 2001.

[25] M. S. Pepper, "Lymphangiogenesis and tumor metastasis: myth or reality?" *Clinical Cancer Research*, vol. 7, no. 3, pp. 462–468, 2001.

[26] C. L. Yang and Y. J. Qi, *Animal Drug of Chinese Traditional Medicine*, Ancient Books Press of Chinese Traditional Medicine, Beijing, China, 1st edition, 2001.

[27] R. Cao, A. Eriksson, H. Kubo, K. Alitalo, Y. Cao, and J. Thyberg, "Comparative evaluation of FGF-2-, VEGF-A-, and VEGF-C-induced angiogenesis, lymphangiogenesis, vascular fenestrations, and permeability," *Circulation Research*, vol. 94, pp. 664–670, 2004.

[28] M. J. Karkkainen, R. E. Ferrell, E. C. Lawrence et al., "Missense mutations interfere with VEGFR-3 signalling in primary lymphoedema," *Nature Genetics*, vol. 25, no. 2, pp. 153–159, 2000.

[29] S. Guo, L. Yu, Y. Cheng et al., "PDGFRβ triggered by bFGF promotes the proliferation and migration of endothelial progenitor cells via p-ERK signalling," *Cell Biology International*, vol. 36, no. 10, pp. 945–950, 2012.

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Research Article

Proapoptotic Role of Potassium Ions in Liver Cells

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Potassium channels are transmembrane proteins that selectively promote the infiltration of potassium ions. The significance of these channels for tumor biology has become obvious. However, the effects of potassium ions on the tumor or normal cells have seldom been studied. To address this problem, we studied the biological effects of L02 and HepG2 cells with ectogenous potassium ions. Cell proliferation, cell cycle, and apoptosis rate were analyzed. Our results indicated that potassium ions inhibited proliferation of L02 and HepG2 cells and promoted their apoptosis. Potassium ions induced apoptosis through regulating Bcl-2 family members and depolarized the mitochondrial membrane, especially for HepG2 cell. These biological effects were associated with channel protein HERG. By facilitating expression of channel protein HERG, potassium ions may prevent it from being shunted to procancerous pathways by inducing apoptosis. These results demonstrated that potassium ions may be a key regulator of liver cell function. Thus, our findings suggest that potassium ions could inhibit tumorigenesis through inducing apoptosis of hepatoma cells by upregulating potassium ions transport channel proteins HERG and VDAC1.

1. Introduction

The plasma membrane (PM) ion channels involve almost all of the basic cellular processes and the malignant phenotype of tumor cells. Ion fluxes regulate cell volume and membrane potential through their ion channels and participate in intracellular signal transduction and controlling cell functions. Moreover, in the process of tumorigenesis development, the differences on tumor gene expression levels are determined by ion channels, which may involve, at least in part, a number of pathophysiological features associated with malignant growth [1–3].

In the ion transport molecular family, based on the biochemical structure and highest variability, potassium channels might be the most likely ones to be designed for the targeted therapy of the channel in cancer [4]. It could be used as a new research direction, providing important clues in the development of new therapeutic agents [5]. Thus, the study of ion channel serving as a new target for the diagnosis and treatment of cancer is very important. In this study, we compared the effect of potassium ions in L02 and HepG2 cells and investigated the regulation mechanism of cell functional changes induced by potassium ions.

The differential expressions of potassium channels are frequently observed in different tumors; these differences make tumors have many advantages in biological behaviors [6, 7]. Expression changes are seen in the genome, transcription, translation, or epigenetic level and can also adjust the expression level of potassium channel through the upstream changes in some cases [8, 9]. Some hormones or

growth factors can activate potassium channels and cause abnormal gene expressions of potassium channels [10]. The changes of cell death, proliferation, adhesion, and migration have a significant impact on life activities. All these changes can affect the tumorigenesis. Therefore, interruption of the expression of potassium channels combined with current treatment may significantly improve the treatment of cancer. In short, interfering with potassium channel expression or activity may offer a new therapy for liver cancer [4].

2. Materials and Methods

2.1. Preparation of Plates Coated with Potassium Ions. PBS with different concentrations of potassium ions was prepared and the abbreviations represent K 0 (0 mmol/L), K 25 (3.75 mmol/L), K 50 (7.5 mmol/L), K 75 (11.25 mmol/L), and K 100 (15 mmol/L). The dispersed PBS were added to 6-well plates (add 200 μL per well) or 96-well plates (add 10 μL per well) and then dried at 100°C for 2 hours in air, sterilized by ultraviolet irradiation for half an hour.

2.2. Cell Culture and Treatment. Hepatic cells line L02 cell and hepatoma cells line HepG2 cell were cocultured with different concentrations of potassium ions and cultured in DMEM supplemented with 10% FBS (Gibco, Carlsbad, CA, USA) in 5% CO_2 at 37°C.

2.3. Cell Proliferation and Viability. Cell counting kit was purchased from DOJINDO (EQ645; Kumamoto, Japan) to determine cell proliferation. To conduct the assay, L02 (3×10^3) and HepG2 (3×10^3) cells cultured in 96-well plates were treated using the above methods. 10 μL CCK-8 solutions were added to each well. Cells were incubated at 37°C incubator for 1 hr. A microplate reader (Thermo Scientific, Waltham, PA, USA) was used to detect the absorbance values at 450 nm. Each group consists of five replicates. The data was repeated in five independent experiments and the proliferation of cells was observed at different time points as indicated.

For cell counting, L02 (3×10^5) and HepG2 cells (3×10^5) were added to 6-well plates treated by the above methods with 2 mL of growth medium, and then the cells were observed according to the general protocol by optical microscope. The total cellular scores were counted by cell counter plate after 48 hrs.

2.4. Annexin V-FITC/7-AAD Staining Assay. L02 and HepG2 cells were treated with potassium ions as described in the above method in 6-well plates. The cells were then collected and washed twice with PBS. After centrifugation, cells were resuspended in 100 μL of binding buffer and then stained by Annexin V-FITC/7-AAD (640906/420404; BioLegend, CA, USA) for analysis of the apoptosis of cells.

2.5. Cells Cycle Analysis. L02 and HepG2 cells were cultivated in 6-well plates treated by the above methods and then cultured for 48 hrs. The cells were collected, fixed with 70% ethanol overnight and then resuspended in cold PBS, and then incubated with 1 mg/mL 7-AAD (420404; BioLegend, CA, USA) at 37°C for 15 min. The samples were detected by using FACScalibur flow cytometer (BD Biosciences, San Diego, CA, USA), and the proportions of cells in the G1, S, and G2 phases were investigated by using ModFit LTTM software (BD Biosciences, San Diego, USA).

2.6. Western Blotting and Antibodies. The total protein concentration was detected using the Pierce BCA assay (23225; Thermo Scientific, Waltham, PA, USA) and a microplate reader (Thermo Scientific, Waltham, PA, USA) at 562 nm. Cell lysates were resolved in 10% SDS-PAGE and transferred onto a PVDF membrane (ISEQ00010; Millipore, MA, USA). Target proteins were detected by using specific antibodies. The primary antibodies were used in a 1 : 1500 dilution. The bands were investigated using the enhanced chemiluminescence system (32209; Thermo Scientific, Waltham, PA, USA). All the data were quantified by using Bio-Profil BiolD software (S:11.640150; VILBER, France). The primary antibodies are anti-Bax (sc-493; Santa Cruz, TX, USA), anti-caspase-3 (sc-7148; Santa Cruz, TX, USA), anti-P53 (sc-126; Santa Cruz, TX, USA), anti-HERG (sc-48428; Santa Cruz, TX, USA), anti-VDAC1/Porin antibody (ab15895; Abcam, Cambridge, MA), anti-ACSS1 (ab69270; Abcam, Cambridge, MA), anti-Bcl-2 (2870; CST, Danvers, MA, USA), and anti-GAPDH (5174; CST, Danvers, MA, USA).

2.7. Mitochondrial Membrane Depolarization. JC-10 (Enzo Life Sciences, New York, USA) was used to detect the mitochondrial membrane depolarization of cells. The absorbing value of JC-10 lies on the polarization level of mitochondrial membrane. Hyperpolarized mitochondria was complicated with higher absorbent of JC-10 compared to depolarization. After treatment, L02 and HepG2 cells were washed once with PBS and then incubated with 500 μL of 1x JC-10 dye-loading solution for 40 minutes. After incubation, the cells were subjected to flow cytometry.

2.8. Caspase-3/7 Activity Assay. L02 and HepG2 cells were cultured in 96-well plates treated by the above methods and incubated for 48 hrs. After the treatment with potassium ion, caspase-3/7 activity in liver cells was analyzed using caspase-Glo 3/7 assay kit.

2.9. Statistics. All data were showed as mean ± SD, from at least three averaged replicates of independent experiments. Statistical comparison of quantitative data in the group was determined by one way ANOVA or Student's t-test. To determine differences between groups not normally distributed, medians were compared using Kruskal-Wallis analysis of variance. SPSS 19.0 software (SPSS Inc., Chicago, USA) was used for data analysis. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. The Potassium Ions Inhibited Cell Proliferation in L02 and HepG2 Cells. To examine the effects of potassium ions on cell proliferation, cells were treated with increasing

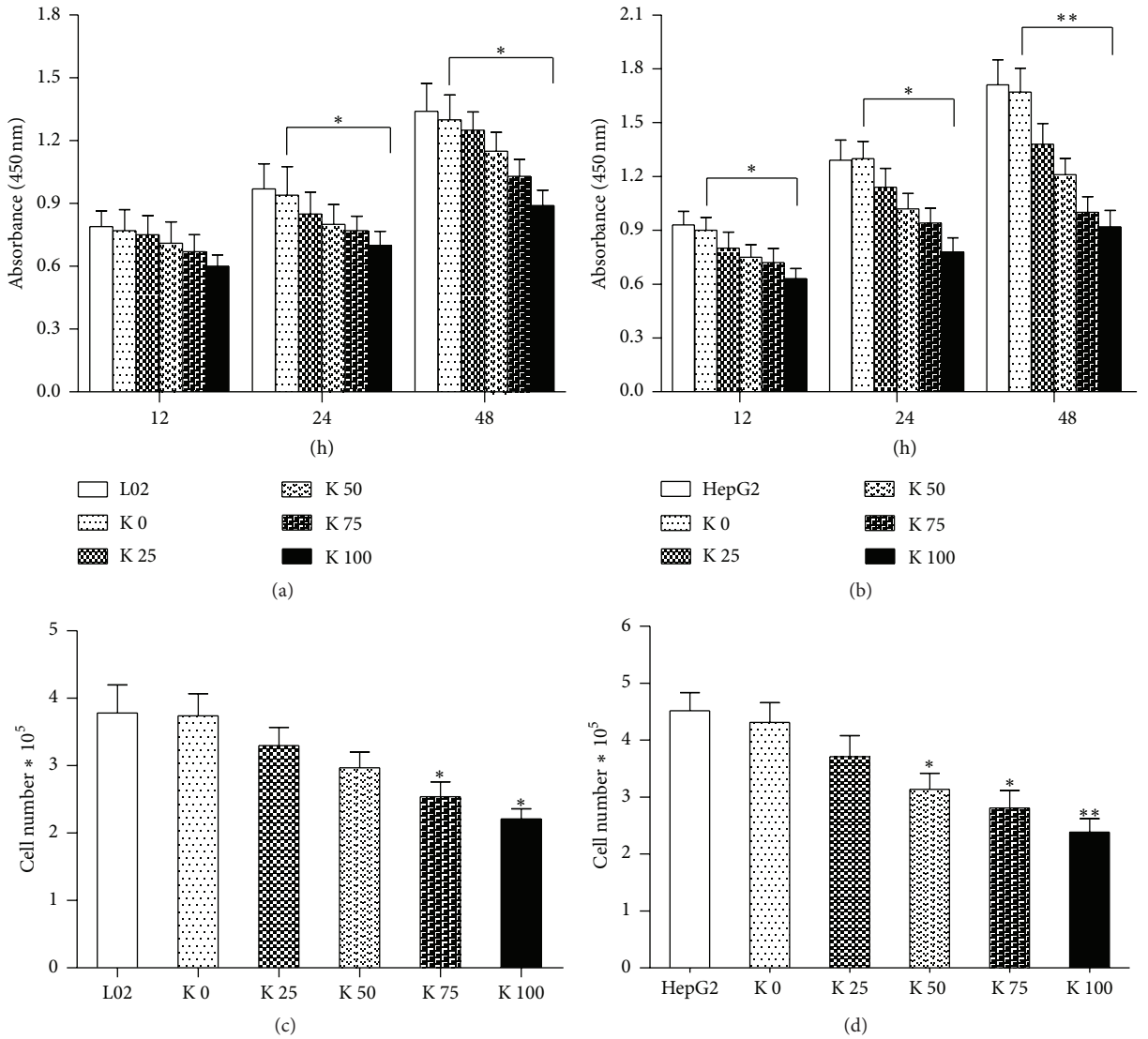


FIGURE 1: Potassium ions inhibited proliferation and growth of liver cells. L02 cells (3×10^3) and HepG2 cells (3×10^3) were added to 96-well plates cocultured with different concentrations of potassium ions and cultured at different time points (12, 24, and 48 hrs), respectively. We conduct the CCK-8 assay to assess how the potassium ions affected proliferation of L02 and HepG2 cells. (a) The absorbance of L02 cells decreased significantly at 24 hrs and 48 hrs ($P < 0.05$) after culture with potassium ions. (b) The absorbance value of HepG2 cells decreased significantly at 12 hrs and 24 hrs ($P < 0.05$) after being cultured with potassium ions and especially for 48 hrs ($P < 0.01$). L02 (3×10^5) and HepG2 cells (3×10^5) were added to 6-well plates cocultured with different concentrations of potassium ions and cultured for 48 hrs. (c) The cells number of L02 treated with potassium ions decreased after being cultured for 48 hrs ($P < 0.05$). (d) The cells number of HepG2 treated with potassium ions decreased significantly after culture for 48 hrs ($P < 0.01$). All data are represented as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$.

concentrations of potassium for indicated time points. By the CCK-8 assay, the results showed that potassium ions could inhibit the proliferation of L02 (Figure 1(a)) and HepG2 cells (Figure 1(b)), especially for HepG2 cells. The inhibition was both time and dose dependent. The proliferation of L02 cells cocultured with potassium ions decreased obviously after culture for 48 hrs ($P < 0.05$). The proliferation of HepG2 cells cocultured with potassium ions decreased especially at 48 hrs.

On the other hand, cell growth was quantified with total cell count. L02 and HepG2 cells were added to 6-well plates treated by the above methods and cultured for 48 hrs. As shown in Figure 1, the cell count for L02 (Figure 1(c)) and HepG2 (Figure 1(d)) was low with increasing concentration of potassium ions. The decreasing trend is more obvious for HepG2 than L02 cells.

3.2. Potassium Ions Affected the Cells Cycle of L02 and HepG2. To test the effects of potassium ions on the cell cycle of L02 and HepG2 cells, we conducted flow cytometry assay. The results indicated that the effects of potassium ions on cell cycle of L02 cells (Figure 2(a)) and HepG2 cells (Figure 2(b)) were dose dependent. The cell number of the S phase in L02 and HepG2 cells both decreased, while it increased significantly in G2/M phase. These results demonstrated that

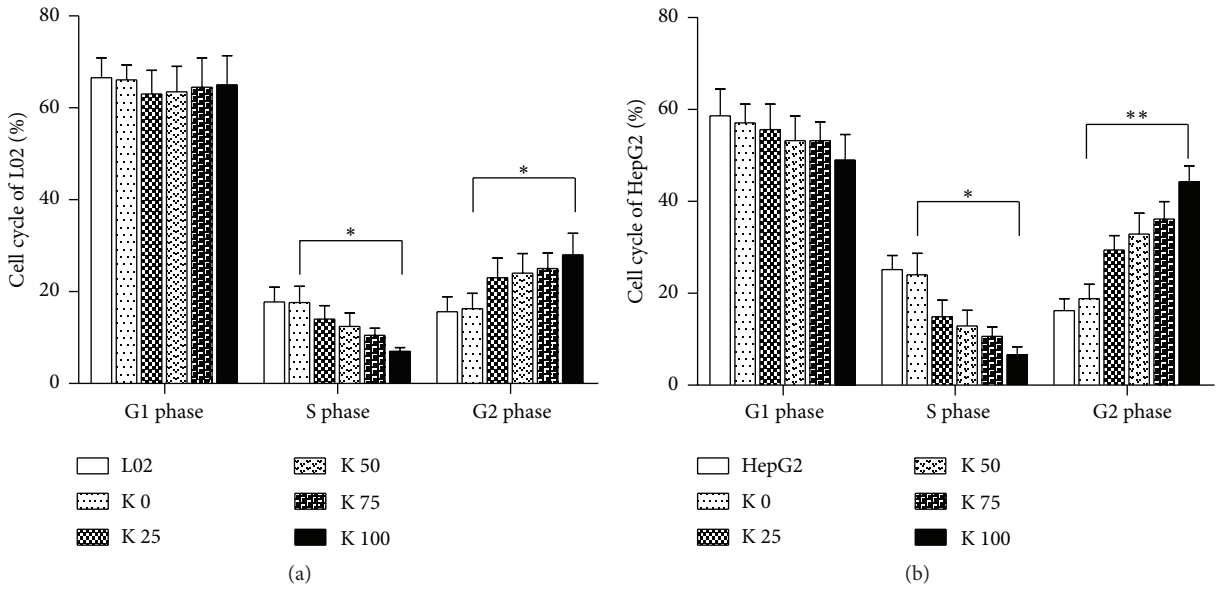


FIGURE 2: Potassium ions affected cell cycle of liver cells. L02 (3×10^5) and HepG2 (3×10^5) cells were added to 6-well plates cocultured with different concentrations of potassium ions for 48 hrs. Then the effects of potassium ions on cell cycle of L02 and HepG2 were determined by flow cytometry. (a) In L02 cells, potassium ions induced significantly decrease of S phase and increase of G2 phase in a dose-dependent manner ($P < 0.05$). (b) In HepG2 cells, potassium ions induced significantly decrease of S phase and increase of G1 phase in a dose-dependent manner ($P < 0.01$). All data are represented as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$.

potassium ions could arrest cell cycle at S phase and suppress the growth of L02 and HepG2 by preventing proper DNA replication. And the arrest is more significant for HepG2 than L02 cells.

3.3. Potassium Ion Induces Apoptosis of L02 and HepG2 Cells through Regulating Antiapoptotic Bcl-2 Family Members and Mitochondrial Proteins and Potassium Channel Related Protein. After being cocultured with potassium ions for 48 hrs, Annexin V-FITC/7-AAD staining was performed. And the numbers of Annexin V positive cells increased significantly. The results showed that the Annexin V positive cells of L02 (Figures 3(a) and 3(b)) and HepG2 (Figures 3(c) and 3(d)) both increased in a concentration dependence manner, especially for HepG2 cells.

As shown in Figure 4(a), the expression level of Bcl-2 downregulated in L02 cells, but Bax and caspase-3 upregulated. We could see the same trends in HepG2 cells (Figure 4(c)), but the trends are more obvious than those in L02 cell. Moreover, in K 100 group, the expression levels of Bax and caspase-3 downregulated. We determined the Bcl-2/Bax ratio in L02 (Figure 4(b)) and HepG2 cells (Figure 4(d)) at the protein level, which increased significantly in a concentration dependence manner, especially for HepG2 cells. They may be responsible for the apoptosis induced by potassium ions observed in L02 and HepG2 cells.

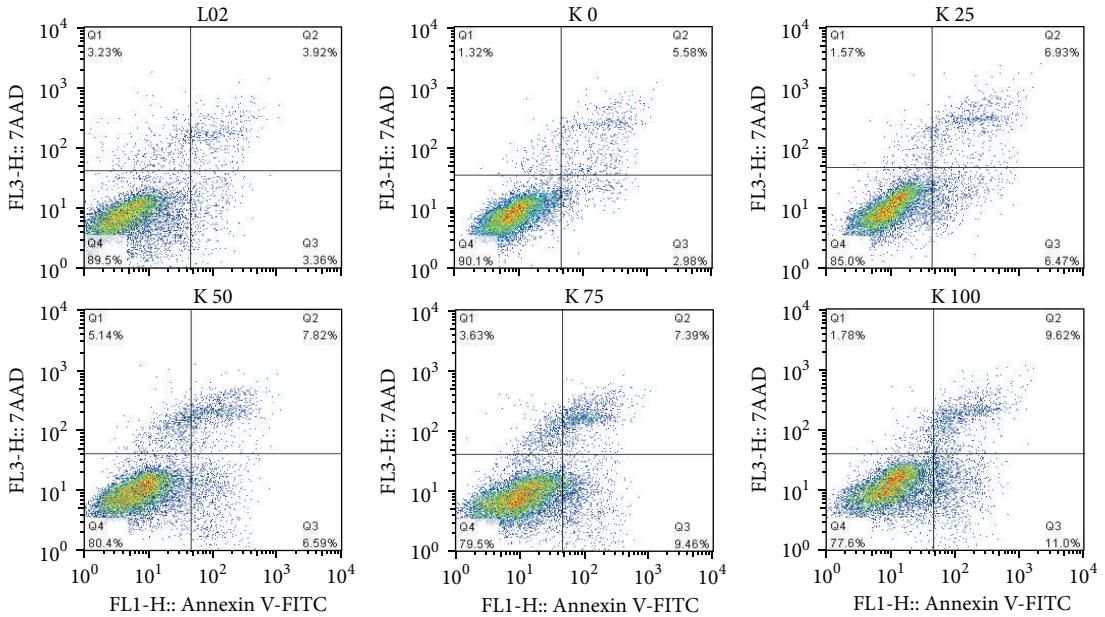
We performed caspase-3/7 activation detection to identify the apoptotic role of potassium ions on L02 and HepG2 cells. Potassium ions induced obvious activation of caspase-3/7 activity in L02 (Figure 5(a)) and HepG2 cells (Figure 5(b)). In summary, the findings demonstrated that potassium ions could reduce cell viability, promote cell apoptosis, and increase caspase-3/7 activity of L02 and HepG2 cells. This is more obvious in HepG2 cells than L02 cells.

In order to assess mitochondrial and potassium channel related genes expression changes, levels of mitochondria related proteins, VDAC1 and ACSS1, and potassium channel related protein HERG were evaluated after treatment of liver cells with potassium ions. As shown in Figures 4(a) and 4(c), the expression of ACSS1 downregulated in a dose-dependent manner, and the expression of VDAC1 and HERG upregulated, especially for HepG2 cells.

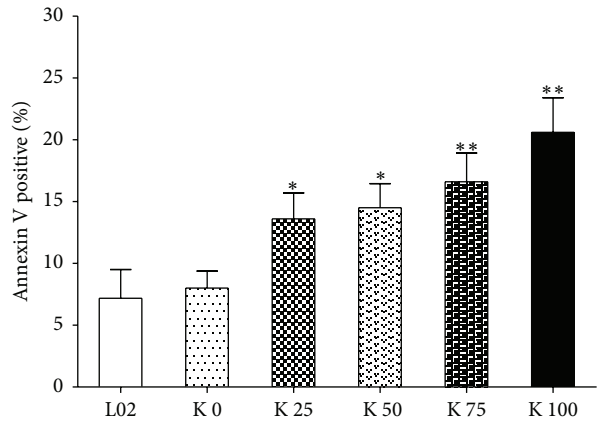
3.4. Potassium Ions Depolarized the Mitochondrial Membrane of L02 and HepG2 Cells. We used JC-10 to measure the mitochondrial membrane polarization in L02 and HepG2 cells. The results showed that the ratios of red to green fluorescence were significantly lower in potassium ions treated L02 cells (Figure 6(a)) and HepG2 cells (Figure 6(b)). And the results demonstrated mitochondrial membrane potential depolarized after exposure to potassium ions in liver cells. Moreover, these changes indicated mitochondrial membrane potential of liver cells depolarized in a concentration dependence manner after exposure to potassium ions, especially for HepG2 cells.

4. Discussions

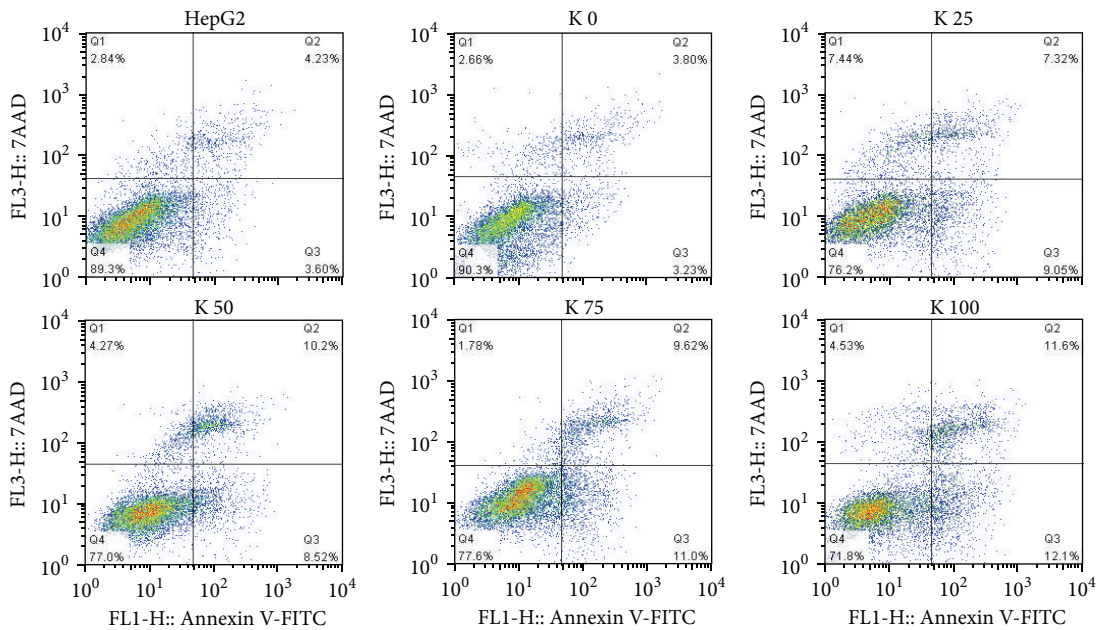
Hepatocellular carcinoma (HCC) is a common malignant tumor of liver, usually in individuals with developing chronic liver disease or cirrhosis. HCC is the common cause of cancer and is ranked fifth while it is also the second commonest



(a)



(b)



(c)

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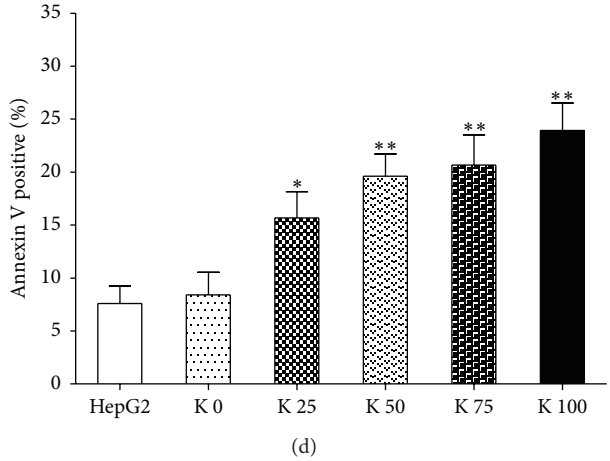

Figure 3: Potassium ions promoted cell apoptosis of liver cells. L02 (3×10^5) and HepG2 (3×10^5) cells were added to 6-well plates and cocultured with different concentrations of potassium ions for 48 hrs. Then, the effects of potassium ions on L02 and HepG2 cell apoptosis were determined by flow cytometry. (a, b) The apoptotic L02 cells treated with potassium ions increased significantly at 48 hrs ($P < 0.05$). (c, d) The apoptotic HepG2 cells treated with potassium ions increased significantly at 48 hrs, too ($P < 0.01$). All data are represented as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$.

cause of cancer deaths among human [11]. Patients with HCC have poor prognosis; thus urgent measures to curb HCC are needed [12–14].

