

# Cancer cell dormancy: mechanisms and implications of cancer recurrence and metastasis

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**Abstract:** More recently, disease metastasis and relapse in many cancer patients several years (even some decades) after surgical remission are regarded as tumor dormancy. However, the knowledge of this phenomenon is cripplingly limited. Substantial quantities of reviews have summarized three main potential models that can be put forth to explain such process, including angiogenic dormancy, immunologic dormancy, and cellular dormancy. In this review, newly uncovered mechanisms governing cancer cell dormancy are discussed, with an emphasis on the cross talk between dormant cancer cells and their microenvironments. In addition, potential mechanisms of reactivation of these dormant cells in certain anatomic sites including lymph nodes and bone marrow are discussed. Molecular mechanism of cellular dormancy in head and neck cancer is also involved.

**Keywords:** cancer cell dormancy, disseminated tumor cells, head and neck cancer, hypoxia, lymph node metastasis

## Introduction

Tumor relapse and metastasis in some cancers can arise years or even decades after treatment, causing huge damage to patients, and are responsible for the vast majority of cancer-related deaths. The inability to treat metastasis is the most important challenge faced by modern oncologists. Recently, the extensive period of time in which patients remain asymptomatic before metastasis and relapse represents the clinical observations known as tumor dormancy. This broadly defined phenomenon has now come into sharp focus. Tumor dormancy was first defined by Willis<sup>1</sup> and then redefined by Hadfield<sup>2</sup> as a temporary mitotic and growth arrest. The mitotic arrest precisely refers to cellular dormancy, suggesting that a G0–G1 arrest can exist in certain cancer cells.<sup>3</sup> The growth arrest means a dormant cancer mass, in which the constituent cancer cells are kept constant by the equilibrium between cell division and apoptosis. In addition, the current literature suggests that the latter process may be due to an angiogenic or/and immunologic dormancy.<sup>4–6</sup> It is widely appreciated that residual cancer cells would continuously encounter different growth-constraining conditions during dissemination and tumorigenic progression, such as hypoxia, nutritional deprivation, and chemotherapy stimuli.<sup>7,8</sup> These cancer cells can release certain factors to modulate their growth-related signaling pathways through the cross talk between residual cancer cells and their microenvironments, leading to a state of dormancy or proliferation. Residual cancer cells can escape immune surveillance and the lethal effect of chemotherapy in hard survival conditions via growth arrest. However, they could exit dormancy and proliferate again in distant organs.

Over the past 2 decades, constant findings have strived to clarify the sources, phenotypes, properties, hosting niches, and signaling pathways of disseminated

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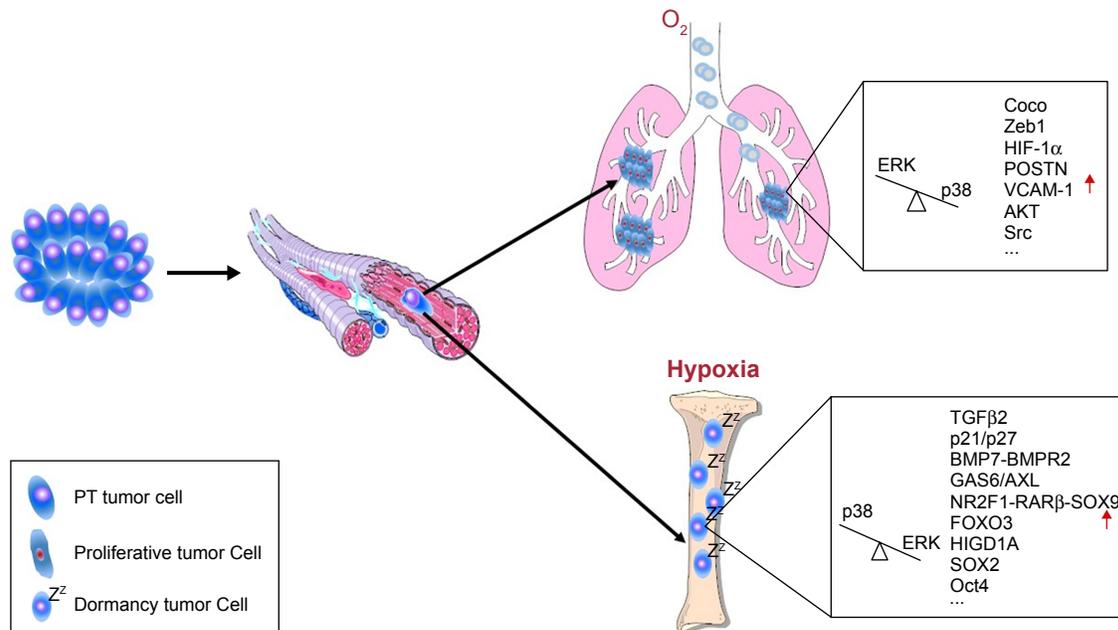
tumor cells (DTCs) that predict the survival, dormancy, and reactivation of minimal residual disease in head and neck cancer (HNC). Large work has been performed to establish DTCs as selection markers and monitoring tools for identifying the early stage of cancers,<sup>9</sup> because of their increasing identification as the cause of metastatic relapse. DTCs are generally detected in the bone marrow (BM). The majority of DTCs remain a state of quiescence.<sup>10</sup> A subgroup of DTCs circulating in the blood is termed circulating tumor cells (CTCs), and some findings indicated that DTCs could hold a stem cell-like phenotype called cancer stem cells (CSCs; Figure 1). A significant body of evidence has demonstrated that DTCs and CTCs could be detected in asymptomatic patients with melanoma, breast cancer, HNC, etc.<sup>11–13</sup> However, how DTCs and CTCs maintain the long-term survival and reactivate to form micrometastases in distant organs is poorly understood. Recently, the underlying mechanisms of DTCs in tumor dormancy have been revealed.

