

## Review Article

# Tumor Budding, Micropapillary Pattern, and Polyploidy Giant Cancer Cells in Colorectal Cancer: Current Status and Future Prospects

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We previously reported that polyploid giant cancer cells (PGCCs) induced by  $\text{CoCl}_2$  could form through endoreduplication or cell fusion. A single PGCC formed tumors in immunodeficient mice. PGCCs are also the key contributors to the cellular atypia and associate with the malignant grade of tumors. PGCCs have the properties of cancer stem cells and produce daughter cells via asymmetric cell division. Compared with diploid cancer cells, these daughter cells express less epithelial markers and acquire mesenchymal phenotype with importance in cancer development and progression. Tumor budding is generally recognized to correlate with a high recurrence rate, lymph node metastasis, chemoresistance, and poor prognosis of colorectal cancers (CRCs) and is a good indicator to predict the metastasis and aggressiveness in CRCs. Micropapillary pattern is a special morphologic pattern and also associates with tumor metastasis and poor prognosis. There are similar morphologic features and molecular phenotypes among tumor budding, micropapillary carcinoma pattern, and PGCCs with their budding daughter cells and all of them show strong ability of tumor invasion and migration. In this review, we discuss the cancer stem cell properties of PGCCs, the molecular mechanisms of their regulation, and the relationships with tumor budding and micropapillary pattern in CRCs.

## 1. Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors and its incidence ranks the third of malignant tumors [1]. The metastasis and relapse of CRC are the main reasons of tumor recurrence and patient death [2]. Twenty percent of CRCs have lymph node and/or distant metastasis at diagnosis [3]. The overall 5-year survival rate for CRC patients is 64% and this rate drops to 12% in metastatic CRC patients [1]. The high death rate of metastatic CRCs is well known. If we can identify characteristic features in the primary lesion which are highly correlated to recurrence and metastasis of CRCs, then these characteristics can be used as prognostic markers to predict the recurrence or metastasis [4, 5]. The essential step in tumor invasion and metastasis is the tumor dedifferentiation and dissociation at the invasion front [6]. However, the degree of tumor differentiation in

CRCs is hard to evaluate and is not exactly in accordance with the metastasis. Recently, increasing evidences confirmed the important role of polyploid giant cancer cells (PGCCs) and tumor budding in predicting the metastasis and patient's prognosis of CRCs [7, 8].

Normal human cells contain 46 chromosomes, but tumors cells contain abnormal numbers (usually between 60 and 90) of chromosomes, with cell-to-cell variability. Structural abnormalities of chromosomes such as inversions, deletions, duplications, and translocations are commonly observed in cancer cells but are rare in normal cells [9, 10]. The cells with abnormal number of chromosomes are named polyploid cells. PGCCs contribute to solid tumor heterogeneity and are the main histological feature of malignant tumor in pathologic diagnosis. Commonly, the number of PGCCs is higher in high-grade malignant tumor than in low-grade malignant tumor, in recurrent tumor after chemotherapy

than in tumor before chemotherapy, and in the metastatic foci than in the primary tumor [11, 12]. PGCCs are previously considered to be at the stage of mitotic catastrophe and believed to be nondividing senescent cells. Polyploid giant cells appear in skeletal muscles, osteoclasts, and senescent cells [13] and can be formed via cell fusion or abortive cell cycles [14]. We previously found that PGCCs isolated from the ovarian cancer and breast cancer cell lines can revert to regular cancer cells through budding [12, 15]. PGCCs can express cancer stem cell markers including CD44 and CD133. The daughter cells budded from PGCCs expressed EMT-related proteins and show strong ability of tumor invasion and migration.

Tumor budding is similar to the morphological features of micropapillary pattern [16–20]. Based on the morphologic features, protein expression, and biologic behaviors, we speculate that these daughter cells budded from PGCCs fall into the broad term of tumor budding and micropapillary cancer pattern [12, 15, 21]. This review will discuss the recent development of PGCCs and its association with tumor budding and micropapillary pattern in CRCs.