The hallmarks of tumor contain the inhibition of programmed cell death and abnormal cell proliferation [2]. Exploring new strategies to induce apoptosis and inhibit proliferation of tumor cells is needed. Researchers found that the first features of potassium channels in nonneural cells were the role of proliferation [1, 2]. Our research also showed that potassium ions could inhibit proliferation of liver cells, especially for HepG2 cells. Moreover, the results of cells cycle analysis indicated that potassium ions could block the S phase of the cell cycle and suppress the growth of L02 and HepG2 cells through preventing proper DNA replication. The antitumor influence of potassium ions has been proved in most human tumor cells through regulation of cell cycle and apoptosis [15, 16]. Inhibition of apoptosis can result from carcinogenesis, so induction of apoptosis may be a better potential antitumor therapeutic strategy [17–20]. However, effects of potassium ions on hepatocellular carcinoma have not been reported. Our results identified that potassium ions promoted cell apoptosis and induced obvious activation of caspase-3/7 activity in both L02 and HepG2 cells, especially for HegG2 cells. Therefore, the specific mechanisms need to be deeply investigated.

Apoptosis is an important mechanism for eliminating both excess normal cells and those cells which have sustained damage. The two common pathways of apoptosis are exogenous (death receptor mediated) and intrinsic (mitochondrial) approach [21, 22]. Potassium channel is the important factor for the changes in membrane potential during the cell-cycle progression. Therefore, blocking the activity of potassium channels could induce antiproliferation effects [23, 24]. Concomitantly, at the initiation of apoptosis, intracellular potassium ions concentration decreased. Potassium channel is a key channel to maintain the stability of cell membrane potential. Because there are many types of these channels mediating dominant potassium efflux, they play a significant role in membrane permeability and cell volume regulation [25, 26]. The tumor cells with negative resting potential are usually smaller than normal cells [27]; thus, inflow of more potassium ions through high level expression of potassium channels in response to the certain physical process is possible. Our results demonstrated that the mitochondrial pathway of apoptosis is important. After being treated with potassium ions for 48 hrs, mitochondrion membrane potential ($\Delta\Psi m$) of HepG2 and L02 cells both decreased, which explained potassium ions induced apoptosis of HepG2 and L02 cells. Potassium ions may inhibit cell-cycle progression and proliferation through reducing the production of ATP and mitochondrial membrane depolarization.

In addition to supplying cellular energy, mitochondria are involved in other tasks, such as signaling, cellular differentiation, and cell death, as well as maintaining control of cell cycle and cell growth. Mitochondrion is an important factor controlling the intrinsic pathway of cell apoptosis, including release of caspase cofactors, such as cytochrome c (Cyt c) and SMAC, generation of apoptotic body, and induction of apoptosis. Furthermore, the Bcl-2 gene family is a key point of the mitochondria pathway [28]. This pathway involves several members including Bax, a proapoptotic protein, and Bcl-2, an antiapoptotic protein, which played a crucial role in regulation of apoptosis [29, 30]. The Bcl-2/Bax ratio has been used to evaluate the cell apoptosis, and its reduction could activate the expression of caspase proteins [31, 32]. By Western Blot detection, we found that Bcl-2/Bax ratio in liver cells decreased after being cocultured with potassium ions for 48 hrs. These data showed that the decrease of the ratio is closely related to liver cells apoptosis induced by potassium ions.

Voltage-dependent anion-selective channel protein 1 (VDAC1) is a voltage-dependent anion channel which is

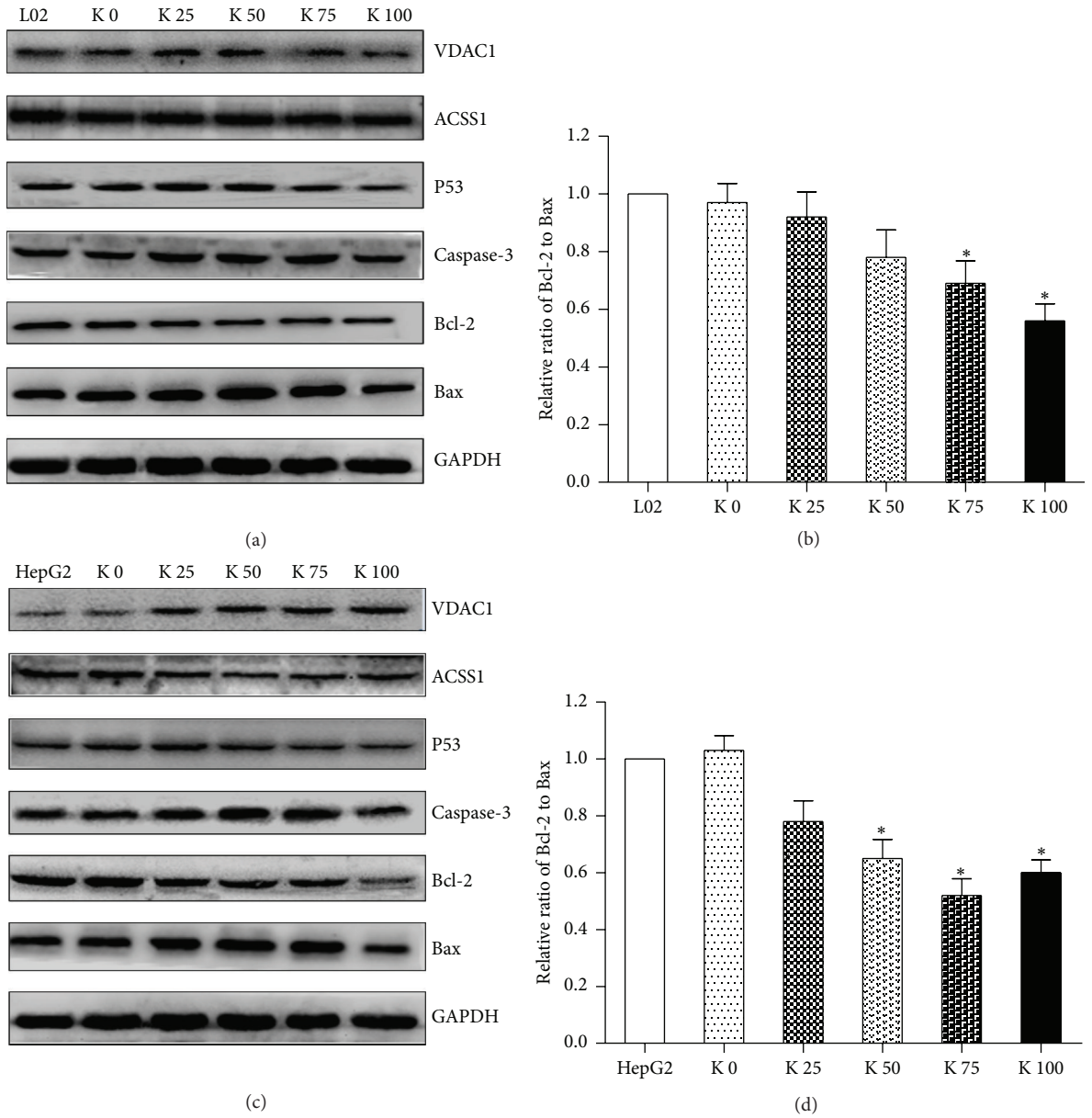

Figure 4: Potassium ions affected expression of apoptosis-related proteins and mitochondrial proteins of liver cells. The expression levels of P53, apoptosis-related proteins Bax, Bcl-2, caspase-3, or mitochondrial protein VDAC1, ACSS1, and K^+ channel protein HERG in liver cells were determined by Western Blotting. (a) In L02 cells, the levels of Bax, caspase-3, VDAC1, and HERG increased after being cocultured with different concentrations of potassium ions in a dose-dependent manner, and the level of Bcl-2 decreased in a dose-dependent manner. The expression level of ACSS1 has no obvious change. (b) The Bcl-2/Bax ratios in L02 cells ($P < 0.05$) showed as relative optical density values of the protein bands normalized to GAPDH decreased in a dose-dependent manner. (c) In HepG2 cells, the expression levels of Bax, caspase-3, VDAC1, and HERG increased after being cocultured with different concentrations of potassium ions in a dose-dependent manner, and the expression level of Bcl-2 and ACSS1 decreased in a dose-dependent manner. Both the increasing and decreasing trends were more obvious than those in L02 cells. (d) The Bcl-2/Bax ratios in HepG2 cells ($P < 0.05$) showed as relative optical densities of the protein bands normalized to GAPDH decreased in a dose-dependent manner and more obviously than in L02 cells. All data are represented as mean ± SEM. $^*P < 0.05$.

involved in the regulation of cell metabolism, mitochondrial apoptosis, and spermatogenesis. VDAC1, mitochondrial porin 1, played a pivotal role in mitochondria mediated apoptosis and transporting various ions or small molecules across the outer mitochondrial membrane. In particular, VDAC1 is the major ion transport channel and is implicated in cancer. The increasing expression of VDACs may be a specific target for treatment of cancer [33]. HK2 binding to VDAC antagonizes cell apoptosis through inhibition of Bax-induced releasing of Cyt c [7, 34, 35] and inhibits the mitochondrial permeability transition [36]. The decomposition product of HK seems to destroy aerobic glycolysis

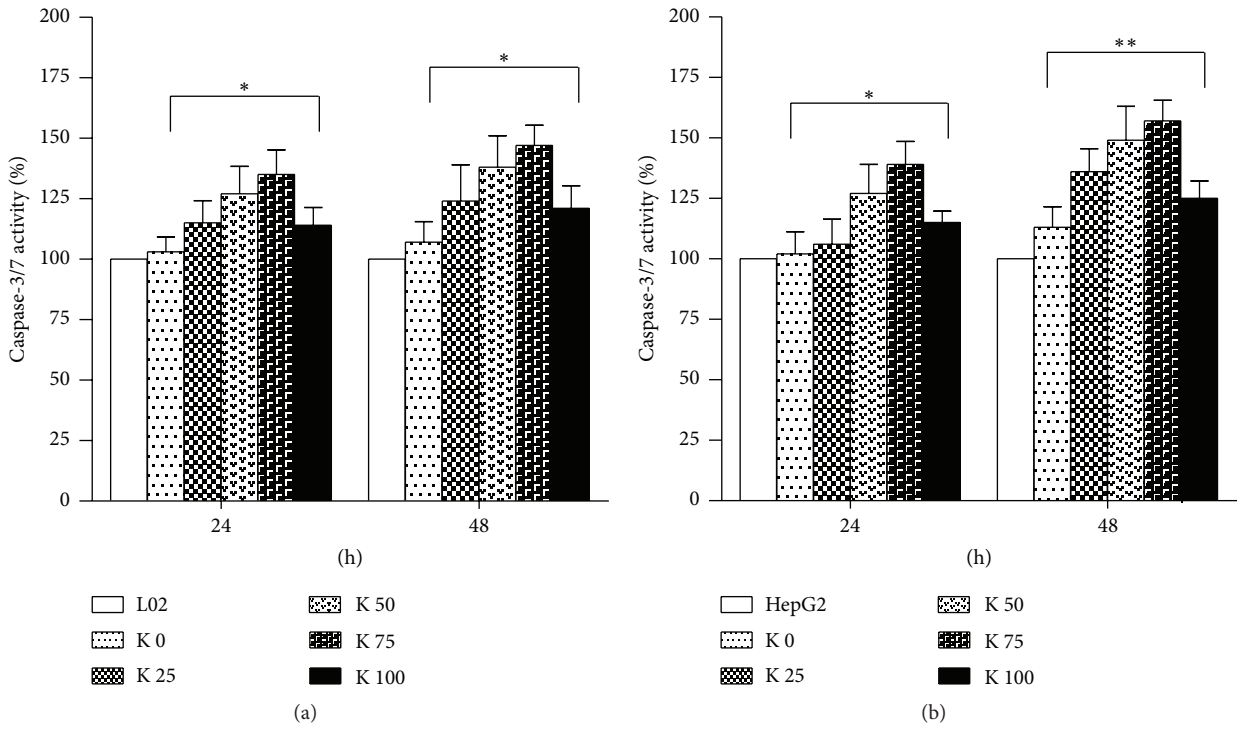

Figure 5: Potassium ions induced activation of caspase-3/7 activity in liver cells. (a) Potassium ions induced activation of caspase-3/7 activity in L02 cells. (b) Potassium ions induced activation of caspase-3/7 activity in HepG2 cells, too. All data are represented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$.

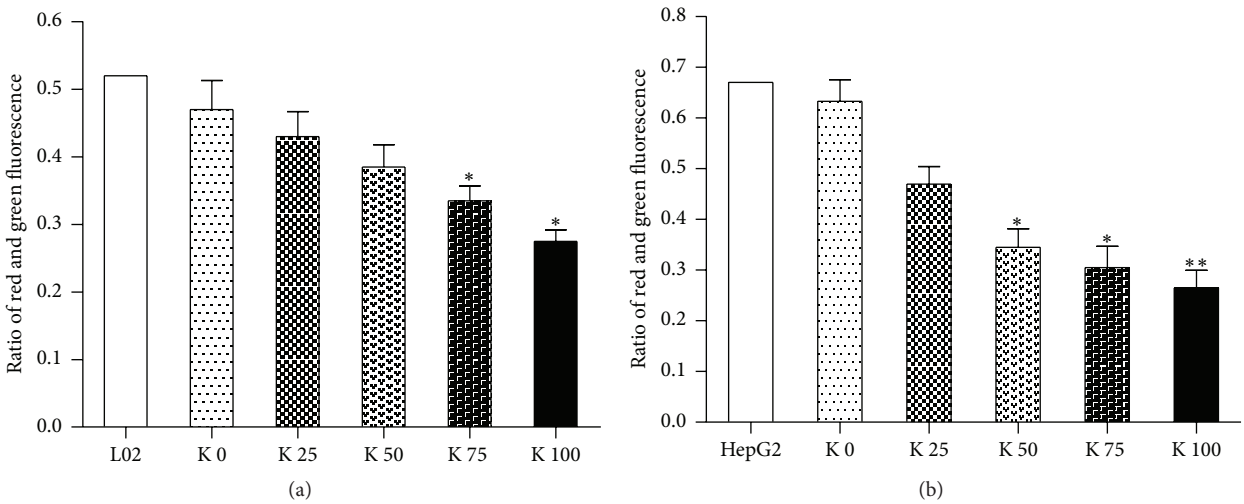

Figure 6: Potassium ions depolarized the mitochondrial membrane potential in liver cells. We measured mitochondrial membrane polarization in L02 and HepG2 cells at 48 hrs using JC-10. The ratio of red to green fluorescence decreased significantly in both L02 and HepG2 cells treated with potassium ions compared to control. (a) In L02 cells, the ratios of red to green fluorescence decreased in a dose-dependent manner ($P < 0.05$). (b) In HepG2, the ratios decreased in a dose-dependent manner cells and more obviously than in L02 cell ($P < 0.05$). All data are represented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$.

and energy balance of cells, regulate the interaction of Bcl-2 protein family, and promote mitochondrial VDAC oligomer formation to induce cell death [37, 38]. Thus, the HK-VDAC complex has become an important target for treatment of cancer [39, 40]. Our results showed that potassium ions upregulated the expression of VDAC1 in a dose-dependent manner. Therefore, potassium ions overbalanced the mitochondrial membrane potential through upregulating VDAC1 or breaking the balance of Bcl-2/Bax ratio and then induced Cyt c released from mitochondria, caspase activation, and caspase-3/7 ration imbalance, finally resulting in cell apoptosis.

Another protein ACSS1 was detected, which is the key protein in mitochondrial respiratory chains and encodes a mitochondrial acetyl-CoA synthetase that it used to produce ATP molecules. Our results indicated that potassium ions induced downregulation of ACSS1, and then mitochondrial energy metabolism was restrained. It caused a reduction of cell proliferation and cell-cycle arrest. Both the mitochondrial membrane depolarization and metabolic disorders are the suppressor of cell cycle. The expression of ACSS1 in HepG2 cells downregulated after being cocultured with potassium ions, but there was no difference in L02 cells. It demonstrated that potassium ions could destroy the mitochondrial respiratory chains and restrain the mitochondrial energy metabolism. Moreover, for the differences between L02 and HepG2 cell, ACSS1 may play a crucial role.

In order to further evaluate the gene expressions change related to potassium channel induced by potassium ions in L02 and HepG2 cells, the expression of HERG was evaluated. HERG is a gene (KCNH2) that encodes a protein known as Kv11.1, the alpha subunit of a potassium ions channel [41]. HERG involves regulation of nervous system functions and also carcinogenesis and development of leukemia tumor [41]. The expression levels of HERG in liver cells upregulated, especially for HepG2 cells. The results indicated that these biological functions affected by potassium ions were associated with channel protein HERG.

It is known that potassium ions play an extensive role. We studied the biological function induced by potassium ions in liver cells and explored their molecular mechanism. By facilitating expression of channel protein HERG, potassium ions may prevent cells from being shunted to procancerous pathways and overbalance the mitochondrial membrane potential through upregulating expression of VDAC1 or breaking the balance of Bcl-2/Bax ratio and then induced cytochrome c released from mitochondria, caspase activation, and caspase-3/7 ration imbalance, finally resulting in cell apoptosis.

In conclusion, our results demonstrated that potassium ions may be a key regulator of liver cell function. Potassium ions could inhibit tumorigenesis through inducing apoptosis of hepatoma cells by upregulating potassium ions transport channel proteins HERG and VDAC1.

Competing Interests

The authors declare that there are no competing interests.

Authors' Contributions

Zhenglin Xia and Xusen Huang contributed equally to this work.

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References

[1] D. Hanahan and R. A. Weinberg, "Hallmarks of cancer: the next generation," *Cell*, vol. 144, no. 5, pp. 646–674, 2011.

[2] D. Urrego, A. P. Tomczak, F. Zahed, W. Stühmer, and L. A. Pardo, "Potassium channels in cell cycle and cell proliferation," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 369, no. 1638, Article ID 20130094, 2014.

[3] T. E. DeCoursey, K. G. Chandy, S. Gupta, and M. D. Cahalan, "Voltage-gated K^+ channels in human T lymphocytes: a role in mitogenesis?" *Nature*, vol. 307, no. 5950, pp. 465–468, 1984.

[4] L. A. Pardo and W. Stühmer, "The roles of K^+ channels in cancer," *Nature Reviews Cancer*, vol. 14, no. 1, pp. 39–48, 2014.

[5] H. Wulff, N. A. Castle, and L. A. Pardo, "Voltage-gated potassium channels as therapeutic targets," *Nature Reviews Drug Discovery*, vol. 8, no. 12, pp. 982–1001, 2009.

[6] S. Pillozzi, M. Masselli, E. De Lorenzo et al., "Chemotherapy resistance in acute lymphoblastic leukemia requires hERG1 channels and is overcome by hERG1 blockers," *Blood*, vol. 117, no. 3, pp. 902–914, 2011.

[7] R. Zhang, P. Tian, Q. Chi et al., "Human ether-à-go-go-related gene expression is essential for cisplatin to induce apoptosis in human gastric cancer," *Oncology Reports*, vol. 27, no. 2, pp. 433–440, 2012.

[8] H. Usman and M. K. Mathew, "Potassium channel regulator KCNRG regulates surface expression of Shaker-type potassium channels," *Biochemical and Biophysical Research Communications*, vol. 391, no. 3, pp. 1301–1305, 2010.

[9] M. Brevet, D. Fucks, D. Chatelain et al., "Deregulation of 2 potassium channels in pancreas adenocarcinomas: implication of kv1.3 gene promoter methylation," *Pancreas*, vol. 38, no. 6, pp. 649–654, 2009.

[10] M. Sciaccaluga, B. Fioretti, L. Catacuzzeno et al., "CXCL12-induced glioblastoma cell migration requires intermediate conductance Ca^{2+}-activated K^+ channel activity," *American Journal of Physiology-Cell Physiology*, vol. 299, no. 1, pp. C175–C184, 2010.

[11] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, and M. J. Thun, "Cancer statistics, 2009," *CA: A Cancer Journal for Clinicians*, vol. 59, no. 4, pp. 225–249, 2009.

[12] Y. Lei, H. Liu, Y. Yang et al., "Interaction of LHBs with C53 promotes hepatocyte mitotic entry: a novel mechanism for HBV-induced hepatocellular carcinoma," *Oncology Reports*, vol. 27, no. 1, pp. 151–159, 2012.

[13] H. Wang, H. Liu, K. Chen et al., "SIRT1 promotes tumorigenesis of hepatocellular carcinoma through PI3K/PTEN/AKT signaling," *Oncology Reports*, vol. 28, no. 1, pp. 311–318, 2012.

[14] Z. Feng, H. Li, S. Liu, J. Cheng, G. Xiang, and J. Zhang, "FAM172A induces S phase arrest of HepG2 cells via Notch 3," *Oncology Reports*, vol. 29, no. 3, pp. 1154–1160, 2013.

[15] H. Thomadaki, A. Scorilas, C. M. Tsiapalis, and M. Havredaki, "The role of cordycepin in cancer treatment via induction or inhibition of apoptosis: implication of polyadenylation in a cell type specific manner," *Cancer Chemotherapy and Pharmacology*, vol. 61, no. 2, pp. 251–265, 2008.

[16] J.-W. Jeong, C.-Y. Jin, C. Park et al., "Induction of apoptosis by cordycepin via reactive oxygen species generation in human

leukemia cells," *Toxicology in Vitro*, vol. 25, no. 4, pp. 817–824, 2011.

[17] N. Kang, J.-H. Zhang, F. Qiu, S.-I. Tashiro, S. Onodera, and T. Ikejima, "Inhibition of EGFR signaling augments oridonin-induced apoptosis in human laryngeal cancer cells via enhancing oxidative stress coincident with activation of both the intrinsic and extrinsic apoptotic pathways," *Cancer Letters*, vol. 294, no. 2, pp. 147–158, 2010.

[18] P. N. Kelly and A. Strasser, "The role of Bcl-2 and its pro-survival relatives in tumourigenesis and cancer therapy," *Cell Death and Differentiation*, vol. 18, no. 9, pp. 1414–1424, 2011.

[19] A. Strasser, S. Cory, and J. M. Adams, "Deciphering the rules of programmed cell death to improve therapy of cancer and other diseases," *The EMBO Journal*, vol. 30, no. 18, pp. 3667–3683, 2011.

[20] S. L. Spencer and P. K. Sorger, "Measuring and modeling apoptosis in single cells," *Cell*, vol. 144, no. 6, pp. 926–939, 2011.

[21] N. Lynam-Lennon, S. G. Maher, and J. V. Reynolds, "The roles of microRNA in cancer and apoptosis," *Biological Reviews*, vol. 84, no. 1, pp. 55–71, 2009.

[22] W. Hu and J. J. Kavanagh, "Anticancer therapy targeting the apoptotic pathway," *The Lancet Oncology*, vol. 4, no. 12, pp. 721–729, 2003.

[23] E. Klimatcheva and W. F. Wonderlin, "An ATP-sensitive K^+ current that regulates progression through early G1 phase of the cell cycle in MCF-7 human breast cancer cells," *Journal of Membrane Biology*, vol. 171, no. 1, pp. 35–46, 1999.

[24] D. J. Blackiston, K. A. McLaughlin, and M. Levin, "Bioelectric controls of cell proliferation: ion channels, membrane voltage and the cell cycle," *Cell Cycle*, vol. 8, no. 21, pp. 3527–3536, 2009.

[25] S. Fulda, L. Galluzzi, and G. Kroemer, "Targeting mitochondria for cancer therapy," *Nature Reviews Drug Discovery*, vol. 9, no. 6, pp. 447–464, 2010.

[26] N. Yagoda, M. von Rechenberg, E. Zaganjor et al., "RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels," *Nature*, vol. 447, no. 7146, pp. 864–868, 2007.

[27] A. A. Marino, I. G. Iliev, M. A. Schwalke, E. Gonzalez, K. C. Marler, and C. A. Flanagan, "Association between cell membrane potential and breast cancer," *Tumor Biology*, vol. 15, no. 2, pp. 82–89, 1994.

[28] A. Burlacu, "Regulation of apoptosis by Bcl-2 family proteins," *Journal of Cellular and Molecular Medicine*, vol. 7, no. 3, pp. 249–257, 2003.

[29] M. C. Wei, W.-X. Zong, E. H.-Y. Cheng et al., "Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death," *Science*, vol. 292, no. 5517, pp. 727–730, 2001.

[30] J. Lindsay, M. D. Esposti, and A. P. Gilmore, "Bcl-2 proteins and mitochondria—specificity in membrane targeting for death," *Biochimica et Biophysica Acta—Molecular Cell Research*, vol. 1813, no. 4, pp. 532–539, 2011.

[31] L. D. Walensky, "BCL-2 in the crosshairs: tipping the balance of life and death," *Cell Death and Differentiation*, vol. 13, no. 8, pp. 1339–1350, 2006.

[32] X.-A. Wang, S.-S. Xiang, H.-F. Li et al., "Cordycepin induces s phase arrest and apoptosis in human gallbladder cancer cells," *Molecules*, vol. 19, no. 8, pp. 11350–11365, 2014.

[33] C. Grills, P. V. Jithesh, J. Blayney, S.-D. Zhang, and D. A. Fennell, "Gene expression meta-analysis identifies VDAC1 as a predictor of poor outcome in early stage non-small cell lung cancer," *PLoS ONE*, vol. 6, no. 1, Article ID e14635, 2011.

[34] A. Wolf, S. Agnihotri, J. Micallef et al., "Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma multiforme," *Journal of Experimental Medicine*, vol. 208, no. 2, pp. 313–326, 2011.

[35] J. G. Pastorino, N. Shulga, and J. B. Hoek, "Mitochondrial binding of hexokinase II inhibits Bax-induced cytochrome c release and apoptosis," *The Journal of Biological Chemistry*, vol. 277, no. 9, pp. 7610–7618, 2002.

[36] F. Chiara, D. Castellaro, O. Marin et al., "Hexokinase II detachment from mitochondria triggers apoptosis through the permeability transition pore independent of voltage-dependent anion channels," *PLoS ONE*, vol. 3, no. 3, Article ID e1852, 2008.

[37] M. C. Shoshan, "3-bromopyruvate: targets and outcomes," *Journal of Bioenergetics and Biomembranes*, vol. 44, no. 1, pp. 7–15, 2012.

[38] V. Shoshan-Barmatz and M. Golan, "Mitochondrial VDAC1: function in cell life and death and a target for cancer therapy," *Current Medicinal Chemistry*, vol. 19, no. 5, pp. 714–735, 2012.

[39] L. Galluzzi, O. Kepp, N. Tajeddine, and G. Kroemer, "Disruption of the hexokinase-VDAC complex for tumor therapy," *Oncogene*, vol. 27, no. 34, pp. 4633–4635, 2008.

[40] E. Simamura, H. Shimada, T. Hatta, and K.-I. Hirai, "Mitochondrial voltage-dependent anion channels (VDACs) as novel pharmacological targets for anti-cancer agents," *Journal of Bioenergetics and Biomembranes*, vol. 40, no. 3, pp. 213–217, 2008.

[41] M. C. Sanguinetti and M. Tristani-Firouzi, "hERG potassium channels and cardiac arrhythmia," *Nature*, vol. 440, no. 7083, pp. 463–469, 2006.

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Review Article

Synergistic Effect and Molecular Mechanisms of Traditional Chinese Medicine on Regulating Tumor Microenvironment and Cancer Cells

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The interaction of tumor cells with the microenvironment is like a relationship between the "seeds" and "soil," which is a hotspot in recent cancer research. Targeting at tumor microenvironment as well as tumor cells has become a new strategy for cancer treatment. Conventional cancer treatments mostly focused on single targets or single mechanism (the seeds or part of the soil); few researches intervened in the whole tumor microenvironment and achieved ideal therapeutic effect as expected. Traditional Chinese medicine displays a broad range of biological effects, and increasing evidence has shown that it may relate with synergistic effect on regulating tumor microenvironment and cancer cells. Based on literature review and our previous studies, we summarize the synergistic effect and the molecular mechanisms of traditional Chinese medicine on regulating tumor microenvironment and cancer cells.

