Cancer Stemness in Tumor Progression

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Cancer stem cells (CSCs) are a population of cells present in malignant tumors that have much in common with normal stem or progenitor cells. The general characteristics of these cells include the ability to self-renew and differentiate into several clones, which leads to activation of tumor growth and heterogeneity. Mutations that occur in the stem cell pool can contribute to the oncogenesis process.

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1. Pluripotency Factors as Intrinsic Factors Regulating Cancer Stemness

CSC function is determined by a dysregulation of stemness-related signaling pathways. A reduced level of tumor differentiation and increased self-renewal are a characteristic of stemness. Transcription factors which are master regulators of self-renewal and pluripotency in embryonic stem cells (ESCs) have been demonstrated to play a keyrole in the regulation of stemness in cancer [1]. These transcription factors include the octamer-binding transcription factor 4 (OCT4), the sex-determining region Y-box 2 (SOX2), the homeobox transcription factor NANOG, the Kruppel-like factor 4 (KLF4), and the proto-oncogene C-MYC [2][3][4].

Expression of these factors can reprogram somatic cells into induced cancer stem cells and promote cell plasticity allowing cancer cells to adapt, survive, grow, and resist therapies. This effect has been demonstrated by a recent study showing acquisition of stemness after induced expression of OCT4, SOX2, and NANOG and high expression of pluripotency genes in advanced prostate, bladder, and renal cancers which was correlated with aggressive disease and drug resistance [5]. In addition, pluripotency factors have been shown to mediate cell plasticity in the TME and of enhance ECM production leading to metastasis [6]. Expression of pluripotency factors also regulate the expression of EMT mediators SNAI1 and SNAI2 [7].

Ectopic expression of OCT4 induced a block of differentiation and dysplasia in epithelial tissues [8][9]. Expression of OCT4 has been found in several cancer types and it contributes to the self-renewal and chemoresistance of CSCs [10][11]. Indeed, OCT4 induces the expression of the drug transporter ABCG2, which is highly expressed in CSCs and responsible for drug resistance [12]. Moreover, a relationship between OCT4 translation and metastasis of colorectal cancer to the liver have been demonstrated [13]. Similarly, it has been shown that OCT4 expression in lung cancer cells promotes the polarization of M2 type macrophages due the macrophage colony-stimulating factor (M-CSF) secretion, which leads to increase in tumor growth and metastasis [14].

SOX2 expression is also associated with cancer stemness [15][16]. Expression of this transcription factor is increased in cells and tumor tissue of patients with triple-negative breast cancer (TNBC). Importantly, inhibition of SOX2 suppresses proliferation and invasion of breast cancer cells, inducing cell apoptosis *in vitro* and inhibiting tumor growth and metastasis *in vivo* [17]. SOX2 knockout in a mouse model of osteosarcoma also induces a sharp decrease in frequency and occurrence of tumors [18]. In addition, SOX2 and CD133 co-expression can be associated with poor outcome in colon, stomach, and ovarian cancers, as well as melanoma and advanced cancers with bone metastases [19].

NANOG is also involved in maintaining embryonic stem cell self-renewal and cancer stemness [20][21][22]. It has been shown that an increase in the number of oral cancer stem-like cells is associated with increase expression of NANOG and increase malignancy [23]. The expression of this transcription factor increases with the degree of dysplasia and is an early predictor of cancer risk in patients with oral cavity malignant diseases [24]. Mutation in the tumor suppressor SPOP and negative regulator of NANOG also leads to increased stemness of prostate cancer and a negative prognosis in prostate cancer [25]. Dehghan Harati et al. have shown that the expression of NANOG is associated with the increased activity of ALDH and radioresistance, as well as with repair of double-strand DNA breaks [26].

Together with other pluripotency genes, KLF4 plays an important role in the regulation of cell growth, proliferation, and differentiation [27]. In embryonic stem cells, KLF4 activates the expression of telomerase reverse transcriptase (*TERT*) and contributes to the maintenance of self-renewal [28]. In cancer, KLF4 can act either as oncogene by inhibiting apoptosis or tumor suppressor by inducing p21-dependent cell cycle arrest. For instance, KLF4 is highly expressed in a subset of human melanomas and ectopic KLF4 expression enhances melanoma cell growth by decreasing apoptosis [29]. It has also been shown that KLF4 expression is associated with stemness of osteosarcoma [30]. However, KLF4 can also function as tumor suppressor and its knockdown can promote migration and invasion of non-small-cell lung carcinoma (NSCLC) [31].