## 2. PGCCs and Cancer Stem Cells

Cancer stem cells, often referred to as tumor-initiating or tumor-propagating cells [22, 23], are capable of generating entire tumor mass. These cells are considered as the seed cells to fuel the development, chemoresistance, and recurrence of human cancer. The history of the cancer stem cell can be traced to Coneheim who proposed the embryonic nest theory of cancer stem cells 150 years ago [24, 25]. The early definitive evidence of cancer stem cells was found in leukemias [26]. Later, Al-Hajj et al. and other groups showed that cancer stem cells were present in solid cancers, including breast carcinoma and glioblastoma [27, 28]. Intensive efforts have been devoted to identifying and characterizing cancer stem cells. To date, stem cell-like populations have been characterized and isolated by flow cytometry using so-called cancer stem cell markers [27–29]. However, these markers were neither pure nor specific for cancer stem cells [30–32]. Furthermore, the phenotypes of these marker-enriched cancer cells were not stable and could change from marker-positive to marker-negative [33]. Thus, characterizations of markers that unequivocally identify a population of cancer stem cells remain challenging. American Association for Cancer Research (AACR) consensus conference workshop described cancer stem cell as “a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor” [34].

PGCCs are often considered as the senescent cells. PGCCs in tumors have not attracted major attention due to lack of extensive study and their poorly understood biology [12]. Actually, PGCCs are the key contributors to cancer heterogeneity and form the basis for differential diagnosis of benign and malignant tumors. They associate with the malignant grade and lymph node metastasis. The relationship between PGCCs and cancer differentiation has long been known, but it is not clear if PGCCs contribute to tumorigenesis or they are only the consequence of malignant transformation [35–37]. Clinical evidence is accumulating in support

of the view that the number of PGCCs positively correlates with the malignant degree of cancer. In cancer, multiple stresses including antimitotic chemotherapy drugs, radiotherapy, hypoxia, or poor microenvironment can increase the number of PGCCs.

We recently reported that PGCCs can be purified from human ovarian and breast cancer cell lines and primary human ovarian tumors with the use of chemical hypoxic mimetic, cobalt chloride ( $\text{CoCl}_2$ ) [12, 15, 21, 36–38], and confirmed that these cells were formed through cell fusion and produced daughter cells via asymmetric cell division. On the other hand, PGCCs are slow-cycling in nature and express stem cell markers. These cells are prone to differentiation into other benign tissues including adipose, cartilage, and bone, which has been confirmed both in vitro and in vivo [12]. A single PGCC can form spheroids in medium and Matrigel in vitro through time-lapse observation. A single PGCC can also form tumor when it was subcutaneously injected into the SCID mice. PGCCs express a distinct signature of proteins involved in hypoxia, invasion, chromatin remodeling, and cell cycle regulation [39–41]. These features of PGCCs suggest that PGCCs may represent a novel type of cancer stem cells which can be defined by size and morphology without using cell surface markers.

PGCCs generate daughter cells via asymmetric cell division which is a hallmark of stem cells. In multicellular eukaryotes, mitosis is the recognized process for somatic cell division, ensuring the accurate separation of genetic material [42, 43]. Asymmetric cell division is important in producing cell diversity during normal tissue development. In contrast to symmetric cell divisions, asymmetric cell division produces two daughter cells including stem cell and non-stem cell which have different cellular fates [44]. Asymmetric cell division is a fundamental process involving many physiological and pathological processes. In a typical outcome, the stem cell generates a copy of itself, and a second daughter cell programmed to differentiate into a non-stem cell type [45, 46]. Asymmetric division is a key mechanism ensuring tissue homeostasis, maintaining the stem and progenitor cell population, and allowing the development of diverse functional cells.

## 3. Tumor Budding and Its Clinical and Pathologic Significances

Prognosis of CRC patients has been associated with several morphological features including the infiltration depth, tumor cell differentiation, and lymph node or distant metastasis. These morphological features are the primary parameters for prognostic evaluation and tumor staging of CRCs in clinics [47]. However, other pathological indicators including tumor budding should be noted in the pathologic records to help the clinicians to judge the prognosis of patients with CRC [48]. Tumor budding is an increasingly recognizable feature to indicate the lymph node metastasis in CRCs [49–53]. “Tumor budding” was first described by Imai who noticed that cells sprouting from the edge of tumor entity are indicative of a tumor with high growth rate [54]. It is generally thought that tumor budding is a histological feature that is