1. Introduction

Tumor microenvironment (TME) plays a pivotal role in the process of cancer development and metastasis. Tumor and its microenvironment are a complex compound including the "seed" and "soil," which was first proposed by Stephen Paget; a hypothesis suggested that the sites where metastases occur were defined not only by the tumor cell (seed) but also by the microenvironment of the secondary metastatic site (soil) [1]. Until recently, the staging and treatment approaches for cancer appeared to be orientated predominantly to both "soil" and "seed." We are currently witnessing an increasing amount of evidence, spanning from clinical to laboratory research, which highlights that cancer growth and metastasis are the result of the dynamic balance between the cancer itself and the impaired function of the TME [2]. Target organs can release various cytokines recruiting tumor cells, promoting cell proliferation, and inducing angiogenesis and eventually form metastases. At the same time, tumor cells can also release various cytokines by paracrine manner, remodeling TME for their own survival. Therefore, the interaction between tumor cells and TME of target organ facilitates a complex metastasis process. Increasing evidence indicates that TME is a key target of tumor therapy research, because of its special physical and chemical properties and the internal relations between inflammation and immune system [3]. The final purpose of target therapy on TME is effectively resisting the interaction between tumor cells and their microenvironment. However, conventional cancer treatments mostly focused on tumor cell and single targets or single mechanism, on the basis of the fact that few researches intervene in the whole TME [4]. In recent years, researches of antitumor drugs focused on remodeling TME emerge endlessly, mainly targeting antiangiogenesis and immunotherapy to overcome the immune tolerance, treatment reversing drug resistance, and so forth. Unexpectedly, antiangiogenesis drugs did not achieve the ideal treatment effect; on the contrary they produce greater toxicity and promote the development of tumor as a result of hypoxia and reduction of transmission chemotherapy drugs to tumor tissue, promoting

tumor drug resistance; tumor cells can get nutrition energy supplement through autophagy from TME and eventually make the antiangiogenesis therapy only show decreasing repair ability of normal tissue [5]. Tumor immunotherapy has become an important means to prevent tumor recurrence and metastasis. Most current tumor immunotherapy has shown good effect of tumor destruction in vitro but did not reach expected effect in vivo, for it cannot overcome the tumor antigen-presenting and immune effectors function inefficiency. Conventional tumor chemotherapy resistance research mainly focused on the genetic changes of endogenous factors. A large number of studies have shown that the TME played an important role in mediating acquired drug resistance [6].

In cancer treatment, traditional Chinese medicine (TCM) emphasizes the overall efficacy, inhibiting tumor cell as well as TME to suppress tumor development and recurrence. So far, many Chinese herbs have been shown to have a good effect in clinical studies, which display a broad range of clinical effects including alleviation of cancer-associated symptoms, prolonging survival rates, decreasing treatment-related toxicity, and preventing recurrence and metastasis [7–15], as shown in Table 1. Furthermore, several Chinese herbs have also been proven to inhibit tumors in fundamental experiments. Although the mechanism of TCM is still unclear, increasing evidence has shown that it may relate with synergistic effect on regulating TME and cancer cells. In this review, we will summarize the synergistic effect and the molecular mechanisms of TCM on regulating TME and cancer cells according to recent researches.

2. TCM Regulates Tumor Microenvironment

The TME encompasses a complex meshwork of nonmalignant cells, structural components, molecules, and chemicals that surround cancer cells. The nonmalignant cells, including endothelial cells, pericytes, fibroblasts, and immune cells, together with the surrounding extracellular matrix (ECM), comprise the supportive stroma of the tumor and modulate the TME [16]. The production of both tumor-promoting and tumor-suppressing signals from these various cell types influences the tumor microenvironment. Recently, targeting TME has opened new avenues in clinical oncology [17]. However, treatment itself activates the microenvironment by damaging a large population of cells, which can drastically exacerbate disease conditions in a cell in a nonautonomous manner, and such off-target effects should be well taken into account when establishing future therapeutic rationale [18]. TCM is a potential treatment strategy.

2.1. TCM Inhibits the Degradation of Extracellular Matrix. The tumor microenvironment consists of an insoluble ECM, a stroma composed of fibroblasts, adipocytes, and endothelial and resident immune cells, and a multitude of growth factors and cytokines [19]. Abnormal changes in the amount and organization of molecules lead to altered biochemical and physical properties of tumor-associated ECM that contributes to tumor progression and resistance to therapy [20, 21]. Matrix metalloproteinases (MMPs) are enzymes that degrade structural components of the ECM, produced by cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), tumor-associated neutrophils (TAN), mast cells (MCs), blood endothelial cells (BECs), lymphatic endothelial cells (LECs), and bone marrow-derived mesenchymal stem cells (MSCs). These enzymes regulate a multitude of physiological processes such as morphogenesis, tissue remodeling, and signaling events [22]. Its activity has been implicated in almost every stage of the metastatic cascade from the primary site to the progression of tumor extravasation, growth, and development. The expression and activity of MMPs against matrix macromolecules have been associated with the development of malignant phenotypes and the promotion of cell invasiveness and metastasis [23]. Several studies show that MMP-2 and MMP-9 are highly expressed in tumors and are associated with poor clinical outcome. Some traditional Chinese medicine monomers and compounds have been reported to have inhibitory effects on the migration and invasion of cancer cells via reducing the expression of MMPs [24, 25].

Deng et al. illustrated YQFS (a standard formulation of Si-Jun-Zi-Tang with the addition of *Myristica fragrans* and five-leaf *Akebia* fruit) extract had an antitumor effect, which could be attributed to ERK1/2-dependent inhibition of MMP-2/9 expression, modulating the ERK/MAPK pathway and its downstream factors by selectively targeting ERK phosphorylation [24]. *Momordica cochinchinensis* (a Chinese herbal called Mu BieZi) has been used for a variety of purposes, showing an anticancer action. Zheng et al. found that extracts of *Momordica cochinchinensis* seeds (ESMCs) revealed strong growth inhibitory effects on ZR-75-30 cells and effectively inhibit ZR-75-30 cell invasion in a dose-dependent manner. ESMC treatment could not only reduce the protein expression but also repress the enzymatic activity of MMP-2 and MMP-9, which suggests that ESMC's anti-invasive action was mediated by diminishing the ability of breast cancer cells to degrade the components of ECM by modulating MMP-2 and MMP-9 expression and activity [26].

2.2. TCM Improves the Hypoxia Microenvironment. Abnormal and dysfunctional blood vessels in tumor tissues are incapable of restoring oxygenation, therefore perpetuating hypoxia, which, in turn, will fuel tumor progression, metastasis, and resistance to antitumor therapies [27]. Increasing evidences indicate that the vasculature is insufficient to supply adequate oxygen when solid tumor diameter is >2 mm, resulting in local hypoxic and anoxic conditions inside the tumor. The level of hypoxia within a tumor increases during tumor progression and is a good indicator of disease outcome because hypoxia selects the most invasive cancer cells and promotes resistance to therapies [28–30]. Factors in the response of tumor cells to this distinct microenvironment are the activities of the hypoxia inducible factor-1α (HIF-1α), which is regulated in an oxygen-dependent manner [31]. HIF-1α signaling pathway is frequently observed in solid tumors and is strongly associated with numerous pathophysiological processes, including the induction of epithelial-mesenchymal

Table 1: Several clinical studies have confirmed the effectiveness of TCM in cancer treatment.

Drug	Type of cancer	Phase	Method	Interventions		Outcomes	Cite
				Control group	Treatment group		
Ginsenoside Rg3	NSCLC	Clinical stages II/IIIa, postoperative patients	RCT, not blinded 4–6 cycles	Chemotherapy (NP/CE/GP)	Shenyi capsule, or it combines with chemotherapy	Improves the life span of patients	[7]
Cantharidinate	NSCLC	Middle-late stage	RCT, not blinded 4 cycles	GP	Combines with cantharidinate	Improves clinical effects and life quality, lowers the toxic/adverse effects of chemotherapy	[8]
Astragalus polysaccharide	NSCLC	Clinical stages IIIB or IV advanced disease	RCT, not blinded 3 cycles	Vinorelbine and cisplatin	Combines with APS	Improves patients' QOL	[9]
Xiaoaiping	Breast cancer	Neoadjuvant chemotherapy	RCT, not blinded duration, 12 weeks	TEC neoadjuvant chemotherapy	Combines with Xiaoaiping injection	Significantly enhances short-term and long-term efficacies of neoadjuvant chemotherapy	[10]
Atractylenolide I	Gastric cancer	Cachexia patients	RCT, not blinded duration, 7 weeks	Nutritional supplementation	Adds atractylenolide I	Improves appetite and KPS status and decreases PIF positive rate	[11]
Jianpi Huayu therapy	Hepatocellular carcinoma	Firstly treated patients	RCT, not blinded duration, 1 year	Hepatectomy and conventional western medicine treatment	Combines with Jianpi Huayu therapy	Reduces postoperative recurrence and metastasis, improves DFS and OS	[12]
Huachansu	Gallbladder carcinoma	Locally advanced or metastatic	Not RCT, not blinded, continued until termination events	Gemcitabine-oxaliplatin	Combines with Huachansu injection	Makes chemotherapy well tolerated and improves the QOL of patients	[13]
Curcumin	Colorectal cancer	Liver metastases	Escalation trial, 12 cycles of chemotherapy	FOLFOX chemotherapy	Combines with curcumin	Prolongs median PFS and improves treatment response	[14]
Compound Zhebei granules	Acute leukemia	Posttreatment and defined as refractory	RCT, double-blind and multicentral concurrent control, 14 days	Multiple chemotherapeu-tic schemes	Combines with compound Zhebei granules	Increases the clinical remission rate	[15]

transition (EMT), a process in which epithelial cells lose cell-cell adhesion and cell polarity and acquire properties of mesenchymal cells, which results in cancer progression, metastasis, and multidrug resistance in cancer [32–35].

Pien Tze Huang (PZH) has been used in China and Southeast Asia for centuries as a remedy for various types of human cancer. It was found that treatment with PZH was observed to significantly decrease the cell migration and invasion rates and inhibit the hypoxia-mediated EMT and HIF-1 signaling, suggesting that PZH concentration dependently inhibits the hypoxia-induced metastasis of colon cancer cells [35]. Another study showed that Oroxylin A remarkably inhibited HIF-1α expression and its stability and also suppressed the downstream targets (e.g., PDK1, LDHA, and HK II) and their mRNA levels under hypoxia. Furthermore, Oroxylin A could decrease the accumulation of ROS, which was benefit for inhibition on glycolytic activity by decreasing ROS-mediated HIF-1 expression. PI3K is an important molecular signal transduction in tumor cells and an upstream signal pathway molecular of HIF-1α [36]. Wogonin, extracted from the TCM herb *Scutellaria baicalensis*, was recently found to be a good candidate for the development of new multidrug resistance (MDR) reversal agent and its reversal mechanism was due to the suppression of HIF-1α expression via inhibiting PI3K/Akt signaling pathway [37, 38].

2.3. TCM Reverses the Immunosuppressive Microenvironment. Suppressive phenotypes of immune cells are regulated and reversed by TCM treatment. Concretely, inflammatory T cells and natural killer T cells (NKT) increasingly proliferate in contrast of the reducing quantity of regulatory T cells and myeloid-derived suppressor cells (MDSCs) after treatment by TCM. Furthermore, suppressive macrophages and dendritic cells (DCs) change their functions to antitumor effects, such as M2 to M1 phenotype reversing and DCs maturation by increasing inflammatory factors expression and immune suppressive cytokines decreasing [16].

The major function of DCs is to process and present antigen for the activation of $CD4^+$ and $CD8^+$ T cells. Endocytosis of antigen by immature DCs drives DCs maturation and the subsequent presentation of antigen to T cells [39]. However, the tumor microenvironment systemically perturbs this process by increasing the accumulation of immature DCs and decreasing DCs maturation. As a result, DCs fail to activate T cells. Defective DCs function has been found in many patients with a variety of cancers, such as pancreatic carcinoma, cervical squamous intraepithelial lesions, hepatocellular carcinoma, and non-small cell lung cancer [39–43]. Multiple conditions and factors within the TME cause DCs abnormalities, including hypoxia, lactic acid build-up, and adenosine accumulation. *Astragalus mongholicus* (AMs) is a common herbal of TCM and has been proved to be effective in treating cancers according to abundant clinical case reports. Tian et al. investigated the effect and mechanism of AMs on human stomach cancer. It turned out that AMs is effective in treating stomach cancer and it might precipitate DCs maturation by regulating TLR4 mediated NF-κB signal transduction against tumor [44].

MDSCs are a major host component contributing to the immune suppressive environment, inhibiting both adaptive and innate antitumor immunity through different ways, such as inhibiting T cell activation and function and suppressing natural killer cell (NK) cytotoxicity [16]. MDSCs represent a heterogeneous population, including immature macrophages, DCs, and granulocytes, generated by and released from the bone marrow in response to a wide array of pathological stimulations like malignant tumor and inflammation, leading to the expansion of MDSCs and contributing to the negative regulation of tumor immune response [45]. The MDSCs can inhibit T cell-mediated tumor acquired immune responses with overexpression of Arg1, iNOS, and Ros, suppress NK cell cytotoxicity by inhibiting NKG2D and IFN-c, and facilitate angiogenesis by releasing vascular endothelial growth factor (VEGF), MMPs, and TGF-β [46]. Wang et al. investigated the effect of ginseng-derived compound K (C-K) on apoptosis, immunosuppressive activity, and proinflammatory cytokine production of MDSCs. It turned out that C-K treatment can significantly increase the percentages of early and late apoptotic MDSCs in vitro, decrease the expressions of immunosuppression-related genes Cox-2 and Arg-1, and suppress the function of IL-1β, IL-6, and IL-17, which implied that C-K can restrain the immunosuppressive effect of MDSCs to inhibit tumor cell proliferation in mice [47].

Tumor-associated macrophages (TAMs) are a heterogeneous population of myeloid cells with a potential to promote cancer cell proliferation and invasion and regulate tumor neovascularization and lymphangiogenesis, as well as cytotoxic T cell function [47]. Under pathological conditions, macrophages acquire distinct phenotypic characteristics through different activation mechanisms [48]. The classical activated macrophage (M1-like) exhibits proinflammatory properties by expressing and secreting proinflammatory molecules (e.g., TNF-α, IL-6, IL-12, IL-1, Type I, IFN-γ, CXCL1–3, CXCL-5, and CXCL8–10), while macrophage could be alternatively activated to M2-like phenotype, which expresses anti-inflammatory factors (e.g., VEGFC/D, VEGFR3, MMPs, IL-10, and IL-13) [49–52]. Several researchers have reported TCM could switch the phenotype of TAMs from M2 to M1, inhibiting tumor progression. Baicalin is a natural flavonoid from medicinal herbs including *Scutellaria baicalensis* Georgi. Several studies have revealed the antitumor action of Baicalin by increased expressions of IFN-γ and IL-12 to activate immune response. Recent study results showed that Baicalin can initiate TAM reprogramming to M1-like macrophage, induce repolarization of TAM and M2-like macrophage through autophagy and transcriptional activation of RelB/p52 pathway, and promote proinflammatory cytokines production [53, 54].

2.4. TCM Inhibits Angiogenesis. The growth and metastasis of the tumor depend on an effective microcirculation. The formation of a microcirculation can occur via the traditionally recognized mechanisms of angiogenesis and

vasculogenic mimicry (VM). It is well established that angiogenesis is necessary for the tumor progression and metastasis [55]. Angiogenesis occurs by complex sequential steps, such as basement membrane degradation by proteases, endothelial cell proliferation and migration/invasion, formation of capillary tubes, and survival of newly formed blood vessels, which is tightly regulated by an intricate balance between stimulators and inhibitors such as VEGF, fibroblast growth factor, and MMPs [56]. Among them, VEGF is the most important angiogenic factor closely associated with neovascularization in human tumors [57]. VM is a novel tumor blood supply in some highly aggressive malignant tumors, provides a special passage without endothelial cells, and is conspicuously different from vasculogenesis [58]. VM has unique ability of highly aggressive tumor cells to express endothelial cell-associated genes and form ECM-rich, patterned tubular networks when cultured on a three-dimensional (3D) matrix and is associated with a poor prognosis for the patients with some aggressive malignant tumors [59, 60].

Ginsenoside Rg3, a saponin extracted from ginseng, has been demonstrated to have anticancer activity in vitro and in vivo with relatively low toxicity, especially on vessels or angiogenesis in tumors [61]. A study aimed to investigate the antiangiogenic effects of Rg3 in patients with acute leukemia. The results showed that Rg3 exhibited antileukemia effect in part due to its antiangiogenic activity via inhibiting PI3K/Akt and ERK1/2 pathways, which act to regulate the expression of HIF-1α and VEGF [62]. The rhizome of *Atractylodes lancea* is extensively used in Chinese medicine as crude extracts/decoctions or a component in various herbal formulations. Numerous studies have reported the anticancer activities of *Atractylodes lancea* [63]. CLT, also named Atractylenolide III, is the major bioactive component of *Atractylodes lancea*. Wang et al. demonstrated CLT inhibited the development of angiogenesis both in vitro and in vivo. Similar to its effects on cancer cells, the inhibitory effect of CLT on ECs was due to its ability to inhibit MMPs expression and VEGF secretion by downregulating Runx2 activation of ECs, which may be associated with interference with BMP signaling in endothelial cells [64]. Abundant studies have demonstrated that PZH could suppress multiple colorectal cancers and associated signaling pathways, leading to the promotion of cancer cell apoptosis and the inhibition of cell proliferation and tumor angiogenesis [65–70].

Most antiangiogenic therapies currently being evaluated target the VEGF pathway. However, the tumor vasculature can acquire resistance to VEGF-targeted therapy by shifting to other angiogenesis mechanisms. Therefore, other therapeutic agents that block non-VEGF angiogenic pathways need to be evaluated. Recent studies have identified fibroblast growth factor 1 (FGF1) as a direct activator of PI3K-Akt, which is a non-VEGF angiogenic pathway to initiate endothelial cell migration, invasion, and differentiation. Ferulic acid (FA), an effective component of many Chinese medicinal herbs, like *Cimicifuga heracleifolia*, *Angelica sinensis*, and *Lignsticum chuangxiong*, exhibits anti-inflammatory and anticancer activities. A study indicated that FA exerted antiangiogenesis activities at a nontoxic dosage via specifically targeting fibroblast growth factor receptor 1 (FGFR1) and its PI3K/Akt signaling pathway in melanoma [71].

2.5. TCM Inhibits Lymphangiogenesis. There is accumulating evidence that tumor-associated lymphangiogenesis is an important feature of tumor progression and may facilitate cancer cell dissemination to the lymph nodes [72, 73]. Accordingly, numerous clinical studies have demonstrated a significant correlation between lymphatic vessel density and lymph node metastasis. Clinical evidence suggests that the VEGF family members VEGF-C and VEGF-D are major lymphangiogenic regulators by binding to VEGFR-2 and VEGFR-3, which are expressed on LECs. Higher VEGF-C expression is associated with higher peritumoral lymphatic vessel density, increased lymphatic invasion, and increased lymph node metastasis [74]. Kimura and Sumiyoshi discovered Wogonin isolated from *Scutellaria baicalensis* roots could inhibit VEGF-C-induced lymphangiogenesis through a reduction in VEGF-C-induced VEGFR-3 phosphorylation [75]. Norcantharidin (NCTD) is a demethylated and low-cytotoxic derivative of cantharidin with antitumor properties, an active ingredient of the traditional Chinese medicine *Mylabris*. It has been reported that NCTD is used selectively in clinic to treat hepatic, gastric, colorectal, and ovarian carcinomas and leucopenia in China because of its effective anticancer activity, fewer side effects, and leukocytosis [76–81]. A study showed that NCTD inhibited tumor growth and lymphangiogenesis of HCACs through "multipoints priming" mechanisms, that is, directly or indirectly downregulating VEGF-A, -C, -D/VEGFR-2, -3 signaling pathways, which strongly suggested that NCTD could serve as a potential antilymphangiogenic agent for tumor lymphangiogenesis [82]. Nagy et al. demonstrated that, in addition to angiogenesis, VEGF-A also induced proliferation of lymphatic endothelium, resulting in the formation of greatly enlarged and poorly functioning lymphatic channels, and abnormal lymphangiogenesis. These findings raise the possibility that abnormal lymphangiogenesis may also be expected in other circumstances such as malignant tumors characterized by VEGF-A overexpression [81].

3. TCM Inhibits the Development of the "Seed"

Just as the role of a seed in growth, cancer cells play a pivotal role in the process of tumor occurrences, developments, and metastasis, which are always the focus and hot areas of tumor researches. It was reported that this "Seed" can proliferate immortally and activate invasions and metastasis [83]. In addition, tumor cells were also deemed to be the initiators of tumor microenvironment formation, which reversely promote the growth of tumor cells [84]. Nowadays, surgery, radiotherapy, and chemotherapy are three major treating strategies of tumors for minishing burden and inhibiting growth, which have been widely used and achieved effects in clinic [85]. However, these targeted treatments were reported to have some degree of adverse effect with them. TCM, as

Table 2: Different mechanisms and channels of TCM inhibiting the growth of tumor cells.

Cancer	Drug	Main mechanisms	Main channels	Related biomarkers	Cite
Lung cancer (A549 Cells)	Xiaoji decoction	Inhibits proliferation and induces apoptosis	Signaling Akt pathway	BAD ↑, caspase-9 ↑	[94]
Lung cancer (A549 Cells)	Oxymatrine	Induces apoptosis	Bcl-2 family	Bax ↑ and Bcl-2 ↓	[88]
Esophageal carcinoma (CaEs-17)	Soups of *Rosa roxburghii* Tratt and *Fagopyrum cymosum*	Inhibits proliferation and induces apoptosis	Ki-67, Bcl-2 family	Bax ↑ and Ki-67, Bcl-2 ↓	[92]
Esophageal carcinoma (KYSE150, Eca-109)	*Marsdenia tenacissima* extract	Inhibits proliferation	MAPK signaling pathway	cyclinD1, p-ERK ↓	[84]
Gastric cancer (SGC-7901)	Sanpi Pingwei formula	Inhibits proliferation and induces apoptosis	Bax, p53, and Bcl-2	Bax, p53 ↑ and Bcl-2 ↓	[90]
Gastric cancer (AGS, MGC803)	Arsenic sulfide	Induces apoptosis	p53	Bax, MDM2 ↑ and Bcl-2 ↓	[96]
Colorectal cancer (SW480)	Jianpi Huayu decoction	Inhibits proliferation and induces apoptosis	G0/G1-phase cell cycle arrest, caspase-cascade activation and execution	p27 ↑ and cyclinD1, cyclinD2, cyclinD3, cyclinE1, CDK4, CDK6, CDK2 ↓	[95]
Colorectal cancer (LOVO)	Emodin	Induces apoptosis	Bcl-2 family	Bax ↑ and Bcl-2 ↓	[85]
Live cancer (Huh7, Hep3B, HA22T)	Bufalin	Inhibits proliferation and triggers autophagy	G2/M phase arrest and JNK pathway	TNF, BECN-1, MAPK, ATG8 ↑ and Bcl-2, Bid ↓	[81]
Breast cancer (MCF-7, MDA-MB-231)	San-Zhong-Kui-Jian-Tang	Inhibits proliferation and induces apoptosis	p21/WAF1 levels, p53, Bcl-2 family	p21, Bax, Bak ↑ and cyclinD1, cyclinD2, Bcl-2 ↓	[93]
Ovarian cancer (OVCAR-3)	Pien Tze Huang	Inhibits proliferation	AKT-mTOR pathway	AKT, (p)-AKT, mTOR p-mTOR proteins, CDK4, CDK6 ↓	[79]

an important complementary strategy, has also been shown to possess therapeutic effects on cancer cells. The followings may be the principle and potential molecular mechanisms for TCM in cancer treatment.

3.1. TCM Inhibits the Growth of Cancer Cells. Proliferation and apoptosis of cells are two critical factors that determine organism development and tissue homeostasis [86, 87]. Precisely, it is the unrestricted proliferation and suppressed apoptosis that make cancer cells grow frantically and malignantly. Accumulating studies have revealed that dysfunction in cell cycle regulation often resulted in abnormal proliferation of cancer cells [88]. The regulation of the cell cycle is influenced by many molecules such as cyclins, CDKs, and CDKIs and through Akt and MAPK signaling pathways [89]. In addition, p53, a major mediator of cell cycle arrest, was reported to mutate or be inactivated in several tumors. If that happened, the apoptotic response is not activated and cell proliferation is allowed [90]. Studies have shown that p53 can upregulate the level of p21 and Gadd45 to mediated cell cycle arrest [91–95]. Others also implicated that p53 upregulated proapoptotic Bax and downregulated prosurvival Bcl-2 to mediate apoptosis [96, 97]. Several mechanisms also demonstrated that overexpression of Bcl-2 increases the activity of AKT and IKK as well as NF-κB transcriptional activity in cancer [98]. TCM has been reported to inhibit the growth of cancer cells in abundant clinical trials. Although the mechanisms are not very clear, increasing data has shown that they maybe relate with above-mentioned regulating biomarkers to inhibit proliferation and induce apoptosis of cancer cells. The confirmed mechanisms of herb compounds and monomers acting common cancers are listed in Table 2 [86, 88, 91, 92, 95, 97, 99–103].

3.2. TCM Prevents Invasion and Metastasis of Tumor Cells. Despite the fact that all available treatments had been implemented, local invasion and metastasis predict a poor prognosis and contribute to more than 90% of tumor mortality [104, 105]. Therefore, it is needed to gain further insight into the molecular mechanisms on invasion and metastasis of tumor

cells and to search for more effective therapies. Extracellular matrix (ECM) is related to the tumor cell invasion and metastasis, and, as we know, the abnormal and absent expression of ECM has been found in many malignant cells [106]. In tumor microenvironment, tumor cells can degrade and remodel the ECM by excessively secreting matrix MMPs such as MMP-2 and MMP-9 [107]. In the meantime tumor cells also conducted epithelial-to-mesenchymal transition (EMT) process, downregulating E-cadherin expression and overexpressing N-cadherin and vimentin [108, 109], which weakens intercellular adhesive attractions. In addition, increased levels of phosphorylated p38α could downregulate fibulin-3 expression through hypermethylation of regulatory sequences of the gene and then facilitated the invasion and metastasis of tumor cells [110].

TCM can prevent invasion and metastasis of tumor cells via inhibiting ECM degradation and EMT process. Fei-Liu-Ping (FLP) ointment is an oral prescription medication that is used to treat lung cancer patients in China, which has been shown to possess anticancer properties [64]. Li et al. revealed that FLP could inhibit A549 cell invasion and metastasis by increasing E-cadherin expression and decreasing the expression of N-cadherin and MMP9. They also found that FLP performed synergistic effect when combined with cyclophosphamide (CTX) [111]. Baicalein, another antineoplastic compound of Chinese herbs, also inhibited the expression of MMP-9 and MMP-2 via reducing expression of protein kinase Cα (PKCα) and p38 mitogen-activated protein kinase (p38 MAPK) levels in poorly differentiated hepatoma cells [112]. The anti-invasive and antimigratory effects of TCM were also found in curcumin researches. Curcumin can prevent the invasion of hepatocellular carcinoma through inhibiting the production of MMP-9. In addition, it was found that curcumin significantly inhibited adhesion and haptotactic migration to fibronectin and laminin without affecting the expression of integrin on the cell surface. Furthermore, it could also affect the formation of actin stress fibers [113].

3.3. TCM Reverses the Immunosuppressive Phenotype of Tumor Cells. As the immune-editing theory says, immune system plays a critical role in maintaining equilibrium between immune recognition and tumor development, with a dual capacity to both promoting and suppressing tumor growth [114]. During cancer immune editing, the immune system is able to recognize and destroy the most immunologically vulnerable cancer cells. Nonetheless, due to genetic instability, constant tumor cell division generates with reduced immunogenicity that can evade immune elimination [115]. Furthermore, tumor cells can also vary several immune phenotypes to impair the capacity of the immune system to eradicate them by immune suppressive effects [116]. In the recent years, studies have shown that, while being treated by TCM, the expression of programmed cell death protein-1 (PD-1) was decreased. Classic major histocompatibility complex (MHC) and Fas molecules were expressed more on tumor cytomembranes, which leaded tumor cells to be recognized easier and killed by the immune system. In conclusion, TCM can exert a biphasic regulation on tumor cells phenotype to enhance antitumor immune responses.

3.3.1. TCM Promotes Classic MHC Molecules Expression. MHC is the important immunological recognition molecule in the process of tumor immune response. Classic MHC molecule can be divided into two subgroups: MHC I and MHC II; both of them reinforce the interactions of cytotoxic T cell (CTL) or NK cells with tumor cells by presenting tumor antigens to them [117]. However, immune and malignant cells in the tumor microenvironment do not express typical MHC molecules but overexpress sHLA and sNKAL, which may lead to killing CTL and NK cell mediated by apoptosis [118]. Increasing data has shown that TCM can efficiently reverse this harmful phenomenon. Li et al. explored the effect of Invigorating Spleen and Detoxification Decoction (ISD) (*Radix Codonopsis*, *Poria*, *Rhizoma Atractylodis Macrocephalae*, *Radix Glycyrrhizae*, *Radix Bupleuri*, *Rhizoma Curcumae*, and herba *Scutellaria barbata*) on MHC molecules in the rat liver cancer tissue and found that ISD could enhance the expression of MHC I and MHC II both in tumor and in liver tissue, besides prolonging the survival time and decreasing the incidence of cachexia [119].