Similarly, enhanced expression of KLF4 by lentiviral transduction increased sensitivity of ovarian cancer cells to the chemotherapeutic drugs paclitaxel and cisplatin [32].

Finally, *C-MYC* coordinates various biological processes in stem cells, such as cell cycle, cell metabolism, self-renewal, differentiation, and apoptosis [33]. Mutations in *MYC* genes have been found in many tumors and C-MYC is upregulated and acts as an oncogene in more than 50% of human cancers [34]. The expression of C-MYC correlates with the level of differentiation in cancer, as expression of C-MYC induces de-differentiation and acquisition of CSC properties, including glutamine metabolic addiction, dormancy and therapeutic resistance [35]. Dysregulation of MYC usually plays an important role in maintaining the number of invasive CSCs. For example, increased expression of *MYC* is associated with glioblastoma CSC-induced cell proliferation and invasion, and apoptosis inhibition [36].

2. Signaling Pathways Modulate Cancer Stemness

Several signaling pathways that are known mediators of juxtacrine (cell–cell) and paracrine extracellular signaling in the local TME have been identified to be key extrinsic players in the regulation of cancer stemness. These include Wnt, Notch, Hedgehog (Hh), Janus kinase/signal transducers and activators of transcription (JAK/STAT), and phosphatidylinositol 3-kinase/serine/threonine-protein kinase/mammalian target of the rapamycin (PI3K/AKT/mTOR) (reviewed in detail in Yang et al.) [1]. Moreover, some of these pathways also participate in epithelial-to-mesenchymal and mesenchymal-toepithelial (MET) transitions, thus regulating cell identity and plasticity [37].

In addition, there are a large number of studies related to other signaling pathways involved in cancer progression, self-renewal, and metastasis of CSCs [1][38]. For example, recent developments to target and inhibit NF-κB in the ovarian cancer or disruption of the NF-κB/IL-8 signaling in breast cancer can potential targeted therapy for CSCs [39][40][41]. Signaling regulation can be complex in different types of tumors, with cross-interaction of pathways participating in the regulation of CSCs [42][43].

2.1. Wnt Signaling

The activation of the Wnt pathway is common in cancer and can be caused by mutations in Wnt signaling components [44][45][46], as well as in downstream targets. Indeed,aberrant activation of Wnt mediators such as APC, β-catenin, Axin, Wnt1, and others are found in many cancers. For instance, thyroid receptor-interacting protein 6 (TRIP6) is an adapter protein that belongs to Lim proteins Zixin family and plays an important role in regulating the function of CSCs in breast cancer through regulation of Wnt/β-Catenin signaling [47]. Similarly, B-cell lymphoma/leukemia 11A (BCL11A) contributes to formation and invasion of tumor cells, stem cell self-renewal and activation of signalling by Wnt/β-Catenin and the EMT pathway. In addition, BCL11A is associated with lung metastasis and increase stemness of breast cancer cells [48].

Interestingly, glioblastoma cells expressing high levels of Wnt demonstrated expression of OCT-4, SOX2, NANOG, NESTIN, and CD133, thus suggesting a role of Wnt signaling in the maintenance of glioma CSCs [49].

2.2. Notch Signaling

The Notch pathway is also important for CSC function, and it is activated in tumors surviving and adapting to their microenvironment. Activation of the Notch pathway contributes to self-renewal, metastasis, and suppression of apoptosis. For example, the aberrant transmission of Notch signals (Notch1 and Notch4) contributes to self-renewal and metastasis of breast CSCs [50]. High levels of Notch1, Notch3, JAG1, JAG2, and the target HES-1 are found in pancreatic and breast cancers [51][52]. Notch signaling is activated under hypoxic conditions in breast cancer mediating chemoresistance and CSC expansion, which can be reversed by treatment with Notch inhibitors [53]. In addition, suppression of Notch1 via miR-34a can lead to an increase in breast cancer cell chemosensitivity to paclitaxel with a reduction in CSC proliferation and expression of the stemness marker ALDH1 [54]. Glioma stem cells are also regulated by activation of Notch1 and they show increased expression of the CSC genes OCT4 and CD133 under hypoxia [55].