In this review, we focus primarily on mechanisms governing cancer cell dormancy and discuss how DTCs and their niche jointly modulate tumor dormancy in the metastatic progression of cancer. We also provide some

insights into lymph node metastasis in patients with HNC based on recent evidence. Importantly, increasing studies of mechanisms of tumor dormancy could bring new hope to neoadjuvant chemotherapy and precision medicine for patients in the future.

## Dormancy-related cancer cells DTCs

Tumor dormancy is a critical step in the development of both primary tumor and metastatic disease. It is strongly conceived that there must be some cancer cells maintain and survive after an apparently successful treatment. In addition, they may even lodge in distant organs at the early stage of cancer and eventually contribute to late recurrence of disease.<sup>14</sup> Intriguingly, DTCs have been routinely detected in BM of patients with different cancers, since the pioneering work of Schlimok et al<sup>15</sup> and Riethmuller and Johnson<sup>16</sup> was published in the 1980s. Moreover, current findings support the suggestion that certain DTCs retain dormant for an extended period of time, which is determined by the lack of proliferating markers (Ki-67, PCNA) accompanied by the lack of apoptotic markers terminal deoxynucleotidyl transferase dUTP nick end



**Figure 1** Schematic view of dormancy-related tumor cells and molecules in tumor development.

**Notes:** PT contains various cancer cells, including proliferative cells, dormant cells, and cancer stem cells. PT microenvironment is also heterogeneous in oxygen concentration and the ECM. When PT cancer cells invade into peripheral blood (named circulating tumor cells [CTCs]), some of them can undergo an epithelial-to-mesenchymal transition (EMT) and obtain a stem-like phenotype. In addition, these PT cancer cells can sequentially disseminate to distant organs such as the BM (named DTCs). These cancer cells experience a genetic, epigenetic, and phenotypic conversion. PT hypoxic microenvironment could induce the expression of dormancy markers in cancer cells and decrease the chemosensitivity. When DTCs arrive at the BM, the permissive niche (TGFβ2, p21/p27, BMP7-BMP2, GAS6/AXL, NR2F1-RARβ-SOX9, FOXO3, HIGD1A, SOX2, Oct4, etc.) can contribute to maintaining a dormant state of DTCs. Conversely, the lung is a restrictive microenvironment to DTC dormancy. A high concentration of oxygen and the special ECM (TGFβ1/3, Coco, Zeb1, HIF-1α, POSTN, VCAM-1, AKT, SFK, etc.) can awaken the dormant cancer cells to form micrometastases in the lung. Especially, the ratio of ERK MAPK/p38 MAPK plays a crucial role in this dormancy and reactivation. A high ratio of p38 MAPK/ERK MAPK can induce DTCs entering into dormancy, and in turn a high ratio of ERK MAPK/p38 MAPK can reactivate dormant cancer cells to proliferate. In addition, dormancy DTCs in the BM also can disseminate to other distant sites and then hide and/or wake up to form secondary tumor at a particular point in time.