3.3.2. TCM Reverses the FasL/Fas Expression of Tumor Cells. FasL and its receptor Fas are membrane-bound glycoproteins; the activation of them plays an important role in cell apoptosis. Physiologically, cytotoxic T lymphocytes ($Fas^{low}FasL^{high}$) express FasL combined with the Fas expressing cells (Fas^{high} $FasL^{low}$), resulting in the activation of the Fas receptor, and then mediate target cells apoptosis [120]. However, loss of Fas and gain of aberrant FasL expression are common features of malignant transformation, such as FasL and sFasL, which in turn combined with Fas express lymphocytes and eliminated their activated immune reactions [121]. Our previous study showed that TCM formula Yang Wei Kang Liu (YWKL) (*Radix Astragali*, *Radix Ginseng*, *Hedyotis diffusa*, *Yunnan Manyleaf Paris Rhizome*, *Radix Notoginseng*, *Radix Paeoniae Rubra*, and *Hematoxylon*) could increase Fas expression, downregulate FasL-mRNA expression in MGC-803 stomach cancer cell model in vitro, and induce the apoptosis of MGC-803 cells. Together with the previous research, it indicated that potential mechanisms of YWKL inducing gastric cancer cells apoptosis might be through regulating Fas/FasL pathway so as to enhance cancer cells' sensitivity to immune response cells like CTL [122].

3.3.3. TCM Decreases the Expression of PD-L1. PD-1, which is expressed on activated T and B cells, natural killer cells, and myeloid cells, is an another immune checkpoint [123]. Two ligands for PD-1 have been identified: PD-L1 and PD-L2. Researches indicated that PD-L1 was overexpressed by various tumor cells, including breast cancer, thyroid carcinomas, lung, colon, ovarian, melanoma, bladder, liver, salivary, stomach, and gliomas [124]. PD-L1 can engage the PD-1 receptor and induce T cell exhaustion and eventually inhibit T cell activation and proliferation. Thus, the interaction between PD-1 and its ligands, especially PD-L1, may contribute to

the immune evasion of cancer cells [124]. Recently, Wang et al. found that *Astragalus* polysaccharide (APS), extracted from TCM herb *Astragalus*, could significantly inhibit the growth of B16-F10 melanoma cells in a transplant model and decreased the expression of both PD-L1 protein and PD-L1 mRNA in tumor. This indicated that mechanism may be related to regulating PD-1/PD-L1 pathway to enhance the antitumor immune activity of T lymphocytes [125].

3.4. TCM Reverses the Drug Resistance of Tumor Cells. Over the past few decades, the efficiency of endless chemotherapies did not reach what we have expected. Some experts attributed this phenomenon to the multidrug resistance (MDR) [126]. It was reported that major mechanical of MDR in tumor cells was the overexpression of a membrane-bound protein, P-glycoprotein (P-gp), and other members of the adenosine triphosphate (ATP) binding cassette (ABC) transporter superfamily [127], which translocate a substrate from the intracellular compartment to the extracellular compartment, leading to a reduced intracellular concentration of the substrate and resistance to antineoplastic drugs [128]. However, several other mechanisms are also involved in the development of MDR in tumor cells, including alterations in drug targets, the activation of detoxifying systems, the interruption of signaling pathways, and alterations in regulators involved in cell cycle control [128].

Recently, accumulated basic researches have proven that TCM could reverse multidrug resistance of tumor cells through several pathways. Yiqi Jianpi Huaji Decoction (YJHD), a traditional Chinese medicinal formula composed of twelve ingredients, has recently been reported to have a good clinical therapeutic effect. Li et al. found that low dose YJHD could reverse MDR and increase sensitivity of cancer cells to chemotherapeutic agents in vitro by downregulating P-gp, MRP, TUBB3, and STMN1 expression [129]. MDR can also be reversed by siRNAs targeting genes involved in MDR. Eid et al. also reported that *Fallopia japonica* (FJ) could modulate the function of ABC drug transporters to overcome multidrug resistance in cancer cells [130].

5-Fluorouracil (5-FU), a common chemotherapeutic agent used for tumor treatment, by itself has inadequate response rates, highlighting the need for improving the effects for these patients [131–134]. Baicalein, a flavonoid derived from the root of *Scutellaria baicalensis*, was reported to increase the sensitivity of AGS cells to 5-FU treatment under hypoxia. In addition, the hypoxia-enhanced glycolytic flux and expression of several critical glycolysis-associated enzymes (HK2, LDH-A, and PDK1) in the AGS cells were suppressed by baicalein. These findings suggested that inhibition of glycolysis via regulation of the PTEN/Akt/HIF-1α signaling pathway might be one of the mechanisms whereby baicalein reverses 5-FU resistance in cancer cells under hypoxia [131].

Sorafenib, a standard first-line therapeutic treatment for patients with advanced hepatocellular carcinoma (HCC), is also demonstrated to be hampered by the development of drug resistance in recent years [134, 135]. The activation of Akt by sorafenib was thought to be responsible for this resistance [136]. It was found that Bufalin, which is the major active ingredient of the traditional Chinese medicine Chan Su, could inhibit Akt activation and reverse drug resistance to sorafenib. Further studies reported that bufalin reversed acquired resistance to sorafenib by downregulating phosphorylated Akt in an ER-stress-dependent manner via the inositol-requiring enzyme 1 (IRE1) pathway [137].

3.5. TCM Attenuates Oncogenicity of CSCs. Cancer stem cells (CSCs) existing in the tumor play a crucial role in carcinogenesis though in a very few quantity [138]. Julius Cohnheim once inferred that tumors might arise from stem cells left over from embryonic development [139]. Recently, researches have further demonstrated that CSCs had the ability of self-renewing, invasion, metastasis, immunosuppressive, and multidrug resistance. Hence, CSCs are proposed to be the main cause of cancer relapse after resisting several therapies [140]. Evidences showed that TCM attenuated oncogenicity of CSCs. When treated with bufalin, the sphere of CSCs could not get attached to the flask and failed to differentiate, which was indicated by the stable expression of stem cell marker CD133 and OCT-4 in the condition permissive to differentiation. Treatment of bufalin also suppressed the single cells isolated from the sphere to form sphere again in the nonadhesive culture system, and a decreased expression of proliferation marker Ki67 was also detected in these cells [141]. Besides, CSCs tumor spheres lowly expressed Fas and highly expressed membrane complement regulatory proteins and Foxp3, which were associated with a high frequency of metastasis [142]. Some medicinal herbs had been proved to reverse CSCs immune suppression and suppress CSCs metastasis via dual-blocking epithelial-mesenchymal transition (EMT) and CSCs properties [143]. Pien Tze Huang (PZH), a well-known and ancient TCM formula, was reported as an operative medicine that significantly and does-dependently inhibited the viability and promoted the apoptosis and differentiation of the colorectal CSCs via suppressing the Notch1 pathway. Studies also revealed that PZH markedly inhibited the mRNA levels of ABCB1 and ABCG2, which are members of the ATP-binding cassette (ABC) transporter superfamily, thereby contributing to the side population phenotype and multidrug resistance [144].

4. Summary

The mutual and interdependent interaction between tumor and its microenvironment is a crucial topic in cancer research. Previously, we have made headways in understanding and preventing tumors, but most of them are focused only on cancer cells. Recently, accumulated data indicates that TME played a crucial role in protecting cancer cells [145]. Studies declare that tumor microenvironment consists of cells, soluble factors, signaling molecules, extracellular matrix, and mechanical cues that can promote neoplastic transformation, support tumor growth and invasion, protect the tumor from host immunity, foster therapeutic resistance, and provide niches for dormant metastases to thrive [145]. Thus, multiple abnormal segments have existed in malignant

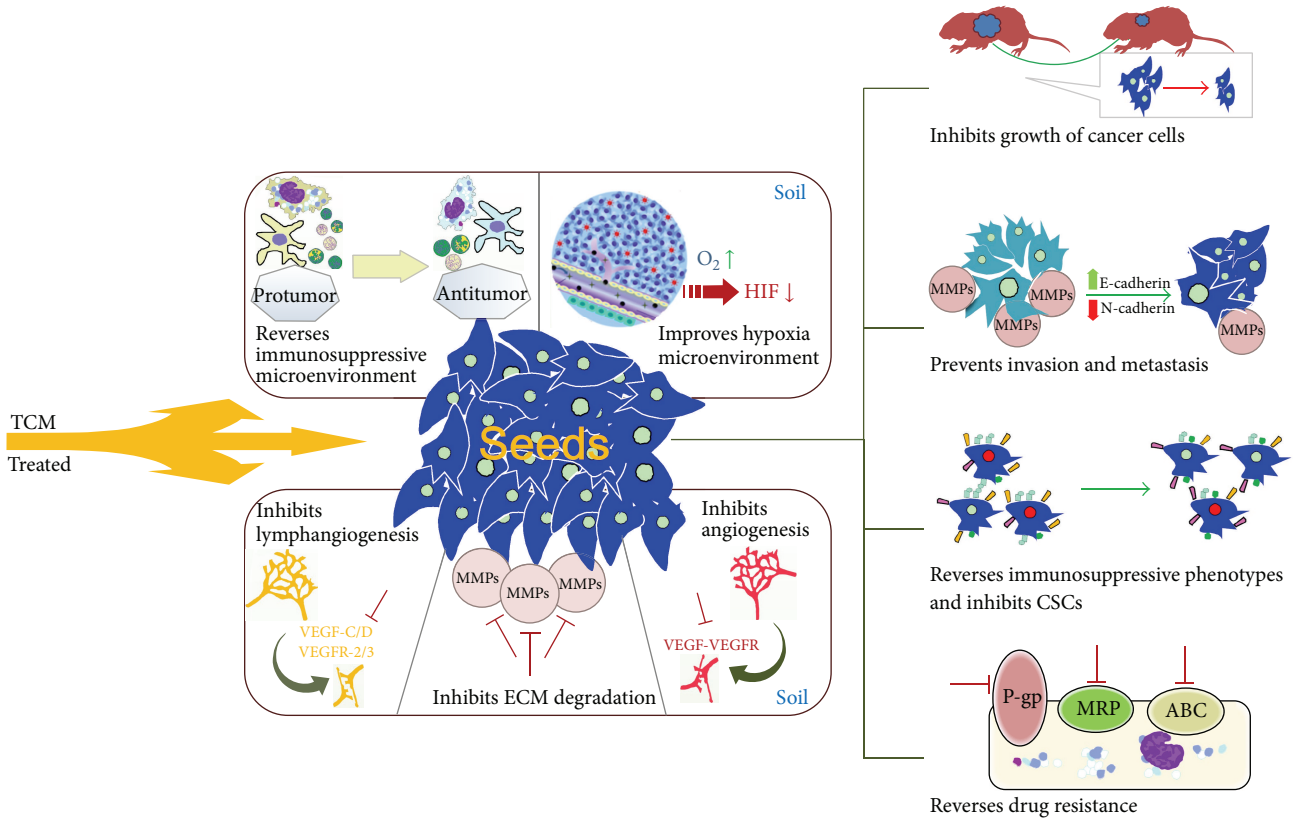

Figure 1: The confirmed mechanism of TCM on tumor cells and microenvironment. TCM can inhibit "seeds" and regulate "soil" to suppress tumor development and recurrence via diverse ways. The mechanism of TCM inhibiting tumor "seeds" includes inhibiting growth, invasion, metastasis of cancer cells, and reversing immunosuppressive phenotypes and drug resistance. Meanwhile, TCM regulates "soil" through remodeling immunosuppressive microenvironment, hypoxia microenvironment, and angiogenesis/lymphangiogenesis, and ECM are also reversed when treated by TCM.

diseases. The synergistic interplay between tumor cells and microenvironment plays a key role in the progress of cancer, for which can offset the actions of soloes target for drugs [146]. So it has become a new trend for researchers to block the interplay between tumor and microenvironment via comprehensive treatments and combined application of drugs.

A better understanding of interplay between tumor cells and microenvironment may be a crucial key to improve therapeutic efficacy. For example, it was well known that radiotherapy directly caused cancer cells death through the induction of DNA damage. In the meantime irradiated tumors stimulated the immune system and caused immunogenic cell death by releasing tumor antigens and damage-associated molecular patterns, which promotes the uptake of dying cells and triggers a cytotoxic T-lymphocyte response [147, 148]. Zegers et al. finally demonstrated that radiotherapy combined with the immune cytokine L19-IL2 could provide long-lasting antitumor effects [148]. Therefore, compared with antitumor immune response that radiotherapy provides alone, the addition of active immunotherapy may increase the therapeutic potential. In addition, it was reported that dying tumor cells through the apoptosis generated potent growth-stimulating signals to stimulate the repopulation of tumors undergoing radiotherapy. And activated caspase 3, a key executioner in apoptosis, was also involved in the growth stimulation [149].

When targeted, cancer cells alter the surrounding tumor microenvironment to protect themselves. Although studies showed that TCM inhibit the cancer cell growth and tumor microenvironment through various mechanisms (Figure 1), the interplay between the tumor cells and microenvironment is not clear. By understanding the mechanisms by which TCM inhibits tumor cells and microenvironment, novel cancer therapeutics can be evolved.

Conflict of Interests

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

Authors' Contribution

Jingnan Xu, Zhuo Song, and Qiujun Guo contributed equally to this work and should be considered as co-first authors.

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References

[1] F. Seretis, C. Seretis, H. Youssef, and M. Chapman, "Colorectal cancer: seed and soil hypothesis revisited," *Anticancer Research*, vol. 34, no. 5, pp. 2087–2094, 2014.

[2] I. J. Fidler, "The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited," *Nature Reviews Cancer*, vol. 3, no. 6, pp. 453–458, 2003.

[3] J. Diao, J. Zhao, E. Winter, and M. S. Cattral, "Tumors suppress in situ proliferation of cytotoxic T cells by promoting differentiation of Gr-1$^+$ conventional dendritic cells through IL-6," *The Journal of Immunology*, vol. 186, no. 9, pp. 5058–5067, 2011.

[4] O. Oluwadara, L. Giacomelli, X. Brant et al., "The role of the microenvironment in tumor immune surveillance," *Bioinformation*, vol. 5, no. 7, pp. 285–290, 2011.

[5] S. Chabot, N. Jabrane-Ferrat, K. Bigot et al., "A novel antiangiogenic and vascular normalization therapy targeted against human CD160 receptor," *The Journal of Experimental Medicine*, vol. 208, no. 5, pp. 973–986, 2011.

[6] E. T. Shinohara and A. Maity, "Increasing sensitivity to radiotherapy and chemotherapy by using novel biological agents that alter the tumor microenvironment," *Current Molecular Medicine*, vol. 9, no. 9, pp. 1034–1045, 2009.

[7] P. Lu, W. Su, Z.-H. Miao, H.-R. Niu, J. Liu, and Q.-L. Hua, "Effect and mechanism of ginsenoside Rg3 on postoperative life span of patients with non-small cell lung cancer," *Chinese Journal of Integrative Medicine*, vol. 14, no. 1, pp. 33–36, 2008.

[8] B. Wang and J. Cui, "Treatment of mid-late stage NSCLC using sodium cantharidinate/vitamin B6/GP regimen in clinic," *Journal of Cancer Research and Therapeutics*, vol. 10, no. 5, pp. 79–81, 2014.

[9] L. Guo, S.-P. Bai, L. Zhao, and X.-H. Wang, "Astragalus polysaccharide injection integrated with vinorelbine and cisplatin for patients with advanced non-small cell lung cancer: effects on quality of life and survival," *Medical Oncology*, vol. 29, no. 3, pp. 1656–1662, 2012.

[10] L. W. Ruan and Y. C. Deng, "Study on effect of Xiaoaiping in enhancing efficacy of neoadjuvant chemotherapy for breast cancer and its mechanism," *Zhongguo Zhong Yao Za Zhi*, vol. 40, no. 4, pp. 749–752, 2015.

[11] Y. Liu, Z. W. Jia, L. Dong, R. Wang, and G. Q. Qiu, "A randomized pilot study of atractylenolide I on gastric cancer cachexia patients," *Evidence-Based Complementary and Alternative Medicine*, vol. 5, no. 3, pp. 337–344, 2008.

[12] C. Zhong, H.-D. Li, D.-Y. Liu et al., "Clinical study of hepatectomy combined with Jianpi Huayu Therapy for hepatocellular carcinoma," *Asian Pacific Journal of Cancer Prevention*, vol. 15, no. 14, pp. 5951–5957, 2014.

[13] T.-J. Qin, X.-H. Zhao, J. Yun, L.-X. Zhang, Z.-P. Ruan, and B.-R. Pan, "Efficacy and safety of gemcitabine-oxaliplatin combined with huachansu in patients with advanced gallbladder carcinoma," *World Journal of Gastroenterology*, vol. 14, no. 33, pp. 5210–5216, 2008.

[14] M. I. James, C. Iwuji, G. Irving et al., "Curcumin inhibits cancer stem cell phenotypes in *ex vivo* models of colorectal liver metastases, and is clinically safe and tolerable in combination with FOLFOX chemotherapy," *Cancer Letters*, vol. 364, no. 2, pp. 135–141, 2015.

[15] D.-R. Lu, D.-Y. Li, X.-Y. Chen, P.-Z. Ye, and S.-D. Tian, "Clinical research of compound Zhebei granules for increasing the therapeutic effect of chemotherapy in refractory acute leukemia patients," *Journal of Traditional Chinese Medicine*, vol. 29, no. 3, pp. 190–194, 2009.

[16] Y. Jia, Q. Guan, Y. Guo, and C. Du, "Reduction of inflammatory hyperplasia in the intestine in colon cancer-prone mice by water-extract of *Cistanche deserticola*," *Phytotherapy Research*, vol. 26, no. 6, pp. 812–819, 2012.

[17] Y. Sun, "Translational horizons in the tumor microenvironment: harnessing breakthroughs and targeting cures," *Medicinal Research Reviews*, vol. 35, no. 2, pp. 408–436, 2015.

[18] J. Li, L. Li, R. Liu, and H.-S. Lin, "Establishing Chinese medicine characteristic tumor response evaluation system is the key to promote internationalization of Chinese medicine oncology," *Chinese Journal of Integrative Medicine*, vol. 18, no. 10, pp. 730–736, 2012.

[19] M. W. Pickup, J. K. Mouw, and V. M. Weaver, "The extracellular matrix modulates the hallmarks of cancer," *EMBO Reports*, vol. 15, no. 12, pp. 1243–1253, 2014.

[20] P. Lu, V. M. Weaver, and Z. Werb, "The extracellular matrix: a dynamic niche in cancer progression," *The Journal of Cell Biology*, vol. 196, no. 4, pp. 395–406, 2012.

[21] M. Giussani, G. Merlino, V. Cappelletti, E. Tagliabue, and M. G. Daidone, "Tumor-extracellular matrix interactions: identification of tools associated with breast cancer progression," *Seminars in Cancer Biology*, vol. 35, pp. 3–10, 2015.

[22] A. W. Holle, J. L. Young, and J. P. Spatz, "*In vitro* cancer cell-ECM interactions inform *in vivo* cancer treatment," *Advanced Drug Delivery Reviews*, vol. 97, pp. 270–279, 2016.

[23] C. A. Sherman-Baust, A. T. Weeraratna, L. B. A. Rangel et al., "Remodeling of the extracellular matrix through overexpression of collagen VI contributes to cisplatin resistance in ovarian cancer cells," *Cancer Cell*, vol. 3, no. 4, pp. 377–386, 2003.

[24] W. Deng, H. Sui, Q. Wang et al., "A Chinese herbal formula, Yi-Qi-Fu-Sheng, inhibits migration/invasion of colorectal cancer by down-regulating MMP-2/9 via inhibiting the activation of ERK/MAPK signaling pathways," *BMC Complementary and Alternative Medicine*, vol. 13, article 65, 2013.

[25] R. Zhou, L. Xu, M. Ye, M. Liao, H. Du, and H. Chen, "Formononetin inhibits migration and invasion of MDA-MB-231 and 4T1 breast cancer cells by suppressing MMP-2 and MMP-9 through PI3K/AKT signaling pathways," *Hormone and Metabolic Research*, vol. 46, no. 11, pp. 753–760, 2014.

[26] L. Zheng, Y.-M. Zhang, Y.-Z. Zhan, and C.-X. Liu, "Momordica cochinchinensis seed extracts suppress migration and invasion of human breast cancer ZR-75-30 cells via down-regulating MMP-2 and MMP-9," *Asian Pacific Journal of Cancer Prevention*, vol. 15, no. 3, pp. 1105–1110, 2014.

[27] G. Kroemer, G. Mariño, and B. Levine, "Autophagy and the integrated stress response," *Molecular Cell*, vol. 40, no. 2, pp. 280–293, 2010.

[28] P. Vaupel, M. Höckel, and A. Mayer, "Detection and characterization of tumor hypoxia using pO_2 histography," *Antioxidants and Redox Signaling*, vol. 9, no. 8, pp. 1221–1235, 2007.

[29] J. L. Yu, J. W. Rak, B. L. Coomber, D. J. Hicklin, and R. S. Kerbel, "Effect of p53 status on tumor response to antiangiogenic therapy," *Science*, vol. 295, no. 5559, pp. 1526–1528, 2002.

[30] X. Yang, D.-D. Yu, F. Yan et al., "The role of autophagy induced by tumor microenvironment in different cells and stages of cancer," *Cell & Bioscience*, vol. 5, article 14, 2015.

[31] Z. Zhou, H. Liu, X. Chi et al., "A protein-corona-free T_1–T_2 dual-modal contrast agent for accurate imaging of lymphatic tumor metastasis," *ACS Applied Materials & Interfaces*, vol. 7, no. 51, pp. 28286–28293, 2015.

[32] 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.

[33] Y. Lv, S. Zhao, J. Han, L. Zheng, Z. Yang, and L. Zhao, "Hypoxia-inducible factor-1α induces multidrug resistance protein in colon cancer," *OncoTargets and Therapy*, vol. 8, pp. 1941–1948, 2015.

[34] E. A. Turley, M. Veiseh, D. C. Radisky, and M. J. Bissell, "Mechanisms of disease: epithelial-mesenchymal transition—does cellular plasticity fuel neoplastic progression?" *Nature Clinical Practice Oncology*, vol. 5, no. 5, pp. 280–290, 2008.

[35] H. Chen, A. Shen, Y. Zhang et al., "Pien Tze Huang inhibits hypoxia-induced epithelial-mesenchymal transition in human colon carcinoma cells through suppression of the HIF-1 pathway," *Experimental and Therapeutic Medicine*, vol. 7, no. 5, pp. 1237–1242, 2014.

[36] Q. Dai, Q. Yin, L. Wei et al., "Oroxylin A regulates glucose metabolism in response to hypoxic stress with the involvement of Hypoxia-inducible factor-1 in human hepatoma HepG2 cells," *Molecular Carcinogenesis*, 2015.

[37] K. Zhao, X. Song, Y. Huang et al., "Wogonin inhibits LPS-induced tumor angiogenesis via suppressing PI3K/Akt/NF-κB signaling," *European Journal of Pharmacology*, vol. 737, pp. 57–69, 2014.

[38] H. Wang, L. Zhao, L.-T. Zhu et al., "Wogonin reverses hypoxia resistance of human colon cancer HCT116 cells via downregulation of HIF-1α and glycolysis, by inhibiting PI3K/Akt signaling pathway," *Molecular Carcinogenesis*, vol. 53, no. 1, pp. E107–E118, 2014.

[39] G. Bellone, A. Carbone, C. Smirne et al., "Cooperative induction of a tolerogenic dendritic cell phenotype by cytokines secreted by pancreatic carcinoma cells," *The Journal of Immunology*, vol. 177, no. 5, pp. 3448–3460, 2006.

[40] B.-N. Lee, M. Follen, G. Rodriquez et al., "Deficiencies in myeloid antigen-presenting cells in women with cervical squamous intraepithelial lesions," *Cancer*, vol. 107, no. 5, pp. 999–1007, 2006.

[41] I. Perrot, D. Blanchard, N. Freymond et al., "Dendritic cells infiltrating human non-small cell lung cancer are blocked at immature stage," *The Journal of Immunology*, vol. 178, no. 5, pp. 2763–2769, 2007.

[42] L. A. Ormandy, A. Färber, T. Cantz et al., "Direct ex vivo analysis of dendritic cells in patients with hepatocellular carcinoma," *World Journal of Gastroenterology*, vol. 12, no. 20, pp. 3275–3282, 2006.

[43] D. Gabrilovich, "Mechanisms and functional significance of tumour-induced dendritic-cell defects," *Nature Reviews Immunology*, vol. 4, no. 12, pp. 941–952, 2004.

[44] Y. Tian, X. Li, H. Li, Q. Lu, G. Sun, and H. Chen, "*Astragalus mongholicus* regulate the Toll-like-receptor 4 meditated signal transduction of dendritic cells to restrain stomach cancer cells," *African Journal of Traditional, Complementary and Alternative Medicines*, vol. 11, no. 3, pp. 92–96, 2014.

[45] J. E. Talmadge and D. I. Gabrilovich, "History of myeloid-derived suppressor cells," *Nature Reviews Cancer*, vol. 13, no. 10, pp. 739–752, 2013.

[46] G. Schiavoni, L. Gabriele, and F. Mattei, "The tumor microenvironment: a pitch for multiple players," *Frontiers in Oncology*, vol. 3, article 90, 2013.

[47] R. Wang, Y. Li, W. Wang, M. Zhou, and Z. Cao, "Compound K suppresses myeloid-derived suppressor cells in a mouse model bearing CT26 colorectal cancer xenograft," *Nan Fang Yi Ke Da Xue Xue Bao*, vol. 35, no. 5, pp. 748–752, 2015.

[48] B. Ruffell, N. I. Affara, and L. M. Coussens, "Differential macrophage programming in the tumor microenvironment," *Trends in Immunology*, vol. 33, no. 3, pp. 119–126, 2012.

[49] B.-Z. Qian and J. W. Pollard, "Macrophage diversity enhances tumor progression and metastasis," *Cell*, vol. 141, no. 1, pp. 39–51, 2010.

[50] J. W. Pollard, "Trophic macrophages in development and disease," *Nature Reviews Immunology*, vol. 9, no. 4, pp. 259–270, 2009.

[51] G. Solinas, G. Germano, A. Mantovani, and P. Allavena, "Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation," *Journal of Leukocyte Biology*, vol. 86, no. 5, pp. 1065–1073, 2009.

[52] S. F. Schoppmann, P. Birner, J. Stöckl et al., "Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis," *The American Journal of Pathology*, vol. 161, no. 3, pp. 947–956, 2002.

[53] H. Y. Tan, N. Wang, K. Man, S. W. Tsao, C. M. Che, and Y. Feng, "Autophagy-induced RelB/p52 activation mediates tumour-associated macrophage repolarisation and suppression of hepatocellular carcinoma by natural compound baicalin," *Cell Death & Disease*, vol. 6, no. 10, Article ID e1942, 2015.

[54] S.-Q. Gong, W. Sun, M. Wang, and Y.-Y. Fu, "Role of TLR4 and TCR or BCR against baicalin-induced responses in T and B cells," *International Immunopharmacology*, vol. 11, no. 12, pp. 2176–2180, 2011.

[55] X. Qi, G. Liu, L. Qiu, X. Lin, and M. Liu, "Marine bromophenol bis(2,3-dibromo-4,5-dihydroxybenzyl) ether, represses angiogenesis in HUVEC cells and in zebrafish embryos via inhibiting the VEGF signal systems," *Biomedicine & Pharmacotherapy*, vol. 75, pp. 58–66, 2015.

[56] Y.-C. Hseu, S.-C. Chen, W.-H. Lin et al., "Toona sinensis (leaf extracts) inhibit vascular endothelial growth factor (VEGF)-induced angiogenesis in vascular endothelial cells," *Journal of Ethnopharmacology*, vol. 134, no. 1, pp. 111–121, 2011.

[57] N. Ferrara, "Vascular endothelial growth factor: basic science and clinical progress," *Endocrine Reviews*, vol. 25, no. 4, pp. 581–611, 2004.