2.3. Hedgehog Signaling

Together with Wnt and Notch signaling, the Hedgehog pathway is involved in embryonic development and organogenesis, including the nervous system, and organs such as lung, heart, and bowel [56]. Abnormal activation of the Hedgehog signaling pathway can be detected in CSCs [57][58]. For instance, it contributes to self-renewal, proliferation, and tumorigenicity of lung adenocarcinoma stem cells [59]. Through activation of the PTCH1 receptor and downstream effector Gli-1, Hedgehog signaling stimulates the transcription of the target genes OCT4, SOX2, NANOG, and C-MYC [60]. Zhu et al. showed that SHH, PTCH1, and Gli-1 are activated by TSPAN8 expression in breast CSCs leading to increased expression of NANOG, OCT4, and ALDHA1 genes, as well as increased stem cell selfrenewal and cell survival after treatment with adriamycin and paclitaxel [61]. Similarly, Hedgehog signaling stimulates self-renewal of glioma CSCs as they overexpress SHH, PTCH11, and GLI1 [62]. The Hedgehog pathway has also been shown to be important for pancreatic CSCs, as inhibition of the ligand SHH by inhibition of sialidase-2 (Neu2) and desialylation leads to a decrease in stemness [63].

2.4. JAK/STAT Signaling

The JAK/STAT pathway promotes survival, self-renewal, hematopoiesis, and neurogenesis of ESCs [64]. This pathway is also activated in CSCs [65]. Among the different subtypes of STAT proteins, activation of STAT3 plays an important role in CSC function by regulating oncogenic signaling pathways. STAT3 is constitutively activated in many different cancers, including pancreatic breast, prostate, ovarian liver, colorectal, and bone cancers, as well as leukemia and melanoma. In addition, STAT3 activation is associated with the generation of glioblastoma stem cells and the metastatic potential of colon CSCs [35]. As well as STAT3, it has been shown that suppression of STAT1 reduces the formation of lung A549 tumor spheres which was maintained by the suppression of factors associated with stemness, such as SOX2, OCT4, and NANOG [66].

2.5. AKT/mTOR Signaling

The PI3K/AKT/mTOR signaling pathway is important for cell proliferation and survival, and abnormal activation of PI3K/mTOR signals is commonly found in cancer [67][68]. The activation of this pathway also increases the migration, invasion, and resistance of the CSCs [69]. The transmission of PI3K/AKT signals is part of the main molecular stemness program both in mouse and human pluripotent stem cells. The oncogenic version of PIK3CA ^{H1047R} in cancer causes constitutive activation of the PI3K pathway and is associated with increased stemness in a dose-dependent manner, as shown in mouse models of breast, lung, and colorectal cancers [70]. Activation of the PI3KCA is also associated with induction of EMT and stem cell plasticity through multiple signals, including TGFβ [71].

3. Influence of the Microenvironment on CSC

Stem cells cannot survive outside their niche environment or in the absence of specific pluripotency factors and signaling pathways that support stem cell function [72]. Importantly, these factors can facilitate the emergence of

stem cells from more differentiated cells, as these retain the ability to dedifferentiate and return to a more primitive developmental state [73].

The plasticity demonstrated by cancer cells is key in cancer as extrinsic factors can promote the acquisition of stemness by reprogramming cancer cells into CSCs. These factors include cytokine and growth factors secreted cells of the TME (mesenchymal stem cells (MSCs), macrophages, tumor-associated fibroblasts (TAFs)), as well as extracellular vesicles (EVs), and hypoxia [74]. In epithelial tissues, the activation of EMT has been linked to the formation of both normal cells and CSCs [75]. Fundamental to the process of gastrulation during embryo development, EMT is activated in the adult during wound healing and in cancer [76].