**Abbreviations:** BM, bone marrow; DTC, disseminated tumor cells; ECM, extracellular matrix; PT, primary tumor.

labeling (TUNEL) assay and (M30) and may be responsible for conventional chemoradiotherapy resistance.<sup>17,18</sup> In addition, new markers including NR2F1, DEC2, and p27 have recently suggested a DTC dormancy.<sup>19</sup> BM is the most common organ for DTCs homing in different epithelial cancers including breast, prostate, colon, HNC, etc. (Figure 1).<sup>20</sup> Also, substantial quantities of evidence suggest that BM niche is permissive to survival and maintenance of dormant DTCs (Figure 1). However, there is an obvious distinction between the early DTCs and late DTCs. Late DTCs are validated to be more tumorigenic than early DTCs. In general, DTCs can be obtained through the BM aspiration. But this approach has not been universal in clinical practice due to being invasive. Up to now, the identification of dormant DTCs primarily depends on two main approaches, including immunological assays using monoclonal antibodies directed against histogenic proteins (GFP fluorescence) and polymerase chain reaction (PCR)-based molecular assays exploiting tissue-specific transcripts (quantitative PCR [qPCR]).

## CTCs

CTCs are usually implicated in metastatic relapse and progression. The detection of CTCs in peripheral blood has been a routine program to indicate patients with cancer metastasis and poor prognosis in breast cancer, prostate cancer, and colorectal cancer.<sup>22–24</sup> Although they have been detected in HNC, CTCs have not yet been widely acknowledged because of the limitation of the small patient cohorts, as well as the poor understanding of the impact of these cells. In addition, thus, CTCs are not in routine clinical practice for patients with HNC.<sup>25,26</sup> It is reported that DTCs have more significant value compared to CTCs with respect to elucidating tumor dormancy. However, CTCs are more easily detected in peripheral blood, and the method brings merely damage to patients than DTCs. The convenience and acceptability of sequential peripheral blood analysis for CTCs are of great potential significance. Detection and enrichment of CTCs are based on the expression of EpCAMs and cytokeratins. CellSearch<sup>®</sup> System has been applied to noninvasive monitoring of CTCs in cancer patient samples as well as the isolation of single cell for genomic analysis with high accuracy.<sup>27</sup> Due to the low CTC counts in peripheral blood, advanced technologies and ultrasensitive methods need to be gradually developed to improve their clinical utility.

## CSCs

CSCs have been demonstrated in several solid tumors, including HNC, melanoma, breast cancer, prostate cancer, colon cancer, and pancreas cancer.<sup>28–32</sup> CSCs not only play

a key role in cancer initiation and maintenance of tumor bulk but also reflect a more aggressive and poorer prognosis.<sup>33</sup> Specific CSC markers for solid tumors mainly include CD44, CD133, ALDH, and EpCAM.<sup>34–36</sup> In addition, CD10 has been identified as a potential marker for CSCs in head and neck squamous cell carcinoma (HNSCC).<sup>37</sup> Increasing studies indicated that a subset of DTCs and CTCs undergoes an epithelial-to-mesenchymal transition (EMT) and obtain a stem-like phenotype. Also, the phenotype is associated with an increased capacity for migration and invasion, as well as resistance to anoikis and apoptosis. In HNSCC, epidermal growth factor receptor (EGFR), neurotrophin receptor B, and interleukin-1 $\beta$  are reported to be involved in EMT and an elevated population of CSCs.<sup>38–40</sup> However, most of these cancer cells seem to be unable to undergo the reverse process of mesenchymal-to-epithelial transition (MET) to form metastases.<sup>41</sup> Chaffer and Weinberg<sup>42</sup> reported that CSCs have greatly enhanced tumor-initiating potential but a temporary growth arrest within a tumor. Dormancy and reactivation of CSCs are closely related to epigenetic reprogramming of these CSCs in HNC.<sup>43,44</sup> A subset of miRNAs is demonstrated to be responsible for self-renewal and differentiation of CSCs in different types of cancers.<sup>45</sup> In HNC CSCs, miR-424, let-7a, miR-6836, and miR-6873 are lower expressed than miR-147b and miR-7152.<sup>46</sup> Especially, miRNA-34a is shown to repress EMT, aldehyde dehydrogenase activity, invasiveness, and clonogenicity of CSCs in HNSCC.<sup>47</sup> However, potential pathways involved in these processes have not been described in detail, and thus further work will be needed in the future. Strikingly, the production of CSCs in HNSCC may be orchestrated by stress-triggered atavistic reprogramming (STAR), and even the HNSCC evolution may be highly dependent on the STAR activities.<sup>48</sup>

## Molecular mechanisms of cancer cell dormancy

Cancer cell dormancy has been confirmed as residual cancer cells that lack proliferative and apoptotic markers and maintain in a state of quiescence without a continuous growth. In this paper, we reviewed the molecular mechanisms of dormant-related cancer cells, with an emphasis on the cross talk between cancer cells and their microenvironments.