[58] C. I. M. Baeten, F. Hillen, P. Pauwels, A. P. de Bruine, and C. G. M. I. Baeten, "Prognostic role of vasculogenic mimicry in colorectal cancer," *Diseases of the Colon and Rectum*, vol. 52, no. 12, pp. 2028–2035, 2009.

[59] B. Sun, S. Zhang, D. Zhang et al., "Vasculogenic mimicry is associated with high tumor grade, invasion and metastasis, and short survival in patients with hepatocellular carcinoma," *Oncology Reports*, vol. 16, no. 4, pp. 693–698, 2006.

[60] M. Li, Y. Gu, Z. Zhang et al., "Vasculogenic mimicry: a new prognostic sign of gastric adenocarcinoma," *Pathology and Oncology Research*, vol. 16, no. 2, pp. 259–266, 2010.

[61] J.-M. Lü, Q. Yao, and C. Chen, "Ginseng compounds: an update on their molecular mechanisms and medical applications," *Current Vascular Pharmacology*, vol. 7, no. 3, pp. 293–302, 2009.

[62] D. F. Zeng, J. L. Wang, P. Y. Kong, C. Chang, J. Li, and J. Li, "Ginsenoside Rg3 inhibits HIF-1α and VEGF expression in patient with acute leukemia via inhibiting the activation of PI3K/Akt and ERK1/2 pathways," *International Journal of Clinical and Experimental Pathology*, vol. 7, no. 5, pp. 2172–2178, 2014.

[63] N. Koonrungsesomboon, K. Na-Bangchang, and J. Karbwang, "Therapeutic potential and pharmacological activities of *Atractylodes lancea* (Thunb.) DC," *Asian Pacific Journal of Tropical Medicine*, vol. 7, no. 6, pp. 421–428, 2014.

[64] S. Wang, R. Cai, J. Ma et al., "The natural compound codonolactone impairs tumor induced angiogenesis by downregulating BMP signaling in endothelial cells," *Phytomedicine*, vol. 22, no. 11, pp. 1017–1026, 2015.

[65] J.-M. Lin, L.-H. Wei, Y.-Q. Chen et al., "Pien Tze Huang (片仔癀)-induced apoptosis in human colon cancer HT-29 cells is associated with regulation of the Bcl-2 family and activation of caspase 3," *Chinese Journal of Integrative Medicine*, vol. 17, no. 9, pp. 685–690, 2011.

[66] Q. Zhuang, F. Hong, A. Shen et al., "Pien Tze Huang inhibits tumor cell proliferation and promotes apoptosis via suppressing the STAT3 pathway in a colorectal cancer mouse model," *International Journal of Oncology*, vol. 40, no. 5, pp. 1569–1574, 2012.

[67] A.-L. Shen, F. Hong, L.-Y. Liu et al., "Effects of Pien Tze Huang on angiogenesis in vivo and in vitro," *Chinese Journal of Integrative Medicine*, vol. 18, no. 6, pp. 431–436, 2012.

[68] A. Shen, F. Hong, L. Liu et al., "Pien Tze Huang inhibits the proliferation of human colon carcinoma cells by arresting G1/S cell cycle progression," *Oncology Letters*, vol. 4, no. 4, pp. 767–770, 2012.

[69] A. Shen, J. Lin, Y. Chen et al., "Pien Tze Huang inhibits tumor angiogenesis in a mouse model of colorectal cancer via suppression of multiple cellular pathways," *Oncology Reports*, vol. 30, no. 4, pp. 1701–1706, 2013.

[70] A. Shen, Y. Chen, F. Hong et al., "Pien Tze Huang suppresses IL-6-inducible STAT3 activation in human colon carcinoma cells through induction of SOCS3," *Oncology Reports*, vol. 28, no. 6, pp. 2125–2130, 2012.

[71] G. W. Yang, J. S. Jiang, and W. Q. Lu, "Ferulic acid exerts anti-angiogenic and anti-tumor activity by targeting fibroblast growth factor receptor 1-mediated angiogenesis," *International Journal of Molecular Sciences*, vol. 16, no. 10, pp. 24011–24031, 2015.

[72] W. Zheng, A. Aspelund, and K. Alitalo, "Lymphangiogenic factors, mechanisms, and applications," *The Journal of Clinical Investigation*, vol. 124, no. 3, pp. 878–887, 2014.

[73] S. Karaman and M. Detmar, "Mechanisms of lymphatic metastasis," *The Journal of Clinical Investigation*, vol. 124, no. 3, pp. 922–928, 2014.

[74] X. Kang, J. Kim, M. Deng et al., "Inhibitory leukocyte immunoglobulin-like receptors: immune checkpoint proteins and tumor sustaining factors," *Cell Cycle*, vol. 15, no. 1, pp. 25–40, 2016.

[75] Y. Kimura and M. Sumiyoshi, "Anti-tumor and anti-metastatic actions of wogonin isolated from *Scutellaria baicalensis* roots through anti-lymphangiogenesis," *Phytomedicine*, vol. 20, no. 3-4, pp. 328–336, 2013.

[76] Y.-Z. Fan, J.-Y. Fu, Z.-M. Zhao, and C.-Q. Chen, "Inhibitory effect of norcantharidin on the growth of human gallbladder carcinoma GBC-SD cells in vitro," *Hepatobiliary & Pancreatic Diseases International*, vol. 6, no. 1, pp. 72–80, 2007.

[77] Y.-Z. Fan, Z.-M. Zhao, J.-Y. Fu, C.-Q. Chen, and W. Sun, "Norcantharidin inhibits growth of human gallbladder carcinoma xenografted tumors in nude mice by inducing apoptosis and blocking the cell cycle in vivo," *Hepatobiliary & Pancreatic Diseases International*, vol. 9, no. 4, pp. 414–422, 2010.

[78] C.-C. Yu, F.-Y. Ko, C.-S. Yu et al., "Norcantharidin triggers cell death and DNA damage through S-phase arrest and ROS-modulated apoptotic pathways in TSGH 8301 human urinary bladder carcinoma cells," *International Journal of Oncology*, vol. 41, no. 3, pp. 1050–1060, 2012.

[79] S.-H. Kok, S.-J. Cheng, C.-Y. Hong et al., "Norcantharidin-induced apoptosis in oral cancer cells is associated with an increase of proapoptotic to antiapoptotic protein ratio," *Cancer Letters*, vol. 217, no. 1, pp. 43–52, 2005.

[80] Y.-P. Ho, K. K. W. To, S. C. F. Au-Yeung, X. Wang, G. Lin, and X. Han, "Potential new antitumor agents from an innovative combination of demethylcantharidin, a modified traditional chinese medicine, with a platinum moiety," *Journal of Medicinal Chemistry*, vol. 44, no. 13, pp. 2065–2068, 2001.

[81] J. A. Nagy, E. Vasile, D. Feng et al., "Vascular permeability factor/vascular endothelial growth factor induces lymphangiogenesis as well as angiogenesis," *The Journal of Experimental Medicine*, vol. 196, no. 11, pp. 1497–1506, 2002.

[82] F. Räßler, U. Voigt, S. Metz, P. Elsner, and S. Schliemann, "Fast-growing tumor of the eyelid," *Journal der Deutschen Dermatologischen Gesellschaft*, vol. 14, no. 1, pp. 81–84, 2016.

[83] D. Hanahan and R. A. Weinberg, "Hallmarks of cancer: the next generation," *Cell*, vol. 144, no. 5, pp. 646–674, 2011.

[84] V. Catalano, A. Turdo, S. Di Franco, F. Dieli, M. Todaro, and G. Stassi, "Tumor and its microenvironment: a synergistic interplay," *Seminars in Cancer Biology*, vol. 23, no. 6, pp. 522–532, 2013.

[85] S. Wang, X. Wu, M. Tan et al., "Fighting fire with fire: poisonous Chinese herbal medicine for cancer therapy," *Journal of Ethnopharmacology*, vol. 140, no. 1, pp. 33–45, 2012.

[86] F. He, H.-N. Wu, M.-Y. Cai et al., "Inhibition of ovarian cancer cell proliferation by Pien Tze Huang via the AKT-mTOR pathway," *Oncology Letters*, vol. 7, no. 6, pp. 2047–2052, 2014.

[87] E. Hervouet, M. Cheray, F. M. Vallette, and P. Cartron, "DNA methylation and apoptosis resistance in cancer cells," *Cells*, vol. 2, no. 3, pp. 545–573, 2013.

[88] C.-M. Hsu, Y. Tsai, L. Wan, and F.-J. Tsai, "Bufalin induces G2/M phase arrest and triggers autophagy via the TNF, JNK, BECN-1 and ATG8 pathway in human hepatoma cells," *International Journal of Oncology*, vol. 43, no. 1, pp. 338–348, 2013.

[89] B. Wang, Q. Han, and Y. Zhu, "Oxymatrine inhibited cell proliferation by inducing apoptosis in human lung cancer A549 cells," *Bio-Medical Materials and Engineering*, vol. 26, pp. S165–S172, 2015.

[90] D. A. Liebermann, B. Hoffman, and D. Vesely, "p53 induced growth arrest versus apoptosis and its modulation by survival cytokines," *Cell Cycle*, vol. 6, no. 2, pp. 166–170, 2007.

[91] F. Liu, J.-G. Wang, S.-Y. Wang, Y. Li, Y.-P. Wu, and S.-M. Xi, "Antitumor effect and mechanism of Gecko on human esophageal carcinoma cell lines in vitro and xenografted sarcoma 180 in Kunming mice," *World Journal of Gastroenterology*, vol. 14, no. 25, pp. 3990–3996, 2008.

[92] L. Ma and W. Li, "Emodin inhibits LOVO colorectal cancer cell proliferation via the regulation of the Bcl-2/Bax ratio and cytochrome c," *Experimental and Therapeutic Medicine*, vol. 8, no. 4, pp. 1225–1228, 2014.

[93] R. M. Mohammad, I. Muqbil, L. Lowe et al., "Broad targeting of resistance to apoptosis in cancer," *Seminars in Cancer Biology*, vol. 35, supplement, pp. S78–S103, 2015.

[94] P. Plastina, D. Bonofiglio, D. Vizza et al., "Identification of bioactive constituents of *Ziziphus jujube* fruit extracts exerting antiproliferative and apoptotic effects in human breast cancer cells," *Journal of Ethnopharmacology*, vol. 140, no. 2, pp. 325–332, 2012.

[95] B. Wang, Q. Han, and Y. Zhu, "Oxymatrine inhibited cell proliferation by inducing apoptosis in human lung cancer A549 cells," *Bio-Medical Materials and Engineering*, vol. 26, supplement 1, pp. S165–S172, 2015.

[96] L. Chu, H. Zhao, J. Fang et al., "The traditional Chinese medicinal formula BDL301 suppresses tumor growth by inhibiting STAT3 pathway and inducing apoptosis in colorectal cancer cells," *DNA and Cell Biology*, vol. 34, no. 3, pp. 178–188, 2015.

[97] X.-Y. Dang, L. Dong, H.-T. Shi, and B.-C. Zou, "Effects of serum containing Chinese medicine Sanpi Pingwei (散癖平胃) formula on proliferation and apoptosis of human SGC-7901 cells," *Chinese Journal of Integrative Medicine*, vol. 19, no. 2, pp. 119–126, 2013.

[98] N. Li, D. Zheng, J. Xue et al., "Cidan inhibits liver cancer cell growth by reducing COX-2 and VEGF expression and cell cycle arrest," *Experimental and Therapeutic Medicine*, vol. 9, no. 5, pp. 1709–1718, 2015.

[99] W. Liu, S. Y. Li, X. E. Huang, J. J. Cui, T. Zhao, and H. Zhang, "Inhibition of tumor growth in vitro by a combination of extracts from Rosa roxburghii Tratt and *Fagopyrum cymosum*," *Asian Pacific Journal of Cancer Prevention*, vol. 13, no. 5, pp. 2409–2414, 2012.

[100] Y.-L. Hsu, M.-H. Yen, P.-L. Kuo et al., "San-Zhong-Kui-Jian-Tang, a traditional Chinese medicine prescription, inhibits the proliferation of human breast cancer cell by blocking cell cycle progression and inducing apoptosis," *Biological & Pharmaceutical Bulletin*, vol. 29, no. 12, pp. 2388–2394, 2006.

[101] X. S. Chai, X. X. Zhang, and W. Y. Wu, "Xiaoji Decoction inhibited cell proliferation and induced apoptosis through Akt signaling pathway in human lung cancer A549 cells," *Chinese Journal of Integrative Medicine*, vol. 20, no. 9, pp. 701–705, 2014.

[102] S.-Y. Xi, Y.-H. Teng, Y. Chen et al., "Jianpi Huayu Decoction inhibits proliferation in human colorectal cancer cells (SW480) by inducing G0/G1-phase cell cycle arrest and apoptosis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 236506, 8 pages, 2015.

[103] L. Zhang, W. Tian, S. Kim, W. Ding, Y. Tong, and S. Chen, "Arsenic sulfide, the main component of realgar, a traditional Chinese medicine, induces apoptosis of gastric cancer cells in vitro and in vivo," *Drug Design Development and Therapy*, vol. 9, pp. 79–92, 2015.

[104] M. Qiao, S. Sheng, and A. B. Pardee, "Metastasis and AKT activation," *Cell Cycle*, vol. 7, no. 19, pp. 2991–2996, 2008.

[105] J. K. Beatty, A. Bhargava, and A. G. Buret, "Post-infectious irritable bowel syndrome: mechanistic insights into chronic disturbances following enteric infection," *World Journal of Gastroenterology*, vol. 20, no. 14, pp. 3976–3985, 2014.

[106] Z. Liu, Z. Liu, X. Zhang, P. Xue, and H. Zhang, "RY10-4 suppressed metastasis of MDA-MB-231 by stabilizing ECM and E-cadherin," *Biomedicine & Pharmacotherapy*, vol. 68, no. 4, pp. 439–445, 2014.

[107] Y. Zhang, J. Liu, J. Kou, J. Yu, and B. Yu, "DT-13 suppresses MDA-MB-435 cell adhesion and invasion by inhibiting MMP-2/9 via the p38 MAPK pathway," *Molecular Medicine Reports*, vol. 6, no. 5, pp. 1121–1125, 2012.

[108] X. Liu, R. Dong, Z. Jiang et al., "MDC1 promotes ovarian cancer metastasis by inducing epithelial-mesenchymal transition," *Tumor Biology*, vol. 36, no. 6, pp. 4261–4269, 2015.

[109] M. S. Tretiakova, J. Hart, M. T. Shabani-Rad, J. Zhang, and Z.-H. Gao, "Distinction of hepatocellular adenoma from hepatocellular carcinoma with and without cirrhosis using E-cadherin and matrix metalloproteinase immunohistochemistry," *Modern Pathology*, vol. 22, no. 8, pp. 1113–1120, 2009.

[110] M. Arechederra, N. Priego, A. Vázquez-Carballo et al., "p38 MAPK down-regulates fibulin 3 expression through methylation of gene regulatory sequences: role in migration and invasion," *The Journal of Biological Chemistry*, vol. 290, no. 7, pp. 4383–4397, 2015.

[111] W. Li, C. Chen, S. M. Saud et al., "Fei-Liu-Ping ointment inhibits lung cancer growth and invasion by suppressing tumor inflammatory microenvironment," *BMC Complementary and Alternative Medicine*, vol. 14, article 153, 2014.

[112] Y.-W. Chiu, T.-H. Lin, W.-S. Huang et al., "Baicalein inhibits the migration and invasive properties of human hepatoma cells," *Toxicology and Applied Pharmacology*, vol. 255, no. 3, pp. 316–326, 2011.

[113] Y. Ohashi, Y. Tsuchiya, K. Koizumi, H. Sakurai, and I. Saiki, "Prevention of intrahepatic metastasis by curcumin in an orthotopic implantation model," *Oncology*, vol. 65, no. 3, pp. 250–258, 2003.

[114] D. S. Vinay, E. P. Ryan, G. Pawelec et al., "Immune evasion in cancer: mechanistic basis and therapeutic strategies," *Seminars in Cancer Biology*, vol. 35, supplement, pp. S185–S198, 2015.

[115] J. B. Swann and M. J. Smyth, "Immune surveillance of tumors," *The Journal of Clinical Investigation*, vol. 117, no. 5, pp. 1137–1146, 2007.

[116] J. W. Shay and I. B. Roninson, "Hallmarks of senescence in carcinogenesis and cancer therapy," *Oncogene*, vol. 23, no. 16, pp. 2919–2933, 2004.

[117] Q. Guo, J. Li, and H. Lin, "Effect and molecular mechanisms of traditional chinese medicine on regulating tumor immunosuppressive microenvironment," *BioMed Research International*, vol. 2015, Article ID 261620, 12 pages, 2015.

[118] M. Campoli and S. Ferrone, "Tumor escape mechanisms: potential role of soluble HLA antigens and NK cells activating ligands," *Tissue Antigens*, vol. 72, no. 4, pp. 321–334, 2008.

[119] Y.-L. Li, B.-G. Sun, T. Xiang, Z.-X. Chen, and S.-J. Zhang, "Effect of invigorating spleen and detoxification decoction on MHC I/MHC II in spleen-deficiency liver cancer rats survival," *Journal of Chinese Medicinal Materials*, vol. 37, no. 3, pp. 454–460, 2014.

[120] M. Villa-Morales and J. Fernández-Piqueras, "Targeting the Fas/FasL signaling pathway in cancer therapy," *Expert Opinion on Therapeutic Targets*, vol. 16, no. 1, pp. 85–101, 2012.

[121] E. Reichmann, "The biological role of the Fas/FasL system during tumor formation and progression," *Seminars in Cancer Biology*, vol. 12, no. 4, pp. 309–315, 2002.

[122] J. Li, G.-Z. Sun, H.-S. Lin et al., "The herb medicine formula 'Yang Wei Kang Liu' improves the survival of late stage gastric cancer patients and induces the apoptosis of human gastric cancer cell line through Fas/Fas ligand and Bax/Bcl-2 pathways," *International Immunopharmacology*, vol. 8, no. 9, pp. 1196–1206, 2008.

[123] M. Kedmi, A. Avigdor, and A. Nagler, "Anti-PD-1-targeted therapies focusing on lymphatic malignancies: biological rationale, clinical challenges and opportunities," *Acta Haematologica*, vol. 133, no. 2, pp. 129–135, 2014.

[124] 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.

[125] J. R. Wang, J. Y. Wang, T. T. Zhang, and X. D. Cheng, "Regulate molecular expression of PD-1/PD-Ls by Astragalus polysaccharides on melanoma mice," *Acta Universitatis Traditionis Medicalis Sinensis Pharmacologiaeque Shanghai*, vol. 28, no. 5, pp. 74–79, 2014.

[126] J. Gu, X. Fang, J. Hao, and X. Sha, "Reversal of P-glycoprotein-mediated multidrug resistance by CD44 antibody-targeted nanocomplexes for short hairpin RNA-encoding plasmid DNA delivery," *Biomaterials*, vol. 45, pp. 99–114, 2015.

[127] J. C. Leighton Jr. and L. J. Goldstein, "P-glycoprotein in adult solid tumors: expression and prognostic significance," *Hematology/Oncology Clinics of North America*, vol. 9, no. 2, pp. 251–273, 1995.

[128] E. K. Fetisova, A. V. Avetisyan, D. S. Izyumov, M. V. Korotetskaya, B. V. Chernyak, and V. P. Skulachev, "Mitochondria-targeted antioxidant SkQR1 selectively protects MDR (Pgp 170)-negative cells against oxidative stress," *FEBS Letters*, vol. 584, no. 3, pp. 562–566, 2010.

[129] W.-B. Li, Y. Li, C. Yu, and Y.-M. He, "Reversal of multidrug resistance by the Chinese medicine Yiqi Jianpi Huaji decoction and the mechanism of action in human gastric cancer SGC7901/VCR Cells," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 390812, 11 pages, 2015.

[130] S. Y. Eid, M. Z. El-Readi, M. L. Ashour, and M. Wink, "*Fallopia japonica*, a natural modulator, can overcome multidrug resistance in cancer cells," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 868424, 8 pages, 2015.

[131] F. Chen, M. Zhuang, C. Zhong et al., "Baicalein reverses hypoxia-induced 5-FU resistance in gastric cancer AGS cells through suppression of glycolysis and the PTEN/Akt/HIF-1α signaling pathway," *Oncology Reports*, vol. 33, no. 1, pp. 457–463, 2015.

[132] H. Sui, L.-H. Zhou, Y.-L. Zhang et al., "Evodiamine suppresses ABCG2 mediated drug resistance by inhibiting p50/NF-κB pathway in colorectal cancer," *Journal of Cellular Biochemistry*, 2015.

[133] C.-Z. Wang, X. Luo, B. Zhang et al., "Notoginseng enhances anti-cancer effect of 5-fluorouracil on human colorectal cancer cells," *Cancer Chemotherapy and Pharmacology*, vol. 60, no. 1, pp. 69–79, 2007.

[134] J. M. Llovet, S. Ricci, V. Mazzaferro et al., "Sorafenib in advanced hepatocellular carcinoma," *The New England Journal of Medicine*, vol. 359, no. 4, pp. 378–390, 2008.

[135] B. Zhai and X.-Y. Sun, "Mechanisms of resistance to sorafenib and the corresponding strategies in hepatocellular carcinoma," *World Journal of Hepatology*, vol. 5, no. 7, pp. 345–352, 2013.

[136] B. Zhai, F. Hu, X. Jiang et al., "Inhibition of Akt reverses the acquired resistance to sorafenib by switching protective autophagy to autophagic cell death in hepatocellular carcinoma," *Molecular Cancer Therapeutics*, vol. 13, no. 6, pp. 1589–1598, 2014.

[137] B. Zhai, F. Hu, H. Yan et al., "Bufalin reverses resistance to sorafenib by inhibiting Akt activation in hepatocellular carcinoma: the role of endoplasmic reticulum stress," *PLoS ONE*, vol. 10, no. 9, Article ID e0138485, 2015.

[138] M. F. Clarke, J. E. Dick, P. B. Dirks et al., "Cancer stem cells—perspectives on current status and future directions: AACR workshop on cancer stem cells," *Cancer Research*, vol. 66, no. 19, pp. 9339–9344, 2006.

[139] Y.-C. He, F.-L. Zhou, Y. Shen, D.-F. Liao, and D. Cao, "Apoptotic death of cancer stem cells for cancer therapy," *International Journal of Molecular Sciences*, vol. 15, no. 5, pp. 8335–8351, 2014.

[140] D. E. Costea, O. Tsinkalovsky, O. K. Vintermyr, A. C. Johannessen, and I. C. Mackenzie, "Cancer stem cells—new and potentially important targets for the therapy of oral squamous cell carcinoma," *Oral Diseases*, vol. 12, no. 5, pp. 443–454, 2006.

[141] Y. Chang, Y. Zhao, H. Zhan, X. Wei, T. Liu, and B. Zheng, "Bufalin inhibits the differentiation and proliferation of human osteosarcoma cell line hMG63-derived cancer stem cells," *Tumor Biology*, vol. 35, no. 2, pp. 1075–1082, 2014.

[142] E. R. Carlson, D. Oreadi, and J. M. McCoy, "Nevoid basal cell carcinoma syndrome and the keratocystic odontogenic tumor," *Journal of Oral and Maxillofacial Surgery*, vol. 73, no. 12, pp. S77–S86, 2015.

[143] W. Li, Q. Wang, Q. Su et al., "Honokiol suppresses renal cancer cells' metastasis *Via* dual-blocking epithelial-mesenchymal transition and cancer stem cell properties through modulating miR-141/ZEB2 signaling," *Molecules and Cells*, vol. 37, no. 5, pp. 383–388, 2014.

[144] L. Wei, P. Chen, Y. Chen et al., "Pien Tze Huang suppresses the stem-like side population in colorectal cancer cells," *Molecular Medicine Reports*, vol. 9, no. 1, pp. 261–266, 2014.

[145] M. A. Swartz, N. Iida, E. W. Roberts et al., "Tumor microenvironment complexity: emerging roles in cancer therapy," *Cancer Research*, vol. 72, no. 10, pp. 2473–2480, 2012.

[146] T. Yin, S. He, T. Ye, G. Shen, Y. Wan, and Y. Wang, "Antiangiogenic therapy using sunitinib combined with rapamycin retards tumor growth but promotes metastasis," *Translational Oncology*, vol. 7, no. 2, pp. 221–229, 2014.

[147] C.-Y. Wu, L.-H. Yang, H.-Y. Yang et al., "Enhanced cancer radiotherapy through immunosuppressive stromal cell destruction in tumors," *Clinical Cancer Research*, vol. 20, no. 3, pp. 644–657, 2014.

[148] C. M. Zegers, N. H. Rekers, D. H. Quaden et al., "Radiotherapy combined with the immunocytokine L19-IL2 provides long-lasting antitumor effects," *Clinical Cancer Research*, vol. 21, no. 5, pp. 1151–1160, 2015.

[149] Q. Huang, F. Li, X. Liu et al., "Caspase 3-mediated stimulation of tumor cell repopulation during cancer radiotherapy," *Nature Medicine*, vol. 17, no. 7, pp. 860–866, 2011.

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Review Article

Prognostic Indications of Elevated MCT4 and CD147 across Cancer Types: A Meta-Analysis

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Background. Metabolism in the tumor microenvironment can play a critical role in tumorigenesis and tumor aggression. Metabolic coupling may occur between tumor compartments; this phenomenon can be prognostically significant and may be conserved across tumor types. Monocarboxylate transporters (MCTs) play an integral role in cellular metabolism via lactate transport and have been implicated in metabolic synergy in tumors. The transporters MCT1 and MCT4 are regulated via expression of their chaperone, CD147. *Methods*. We conducted a meta-analysis of existing publications on the relationship between MCT1, MCT4, and CD147 expression and overall survival and disease-free survival in cancer, using hazard ratios derived via multivariate Cox regression analyses. *Results*. Increased MCT4 expressions in the tumor microenvironment, cancer cells, or stromal cells were all associated with decreased overall survival and decreased disease-free survival ($p < 0.001$ for all analyses). Increased CD147 expression in cancer cells was associated with decreased overall survival and disease-free survival ($p < 0.0001$ for both analyses). Few studies were available on MCT1 expression; MCT1 expression was not clearly associated with overall or disease-free survival. *Conclusion*. MCT4 and CD147 expression correlate with worse prognosis across many cancer types. These results warrant further investigation of these associations.

1. Background

Overview of Monocarboxylate Transporters. Monocarboxylic acids play an important role in cellular metabolism, and the regulation of this system has become a new target for understanding the pathogenesis of abnormal cellular processes such as tumorigenesis. Monocarboxylate transporters (MCTs) are 12-segment transmembrane proteins that symport protons with monocarboxylic acids through the plasma membrane [1]. These monocarboxylic acids include lactate and, to a lesser extent, pyruvate, ketone bodies, and metabolites of branched-chain amino acids. MCT family members have different characteristics regarding transport directionality and substrate specificity.

There are at least 14 members of the MCT family; all are encoded by the solute carrier gene series, *SLC16A*. Of this family, MCTs 1–4 are the best characterized thus far, with particular research emphasis placed on MCT1 and MCT4. The most studied function of MCT1 is transport of lactate into the cell, although in some physiologic conditions MCT1

can mediate lactate efflux [2]. This transporter also has a widespread expression throughout the body [2]. MCT2 has similar function to MCT1 but has a higher affinity for pyruvate and has distinct expression patterns. MCT3 expression is limited to the retinal pigment epithelium where it regulates lactate levels; its mechanism of action is not well characterized [3]. MCT4 is highly expressed in tissues dependent on glycolysis, and it plays an important role in lactate efflux from cells. MCTs 5–10 are not well characterized, although there is evidence of a role for MCT8 in targeting proteins to lysosomes and thyroid hormone transport [4]. MCT1 and MCT4 typically act as lactate importers and exporters, respectively. However, these two transporters have similar regulatory control: CD147 is a chaperone, which is essential for both MCT1 and MCT4 transport to the plasma membrane [5]. MCTs are being studied as cancer therapeutic targets since they regulate glycolytic processes via lactate transport.