observed by pathologists from microscopy. The term “tumor budding” is referred to as a cluster of cancer cells which are located in the invasive front microscopically [55, 56]. Tumor budding is defined as an isolated single cancer cell or a cluster composed of fewer than five cancer cells and has been reported to be highly related to the recurrence rate and poor prognosis in CRCs [48, 49, 57–61]. Tumor cells with minimally differentiated CRCs show strong ability of tumor invasion and migration and lymph node metastasis. The number of tumor budings has negative correlations with the degree of tumor differentiation. There are more tumor budings in minimally differentiated CRCs than in the highly differentiated and moderately differentiated CRCs [57, 62–65]. In clinical practice, Dukes staging system has been widely used for CRC classification for many years because it can effectively predict the disease prognosis. However, some patients with the same Dukes stage have different prognosis and different response to chemotherapy. Comprehensive evaluation system including the degree and type of differentiation, tumor budding, lymph node metastasis, and infiltrative depth should be used to guide the clinical treatment and predict prognosis. The comprehensive evaluation system should be reproducible and have substantial predictive value for the patients with CRC.

Several large studies tried to establish the criteria of clinical significances of tumor budding in CRCs and confirmed that tumor budding correlates with the lymph node or distant metastasis and associates with patient prognosis. Hase et al. studied 663 patients with CRCs; they divided patients into two groups according to the number of budings including none or mild group and moderate or severe group [55]. They found that the presence of moderate-to-severe tumor budding indicated a bad biological behavior of CRCs; they also proposed that meticulous follow-up and adjuvant chemotherapy may be beneficial to patients with moderate-to-severe tumor budding regardless of their Dukes staging. In a multivariate analysis, tumor budding but not diffuse infiltration was identified as an independent prognostic factor. Other studies showed that tumor budding is an independent factor to predict the prognosis of CRCs. Ueno et al. reported that the number of tumor budings is associated with tumor metastasis in CRCs [66–69]. A count of 0 to 9 tumor budings per field (magnification of  $\times 250$ ) was marked as low grade, and a count of 10 or more was considered as high grade [68]. Like the Gleason scores in cancers of the prostate, tumor budding may be one of the important scoring factors to predict the prognosis in CRC patients.

The morphologic characteristics of tumor budding reflect the loss of adhesive epithelial phenotype in cancer cells and are accompanied by the metastasis of cancer [70–73]. Results from Pyke et al. confirmed that laminin-5 is a marker of invading cancer cells because of similar distributions between laminin-5-positive budding cancer cells at the invasive front in CRCs and the receptor for urokinase-type plasminogen activator [74]. It was suggested that laminin-5 might represent a valuable marker for tumor budding. Furthermore, laminin-5 and urokinase-type plasminogen activator receptor colocalized in CRCs could be important in the invasion and metastasis of cancer cells [74].

#### **4. The Role of EMT in the Process of Tumor Budding and PGCCs with Daughter Cells**

EMT is involved in many physiological processes including mesoderm formation and neural tube formation and pathological processes including wound healing and organ fibrosis [75, 76]. It should be pointed out that EMT plays an essential role in cancer metastasis and progression as epithelial cells lose their cell polarity and cell-cell adhesion and become mesenchymal cells with migratory and invasive properties [77]. During EMT, cells lose their epithelial morphology because of the cytoskeleton reorganization. Low cell-cell adhesion resulted from E-cadherin dysfunction and different expression of tight and adherent junction proteins increased invasive properties of cancer cells [78].

EMT has also been demonstrated to play an essential role during the formation of cancer stem cells [79]. Previously, we confirmed that PGCCs can be induced by CoCl<sub>2</sub> and paclitaxel. When cancer cell lines recovered from the treatment of CoCl<sub>2</sub> and paclitaxel, PGCCs could produce daughter cells via asymmetric cell division. Western blot analysis was performed to confirm that cytokeratin (AE1/AE3) expression was lower in the daughter cells than in cancer cells without treatment. The increased expression of mesenchymal markers such as vimentin was evident in daughter cells [12, 15, 21]. Particularly after paclitaxel treatment, the daughter cells developed an elongated, spindle cell, fibroblastic morphology, which was consistent with mesenchymal cells [15]. A high-throughput iTRAQ-based proteomic methodology was used to determine the differentially expressed proteins between PGCCs treated with CoCl<sub>2</sub> and the control cells. Results showed that a panel of stem cell-regulating factors and EMT-related proteins were upregulated in PGCCs [21]. PGCCs with budding daughter cells had higher Snail, TWIST, and Slug expression than the diploid control tumor cells [21].