### Intracellular signals

#### Mitogen-activated protein kinase (MAPK) pathways

MAPK family (also known as Ras–Raf–MEK–ERK pathway) is of paramount importance in converting extracellular stimuli into a wide spectrum of cellular responses, and its functions in cancer development are complex. Some findings

suggest that p38 MAPK signaling is a double-edged sword on cancer cell growth. P38 $\alpha$  negatively regulates cell cycle progression like p38 $\gamma$ , while p38 $\delta$  induces cell proliferation in squamous cell carcinoma.<sup>49</sup> Furthermore, Sosa et al<sup>50</sup> showed a low (ERK MAPK/p38 MAPK) signaling ratio in dormant HEP3 cells in head and neck carcinoma (Figure 1). In addition, then, it has been confirmed in breast cancer, prostate cancer, melanoma, ovarian cancer, and fibrosarcoma, finding that ~90% of the dormant cell lines expressed a similar level of ERK MAPK/p38 MAPK. The alteration in ERK MAPK/p38 MAPK is demonstrated to be arranged by the uPA–uPAR complex (urokinase plasminogen activator binds to uPA receptor) in extracellular matrix (ECM).<sup>51–54</sup> Activated p38 MAPK pathway could induce DTCs to enter into growth arrest via activating p53 and p16 signaling and downregulating cyclin D1 (Table 1).<sup>50</sup> Overall, the equilibrium of ERK MAPK and p38 MAPK is closely related to cancer cell dormancy and the ratio of ERK MAPK/p38 MAPK may be the key determining factor for tumor dormancy. Accordingly, p38 inhibitor should be used with caution as it may carry a potential risk for a cohort of patients with cancer for the reason that p38 $\alpha$ / $\beta$  inhibition after surgery can increase the burden of DTCs in the BM, liver, and spleen.<sup>55</sup>

Jun N-terminal kinase (JNK) is the third major MAPK pathway that has been reported to exert an inverse function compared with p38 MAPK signaling, as JNK can induce proliferation and tumorigenesis of cancer cells.<sup>56</sup> But extensive experiments suggested a contradictory scenario that active JNK was required for growth arrest and could induce dormancy in breast cancer (Table 1).<sup>57</sup> In addition, mitogen-activated protein kinase kinase 4 (MKK4) has been recently demonstrated to induce cancer cells into a transient growth arrest.<sup>58,59</sup>

### PI3K-AKT pathways

PI3K-AKT-mTOR pathway is another well-studied signaling pathway in regulating the cell cycle. Jo et al described a cancer cell-secreted regulatory system that mediated the

PI3K-AKT-mTOR pathway within nutritional deprivation stress and demonstrated that reduced PI3K-AKT signaling could result in quiescence and autophagy.<sup>60</sup> In addition, such phenomenon was further validated in dormant HNSCC cells.<sup>61</sup> Glucocorticoid-induced leucine zipper (GILZ) is an important upstream target of PI3K/AKT signaling pathway, and AKT can be downregulated by the repression of GILZ in dormant cancer cells. And FOXO3A (one key substrate of PI3K/AKT pathway) is consequently diminished and p21 increased and eventually the cancer cell maintain dormancy.<sup>62</sup> Inhibiting AKT can trigger EGFR autophosphorylation, which also can lead to a growth arrest in cancer cells (Table 1).<sup>63</sup>