Cancer Metabolism and the Tumor Microenvironment. Cancer metabolism involves a complex array of intracellular and intercellular interactions within the tumor microenvironment; understanding and intervening in these processes have allowed exploration of novel anticancer therapy approaches. A "seed and soil" hypothesis of tumor growth, which states that cancer flourishes in a favorable environment, was originally proposed by Paget [7]. Recent investigations of the metabolic microenvironment of tumors have brought this theory back to light. One of the best-known differences between cancer cell metabolism and that of healthy tissue is that tumor cells utilize glycolysis despite oxygen being present, which is termed the "Warburg Effect" ([8]; Figure 1(c)). This metabolic adaptation is postulated to confer a biosynthetic advantage for tumor development and progression due to increased carbon utilization, hypoxic adaptation, and increased rate of ATP production [9–11]. This unique glycolytic feature of tumors is the basis of fluoro-2-deoxy-glucose positron emission tomography (FDG-PET) imaging. This theory has been expanded by evidence that proliferating cancer cells may benefit from a "Reverse Warburg Effect" (Figure 1(b)) by inducing glycolysis in the surrounding tissue and deriving nutrients such as lactate from cancer-associated fibroblasts [12–14]. In a recently proposed model, the Reverse Warburg Effect is further dissected to include different populations of cancer cells: highly proliferative cancer cells and less proliferative cancer cells [6]. This Multicompartment Metabolism Model (Figure 1(a)) hypothesizes that highly proliferative cancer cells derive their lactate substrate not only from stromal cells, but also from surrounding nonproliferative cancer cells. Thus, the leading edge of the tumor with highly proliferative cancer cells takes advantage of the favorable microenvironment provided by both stroma and less proliferative cancer cells. The highly proliferative cancer cells are poorly differentiated and are believed to arise from basal stem cells, representing a group of cancer stem cells [6]. The less proliferative cancer cells have little to no expression of Ki-67, a proliferation marker; this population is also more differentiated and mitochondrially poor [6]. The tumor microenvironment is composed of proliferative cancer cells, nonproliferative cancer cells, adjacent epithelial cells, stromal cells, immune cells, and surrounding matrix. Tumor cell engraftment requires that cancer cells metabolically reprogram their microenvironment to form a suitable "nest" for tumor cell growth. This reprogramming can be explained by hydrogen peroxide secretion and HIFα and NFκB signaling from cancer cells which induces aerobic glycolysis in surrounding tissue [12, 15]. The surrounding fibroblasts and cancer cells then are able to supply metabolic catabolites of glycolysis such as lactate and pyruvate. This "lactate shuttle" is an efficient transfer of high-energy nutrients from fibroblasts and nonproliferative cancer cells to proliferative cancer cells [6, 16, 17]. A metabolic symbiosis occurs, where fibroblasts upregulate MCT4 for lactate and ketone body export [16–18], and proliferative cancer cells import these metabolic fuels via MCT1 [16]. This type of metabolic symbiosis has been described in many different epithelial cancer types [6, 15, 19–21] and creates an environment favorable to growth, survival, and metastatic spread [13, 14]. One model proposes multiple compartments with a proliferative cancer cell population which expresses MCT1 at the tumor front with a deeper population of MCT4+ cancer cells and MCT4+ cancer-associated fibroblasts, which serve as the driving force for cancer cells to proliferate via a lactate shuttle (Figure 1, [6]).

Monocarboxylate Transporter Expression and Cancer Prognosis. We hypothesize that altered metabolism induces tumor progression by a similar mechanism in many cancer types that involves MCT1, MCT4, and CD147 expression. Tumoral and peritumoral expression of these three functional proteins correlate with poor prognosis in various cancers. To date, there have been no analyses correlating overall survival or disease-free survival with expression of these markers across cancer types. Though each cancer is unique, it is important to determine general oncologic principles that can be used for expansion of therapeutic trials. There are CD147 and MCT inhibitors in clinical trials in specific cancer patient populations [22, 23]. By understanding common features among different cancer types, these potential therapies can be applied more broadly.

2. Materials and Methods

A PubMed search for the keywords ("MCT1" OR "MCT4" OR "monocarboxylate transporters" OR "CD147" OR "EMMPRIN" OR "Basigin") AND ("survival" OR "prognosis") was performed. Clinical investigations into the prognostic value of MCT1, MCT4, and CD147 were selected as entries for the present study. Studies included in Forest Plot analyses were limited to those in which multivariate analysis Cox regression hazard ratio data on overall survival or disease-free survival was available. Forest Plots were constructed using RevMan 5.3 software (The Cochrane Collaboration). Forest Plot specifications were generic inverse variance for data type, fixed effect for analysis method, and hazard ratio for effect measure.

When SEM was not provided directly by the studies, they were calculated from the 95% confidence intervals by

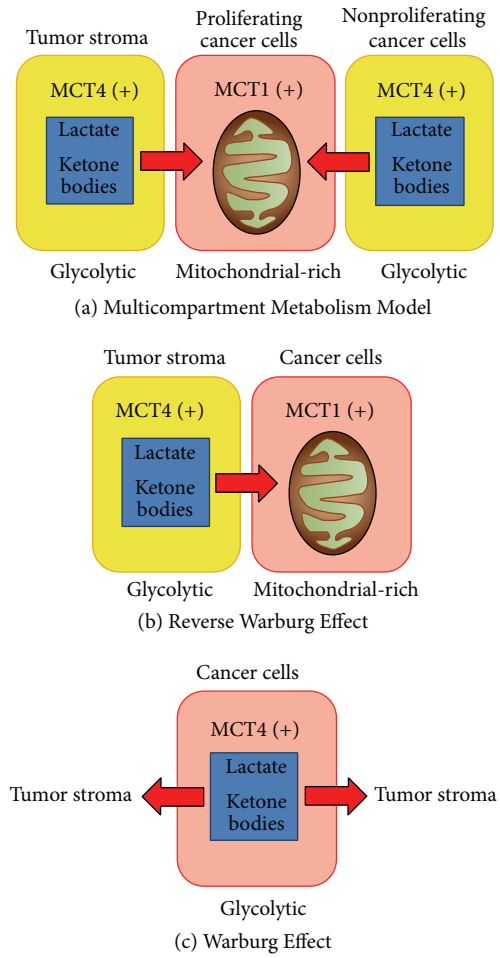


FIGURE 1: Multicompartment metabolism model in cancer. Modified with permission from Curry et al. [6].

the formula SEM = (ln(Upper CI limit) − ln(Lower CI limit))/3.92 [24].

3. Results

3.1. Increased MCT4 Expression Is Associated with Decreased Overall Survival. MCT4 expression anywhere in the tumor microenvironment was associated with decreased overall survival (OS, Figure 2(a)). The 12 included studies showed that elevated MCT4 expression was associated with decreased OS by a factor of 1.82 ($p < 0.00001$, Figure 1(a)). This analysis included studies that reported either cancer cell or stromal cell MCT4 expression. Cancer types represented are pancreas (cancer and stroma) [25], breast (cancer x2) [26], phyllodes (stroma) [27], oral squamous cell carcinoma (oral SCC, cancer) [28], hepatocellular carcinoma (HCC, cancer x2, stroma) [29–31], gastric (stroma x2) [19, 20, 32], and colorectal carcinoma (CRC, cancer) [33].

High MCT4 expression specifically in cancer cells was associated with decreased OS (Figure 2(b)). The 7 included studies showed that elevated MCT4 expression was associated with decreased OS by a factor of 1.98 ($p < 0.00001$, Figure 2(b)). Cancer types included were pancreas [25], breast [26], oral SCC [28], HCC [29–31], and CRC [33]. There were 11 studies that did not have multivariate analysis data available [19, 20, 29, 30, 34–43]. Of these, 6 had statistically significant univariate analysis of elevated cancer cell MCT4 correlating with decreased OS ([29, 30, 34–36, 42, 43], see Supplementary Table 1 in Supplementary Material available online at http://dx.doi.org/10.1155/2015/242437). The other studies failed to show a significant association between elevated MCT4 and decreased OS [19, 20, 38–41].

Elevated MCT4 expression specifically by tumor-associated stroma was also associated with decreased OS (Figure 2(c)). The 5 included studies showed that elevated MCT4 expression was associated with decreased OS by a factor of 1.67 ($p < 0.00001$, Figure 2(c)). Cancer types represented are pancreas [25], phyllodes [27], gastric [19, 20, 32], and HCC [31]. There were 2 studies without multivariate analysis: one which showed no association between MCT4 expression and OS in non-small-cell lung cancer [42] and one that showed that stromal MCT4 expression correlated with decreased OS in triple-negative breast cancer under univariate analysis ($p < 0.0001$, [41]; Supplementary Table 1).

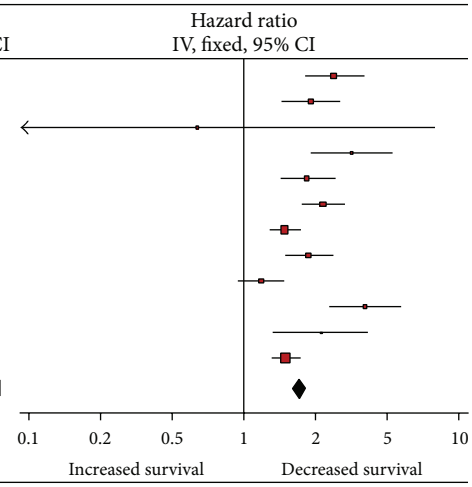
3.2. Increased MCT4 Expression Is Associated with Decreased Disease-Free Survival. MCT4 expression in the tumor microenvironment was associated with decreased disease-free survival (DFS, Figure 3(a)). The 11 studies included showed that elevated MCT4 expression was associated with decreased disease-free survival by a factor of 1.75 ($p < 0.00001$, Figure 3(a)). This analysis included studies that reported either cancer cell or stromal cell MCT4 expression. Cancer types represented are breast (cancer) [26], phyllodes (stroma) [27], oral SCC (cancer) [28], HCC (cancer) [29–31], gastric (cancer and stroma) [19, 20, 32], head and neck squamous cell carcinoma (HNSCC, cancer) [6], bladder (cancer) [44], and lacrimal gland adenoid cystic carcinoma (lacrimal gland ACC, cancer) [36].

Elevated MCT4 expression specifically by cancer cells was associated with decreased DFS (Figure 3(b)). The 8 included studies showed that elevated MCT4 expression was associated with decreased DFS by a factor of 1.68 ($p < 0.00001$, Figure 3(b)). Cancer types represented were breast [26], oral SCC [28], HCC [29–31], bladder [44], lacrimal gland ACC [36], and HNSCC [6]. There were 12 studies that did not have multivariate analysis data available ([19, 20, 29, 30, 34–43]; Supplementary Table 2). Of these, 6 had statistically significant univariate analysis of elevated tumoral MCT4 correlating with decreased DFS in renal cell carcinoma [34], soft tissue sarcoma [35], hepatocellular carcinoma [29, 30], Lacrimal gland adenoid cystic carcinoma [36], non-small-cell lung cancer [42], and glioblastoma multiforme [43]. The other studies did not show an association between elevated MCT4 and decreased DFS [19, 20, 37–41].

Elevated MCT4 expression in tumor-associated stroma was also associated with decreased DFS (Figure 3(c)). The 3 studies included showed that elevated MCT4 expression was

Study or subgroup	n	log [Hazard ratio]	SE	Weight	Hazard ratio IV, fixed, 95% CI
Breast—cohort A (Doyen et al., 2014)	643	0.966984	0.155713	5.4%	2.63 [1.94, 3.57]
Breast—cohort B (Doyen et al., 2014)	127	0.71295	0.156994	5.3%	2.04 [1.50, 2.78]
Breast—phyllodes stroma (Kwon et al., 2013)	207	−0.51083	1.302045	0.1%	0.60 [0.05, 7.70]
Colorectal (Nakayama et al., 2012)	105	1.151521	0.215189	2.8%	3.16 [2.07, 4.82]
Gastric stroma (Yan et al., 2014)	143	0.673964	0.142325	6.5%	1.96 [1.48, 2.59]
Gastric stroma (Zhao et al., 2014)	113	0.835081	0.108734	11.1%	2.31 [1.86, 2.85]
Hepatocellular (Gao et al., 2014)	318	0.438255	0.075272	23.2%	1.55 [1.34, 1.80]
Hepatocellular (Ohno et al., 2014)	225	0.693147	0.128234	8.0%	2.00 [1.56, 2.57]
Hepatocellular stroma (Ohno et al., 2014)	225	0.182322	0.121715	8.9%	1.20 [0.95, 1.52]
Oral SCC (Zhu et al., 2014)	99	1.292258	0.190475	3.6%	3.64 [2.51, 5.29]
Pancreatic (Baek et al., 2014)	205	0.817133	0.258214	2.0%	2.26 [1.36, 3.76]
Pancreatic stroma (Baek et al., 2014)	205	0.442761	0.075234	23.2%	1.56 [1.34, 1.80]
Total (95% CI)	**2615**			**100.0%**	**1.82 [1.69, 1.95]**

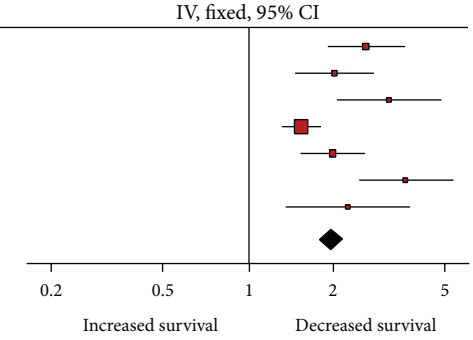
Heterogeneity: $\chi^2 = 53.51$, df = 11 ($p < 0.00001$); $I^2 = 79\%$
Test for overall effect: $Z = 16.49$ ($p < 0.00001$)



(a) TME MCT4 expression and overall survival

Study or subgroup	n	log [Hazard ratio]	SE	Weight	Hazard ratio IV, fixed, 95% CI
Breast—cohort A (Doyen et al., 2014)	643	0.966984	0.155713	10.8%	2.63 [1.94, 3.57]
Breast—cohort B (Doyen et al., 2014)	127	0.71295	0.156994	10.6%	2.04 [1.50, 2.78]
Colorectal (Nakayama et al., 2012)	105	1.151521	0.215189	5.6%	3.16 [2.07, 4.82]
Hepatocellular (Gao et al., 2014)	318	0.438255	0.075272	46.0%	1.55 [1.34, 1.80]
Hepatocellular (Ohno et al., 2014)	225	0.693147	0.128234	15.9%	2.00 [1.56, 2.57]
Oral SCC (Zhu et al., 2014)	99	1.292258	0.190475	7.2%	3.64 [2.51, 5.29]
Pancreatic (Baek et al., 2014)	205	0.819634	0.258214	3.9%	2.27 [1.37, 3.76]
Total (95% CI)	**1722**			**100.0%**	**1.98 [1.79, 2.18]**

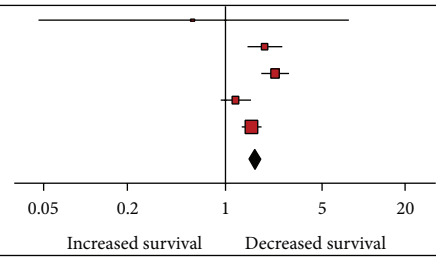
Heterogeneity: $\chi^2 = 29.19$, df = 6 ($p < 0.0001$); $I^2 = 79\%$
Test for overall effect: $Z = 13.34$ ($p < 0.00001$)



(b) Cancer cell MCT4 expression and overall survival

Study or subgroup	n	log [Hazard ratio]	SE	Weight	Hazard ratio IV, fixed, 95% CI
Breast—phyllodes stroma (Kwon et al., 2013)	207	−0.51083	1.302045	0.2%	0.60 [0.05, 7.70]
Gastric stroma (Yan et al., 2014)	143	0.673964	0.142325	13.0%	1.96 [1.48, 2.59]
Gastric stroma (Zhao et al., 2014)	113	0.835081	0.108734	22.3%	2.31 [1.86, 2.85]
Hepatocellular stroma (Ohno et al., 2014)	225	0.182322	0.121715	17.8%	1.20 [0.95, 1.52]
Pancreatic stroma (Baek et al., 2014)	205	0.442761	0.075234	46.7%	1.56 [1.34, 1.80]
Total (95% CI)	**893**			**100.0%**	**1.67 [1.51, 1.85]**

Heterogeneity: $\chi^2 = 18.92$, df = 4 ($p = 0.0008$); $I^2 = 79\%$
Test for overall effect: $Z = 9.98$ ($p < 0.00001$)



(c) Stromal cell MCT4 expression and overall survival

Figure 2: Elevated MCT4 expression is associated with decreased overall survival. (a) Elevated MCT4 expression in the tumor microenvironment is associated with decreased OS. (b) Elevated MCT4 expression in cancer cells is associated with decreased OS. (c) Elevated MCT4 expression in stromal cells is associated with decreased OS. SCC: squamous cell carcinoma.

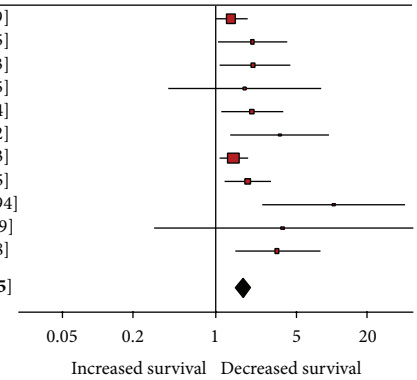
associated with decreased DFS by a factor of 2.35 ($p = 0.0004$, Figure 3(c)). Cancer types represented were phyllodes [27] and gastric [19, 20, 32]. There were 2 studies without multivariate analysis available, one which showed that stromal MCT4 expression was significantly correlated with decreased DFS under univariate analysis in triple-negative breast cancer [41] and one which showed no such association in non-small-cell breast cancer ([42]; Supplementary Table 2).

3.3. Increased CD147 Expression in Cancer Cells Is Associated with Decreased Overall Survival. Elevated CD147 expression in cancer cells was associated with decreased OS (Figure 4(a)). The 25 included studies showed that elevated CD147 expression was associated with decreased OS by a factor of 2.16 ($p < 0.00001$, Figure 4(a)) [29, 30, 45–69]. This analysis included studies that reported only cancer cell CD147 expression. No studies had multivariate analysis of stromal

Study or subgroup	n	log [Hazard ratio]	SE	Weight	Hazard ratio IV, fixed, 95% CI
Bladder (Choi et al., 2014)	360	0.329304	0.155987	27.0%	1.39 [1.02, 1.89]
Breast—cohort A (Doyen et al., 2014)	205	0.751416	0.342787	5.6%	2.12 [1.08, 4.15]
Breast—cohort B (Doyen et al., 2014)	205	0.774727	0.339998	5.7%	2.17 [1.11, 4.23]
Breast—phyllodes stroma (Kwon et al., 2013)	207	0.587787	0.751132	1.2%	1.80 [0.41, 7.85]
Gastric (Yan et al., 2014)	143	0.732849	0.299148	7.3%	2.08 [1.16, 3.74]
Gastric stroma (Zhao et al., 2014)	113	1.277037	0.476161	2.9%	3.59 [1.41, 9.12]
Hepatocellular (Gao et al., 2014)	318	0.378436	0.143421	31.9%	1.46 [1.10, 1.93]
Hepatocellular (Ohno et al., 2014)	222	0.641854	0.225099	13.0%	1.90 [1.22, 2.95]
HNSCC (Curry et al., 2013)	42	2.337952	0.713386	1.3%	10.36 [2.56, 41.94]
Lacrimal gland adenoid cystic CA (Koo and Yoon, 2015)	69	1.345993	1.287228	0.4%	3.84 [0.31, 47.89]
Oral SCC (Zhu et al., 2014)	99	1.230517	0.418936	3.7%	3.42 [1.51, 7.78]
Total (95% CI)	**1983**			**100.0%**	**1.75 [1.49, 2.05]**

Heterogeneity: $\chi^2 = 16.38$, df = 10 ($p = 0.09$); $I^2 = 39\%$

Test for overall effect: $Z = 6.89$ ($p < 0.00001$)

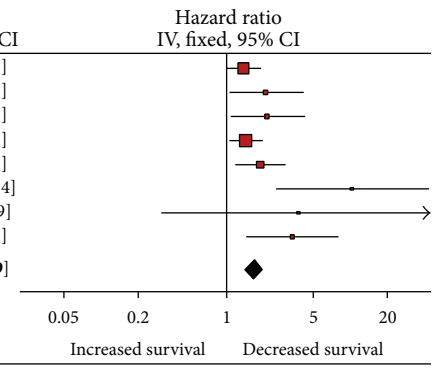


(a) TME MCT4 expression and disease-free survival

Study or subgroup	n	log [Hazard ratio]	SE	Weight	Hazard ratio IV, fixed, 95% CI
Bladder (Choi et al., 2014)	360	0.329304	0.155987	30.5%	1.39 [1.02, 1.89]
Breast—cohort A (Doyen et al., 2014)	205	0.751416	0.342787	6.3%	2.12 [1.08, 4.15]
Breast—cohort B (Doyen et al., 2014)	205	0.774727	0.339998	6.4%	2.17 [1.11, 4.23]
Hepatocellular (Gao et al., 2014)	318	0.378436	0.143421	36.0%	1.46 [1.10, 1.93]
Hepatocellular (Ohno et al., 2014)	222	0.641854	0.225099	14.6%	1.90 [1.22, 2.95]
HNSCC (Curry et al., 2013)	42	2.337952	0.713386	1.5%	10.36 [2.56, 41.94]
Lacrimal gland adenoid cystic CA (Koo and Yoon, 2015)	69	1.345993	1.287228	0.4%	3.84 [0.31, 47.89]
Oral SCC (Zhu et al., 2014)	99	1.230517	0.418936	4.2%	3.42 [1.51, 7.78]
Total (95% CI)	**1520**			**100.0%**	**1.68 [1.42, 1.99]**

Heterogeneity: $\chi^2 = 13.56$, df = 7 ($p = 0.06$); $I^2 = 48\%$

Test for overall effect: $Z = 6.04$ ($p < 0.00001$)

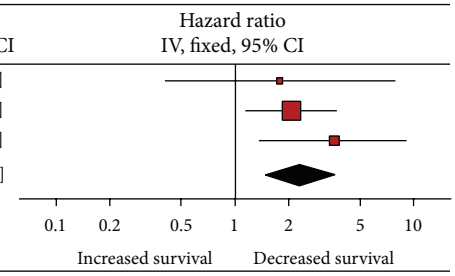


(b) Cancer cell MCT4 expression and disease-free survival

Study or subgroup	n	log [Hazard ratio]	SE	Weight	Hazard ratio IV, fixed, 95% CI
Breast—phyllodes stroma (Kwon et al., 2013)	207	0.587787	0.751132	10.2%	1.80 [0.41, 7.85]
Gastric stroma (Yan et al., 2014)	143	0.732849	0.299148	64.4%	2.08 [1.16, 3.74]
Gastric stroma (Zhao et al., 2014)	113	1.277037	0.476161	25.4%	3.59 [1.41, 9.12]
Total (95% CI)	**463**			**100.0%**	**2.35 [1.47, 3.77]**

Heterogeneity: $\chi^2 = 1.08$, df = 2 ($p = 0.58$); $I^2 = 0\%$

Test for overall effect: $Z = 3.57$ ($p = 0.0004$)



(c) Stromal cell MCT4 expression and disease-free survival

Figure 3: Elevated MCT4 expression is associated with decreased disease-free survival. (a) Elevated MCT4 expression in the tumor microenvironment is associated with decreased DFS. (b) Elevated MCT4 expression in cancer cells is associated with decreased DFS. (c) Elevated MCT4 expression in stromal cells is associated with decreased DFS. HNSCC: head and neck squamous cell carcinoma; CA: carcinoma; and SCC: squamous cell carcinoma.

CD147 expression. Of note, only 2 studies showed an increase in OS with elevated CD147 expression [57, 68].

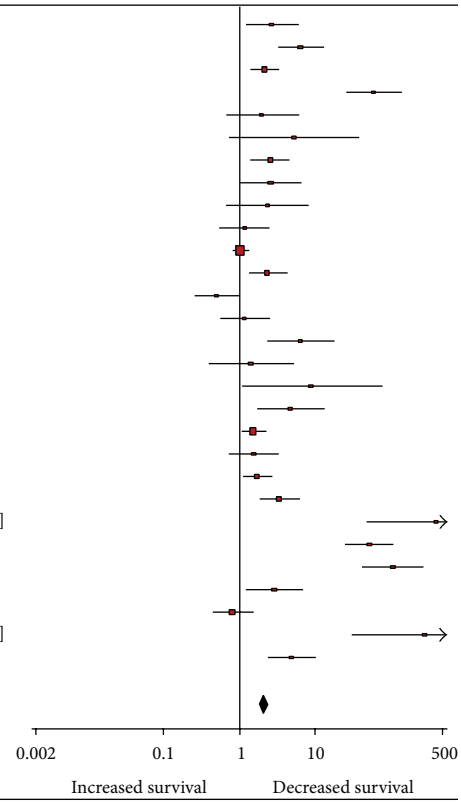
There were 30 studies that did not have adequate multivariate analysis data available ([26, 35, 38, 44, 55–57, 66, 70–93]; Supplementary Table 3). Three of these studies had multivariate p values reported without the necessary hazard ratios necessary for meta-analysis [77, 78, 80]. Of these studies, 17 have statistically significant univariate analysis of elevated CD147 in cancer cells correlating with decreased OS ([44, 55–57, 66, 70–82]; Supplementary Table 3). The other studies did not show an association between elevated CD147 and OS ([26, 35, 38, 83–93]; Supplementary Table 3).