#### **5. Wnt/β-Catenin Signal Pathway in Tumor Budding**

Jass et al. reported that APC (adenomatous polyposis coli) mutation was much less frequent in sporadic microsatellite instability-high (MSI-H) cancers than in MSI-low or microsatellite stable cancers [7]. APC can regulate the expression of β-catenin and is an important component of β-catenin degradation complex. Tumor budding was characterized by increased expression for both β-catenin and p16 (cyclin-dependent kinase inhibitor 2A) [68]. β-Catenin is important for linkage of E-cadherins to the cytoskeleton [80, 81]. The downregulation of E-cadherin in carcinoma cells is associated with increased invasive ability of cancer cells [82, 83]. Furthermore, epithelial cell adhesion molecule (Ep-CAM) has been confirmed to be involved in tumor budding [84]. Loss of membranous Ep-CAM regulates the β-catenin subcellular localization. When β-catenin translocates from the cytoplasm to the nucleus, epithelial adhesion was reduced and migratory potential increased. Loss of Ep-CAM of CRC cell membrane is highly correlated with tumor budding, cancer grade, and local recurrence [84].

## 6. Tumor Budding, Micropapillary Pattern, and PGCCs in CRCs

Micropapillary pattern was first reported in breast cancer, in which a small cluster of tumor cells appear in tumor tissue and there are no vessels and stromal cells in the middle of tumor cluster [16]. Micropapillary pattern has been detected in about 20% of CRCs, a phenomenon analogous to the more familiar one seen in some carcinomas of breast, bladder, lung, pancreas, ovary, urothelial tract, and stomach [85–88]. Micropapillary pattern in carcinomas is associated with a greater frequency of lymphovascular invasion and lymph node metastases and poor prognosis [89]. Tang et al. reported that the average number of metastatic lymph nodes, the proportion of CRC with distant metastasis, and the number of cases with lymphovascular tumor emboli were significantly higher in CRCs with micropapillary pattern compared to those in CRCs without micropapillary pattern. Furthermore, micropapillary pattern often appears in tumor with minimal differentiation and the expression of E-cadherin in tumor cells of micropapillary pattern is lower than that in regular tumor cells [89]. The difference between tumor budding and micropapillary pattern is mainly based on the location and cell number. Micropapillary pattern can appear in both the edge and center of tumor mass, and the cell number is often more than five. Tumor budding is located in the invasion front and the cell number is less than five. Verdú et al. reported that there is an “inside-out” MUC1 immunohistochemical staining feature in micropapillary pattern [90]. MUC1 plays an important role in the detachment of cells from the stroma and determines the characteristic morphological features of the invasive potential and aggressive behavior via regulating the intercellular adherence [91]. Micropapillary pattern has similar morphological features to tumor budding in the invasion front of CRCs [90].

PGCCs appear in most of micropapillary carcinoma patterns and tumor budding. In some micropapillary patterns, the absence of PGCCs may be due to the five-micrometer thickness for slide processing. According to the morphological observations in CRCs with large sample, we speculate that tumor budding and micropapillary pattern may have the same origin, which derive from PGCCs with their budding daughter cells. We previously reported that single PGCCs which appeared after paclitaxel treatment from an invasive breast cancer cell line MCF-7 formed cancer organotypic structure (COS) including glandular, vessel-like, and papilla-like structures *in vitro* [15]. The papilla-like structures derived from single PGCCs were made of PGCCs and their daughter cells. The morphology of the papilla-like structure resembled that of micropapillary pattern in human CRCs [15]. As described above, daughter cells budded from PGCCs in tumors have strong ability to invade and migrate. Both tumor budding and micropapillary pattern originated from PGCCs with budding daughter cells show strong invasive ability.