### Extracellular signals

#### Transforming growth factor- $\beta$ (TGF- $\beta$ ) family

TGF- $\beta$  family is an intricate cytokine network that modulates an array of cell viabilities including cell proliferation, morphogenesis, migration, ECM production, cytokine secretion, and apoptosis. Over the past 2 decades, TGF- $\beta$  family has been found in the process of EMT, angiogenesis, and cancer cell dormancy. Latent TGF- $\beta$ -binding protein 2 (LTBP-2) has recently been suggested to promote dormancy against metastatic growth and restrain the proliferation in nasopharyngeal carcinoma and esophageal squamous cell carcinoma (ESCC). But, it can also augment cancer cell adhesion and migration in melanoma; therefore, the function of LTBP-2 is related to specific tumor types and environments.<sup>64</sup> TGF- $\beta$ 1 can suppress tumor progression in precancerous lesions and early stage of cancers, but it can promote tumor growth in advanced-stage cancers.<sup>65</sup> TGF- $\beta$ 3 has recently been demonstrated to accelerate the growth, migration, and invasion of HNC through inducing matrix-specific protein periostin (POSTN).<sup>66</sup> However, TGF- $\beta$ 2, upregulated within all-trans retinoic acid (atRA) niche can induce a dormant phenotype in HNSCC via p38 MAPK-dependent pathway.<sup>67</sup> In addition, TGF- $\beta$ 2-induced cancer cell dormancy also requires AXL and GAS6.<sup>68</sup> When Coco (an antagonist of TGF- $\beta$  ligands) overexpresses in metastatic site, cancer cells can escape

**Table 1** Molecular mechanism of cancer cell dormancy and reactivation

| Molecular mechanism   | Dormancy   | Reactivation  | Uncertain   |
|-----------------------|--|---|---|
| Intracellular signals | p38MAPK, <sup>50</sup> p16, <sup>50</sup> p21, <sup>62</sup> p27, <sup>19</sup> p53, <sup>50</sup> MKK4, <sup>58,59</sup> NR2F1/RAR $\beta$ /SOX9 <sup>67</sup>  | ERKMAPK, <sup>50</sup> PI3K/AKT, <sup>60</sup> GILZ, <sup>62</sup> FOXO3A <sup>62</sup>   | JNK <sup>56,57</sup>  |
| Extracellular signals | TGF $\beta$ 2/TGF $\beta$ -RIII, <sup>68</sup> BMP7/BMPR2, <sup>73</sup> BMP4, <sup>76</sup> AXL/GAS6, <sup>82–84</sup> TBK1, <sup>88</sup> miR122(3)/miR23b, <sup>91,92</sup> LIFR/STAT3/SOCS3, <sup>108</sup> DEC2 <sup>19</sup> | TGF- $\beta$ 3, <sup>66</sup> MERTK, <sup>85</sup> POSTN, <sup>89</sup> SFK, <sup>125</sup> Fra-1, <sup>87</sup> Coco <sup>69</sup> | LTBP-2, <sup>64</sup> Tyro3, <sup>87</sup> TGF- $\beta$ 1 <sup>65</sup> |
| Bone marrow niche     | atRA, <sup>67</sup> HIGD1A, <sup>110,111</sup> PP2A <sup>112</sup>   | VCAM-1, <sup>79</sup> Zeb1, <sup>120</sup> HIF-1 $\alpha$ , <sup>21</sup> LOXL2 <sup>102</sup>                                      |   |

dormancy.<sup>69</sup> Therefore, it is concluded that TGF- $\beta$  family paradoxically acts on tumor progression (Table 1).

Wendt et al<sup>70</sup> indicated that the multifunction of TGF- $\beta$  largely reflects its ability to govern the expression levels of epithelial cadherin, a hallmark of a fully differentiated epithelium that scarcely proliferates or migrates which is implicated in the process of EMT. Nevertheless, the underlying mechanisms are masked by a complex interplay between diverse cytokines. For example, Denys et al<sup>71</sup> proved in a colon cancer model that cancer cells could remain dormant and did not proliferate on a basement membrane extract, which is composed mainly of various growth factors and other components.<sup>72</sup>

### Bone morphogenetic proteins (BMPs)

Bone morphogenetic protein 7 (BMP7) is one branch of the TGF- $\beta$  family secreted from normal BM stromal cells. It exerts diverse functions of embryonic patterning and organogenesis and tissue remodeling and repair, especially skeletal tissue. Previously, BMP7 has been demonstrated to induce a low ERK MAPK/p38 MAPK signaling ratio in dormant HEP3.<sup>50</sup> Recently, BMP7 is also reported to induce CSC dormancy by activating p38 MAPK, p21, and N-myc downstream-regulated gene 1 (*NDRG1*) in a BMP receptor 2 (BMPR2)-dependent manner. Knockdown of BMPR2 inhibited BMP7 activation, and BMPR2 expression was found to be inversely connected with cancer relapse and bone metastasis.<sup>73</sup> Therefore, it is an attractive possibility that a BMPR2-dependent BMP7-induced dormancy exists in cancer cells (Table 1). Buijs et al<sup>74</sup> have demonstrated the hypothesis about an antagonistic role of BMP7 in Smad-mediated effects of TGF- $\beta$  and suggested that BMP7 may be a promising target to inhibit local cancer progression and bone metastases. However, BMP7 also can induce MET, a process that has been validated to play a critical role in predicting cancer cells that grow into macrometastasis. Therefore, BMP7 might increase the growth of cancer cells in advanced metastasis.<sup>75</sup> Fang et al<sup>76</sup> found that the downregulation of BMP4 by elevated SOX2 could promote the growth of cancer cells. In addition, SOX2 silencing could mediate cancer cell dormancy, and this process was accompanied by the upregulation of BMP4.