3.4. Elevated CD147 Expression in Cancer Cells Is Associated with Decreased Disease-Free Survival. Elevated CD147 expression in cancer cells was associated with decreased DFS (Figure 4(b)). The 11 included studies showed that elevated CD147 expression was associated with decreased DFS by a factor of 3.14 ($p < 0.00001$, Figure 4(b)). This analysis included studies that reported only cancer cell CD147 expression. No studies reported had multivariate analysis of stromal CD147 expression and survival. Cancer types represented include esophageal SCC [94], salivary gland cancer [67], breast cancer [48], triple-negative breast cancer [51, 63, 64], osteosarcoma [61], colorectal cancer [95], ovarian epithelial

Study or subgroup	n	log [Hazard ratio]	SE	Weight	Hazard ratio IV, fixed, 95% CI
Adenoid cystic CA (Yang et al., 2010)	72	1.022451	0.40811	2.0%	2.78 [1.25, 6.19]
Astrocytoma (Tian et al., 2013)	136	1.888584	0.33023	3.0%	6.61 [3.46, 12.63]
Bladder (Als et al., 2007)	124	0.802002	0.215648	7.1%	2.23 [1.46, 3.40]
Bladder (Han et al., 2010)	58	4.148896	0.421398	1.9%	63.36 [27.74, 144.72]
Breast (Liu et al., 2010)	186	0.715887	0.544078	1.1%	2.05 [0.70, 5.94]
Breast—basal-like (Liu et al., 2013)	189	1.687694	1.014606	0.3%	5.41 [0.74, 39.50]
Breast—infiltrating ductal (Li et al., 2009)	106	0.958584	0.303404	3.6%	2.61 [1.44, 4.73]
Breast—TNBC (Zhao et al., 2013a)	127	0.986936	0.463446	1.5%	2.68 [1.08, 6.65]
Cervical (Ju et al., 2008)	82	0.864997	0.628134	0.8%	2.37 [0.69, 8.13]
Esophageal SCC (Zhu et al., 2011)	86	0.171	0.393393	2.1%	1.19 [0.55, 2.57]
Gastric (Zheng et al., 2006)	219	0.053541	0.111528	26.5%	1.06 [0.85, 1.31]
Glioblastoma multiforme (Yang et al., 2013)	206	0.883768	0.288318	4.0%	2.42 [1.38, 4.26]
Hepatocellular (Zhu et al., 2015)	50	−0.69315	0.332	3.0%	0.50 [0.26, 0.96]
Laryngeal (Gou et al., 2014)	92	0.181488	0.371	2.4%	1.20 [0.58, 2.48]
Lung—NSCLC (Xu et al., 2013)	136	1.891906	0.506654	1.3%	6.63 [2.46, 17.90]
Oral SCC (Monteiro et al., 2014)	74	0.359437	0.642169	0.8%	1.43 [0.41, 5.04]
Osteosarcoma (Futamura et al., 2014)	166	2.24071	1.080064	0.3%	9.40 [1.13, 78.07]
Osteosarcoma (Lu et al., 2013)	55	1.588209	0.507593	1.3%	4.89 [1.81, 13.24]
Ovarian (Gao et al., 2014)	92	0.433729	0.176005	10.6%	1.54 [1.09, 2.18]
Ovarian epithelial CA (Zhao et al., 2013b)	242	0.43308	0.368265	2.4%	1.54 [0.75, 3.17]
Ovarian epithelial CA (Zhao et al., 2013c)	146	0.564177	0.203604	7.9%	1.76 [1.18, 2.62]
Pediatric medulloblastoma (Chu et al., 2011)	56	1.252763	0.295724	3.8%	3.50 [1.96, 6.25]
Penile (Han et al., 2010)	17	6.041121	1.07556	0.3%	420.36 [51.06, 3460.54]
Prostate (Han et al., 2010)	101	3.991536	0.373324	2.4%	54.14 [26.05, 112.53]
Renal cell (Han et al., 2010)	52	4.703612	0.46709	1.5%	110.35 [44.17, 275.64]
Salivary gland (Piao et al., 2012)	35	1.082483	0.437285	1.7%	2.95 [1.25, 6.96]
Seminoma (Bi et al., 2012)	65	−0.17913	0.303428	3.6%	0.84 [0.46, 1.52]
Testicular (Han et al., 2010)	17	5.681308	1.11275	0.3%	293.33 [33.13, 2597.38]
Thyroid (Tan et al., 2008)	156	1.629241	0.355098	2.6%	5.10 [2.54, 10.23]
Total (95% CI)	**3511**			**100.0%**	**2.16 [1.93, 2.42]**

Heterogeneity: $\chi^2 = 366.28$, df = 28 ($p < 0.00001$); $I^2 = 92\%$

Test for overall effect: $Z = 13.46$ ($p < 0.00001$)

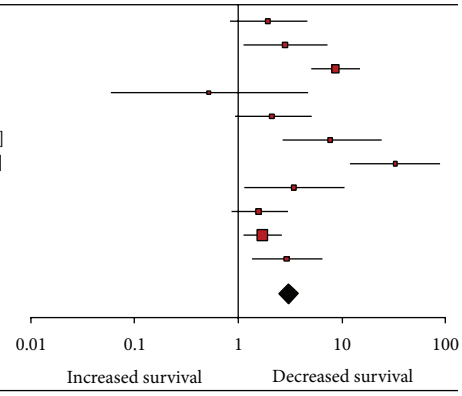


(a) CD147 expression and overall survival

Study or subgroup	n	log [Hazard ratio]	SE	Weight	Hazard ratio IV, fixed, 95% CI
Breast (Liu et al., 2010)	186	0.696641	0.429533	6.5%	2.01 [0.86, 4.66]
Breast—TNBC (Zhao et al., 2013a)	127	1.053266	0.46213	5.6%	2.87 [1.16, 7.09]
Colorectal (Zhu et al., 2013)	328	2.183802	0.261353	17.6%	8.88 [5.32, 14.82]
Endometrial (Ueda et al., 2007)	112	−0.63488	1.09855	1.0%	0.53 [0.06, 4.56]
Esophageal SCC (Ishibashi et al., 2004)	101	0.788457	0.425536	6.6%	2.20 [0.96, 5.07]
Hepatocellular (Zhang et al., 2006)	82	2.081938	0.534634	4.2%	8.02 [2.81, 22.87]
Hepatocellular (Zhang et al., 2007)	111	3.505257	0.510537	4.6%	33.29 [12.24, 90.55]
Osteosarcoma (Futamura et al., 2014)	166	1.258461	0.557618	3.9%	3.52 [1.18, 10.50]
Ovarian epithelial CA (Zhao et al., 2013b)	242	0.485508	0.311694	12.3%	1.63 [0.88, 2.99]
Ovarian epithelial CA (Zhao et al., 2013c)	146	0.564177	0.2	30.0%	1.76 [1.19, 2.60]
Salivary gland (Piao et al., 2012)	35	1.1036	0.39506	7.7%	3.02 [1.39, 6.54]
Total (95% CI)	**1636**			**100.0%**	**3.14 [2.54, 3.90]**

Heterogeneity: $\chi^2 = 57.66$, df = 10 ($p < 0.00001$); $I^2 = 83\%$

Test for overall effect: $Z = 10.46$ ($p < 0.00001$)



(b) CD147 expression and disease-free survival

Figure 4: Elevated CD147 expression is associated with decreased survival. (a) Elevated CD147 expression in cancer cells is associated with decreased overall survival. (b) Elevated CD147 expression in cancer cells is associated with decreased disease-free survival. CA: carcinoma; TNBC: triple-negative breast cancer; SCC: squamous cell carcinoma; and NSCLC: non-small-cell lung cancer.

cancer [63, 64], endometrial cancer [96], and hepatocellular carcinoma [97, 98]. Of note, only one study showed an increase in DFS with elevated CD147 expression [96]. There were 13 studies that did not have multivariate analysis data available ([26, 57, 60, 62, 71, 72, 77, 82, 84, 91, 92, 99, 100]; Supplementary Table 4). Of these, 8 had statistically significant univariate analysis revealing that elevated CD147 correlates with decreased DFS ([26, 57, 62, 71, 72, 82, 99, 100]; Supplementary Table 4). The other studies showed no association between elevated MCT4 and DFS ([60, 77, 84, 91, 92]; Supplementary Table 4).

3.5. MCT1 Expression and Prognosis. There is currently insufficient high-quality data available to conduct a meta-analysis

Study or subgroup	n	log [Hazard ratio]	SE	Weight	Hazard ratio IV, fixed, 95% CI
Bladder (Choi et al., 2014)	360	0.81093	0.37915	16.1%	2.25 [1.07, 4.73]
Lung—NSCLC (Eilertsen et al., 2014)	335	−0.64245	0.195754	60.3%	0.53 [0.36, 0.77]
Lung—SCLC (Polanski et al., 2014)	47	−0.43696	0.449084	11.5%	0.65 [0.27, 1.56]
Renal cell (Kim et al., 2014)	180	1.453953	0.436662	12.1%	4.28 [1.82, 10.07]
Total (95% CI)	**922**			**100.0%**	**0.88 [0.65, 1.18]**

Heterogeneity: $\chi^2 = 26.64$, df = 3 ($p < 0.00001$); $I^2 = 89\%$

Test for overall effect: $Z = 0.86$ ($p = 0.39$)

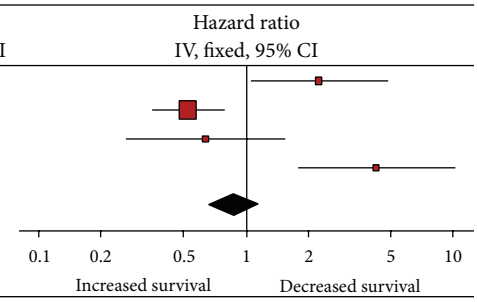


Figure 5: MCT1 expression in the tumor microenvironment is not associated with overall survival. NSCLC: non-small-cell lung cancer; SCLC: small-cell lung cancer.

of studies examining the correlation of OS or DFS with cancer cell expression of MCT1. However, a review of the literature on MCT1's impact on cancer prognosis is provided here.

Multivariate analysis on MCT1 expression and OS was only available in 4 studies (Figure 5). Increased MCT1 expression in cancer cells was associated with decreased OS in bladder cancer and renal cell carcinoma [34, 44]. Increased MCT1 expression was shown to either increase or have no effect on OS in NSCLC and SCLC [39, 42].

An additional five studies that analyzed MCT1 expression and OS did not have multivariate analysis data available ([19, 20, 25, 38, 71, 101]; Supplementary Table 5). Of these, only 2 studies showed a significant decrease in OS associated with elevated MCT1 expression ($p = 0.021$, [35]; $p = 0.014$, [19, 20]). The remainder of the studies failed to show a statistically significant change in survival associated with MCT1 expression ([25, 38, 71]; Supplementary Table 5). A single study evaluated elevated MCT1 expression and DFS in bladder cancer, but this univariate analysis failed to show a significant association ($p = 0.065$, [71]).

Interestingly, only one study examined cancer and stromal cell expression of MCT1 individually. In a study of 335 cases of NSCLC, univariate analysis revealed that increased MCT1 expression in stromal cells corresponded significantly with poor disease-specific survival ($p = 0.003$), but increased MCT1 expression in cancer cells corresponded with a favorable DFS ($p = 0.020$). Both these associations held in multivariate analysis ($p = 0.001$, 0.016, resp., [42]).

4. Discussion

The "Reverse Warburg" model of tumor metabolism hypothesizes a compartmentalized metabolic tumor microenvironment. The transfer of molecules between compartments allows for highly proliferative cancer cells to maintain oxidative phosphorylation while CAFs and less proliferative cancer cells provide metabolic fuels generated by glycolysis. The monocarboxylate transporter system allows the intercellular exchange of metabolites that fuel different tumoral compartments. In particular, MCT1 and MCT4 play crucial roles in the influx and efflux, respectively, of lactate, pyruvate, and other metabolites. CD147 serves as a chaperone for MCT1 and MCT4 and is essential in their expression [5]. MCT1, MCT4, and CD147 are functional biomarkers for metabolic compartmentalization in cancer, and their presence has implications for tumor aggressiveness and prognosis.

The monocarboxylate transporter system has been studied in various cancer types, and here we show that the association between MCT1, MCT4, and CD147 is similar across many types of cancer. This is the first study to investigate the significance of these biomarkers across such varied types of cancer, and, although each cancer is biologically unique, the data presented here suggests that tight metabolic coupling with catabolite transfer between different tumor cells is associated with outcomes. Cancer cells often exploit previously existing cellular functions in order to fuel their own growth; a system of energy transfer may play a role in promoting tumorigenesis in many types of cancer, just as TP53 mutations have been shown to promote growth and suppress apoptosis in many cancers. We provide evidence that expression of MCT4 and CD147 predicts clinical behavior in many different cancers, even if their particular role in each type of cancer is not yet well described. To date, there have been few studies examining the MCT system across cancer types, and none which examine the breadth of cancer types were analyzed in this study. The reviews that cover this subject have been limited to the molecular mechanisms of lactate transporters in tumor metabolism [102, 103]. The current study bolsters the external validity of studies on expression of MCT4 and CD147 and prognosis.

In determining the impact of MCT1, MCT4, and CD147 expression on outcomes, attention must be paid to expression levels as well as expression patterns. In the Multicompartment Metabolism Model, a highly proliferative population of cancer cells express MCT1 strongly, and, in fact, much stronger than the less proliferative cancer cells and stromal cells around them. In contrast, these less proliferative cancer cells express MCT4 strongly, while MCT4 expression in highly proliferative cancer cells is low. Thus, the reported location of MCT4 staining is important when considering its effect on tumor biology and prognosis. There are differences in transporter expression in cancer cells in the tumor leading edge versus other cancer cells and stromal cells. The specific combined expression pattern of MCT1 in cancer cells and MCT4 in stromal cells was associated with decreased DFS in prostate cancer [104, 105]. Expression patterns and colocalization of MCT1, MCT4, and CD147 are also discussed in breast cancer, ovarian cancer, colorectal cancer, and lung cancer

[106]. Understanding the similarities and differences of these patterns across cancer types indicates their significance for prognosis.

This meta-analysis highlights the association between stromal cells and aggressive cancer. Tumor stroma is composed of cancer-associated fibroblasts (CAFs), infiltrating immune cells, and angiogenic vascular cells. The studies in this analysis use various definitions of stromal cells and include stroma between cancer cells and stroma surrounding foci of cancer cells. These studies typically do not differentiate between CAFs and other stromal components, but, in general, define cancer associated stroma as noncancerous cells in proximity to cancer cells. Our results corroborate the recent literature asserting the importance of stromal cells in carcinogenesis, specifically by altering cellular energetics. CAFs have been implicated in carcinogenesis by sustaining proliferative signaling, evading growth suppression, avoiding immune destruction, activating invasion, inducing angiogenesis, resisting cell death, and deregulating cellular energetics [107]. CAFs are also thought to detoxify the tumor microenvironment and provide nutrients to cancer cells [108]. Some researchers have also found that cancer cells produce reactive oxygen species, which influences CAFs to undergo mitophagy and switch to glycolytic metabolism [109]. The role of CAFs in cancer progression and as a therapeutic target is being studied extensively.

Current models of cancer metabolism attempt to encompass not only cancer cells but also the local environment, including the surrounding stromal cells and extracellular matrix. There are a wide array of metabolic changes that occur during carcinogenesis involving a complex coordination between intracellular and intercellular pathways [110], with mitochondrial metabolism changes at the hub of many of these alterations [111]. The interactions between these compartments are fundamental in understanding carcinogenesis and cancer progression. The Warburg Effect (Figure 1(a)) posits that cancer cells utilize glycolysis despite the presence of oxygen and export the lactate produced into the surrounding environment. The Reverse Warburg Effect (Figure 1(b)) describes a metabolic interaction between cancer and stromal cells where glycolysis performed by stromal cells produces lactate which is then shuttled via a monocarboxylate transport system to cancer cells which then have an ample fuel supply to produce energy via oxidative phosphorylation. A newer model, termed the Multicompartment Metabolism Model (Figure 1(c)), is described similarly to the Reverse Warburg Effect; however, it divides the cancer cell compartment into a highly proliferative population and a relatively less proliferative population. Stromal and cancer cells with low proliferation rates provide nutrients for proliferative cancer cells in the Multicompartment Metabolism Model.

The evidence in this meta-analysis supports the Multicompartment Metabolism Model as MCT4 and CD147 expression decreased survival in all scenarios, whether the increased expression levels were found in cancer cells or stromal cells. The decreased survival rates associated with increased MCT4 expression in cancer cells are not fully explained by the Reverse Warburg Effect as this model would lead one to expect that only stromal cell MCT4 expression could contribute to cancer progression. Some studies which associate increased MCT4 expression in cancer cells with decreased survival using the Warburg Effect as a model attribute decreased survival to an acidic microenvironment [26, 29–31, 33] provided by MCT4-mediated lactate efflux causing matrix metalloproteinase activation [29, 30], cathepsin activation [29, 30], decreased natural killer cell activation [29, 30], decreased effectiveness of chemotherapy [26], and increased integrin interactions [33]. Another proposed mechanism which is in concert with the Warburg Effect is the activation of AKT and MEK-ERK pathways in cancer cells with increased MCT4 expression contributing to cancer progression [28–30]. While microenvironment acidification and downstream intracellular pathways may play a role in tumor progression with cancer cell MCT4 expression, we submit that the lactate efflux has additional effects through providing substrates which aid proliferative cancer cell populations. In fact, some studies on the Warburg Effect suggest that cancer cell MCT4 expression may provide enrichment to cancer stem cells [26, 29, 30] and Choi et al. mention that peripheral tumor cells may import this lactate via MCT1 [44]. These descriptions are in line with the Multicompartment Metabolism Model in which MCT4+ nonproliferative cancer cells provide substrates for proliferative MCT1+ cancer cells. There is further evidence that lactate catabolism in cancer may involve MCT1, with lactate uptake specific to aerobic tumor regions [112]. The studies provided are heterogeneous in nature, and hence it is possible that multiple types of metabolism models are found in the different cancer types and even between different areas within a single tumor.

As more is discovered about MCT1, MCT4, and CD147 as functional biomarkers, they become attractive targets for anticancer therapies. Many cancers become increasingly drug-resistant as therapies are initiated and continued, and these new therapeutic targets could prove invaluable in improving patient outcomes [51, 63, 64]. In fact, CD147 coexpression with MCT1 or MCT4 is associated with increased likelihood of multidrug resistance markers [100]. Currently, the main targets for such pharmacologic intervention are the family of monocarboxylate transporters and their regulatory proteins.

A recent study by Amorim et al. addresses the effects of lactate transport inhibition in human colorectal cancer cell lines using the compounds α-cyano-4-hydroxycinnamate (CHC), DIDS (a stilbene derivative), and quercetin, a bioflavonoid, which are known to inhibit lactate transport. They demonstrated that MCT activity inhibition inhibited CRC cells biomass in a dose-dependent manner, increased cell death and decreased cell proliferation, and potentiated the cytotoxicity of 5-fluorouracil in CRC cells pretreated with the MCT inhibitors [113]. However, historically, MCT inhibitors have lacked specificity; α-cyano-4-hydroxycinnamate (CHC), stilbene disulfonates, phloretin, quercetin, and organomercurial reagents were often more potent at inhibiting other cellular functions than plasma membrane lactate transport. More recently, however, new high-affinity MCT inhibitors have been developed and are being investigated both *in vitro* and *in vivo* as anticancer agents.

Draoui et al. investigated 7-aminocarboxycoumarin (7ACC) in xenograft models of cervical, breast, and bladder cancers [114]. 7ACC inhibits lactate influx but not efflux in cells expressing MCT1 and MCT4; in cancer types that express MCT1 and MCT4, 7ACC decreased xenograft tumor growth. In prostate cancer research, AR-C155858, an inhibitor of MCT1 and MCT2, has been shown to result in a significant decrease in proliferation and increased apoptosis in murine tumor tissues with no significant effect on benign tissue [115]. Currently, AZD3965, which is an orally administrable second-generation MCT1/MCT2 inhibitor, is being investigated in a Phase I clinical trial for the treatment of advanced solid tumors, particularly prostate cancer, gastric cancer, and diffuse large B cell lymphoma [23].

Metformin and other biguanides have much cross-reactivity with the lactate transport system as oxidative phosphorylation inhibitors. These biguanides have received much attention in anticancer therapy recently and have been shown to have a synergistic anticancer effect when combined with inhibition of MCT1, MCT4, or CD147 [116–118]. There are currently many clinical trials evaluating the effect of metformin on cancer progression. One study is specifically evaluating whether metformin can interrupt the metabolic coupling between stroma and epithelial cancer cells in head and neck squamous cell carcinoma [119].

MCT4 is a promising target for cancer pharmacotherapy, but there is no published data on specific MCT4 inhibitors to date. There is currently a Small Business Innovation Research Grant awarded to Vettore LLC to develop such an inhibitor [120]. Other agents which decrease MCT4 levels, such as siRNA [28, 37], shRNA [43], and N-acetylcysteine [17], have shown promise in decreasing MCT4 and are being studied as anticancer therapies.

CD147 has been evaluated as a therapeutic target most extensively in hepatocellular carcinoma. Metuximab, a monoclonal antibody specific to CD147, has been shown to decrease HCC recurrence after liver transplantation [121] or radiofrequency ablation [122] and increased OS in HCC patients when combined with chemoembolization [123, 124]. Metuximab is being currently studied in a clinical trial to assess its efficacy in preventing HCC recurrence [22]. Anti-CD147 antibodies have also shown promise in an *ex vivo* HNSCC model [125]. Other cancer types, such as oral SCC [126], HNSCC [127], pancreatic cancer [128], melanoma [129], and colorectal carcinoma [86, 87], have shown to be affected by CD147 levels *in vitro* and *in vivo*; however, these results have not resulted in clinical trials to date.

While our data show that both increased MCT4 expression in the tumor microenvironment, stroma, or cancer and increased CD147 expression in cancer cells are both associated with decreased OS and DFS, our analysis is limited by the fact that studies that do not demonstrate statistical significance are less likely to have published data and that some studies are not amenable to further statistical analysis. Indeed several papers reported associations between the studied biomarkers and OS or DFS simply as "not significant."

Another limitation is the lack of uniformity in the calculation of positivity of biomarker expression. There was significant variation in the methodology by which the included studies designated specimens as positive for a given marker—some using a binary system and others grading along a spectrum. Additionally, characterization of the intensity and density of immunohistochemical staining was also subject to variability, with some studies using a computed algorithm and others relying on the graded observations of one or several pathologists.

An additional limitation is that weighted hazard ratios cannot be compared due to heterogeneity of the data. For example, we cannot assess whether MCT4 has a greater prognostic value when high expression is found in cancer cells versus stromal cells.

Looking to future studies, the tumor microenvironment metabolism will be better understood as more data on both cancer cell and stromal cell marker expression become available. Further investigation into the interaction among these biomarkers in the tumor microenvironment will be necessary to better qualify them as therapeutic targets. For example, CD147 has multiple potential mechanisms of actions to induce cancer aggressiveness. For example, CD147 increases angiogenesis via upregulation of VEGF and metalloproteinases [130, 131], increased EGFR expression [127], and increased invasion and metastasis via MMP upregulation [132]. However, multiple studies have shown cancer-modifying behaviors of CD147 are intricately related with expression of MCT1 and MCT4 [86, 87, 128]. Additionally, cytoplasmic versus membranous CD147 expression may complicate the prognostic effects of this protein [85, 92].

In conclusion, this meta-analysis of published studies identifies elevated MCT4 and CD147 as poor prognostic biomarkers across many cancers. The potential to exploit these findings to develop novel, effective treatments warrants more large-scale and standardized investigations.

Conflict of Interests

The authors declare no conflict of interests.

Authors' Contribution

Ubaldo Martinez-Outschoorn is an equal contributing last author.

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References

[1] A. P. Halestrap and N. T. Price, "The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation," *Biochemical Journal*, vol. 343, no. 2, pp. 281–299, 1999.

[2] A. P. Halestrap and M. C. Wilson, "The monocarboxylate transporter family—role and regulation," *IUBMB Life*, vol. 64, no. 2, pp. 109–119, 2012.

[3] N. J. Philp, H. Yoon, and L. Lombardi, "Mouse MCT3 gene is expressed preferentially in retinal pigment and choroid plexus epithelia," *American Journal of Physiology—Cell Physiology*, vol. 280, no. 5, pp. C1319–C1326, 2001.

[4] E. C. H. Friesema, S. Ganguly, A. Abdalla, J. E. M. Fox, A. P. Halestrap, and T. J. Visser, "Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter," *The Journal of Biological Chemistry*, vol. 278, no. 41, pp. 40128–40135, 2003.

[5] P. Kirk, M. C. Wilson, C. Heddle, M. H. Brown, A. N. Barclay, and A. P. Halestrap, "CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression," *The EMBO Journal*, vol. 19, no. 15, pp. 3896–3904, 2000.

[6] J. M. Curry, M. Tuluc, D. Whitaker-Menezes et al., "Cancer metabolism, stemness and tumor recurrence: MCT1 and MCT4 are functional biomarkers of metabolic symbiosis in head and neck cancer," *Cell Cycle*, vol. 12, no. 9, pp. 1371–1384, 2013.

[7] S. Paget, "The distribution of secondary growths in cancer of the breast," *The Lancet*, vol. 133, no. 3421, pp. 571–573, 1889.

[8] O. Warburg, "On the origin of cancer cells," *Science*, vol. 123, no. 3191, pp. 309–314, 1956.

[9] S. Y. Lunt and M. G. Vander Heiden, "Aerobic glycolysis: meeting the metabolic requirements of cell proliferation," *Annual Review of Cell and Developmental Biology*, vol. 27, pp. 441–464, 2011.

[10] T. Pfeiffer, S. Schuster, and S. Bonhoeffer, "Cooperation and competition in the evolution of ATP-producing pathways," *Science*, vol. 292, no. 5516, pp. 504–507, 2001.

[11] O. Feron, "Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells," *Radiotherapy and Oncology*, vol. 92, no. 3, pp. 329–333, 2009.

[12] U. E. Martinez-Outschoorn, Z. Lin, C. Trimmer et al., "Cancer cells metabolically 'fertilize' the tumor microenvironment with hydrogen peroxide, driving the Warburg effect: implications for PET imaging of human tumors," *Cell Cycle*, vol. 10, no. 15, pp. 2504–2520, 2011.

[13] S. Pavlides, D. Whitaker-Menezes, R. Castello-Cros et al., "The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma," *Cell Cycle*, vol. 8, no. 23, pp. 3984–4001, 2009.

[14] U. E. Martinez-Outschoorn, F. Sotgia, and M. P. Lisanti, "Power surge: supporting cells 'fuel' cancer cell mitochondria," *Cell Metabolism*, vol. 15, no. 1, pp. 4–5, 2012.

[15] U. E. Martinez-Outschoorn, F. Sotgia, and M. P. Lisanti, "Metabolic asymmetry in cancer: a 'balancing act' that promotes tumor growth," *Cancer Cell*, vol. 26, no. 1, pp. 5–7, 2014.

[16] T. Fiaschi, A. Marini, E. Giannoni et al., "Reciprocal metabolic reprogramming through lactate shuttle coordinately influences tumor-stroma interplay," *Cancer Research*, vol. 72, no. 19, pp. 5130–5140, 2012.

[17] U. E. Martinez-Outschoorn, J. M. Curry, Y.-H. Ko et al., "Oncogenes and inflammation rewire host energy metabolism in the tumor microenvironment: RAS and NFκB target stromal MCT4," *Cell Cycle*, vol. 12, no. 16, pp. 2580–2597, 2013.

[18] M. S. Ullah, A. J. Davies, and A. P. Halestrap, "The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism," *The Journal of Biological Chemistry*, vol. 281, no. 14, pp. 9030–9037, 2006.

[19] Z. Zhao, F. Han, Y. He et al., "Stromal-epithelial metabolic coupling in gastric cancer: stromal MCT4 and mitochondrial TOMM20 as poor prognostic factors," *European Journal of Surgical Oncology*, vol. 40, no. 10, pp. 1361–1368, 2014.

[20] Z. Zhao, M.-S. Wu, C. Zou et al., "Downregulation of MCT1 inhibits tumor growth, metastasis and enhances chemotherapeutic efficacy in osteosarcoma through regulation of the NF-κB pathway," *Cancer Letters*, vol. 342, no. 1, pp. 150–158, 2014.

[21] U. Martinez-Outschoorn, F. Sotgia, and M. P. Lisanti, "Tumor microenvironment and metabolic synergy in breast cancers: critical importance of mitochondrial fuels and function," *Seminars in Oncology*, vol. 41, no. 2, pp. 195–216, 2014.

[22] Tianjin Medical University Cancer Institute and Hospital, "A Clinical Trail of Iodine[131I] Metuximab Injection With CIK Cells for Preventing Relapse and Metastasis of Hepatocellular Carcinoma," ClinicalTrials.gov, NLM Identifier: NCT01758679, National Library of Medicine, Bethesda, Md, USA, 2000, https://clinicaltrials.gov/ct2/show/NCT01758679.

[23] Cancer Research UK, *A Cancer Research United Kingdom Phase I Trial of AZD3965, a Monocarboxylate Transporter 1 Inhibitor (MCT1) in Patients with Advanced Cancer*, ClinicalTrials.gov, National Library of Medicine, Bethesda, Md, USA, 2000, https://clinicaltrials.gov/ct2/show/NCT01791595.

[24] J. P. T. Higgins and S. Green, "7.7.7.3 standard errors from confidence intervals and P values: ratio measures," in *Cochrane Handbook for Systematic Reviews of Interventions*, The Cochrane Collaboration, 2011.

[25] G. H. Baek, Y. F. Tse, Z. Hu et al., "MCT4 defines a glycolytic subtype of pancreatic cancer with poor prognosis and unique metabolic dependencies," *Cell Reports*, vol. 9, no. 6, pp. 2233–2249, 2014.

[26] J. Doyen, C. Trastour, F. Ettore et al., "Expression of the hypoxia-inducible monocarboxylate transporter MCT4 is increased in triple negative breast cancer and correlates independently with clinical outcome," *Biochemical and Biophysical Research Communications*, vol. 451, no. 1, pp. 54–61, 2014.

[27] J. E. Kwon, W.-H. Jung, and J. S. Koo, "The expression of metabolism-related proteins in phyllodes tumors," *Tumor Biology*, vol. 34, no. 1, pp. 115–124, 2013.

[28] J. Zhu, Y.-N. Wu, W. Zhang et al., "Monocarboxylate transporter 4 facilitates cell proliferation and migration and is associated with poor prognosis in oral squamous cell carcinoma patients," *PLoS ONE*, vol. 9, no. 1, Article ID e87904, 2014.

[29] H.-J. Gao, M.-C. Zhao, Y.-J. Zhang et al., "Monocarboxylate transporter 4 predicts poor prognosis in hepatocellular carcinoma and is associated with cell proliferation and migration," *Journal of Cancer Research and Clinical Oncology*, vol. 141, no. 7, pp. 1151–1162, 2015.

[30] J. Gao, Z. Hu, J. Liu et al., "Expression of CD147 and Lewis y antigen in ovarian cancer and their relationship to drug resistance," *Medical Oncology*, vol. 31, no. 5, p. 920, 2014.

[31] A. Ohno, K. Yorita, Y. Haruyama et al., "Aberrant expression of monocarboxylate transporter 4 in tumour cells predicts an unfavourable outcome in patients with hepatocellular carcinoma," *Liver International*, vol. 34, no. 6, pp. 942–952, 2014.