There is a close association among tumor differentiation, tumor budding, PGCCs, and micropapillary carcinoma pattern. Lv et al. also reported that single stromal PGCCs

with their budding daughter cells were often associated with tumor metastasis in OSCs [92]. The number of single stromal PGCCs between low-grade and high-grade OSCs was different and statistically significant. High-grade OSCs have more single stromal PGCCs number [41, 92]. Single PGCCs generating daughter cells via budding can be observed in paraffin-embedded CRCs slides. Tumor tissue with single stromal PGCCs is more aggressive than tumor tissue without single stromal PGCCs. In another unpublished paper by us, we have confirmed that there are more tumor budings and PGCCs in minimally differentiated CRCs than in highly differentiated and moderately differentiated CRCs. PGCCs can generate daughter cells via budding (Figures 1(a) and 1(b)). Because of the fact that the cell number in micropapillary pattern is more than that in tumor budding, there is a space separation between the tumor tissue and mesenchymal tissue. However, PGCCs always appear in micropapillary pattern (Figures 1(c) and 1(d)) and tumor budding (Figures 1(e) and 1(f)). Thus, the criterion of using single stoma PGCCs with their budding daughter cells may be better representative than tumor budding or micropapillary pattern. The association among tumor differentiation, tumor budding, PGCCs, and micropapillary pattern can help us further understand tumor metastasis and patient’s prognosis.

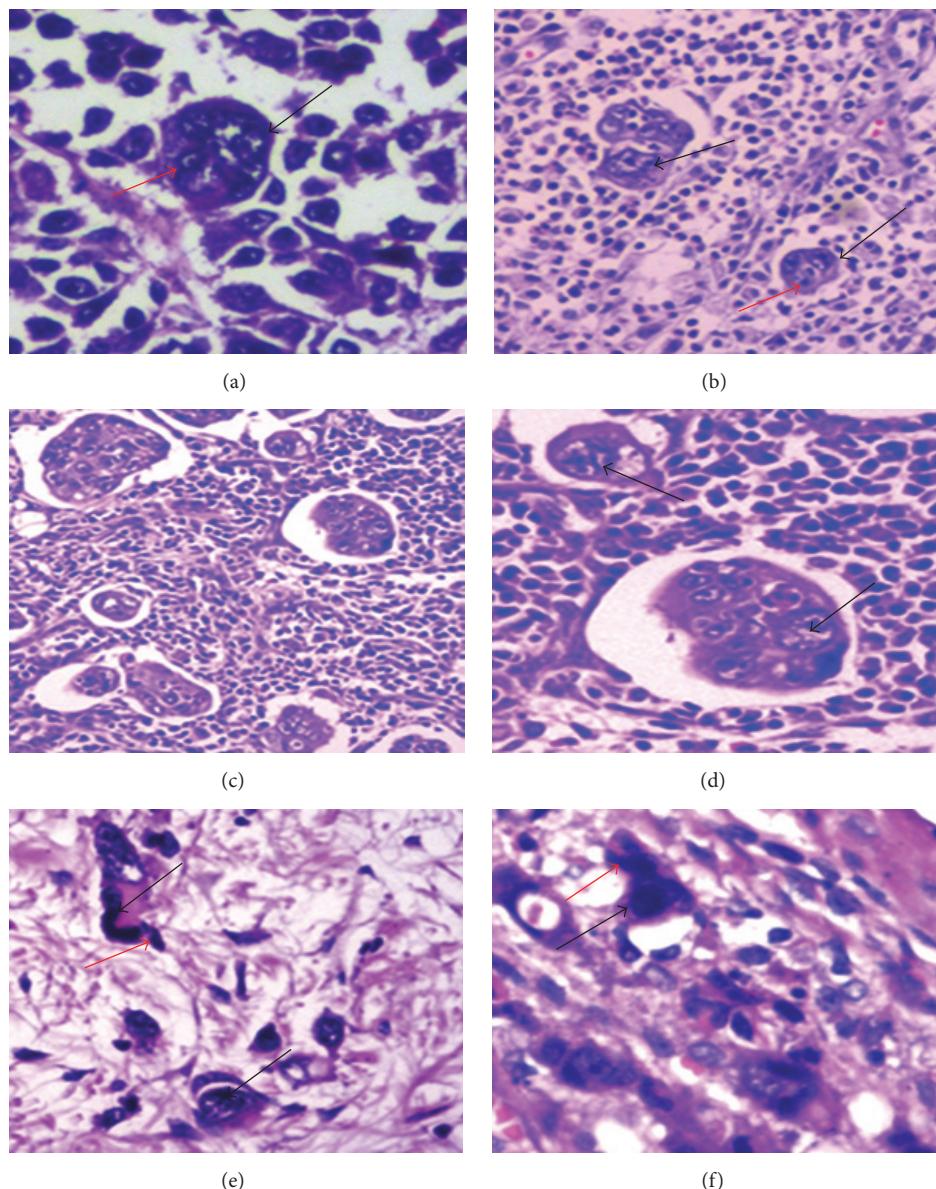
## 7. Future Prospects of Tumor Budding and Single Stromal PGCCs