### BM-derived stromal cell niche

Hematopoietic stem cell (HSC) niche was first proposed by Schofield.<sup>77</sup> HSCs mainly reside in the BM, and HSC niche has been indicated as a fertile ground for the survival and development of DTCs. Recent work by Shiozawa et al<sup>78</sup> has

demonstrated that DTCs can target and displace HSCs and establish pre-metastatic niche within this new home. Blood-vessel-derived signals could modulate the dormant phenotype of DTCs in certain tumor models. Vascular cell adhesion protein-1 (VCAM-1), for instance, has been demonstrated to predict the metastasis progression by interacting with integrin  $\alpha 4\beta 1$  expressed on osteoclasts (Table 1).<sup>79</sup> Obviously, HSC niche not only promotes the survival of DTCs but also could be a dangerous element for the reactivation of DTCs. It is suggested that atRA is abundant in the BM and peri-vascular niche and can regulate HSC renewal.<sup>80</sup> So, it is convinced that atRA may make coalescing contribution to cancer cell dormancy with TGF- $\beta 2$  and BMP7 in the BM (Table 1).<sup>81</sup> Treatment of T-HEP3 cells with atRA induced an elevated expression of NR2F1-RAR $\alpha$ -SOX9 signaling and TGF- $\beta 2$ , eventually pushing the cancer cells into a state of dormancy.<sup>67</sup>

Osteoblasts and osteoclasts in the BM can secrete many ECM-related factors to manipulate metastatic dormancy and reactivation. Recent studies have shown that prostate cancer cell expresses the annexin II receptor, which can bind to annexin II expressed on osteoblast cell surface. In addition, this binding can induce the expression of AXL, Sky, and Mer and eventually induce cancer cell dormancy via AXL-GAS6 signaling pathway. It is validated that the conversion of solitary DTC to dormant cancer cell is regulated by AXL-GAS6 signaling pathway, whereas cancer cells, which grow rapidly in the BM milieu, can express less GAS6.<sup>82–84</sup> However, Cackowski et al<sup>85</sup> have further investigated Mer tyrosine kinase (MERTK) in prostate cancer DTCs and noted that knockdown of MERTK could induce a low ratio of ERK MAPK/p38 MAPK, an increased expression of NR2F1 and p27 and a G0–G1 arrest. Comparing with AXL, Tyro3 (a subfamily of AXL) overexpresses in more proliferative cancer cells of the primary tumor, and high levels of Tyro3 is regarded as a marker for poor prognosis in prostate cancer.<sup>86</sup> Besides, Fra-1 that shares 50% correlation of coexpression with AXL highly expressed in multiple cancers, including HNC. But the overexpression of Fra-1 can directly deplete CSC dormancy and thereby promote cancer cell chemosensitivity.<sup>87</sup> Furthermore, Kim et al<sup>88</sup> found that binding with osteoblasts can induce the expression of TANK-binding kinase 1 (TBK1) in DTCs, which inhibits mTOR, and finally results in cell cycle arrest.

In vivo experiments reveal that dormant cancer cells are usually found near the perivascular niche. In addition, low levels of POSTN and TGF- $\beta 1$  may contribute to DTCs to maintain a dormancy state (Table 1).<sup>89</sup> Mesenchymal

stem/stromal cells (MSCs) are proposed to be first encountered with DTCs within the BM, as they are anatomically located at the abluminal surface of the central vasculature in the cavity.<sup>90</sup> Studies performed by Bliss et al<sup>91</sup> have shown that DTCs could instruct MSCs to release exosomes with distinct miRNAs, such as miR222/223 and miR23b, leading to cycling dormancy of certain DTCs (Table 1).<sup>92</sup> Intriguingly, MSC cannibalism in BM recently can be a unique mechanism supporting cancer cell dormancy via transfer of cell cycle inhibitory miRNA through gap junctions and/or exosomes.<sup>93,94</sup> Consequently, MSCs may be a promising “vehicle” to modulate cancer cell dormancy within the BM.