EMT is a reversible process with cells changing phenotypes from epithelial to mesenchymal and then back to epithelial through MET. These highly dynamic processes are regulated by paracrine signaling, most notably TGF-β, Wnt, and others involved in maintaining stem cell function, as described above. These pathways then induce expression of factors triggering EMT, including transcription factors of the TWIST, SNAIL, and ZEB families, splicing factors and microRNAs (e.g., miR34, miR200) which drive the loss of expression of adhesion molecules such as E-cadherin (encoded by the *CDH1* gene), as well as the acquisition of mesenchymal markers, such as Vimentin [77].

Phenotypic plasticity linked to EMT has important implications for CSCs and their cellular origin in different tumor types. For instance, both epithelial and mesenchymal cells in the human breast can adopt a CSC phenotype and co-exist in tumor. Indeed, epithelial CSCs are proliferative and express ALDH, whereas mesenchymal CSCs are mostly quiescent and display a CD44hi/CD24- profile [78]. This dynamic equilibrium is regulated by the TME and the resulting heterogeneity is at the basis of the existence of different disease molecular and pathological subtypes in most solid tumors [79].

Factors associated with inflammation, such as tumor necrosis factor (TNF), interleukin6 (IL-6), and IL-1β, can activate EMT [80]. For instance, IL-6 serum levels are high in osteosarcoma patients and the cytokine stimulates osteosarcoma stemness as measured in a self-renewal spheroid assay [81]. It was also found that IL-1β can increase the formation of colon cancer spheres, which show an up-regulation of stemness factor genes and increased drug resistance [82]. Finally, tumor necrosis factor (TNF)-α promotes HPV-associated oral carcinogenesis by increasing stemness [83].

These signaling pathways are also involved in the communication between cancerassociated fibroblasts (CAFs) present in the tissue stroma and cancer cells. Indeed, CAFs can activate signaling promoting cancer stemness through activation of Wnt and Notch
signaling. CSCs, in turn, can influence CAFs through activation of signals involved in cancer progression, including the Hedgehog pathway [84]. Inter-related signaling pathways also link hypoxia with EMT. Indeed, hypoxia can directly induce EMT via the activation of the hypoxia-inducible factor (HIF)-1α through cross-talk with TGFβ and Wnt/β catenin pathways. In addition, hypoxia can also induce EMT via HIF-independent pathways which include AMPK, PIK/AKT, MAPK, NF-kB, and Notch signalling [85].

Other non-cellular components of the TME can modulate CSCs, including ECM and EVs. Among ECM molecules, tenascin-C is involved in the stimulation of self-renewal of CSCs. In breast cancer, it promotes stemness through upregulation of the CSC marker LRG5 [86] and it is also associated with poor prognosis in glioblastoma and represents a candidate CSC markers in this cancer type [87]. In addition, the ECM provides a physical barrier to CSCs from cytotoxic drugs and may promote EMT, self-renewal, expression of CSC markers, and drug resistance. ECM properties such as stiffness and porosity affect various CSC functions. The rigidity of the ECM is involved in the regulation of selfrenewal and differentiation of stem cells [88][89]. Tumor ECM is usually more rigid than normal tissue ECM due to overexpression of collagens, proteoglycans, and ECM-modifying enzymes (lysyl oxidases) [90].

Finally, EVs isolated from tumor and stromal cells are involved in various stages of tumor progression such as proliferation, angiogenesis, metastasis, and drug resistance [91]. Tumor cells secrete a heterogeneous set of EVs, which differ in size, biogenesis, and molecular composition, which include cytoplasmic proteins, proteins interacting with lipid rafts, DNA, and RNA [92]. Communication through EVs is important for the maintenance of CSCs. For instance, Evs released by glioblastoma stem cells promote self-renewal and angiogenesis through endothelial tube formation [93]. Similarly, exosomes derived from TAFs promote the formation of colorectal cancer spheres by activating Wnt signaling and ultimately increasing the number of CSCs [94]. Gonzalez et al. also showed that stem/progenitor-enriched mammospheres from primary mammary epithelial cells can secrete extracellular vesicles that are capable of altering the expression levels of genes involved in EMT and stem cell markers [95].

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