[32] P. Yan, Y.-H. Li, Z.-J. Tang, X. Shu, and X. Liu, "High monocarboxylate transporter 4 protein expression in stromal cells predicts adverse survival in gastric cancer," *Asian Pacific Journal of Cancer Prevention*, vol. 15, no. 20, pp. 8923–8929, 2014.

[33] Y. Nakayama, T. Torigoe, Y. Inoue et al., "Prognostic significance of monocarboxylate transporter 4 expression in patients with colorectal cancer," *Experimental and Therapeutic Medicine*, vol. 3, no. 1, pp. 25–30, 2012.

[34] Y. Kim, J.-W. Choi, J.-H. Lee, and Y.-S. Kim, "Expression of lactate/H^+ symporters MCT1 and MCT4 and their chaperone CD147 predicts tumor progression in clear cell renal cell carcinoma: immunohistochemical and the Cancer Genome Atlas data analyses," *Human Pathology*, vol. 46, no. 1, pp. 104–112, 2014.

[35] C. Pinheiro, V. Penna, F. Morais-Santos et al., "Characterization of monocarboxylate transporters (MCTs) expression in soft tissue sarcomas: distinct prognostic impact of MCT1 subcellular localization," *Journal of Translational Medicine*, vol. 12, article 118, 2014.

[36] J. S. Koo and J. S. Yoon, "Expression of metabolism-related proteins in lacrimal gland adenoid cystic carcinoma," *American Journal of Clinical Pathology*, vol. 143, no. 4, pp. 584–592, 2015.

[37] M. Gerlinger, C. R. Santos, B. Spencer-Dene et al., "Genome-wide RNA interference analysis of renal carcinoma survival regulators identifies MCT4 as a Warburg effect metabolic target," *Journal of Pathology*, vol. 227, no. 2, pp. 146–156, 2012.

[38] L. Sweeny, Z. Liu, B. D. Bush, Y. Hartman, T. Zhou, and E. L. Rosenthal, "CD147 and AGR2 expression promote cellular proliferation and metastasis of head and neck squamous cell carcinoma," *Experimental Cell Research*, vol. 318, no. 14, pp. 1788–1798, 2012.

[39] R. Polanski, C. L. Hodgkinson, A. Fusi et al., "Activity of the monocarboxylate transporter 1 inhibitor azd3965 in small cell lung cancer," *Clinical Cancer Research*, vol. 20, no. 4, pp. 926–937, 2014.

[40] T. W. H. Meijer, O. C. J. Schuurbiers, J. H. A. M. Kaanders et al., "Differences in metabolism between adeno- and squamous cell non-small cell lung carcinomas: spatial distribution and prognostic value of GLUT1 and MCT4," *Lung Cancer*, vol. 76, no. 3, pp. 316–323, 2012.

[41] A. K. Witkiewicz, D. Whitaker-Menezes, A. Dasgupta et al., "Using the 'reverse Warburg effect' to identify high-risk breast cancer patients: stromal MCT4 predicts poor clinical outcome in triple-negative breast cancers," *Cell Cycle*, vol. 11, no. 6, pp. 1108–1117, 2012.

[42] M. Eilertsen, S. Andersen, S. Al-Saad et al., "Monocarboxylate transporters 1-4 in NSCLC: MCT1 is an independent prognostic marker for survival," *PLoS ONE*, vol. 9, no. 9, Article ID e105038, 2014.

[43] K. S. Lim, K. J. Lim, A. C. Price, B. A. Orr, C. G. Eberhart, and E. E. Bar, "Inhibition of monocarboxylate transporter-4 depletes stem-like glioblastoma cells and inhibits HIF transcriptional response in a lactate-independent manner," *Oncogene*, vol. 33, pp. 4433–4441, 2014.

[44] J.-W. Choi, Y. Kim, J.-H. Lee, and Y.-S. Kim, "Prognostic significance of lactate/proton symporters mct1, mct4, and their chaperone CD147 expressions in urothelial carcinoma of the bladder," *Urology*, vol. 84, no. 1, pp. 245.e9–245.e15, 2014.

[45] X. Yang, J. Dai, T. Li et al., "Expression of EMMPRIN in adenoid cystic carcinoma of salivary glands: correlation with tumor progression and patients' prognosis," *Oral Oncology*, vol. 46, no. 10, pp. 755–760, 2010.

[46] L. Tian, Y. Zhang, Y. Chen, M. Cai, H. Dong, and L. Xiong, "EMMPRIN is an independent negative prognostic factor for patients with astrocytic glioma," *PLoS ONE*, vol. 8, no. 3, Article ID e58069, 2013.

[47] A. B. Als, L. Dyrskjøt, H. von der Maase et al., "Emmprin and survivin predict response and survival following cisplatin-containing chemotherapy in patients with advanced bladder cancer," *Clinical Cancer Research*, vol. 13, no. 15, pp. 4407–4414, 2007.

[48] F. Liu, L. Cui, Y. Zhang et al., "Expression of HAb18G is associated with tumor progression and prognosis of breast carcinoma," *Breast Cancer Research and Treatment*, vol. 124, no. 3, pp. 677–688, 2010.

[49] Y. Liu, T. Xin, Q.-Y. Jiang et al., "CD147, MMP9 expression and clinical significance of basal-like breast cancer," *Medical Oncology*, vol. 30, article 366, 2013.

[50] Y. Li, J. Xu, L. Chen et al., "HAb18G (CD147), a cancer-associated biomarker and its role in cancer detection," *Histopathology*, vol. 54, no. 6, pp. 677–687, 2009.

[51] S.-H. Zhao, Y. Wang, L. Wen et al., "Basigin-2 is the predominant basigin isoform that promotes tumor cell migration and invasion and correlates with poor prognosis in epithelial ovarian cancer," *Journal of Translational Medicine*, vol. 11, no. 1, p. 92, 2013.

[52] X.-Z. Ju, J.-M. Yang, X.-Y. Zhou, Z.-T. Li, and X.-H. Wu, "EMMPRIN expression as a prognostic factor in radiotherapy of cervical cancer," *Clinical Cancer Research*, vol. 14, no. 2, pp. 494–501, 2008.

[53] S. Zhu, Y. Li, L. Mi et al., "Clinical impact of HAb18G/CD147 expression in esophageal squamous cell carcinoma," *Digestive Diseases and Sciences*, vol. 56, no. 12, pp. 3569–3576, 2011.

[54] H.-C. Zheng, H. Takahashi, Y. Murai et al., "Upregulated EMMPRIN/CD147 might contribute to growth and angiogenesis of gastric carcinoma: a good marker for local invasion and prognosis," *British Journal of Cancer*, vol. 95, no. 10, pp. 1371–1378, 2006.

[55] M. Yang, Y. Yuan, H. Zhang et al., "Prognostic significance of CD147 in patients with glioblastoma," *Journal of Neuro-Oncology*, vol. 115, no. 1, pp. 19–26, 2013.

[56] Q. Yang, Y. Liu, Y. Huang et al., "Expression of COX-2, CD44v6 and CD147 and relationship with invasion and lymph node metastasis in hypopharyngeal squamous cell carcinoma," *PLoS ONE*, vol. 8, no. 9, Article ID e71048, 2013.

[57] L.-C. Zhu, J. Gao, Z.-H. Hu et al., "Membranous expressions of Lewis y and CAM-DR-related markers are independent factors of chemotherapy resistance and poor prognosis in epithelial ovarian cancer," *American Journal of Cancer Research*, vol. 5, no. 2, pp. 830–843, 2015.

[58] X. Gou, H. Chen, F. Jin et al., "Expressions of CD147, MMP-2 and MMP-9 in laryngeal carcinoma and its correlation with poor prognosis," *Pathology and Oncology Research*, vol. 20, no. 2, pp. 475–481, 2014.

[59] X.-Y. Xu, N. Lin, Y.-M. Li, C. Zhi, and H. Shen, "Expression of HAb18G/CD147 and its localization correlate with the progression and poor prognosis of non-small cell lung cancer," *Pathology Research and Practice*, vol. 209, no. 6, pp. 345–352, 2013.

[60] L. S. Monteiro, M. L. Delgado, S. Ricardo et al., "EMMPRIN expression in oral squamous cell carcinomas: correlation with tumor proliferation and patient survival," *BioMed Research International*, vol. 2014, Article ID 905680, 9 pages, 2014.

[61] N. Futamura, Y. Nishida, H. Urakawa et al., "EMMPRIN co-expressed with matrix metalloproteinases predicts poor prognosis in patients with osteosarcoma," *Tumor Biology*, vol. 35, no. 6, pp. 5159–5165, 2014.

[62] Q. Lu, G. Lv, A. Kim, J.-M. Ha, and S. Kim, "Expression and clinical significance of extracellular matrix metalloproteinase inducer, EMMPRIN/CD147, in human osteosarcoma," *Oncology Letters*, vol. 5, no. 1, pp. 201–207, 2013.

[63] S. Zhao, W. Ma, M. Zhang et al., "High expression of CD147 and MMP-9 is correlated with poor prognosis of triple-negative breast cancer (TNBC) patients," *Medical Oncology*, vol. 30, no. 1, article 335, 2013.

[64] Y. Zhao, S. Chen, W. Gou et al., "The role of EMMPRIN expression in ovarian epithelial carcinomas," *Cell Cycle*, vol. 12, no. 17, pp. 2899–2913, 2013.

[65] T. Chu, X. Chen, J. Yu, J. Xiao, and Z. Fu, "Extracellular matrix metalloproteinase inducer is a negative prognostic factor of pediatric medulloblastoma," *Pathology and Oncology Research*, vol. 17, no. 3, pp. 705–711, 2011.

[66] Z.-D. Han, H.-C. He, X.-C. Bi et al., "Expression and clinical significance of CD147 in genitourinary carcinomas," *Journal of Surgical Research*, vol. 160, no. 2, pp. 260–267, 2010.

[67] S. Piao, S. Zhao, F. Guo et al., "Increased expression of CD147 and MMP-9 is correlated with poor prognosis of salivary duct carcinoma," *Journal of Cancer Research and Clinical Oncology*, vol. 138, no. 4, pp. 627–635, 2012.

[68] X.-C. Bi, J.-M. Liu, H.-C. He et al., "Extracellular matrix metalloproteinase inducer: a novel poor prognostic marker for human seminomas," *Clinical and Translational Oncology*, vol. 14, no. 3, pp. 190–196, 2012.

[69] H. Tan, K. Ye, Z. Wang, and H. Tang, "CD147 expression as a significant prognostic factor in differentiated thyroid carcinoma," *Translational Research*, vol. 152, no. 3, pp. 143–149, 2008.

[70] Y. Kim, J.-W. Choi, J.-H. Lee, and Y.-S. Kim, "Expression of lactate/H^+ symporters MCT1 and MCT4 and their chaperone CD147 predicts tumor progression in clear cell renal cell carcinoma: immunohistochemical and the Cancer Genome Atlas data analyses," *Human Pathology*, vol. 46, no. 1, pp. 104–112, 2015.

[71] J. Afonso, L. L. Santos, V. Miranda-Gonçalves et al., "CD147 and MCT1-potential partners in bladder cancer aggressiveness and cisplatin resistance," *Molecular Carcinogenesis*, vol. 54, no. 11, pp. 1451–1466, 2015.

[72] K. Kaira, K. Arakawa, K. Shimizu et al., "Relationship between CD147 and expression of amino acid transporters (LAT1 and ASCT2) in patients with pancreatic cancer," *American Journal of Translational Research*, vol. 7, no. 2, pp. 356–363, 2015.

[73] Y.-X. Liang, H.-C. He, Z.-D. Han et al., "CD147 and VEGF expression in advanced renal cell carcinoma and their prognostic value," *Cancer Investigation*, vol. 27, no. 7, pp. 788–793, 2009.

[74] B. Davidson, I. Goldberg, A. Berner, G. B. Kristensen, and R. Reich, "EMMPRIN (extracellular matrix metalloproteinase inducer) is a novel marker of poor outcome in serous ovarian carcinoma," *Clinical & Experimental Metastasis*, vol. 20, no. 2, pp. 161–169, 2003.

[75] A. G. Liu, Q. Hu, H. F. Tao, S. Y. Liu, L. Q. Zhang, and Y. Hu, "Expression of CD147 and matrix metalloproteinase-9 in children with non-Hodgkin's lymphoma and its correlation with prognosis," *Zhonghua Er Ke Za Zhi*, vol. 47, no. 10, pp. 785–788, 2009.

[76] Y. Wan and X. Y. Wu, "Expression and clinical significance of DAPK1 and CD147 in esophageal squamous cell carcinoma," *Zhonghua Zhong Liu Za Zhi*, vol. 34, no. 1, pp. 44–48, 2012.

[77] Y.-H. Yu, J. Morales, L. Feng, J. J. Lee, A. K. El-Naggar, and N. Vigneswaran, "CD147 and Ki-67 overexpression confers poor prognosis in squamous cell carcinoma of oral tongue: a tissue microarray study," *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, vol. 119, no. 5, pp. 553–565, 2015.

[78] Z. Huang, H. Huang, H. Li, W. Chen, and C. Pan, "EMMPRIN expression in tongue squamous cell carcinoma," *Journal of Oral Pathology and Medicine*, vol. 38, no. 6, pp. 518–523, 2009.

[79] C. Huang, Z. Sun, Y. Sun et al., "Association of increased ligand cyclophilin A and receptor CD147 with hypoxia, angiogenesis, metastasis and prognosis of tongue squamous cell carcinoma," *Histopathology*, vol. 60, no. 5, pp. 793–803, 2012.

[80] Z.-M. Du, C.-F. Hu, Q. Shao et al., "Upregulation of caveolin-1 and CD147 expression in nasopharyngeal carcinoma enhanced tumor cell migration and correlated with poor prognosis of the patients," *International Journal of Cancer*, vol. 125, no. 8, pp. 1832–1841, 2009.

[81] H. Y. Wang, B. Y. Sun, Z. H. Zhu et al., "Eight-signature classifier for prediction of nasopharyngeal [corrected] carcinoma survival," *Journal of Clinical Oncology*, vol. 29, no. 34, pp. 4516–4525, 2011.

[82] K. Nakamura, J. Kodama, A. Hongo, and Y. Hiramatsu, "Role of emmprin in endometrial cancer," *BMC Cancer*, vol. 12, article 191, 2012.

[83] W.-C. Tsai, Y. Chen, L.-C. Huang et al., "EMMPRIN expression positively correlates with WHO grades of astrocytomas and meningiomas," *Journal of Neuro-Oncology*, vol. 114, no. 3, pp. 281–290, 2013.

[84] D. Buergy, T. Fuchs, P. Kambakamba et al., "Prognostic impact of extracellular matrix metalloprotease inducer: immunohistochemical analyses of colorectal tumors and immunocytochemical screening of disseminated tumor cells in bone marrow from patients with gastrointestinal cancer," *Cancer*, vol. 115, no. 20, pp. 4667–4678, 2009.

[85] W. Sienel, B. Polzer, K. Elshawi et al., "Cellular localization of EMMPRIN predicts prognosis of patients with operable lung adenocarcinoma independent from MMP-2 and MMP-9," *Modern Pathology*, vol. 21, no. 9, pp. 1130–1138, 2008.

[86] L. Li, W. Tang, X. Wu et al., "HAb18G/CD147 promotes pSTAT3-mediated pancreatic cancer development via CD44s," *Clinical Cancer Research*, vol. 19, no. 24, pp. 6703–6715, 2013.

[87] R. Li, Y. Pan, B. He et al., "Downregulation of CD147 expression by RNA interference inhibits HT29 cell proliferation, invasion and tumorigenicity in vitro and in vivo," *International Journal of Oncology*, vol. 43, no. 6, pp. 1885–1894, 2013.

[88] H. Z. Zeng, Y. Q. Qu, A. B. Liang et al., "Expression of CD147 in advanced non-small cell lung cancer correlated with cisplatin-based chemotherapy resistance," *Neoplasma*, vol. 58, no. 5, pp. 449–454, 2011.

[89] E. L. Rosenthal, S. Shreenivas, G. E. Peters, W. E. Grizzle, R. Desmond, and C. L. Gladson, "Expression of extracellular matrix metalloprotease inducer in laryngeal squamous cell carcinoma," *Laryngoscope*, vol. 113, no. 8, pp. 1406–1410, 2003.

[90] W.-C. Tsai, Y.-C. Chao, W.-H. Lee, A. Chen, L.-F. Sheu, and J.-S. Jin, "Increasing EMMPRIN and matriptase expression in hepatocellular carcinoma: tissue microarray analysis of immunohistochemical scores with clinicopathological parameters," *Histopathology*, vol. 49, no. 4, pp. 388–395, 2006.

[91] H.-G. Li, D.-R. Xie, X.-M. Shen, H.-H. Li, H. Zeng, and Y.-J. Zeng, "Clinicopathological significance of expression of paxillin, syndecan-1 and EMMPRIN in hepatocellular carcinoma," *World Journal of Gastroenterology*, vol. 11, no. 10, pp. 1445–1451, 2005.

[92] S. Sillanpää, M. Anttila, K. Suhonen et al., "Prognostic significance of extracellular matrix metalloproteinase inducer and matrix metalloproteinase 2 in epithelial ovarian cancer," *Tumor Biology*, vol. 28, no. 5, pp. 280–289, 2007.

[93] W.-C. Tsai, Y.-C. Chao, L.-F. Sheu et al., "EMMPRIN and fascin overexpression associated with clinicopathologic parameters of pancreatobiliary adenocarcinoma in Chinese people," *APMIS*, vol. 115, no. 8, pp. 929–938, 2007.

[94] Y. Ishibashi, T. Matsumoto, M. Niwa et al., "CD147 and matrix metalloproteinase-2 protein expression as significant prognostic factors in esophageal squamos cell carcinoma," *Cancer*, vol. 101, no. 9, pp. 1994–2000, 2004.

[95] S. Zhu, D. Chu, Y. Zhang et al., "EMMPRIN/CD147 expression is associated with disease-free survival of patients with colorectal cancer," *Medical Oncology*, vol. 30, article 369, 2013.

[96] K. Ueda, K. Yamada, M. Urashima et al., "Association of extracellular matrix metalloproteinase inducer in endometrial carcinoma with patient outcomes and clinicopathogenesis using monoclonal antibody 12C3," *Oncology Reports*, vol. 17, no. 4, pp. 731–735, 2007.

[97] Q. Zhang, X. Chen, J. Zhou et al., "CD147, MMP-2, MMP-9 and MVD-CD34 are significant predictors of recurrence after liver transplantation in hepatocellular carcinoma patients," *Cancer Biology and Therapy*, vol. 5, no. 7, pp. 808–814, 2006.

[98] Q. Zhang, J. Zhou, X.-M. Ku et al., "Expression of CD147 as a significantly unfavorable prognostic factor in hepatocellular carcinoma," *European Journal of Cancer Prevention*, vol. 16, no. 3, pp. 196–202, 2007.

[99] X.-Q. Huang, X. Chen, X.-X. Xie et al., "Co-expression of CD147 and GLUT-1 indicates radiation resistance and poor prognosis in cervical squamous cell carcinoma," *International Journal of Clinical and Experimental Pathology*, vol. 7, no. 4, pp. 1651–1666, 2014.

[100] H. Chen, L. Wang, J. Beretov, J. Hao, W. Xiao, and Y. Li, "Co-expression of CD147/EMMPRIN with monocarboxylate transporters and multiple drug resistance proteins is associated with epithelial ovarian cancer progression," *Clinical & Experimental Metastasis*, vol. 27, no. 8, pp. 557–569, 2010.

[101] C. Pinheiro, V. Penna, F. Morais-Santos et al., "Characterization of monocarboxylate transporters (MCTs) expression in soft tissue sarcomas: distinct prognostic impact of MCT1 subcellular localization," *Journal of Translational Medicine*, vol. 12, article 118, 2013.

[102] C. Pinheiro, A. Longatto-Filho, J. Azevedo-Silva, M. Casal, F. C. Schmitt, and F. Baltazar, "Role of monocarboxylate transporters in human cancers: state of the art," *Journal of Bioenergetics and Biomembranes*, vol. 44, no. 1, pp. 127–139, 2012.

[103] E. P. Spugnini, P. Sonveaux, C. Stock et al., "Proton channels and exchangers in cancer," *Biochimica et Biophysica Acta—Biomembranes*, vol. 1848, no. 10, pp. 2715–2726, 2015.

[104] N. Pértega-Gomes, J. R. Vizcaíno, J. Attig, S. Jurmeister, C. Lopes, and F. Baltazar, "A lactate shuttle system between tumour and stromal cells is associated with poor prognosis in prostate cancer," *BMC Cancer*, vol. 14, article 352, 2014.

[105] S. Andersen, Ø. Solstad, L. Moi et al., "Organized metabolic crime in prostate cancer: the coexpression of MCT1 in tumor and MCT4 in stroma is an independent prognosticator for biochemical failure," *Urologic Oncology: Seminars and Original Investigations*, vol. 33, no. 8, pp. 338.e9–338.e17, 2015.

[106] C. Pinheiro, R. M. Reis, S. Ricardo, A. Longatto-Filho, F. Schmitt, and F. Baltazar, "Expression of monocarboxylate transporters 1, 2, and 4 in human tumours and their association with CD147 and CD44," *Journal of Biomedicine and Biotechnology*, vol. 2010, Article ID 427694, 7 pages, 2010.

[107] D. Hanahan and L. M. Coussens, "Accessories to the crime: functions of cells recruited to the tumor microenvironment," *Cancer Cell*, vol. 21, no. 3, pp. 309–322, 2012.

[108] I. L. Romero, A. Mukherjee, H. A. Kenny, L. M. Litchfield, and E. Lengyel, "Molecular pathways: trafficking of metabolic resources in the tumor microenvironment," *Clinical Cancer Research*, vol. 21, no. 4, pp. 680–686, 2015.

[109] D. C. Wallace, "Mitochondria and cancer," *Nature Reviews Cancer*, vol. 12, no. 10, pp. 685–698, 2012.

[110] E. Obre and R. Rossignol, "Emerging concepts in bioenergetics and cancer research: metabolic flexibility, coupling, symbiosis, switch, oxidative tumors, metabolic remodeling, signaling and bioenergetic therapy," *International Journal of Biochemistry and Cell Biology*, vol. 59, pp. 167–181, 2015.

[111] C. S. Ahn and C. M. Metallo, "Mitochondria as biosynthetic factories for cancer proliferation," *Cancer & Metabolism*, vol. 3, no. 1, 2015.

[112] K. M. Kennedy, P. M. Scarbrough, A. Ribeiro et al., "Catabolism of exogenous lactate reveals it as a legitimate metabolic substrate in breast cancer," *PLoS ONE*, vol. 8, no. 9, Article ID e75154, 2013.

[113] R. Amorim, C. Pinheiro, V. Miranda-Gonçalves et al., "Monocarboxylate transport inhibition potentiates the cytotoxic effect of 5-fluorouracil in colorectal cancer cells," *Cancer Letters*, vol. 365, no. 1, pp. 68–78, 2015.

[114] N. Draoui, O. Schicke, E. Seront et al., "Antitumor activity of 7-aminocarboxycoumarin derivatives, a new class of potent inhibitors of lactate influx but not efflux," *Molecular Cancer Therapeutics*, vol. 13, no. 6, pp. 1410–1418, 2014.

[115] N. Pértega-Gomes, S. Felisbino, C. E. Massie et al., "A glycolytic phenotype is associated with prostate cancer progression and aggressiveness: a role for monocarboxylate transporters as metabolic targets for therapy," *The Journal of Pathology*, vol. 236, no. 4, pp. 517–530, 2015.

[116] I. Marchiq, R. Le Floch, D. Roux, M. P. Simon, and J. Pouyssegur, "Genetic disruption of lactate/H^+ symporters (MCTs) and their subunit CD147/BASIGIN sensitizes glycolytic tumor cells to phenformin," *Cancer Research*, vol. 75, no. 1, pp. 171–180, 2015.

[117] S. Granja, I. Marchiq, R. L. Floch, C. S. Moura, F. Baltazar, and J. Pouysségur, "Disruption of BASIGIN decreases lactic acid export and sensitizes non-small cell lung cancer to biguanides independently of the LKB1 status," *Oncotarget*, vol. 6, no. 9, pp. 6708–6721, 2015.

[118] M. Pollak, "Potential applications for biguanides in oncology," *The Journal of Clinical Investigation*, vol. 123, no. 9, pp. 3693–3700, 2013.

[119] Thomas Jefferson University, "Pilot Study of Metformin in Head and Neck Squamous Cell Cancer and Its Effects on Stromal-epithelial Metabolic Uncoupling," ClinicalTrials.gov, NLM Identifier: NCT02083692, National Library of Medicine, Bethesda, Md, USA, 2000, https://clinicaltrials.gov/ct2/show/NCT02083692.

[120] NIH Research Portfolio Online Reporting Tools, *Project Information 1R43CA189391-01: Inhibitors of Lactic Acid*

Transporter MCT4, NIH Research Portfolio Online Reporting Tools, 2014, http://projectreporter.nih.gov/project_info_description.cfm?aid=8780410&icde=26325553&ddparam=&ddvalue=&ddsub=&cr=1&csb=default&cs=ASC.

[121] J. Xu, Z.-Y. Shen, X.-G. Chen et al., "A randomized controlled trial of licartin for preventing hepatoma recurrence after liver transplantation," *Hepatology*, vol. 45, no. 2, pp. 269–276, 2007.

[122] H. Bian, J.-S. Zheng, G. Nan et al., "Randomized trial of [131I] metuximab in treatment of hepatocellular carcinoma after percutaneous radiofrequency ablation," *Journal of the National Cancer Institute*, vol. 106, no. 9, 2014.

[123] Q. He, W.-S. Lu, Y. Liu, Y.-S. Guan, and A.-R. Kuang, "131I-labeled metuximab combined with chemoembolization for unresectable hepatocellular carcinoma," *World Journal of Gastroenterology*, vol. 19, no. 47, pp. 9104–9110, 2013.

[124] L. Wu, Y. F. Yang, N. J. Ge et al., "Hepatic artery injection of ^{131}I-labelled metuximab combined with chemoembolization for intermediate hepatocellular carcinoma: a prospective non-randomized study," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 39, no. 8, pp. 1306–1315, 2012.

[125] N. Dean, E. Helman, J. Aldridge, W. Carroll, S. Magnuson, and E. Rosenthal, "Anti-EMMPRIN treatment of HNSCC in an ex vivo model," *Laryngoscope*, vol. 120, supplement 4, article S146, 2010.

[126] Y.-H. Kuang, X. Chen, J. Su et al., "RNA interference targeting the CD147 induces apoptosis of multi-drug resistant cancer cells related to XIAP depletion," *Cancer Letters*, vol. 276, no. 2, pp. 189–195, 2009.

[127] S. Suzuki and K. Ishikawa, "Combined inhibition of EMMPRIN and epidermal growth factor receptor prevents the growth and migration of head and neck squamous cell carcinoma cells," *International Journal of Oncology*, vol. 44, no. 3, pp. 912–917, 2014.

[128] W. Schneiderhan, M. Scheler, K.-H. Holzmann et al., "CD147 silencing inhibits lactate transport and reduces malignant potential of pancreatic cancer cells in in vivo and in vitro models," *Gut*, vol. 58, no. 10, pp. 1391–1398, 2009.

[129] J. Su, X. Chen, and T. Kanekura, "A CD147-targeting siRNA inhibits the proliferation, invasiveness, and VEGF production of human malignant melanoma cells by down-regulating glycolysis," *Cancer Letters*, vol. 273, no. 1, pp. 140–147, 2009.

[130] S. Riethdorf, N. Reimers, V. Assmann et al., "High incidence of EMMPRIN expression in human tumors," *International Journal of Cancer*, vol. 119, no. 8, pp. 1800–1810, 2006.

[131] Y. Tang, M. T. Nakada, P. Kesavan et al., "Extracellular matrix metalloproteinase inducer stimulates tumor angiogenesis by elevating vascular endothelial cell growth factor and matrix metalloproteinases," *Cancer Research*, vol. 65, no. 8, pp. 3193–3199, 2005.

[132] L. Yan, S. Zucker, and B. P. Toole, "Roles of the multifunctional glycoprotein, emmprin (basigin; CD147), in tumour progression," *Thrombosis and Haemostasis*, vol. 93, no. 2, pp. 199–204, 2005.