## Hypoxia and cancer cell dormancy

Hypoxia occurs frequently in human solid tumors. The environment is associated with poor survival and increased metastatic incidence and tumor burden in patients with various cancer, including HNC, prostate cancer, cervical cancer, breast cancer, etc.<sup>95–97</sup> Under low oxygen tensions, cancer cells have predilection for becoming invasive and disseminating to distant sites through hypoxia-associated transcriptional activation, including the hypoxia-inducible factor (HIF), transcriptional regulators, mTOR complex 1, autophagy and endoplasmic reticulum stress responses, etc.<sup>98,99</sup> Also, it is increasingly suggested that hypoxia is responsible for therapeutic resistance to both chemotherapy and radiotherapy because cancer cells may remain a state of dormancy or growth arrest in such microenvironment. For instance, forkhead box M1 (FOXO1), which is demonstrated as a regulator of cellular redox reaction and radiation response, was detected much lower in quiescent cancer cells than proliferating cancer cells in HNSCC (Table 1).<sup>100</sup> These data provide an underlying mechanism by which cancer cells enter into dormancy or are reactivated by the hypoxic environment.<sup>101</sup> Msaki et al<sup>21</sup> found that HIF-1 $\alpha$  in late DTCs of mammary cancer was highly expressed and suggested that HIF-1 $\alpha$  favored to enhance tumorigenic abilities of DTCs (Table 1). Interestingly, it is reported that conditional hypoxia could induce the expression of endogenous LOXL2, and then promote EMT in dormant MCF-7 cells, drive these cells to express CSC-like phenotypes, and eventually escape from dormancy to metastatic outgrowth.<sup>102</sup> Hypoxia is suggested to induce autophagy activities in a HIF-1 $\alpha$ -dependent manner, and the process may lead to a state of temporary dormancy in the early stage of cancer or therapy-induced microenvironments in HNSCC.<sup>103–105</sup> The leukemia inhibitory factor (LIF) receptor, the ligand LIF belongs to the IL-6 family of pro-inflammatory cytokines, has been recently recognized

as a cancer distant metastasis suppressor and a dormant cancer cell promoter.<sup>106–108</sup> Hypoxia could induce DTCs in the BM to spontaneously exit dormancy and reactivate through downregulating LIFR:STAT3:SOCS3 signaling pathway (Table 1).<sup>108</sup> In addition, hypoxia can increase uPAR expression, which could trigger cancer cell dissemination, invasion, EMT, and release from dormancy via ERK MAPK/p38 MAPK signaling pathway.<sup>109</sup>

In another scenario, however, hypoxia seems to suppress cancer invasion and progression via inducing cancer cells into dormancy. Hypoxia-inducible gene domain family member 1A (HIGD1A), for example, can promote survival and dormancy in a HIF-independent manner (Table 1).<sup>110,111</sup> It has been confirmed that severe hypoxia also could induce protein phosphatase 2A (PP2A) activity that mediated growth inhibition, and PP2A was positively correlated with HIF-1 expression.<sup>112</sup> Furthermore, hypoxia was demonstrated to upregulate dormancy markers including NR2F1 and DEC2.<sup>67</sup> Therefore, whether hypoxia promotes or inhibits DTC dormancy needs further work to validate.

## Lymph node metastasis and dormancy in HNC

Regional lymph node metastasis and distant metastasis are two major metastatic models in epithelial cancer patients. In addition, lymph node metastasis is suggested the most common and adverse event in patients with HNSCC that the presence reduces survival by 50%.<sup>113</sup> Tumor-induced lymph angiogenesis had been reported to be involved in tumor growth and metastasis, not only at the primary site but also in lymph nodes and distant sites.<sup>114,115</sup> Beasley et al<sup>116</sup> provided evidence in HNC that active lymphatic formation occurred in more invasive tumors and a high intra-tumoral lymph vessel concentration was significantly related to cervical node metastasis. Similarly, tumor lymph angiogenesis had been observed to take part in the initiation of lymphatic metastasis.<sup>117</sup> Substantial quantities of evidence suggested that tumor cells lodged in BM of patients with breast cancer maintained in a state of dormancy, as well as in HNSCC.<sup>15</sup> Although obvious differences in biology between the two kinds of cancer cells exist, similar micro-metastasis rates (30%–40%) were reported within the BM of patients with breast cancer and HNSCC. Accordingly, we can raise the possibility that there are some dormant cancer cells reside in lymph nodes of HNC patients, and these cells may be responsible for lymph angiogenesis, lymph node metastasis, and even for distant metastasis. In addition, occult lymph nodes in HNC may be explained by such scenario as well.