CRCs with tumor budding were identified to respond to chemoradiotherapy poorly and have adverse prognosis. Tumor budding is mostly unreported in daily diagnostic practice due to the lack of a standardized evaluating system. Karamitopoulou et al. suggested a method using 10 high-power fields to assess tumor budding at the invasive front. Their opinion showed that using 10 high-power fields to evaluate tumor budding had independent prognostic value and could show good interobserver consistency [93]. Jass et al. described “discrete clusters” of cells as buds [5]; Hase et al. gave definition of buds as clusters “appearing to bud from a larger gland” [55]. However, the identification of tumor budding is often confused with the fibroblasts, histiocytes masquerading, and fragmentation of a larger gland.

Since tumor budding is related to the prognosis of CRC patients, the standardized evaluating index including tumor budding and PGCCs could improve the grading of CRCs. Thus, it is worth paying more attention to the relationships between PGCCs with their newly budding daughter cells and tumor budding. The molecular mechanisms of tumor budding are involved in the expression of EMT-related proteins and Wnt/β-catenin signaling pathway which may provide clinicians with new hints to treat tumor with high-grade malignancy through targeting these proteins involved in tumor budding and PGCCs formation.

## Competing Interests

The authors have no competing interests.



**FIGURE 1:** Tumor budding, PGCCs, and micropapillary carcinoma pattern in CRCs. ((a) and (b)) PGCCs with budding appear in minimally differentiated CRCs. Black arrows indicate PGCCs and red arrows indicate daughter cells generated by PGCCs (HE,  $\times 200$ ). (c) Micropapillary patterns appear in minimally differentiated CRC (HE,  $\times 100$ ). (d) PGCCs appear in micropapillary patterns (HE,  $\times 100$ , black arrows). ((e) and (f)) Single PGCCs with their budding daughter cells in CRCs; and the structure is similar to tumor budding. Black arrows indicate PGCCs and red arrows indicate daughter cells budded by PGCCs (HE,  $\times 200$ ).

## Authors' Contributions

Dan Zhang and Zhengduo Yang contributed to collection of literatures. Shiwu Zhang and Xipeng Zhang contributed to conception and design, financial support, and manuscript writing. All authors read and approved the final manuscript.

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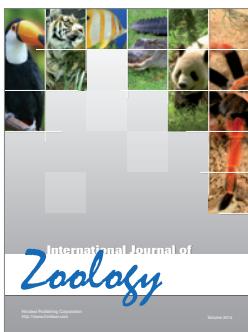
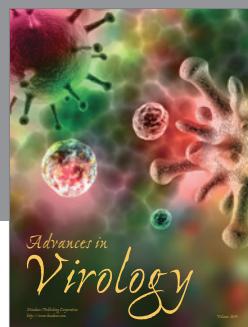
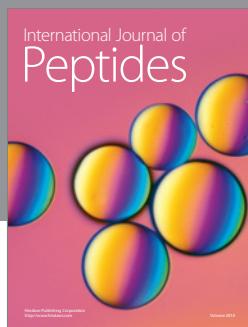
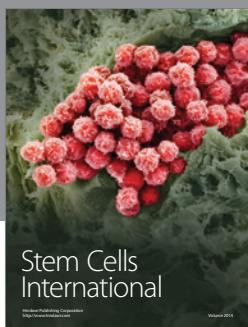
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