Mobilization of BM-derived cells (BMDCs) and their recruitment to metastatic niches by tumor-derived factors are the potential mechanisms of lymph node metastasis and tumor-induced lymph angiogenesis after a period of dormancy in clinical observation. VEGF-A, for example, not only directly mobilizes BMDCs in the BM, but indirectly recruits BMDCs to metastatic sites via inducing the expression of the pro-inflammatory S100A8 and S100A9 cytokines. In addition, that in turn induces the expression of serum amyloid A proteins.<sup>118,119</sup> VEGF-C and VEGF-D, another two members of the VEGF family, were also shown to induce proliferation of local lymphatic vessels. Such tumor-induced lymph angiogenesis then may be induced intra- or peri-tumorally, even remotely in the distant draining lymph systems.<sup>120</sup> However, whether such tumor-derived factors can reactivate the dormant cancer cells in lymph nodes of HNC patients remains unclear.

Lymph nodes are frequently glutted with an inflammation milieu because of their anatomic location, and recently a report by De Cock et al<sup>121</sup> has unveiled a potential effect of inflammation on dormant DTCs. He noted that the escape from dormancy is regulated by a Zeb1-dependent pathway, which is a key regulator of the EMT, so it is highly plausible that other inflammatory stimuli may similarly contribute to the escape process (Table 1). In addition, this mechanism may be implicated in the phenomenon that lymph node metastasis is most frequent in HNSCC patients.

CSCs also can contribute to tumor-induced lymph angiogenesis via direct trans-differentiating to lymphatic endothelial cells and generating various lymph-angiogenic factors. Understanding the underlying mechanisms of cancer cell dormancy in draining lymph nodes and distant metastasis would improve the prognosis and survival of patients with HNC. Inactivating DTCs in lymph nodes by driving them into dormancy through targeting these tumor-induced factors is a promising neoadjuvant chemotherapy, which may become one part of precision medicine. Even it is necessary to reemphasize or de-emphasize the therapeutic effect of preventive neck dissection in the future.

## Therapeutic implications and outlooks

Tumor dormancy and dormancy-related cancer cells have now come into sharp focus as contributions to metastasis and relapse. The studies discussed earlier mainly involved in molecular mechanisms of dormant cancer cells and provided a part of the framework to understand the process of tumor dormancy. Dormancy-related cancer cells are the causes of

cancer metastasis and relapse, including DTCs, CTCs, and CSCs. In addition, a bulk of evidence yields that the vast majority of them can enter into a state of quiescence, temporarily or permanently. Strikingly, fluorouracil – the most frequently medicine in cancer treatment – has been found to increase the burden of dormant cancer cells and enrich the population of CSCs, and these cancer cells in turn to be involved in chemotherapy resistance.<sup>122,123</sup> Also, hormonal therapy has also been demonstrated to promote the generation of CSCs in luminal breast cancer.<sup>124</sup> Therefore, it is anxious to confirm the effect on certain chemotherapies.

In this mini-review, we discussed intracellular, extracellular, and BM-derived factors that are associated with dormancy regulation system and summarized a bulk of potential treatment targets. In addition, we discussed the interaction between cancer cells and their niche. Also, we hypothesized the underlying interplay between lymph node metastasis and tumor dormancy in HNSCC.

Mechanisms of cancer cell dormancy will provide new insights into the complex biology of relapse and metastasis with important implications for the clinical management of cancer patients – either eradicate dormant cancer cells or maintain them. Furthermore, maintaining the state of cancer cell dormancy may be insufficient, but it is also required to suppress their survival. A combination of Src inhibitor and ERK MEK inhibitor, for instance, has been recommended in preventing breast cancer recurrence.<sup>125</sup> However, some studies also suggest to awake dormant cancer cells into the cell cycle and eventually to eliminate more cancer cells. For instance, in HNSCC, LB1 (an inhibitor of PP2A) can enhance the cytotoxic sensitivity to radiation or chemotherapy in quiescent cancer cells via promoting them from dormancy into the cell cycle.<sup>126</sup> Knockdown of TBK1 also can decrease dormant cancer cells and diminish drug resistance.<sup>86</sup> In conclusion, to identify the critical players and more responsive molecules that regulate the cancer cell dormancy which eventually turn to novel therapeutic targets. Furthermore, it is thus promising to expect that cancer cell dormancy may be the “Trojan horse” of the cancer therapy.

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## Disclosure

The authors report no conflicts of interest in this work.

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