Cell. Mol. Life Sci. (2010) 67:2605–2618
DOI 10.1007/s00018-010-0338-2

Cellular and Molecular Life Sciences

REVIEW

Molecular signaling of the epithelial to mesenchymal transition in generating and maintaining cancer stem cells

Gaoliang Ouyang · Zhe Wang · Xiaoguang Fang · Jia Liu · Chaoyong James Yang

Received: 12 January 2010 / Revised: 23 February 2010 / Accepted: 26 February 2010 / Published online: 18 March 2010

Abstract The epithelial to mesenchymal transition (EMT) is a highly conserved cellular program that allows polarized, well-differentiated epithelial cells to convert to unpolarized, motile mesenchymal cells. EMT is critical for appropriate embryogenesis and plays a crucial role in tumorigenesis and cancer progression. Recent studies revealed that there is a direct link between the EMT program and the gain of epithelial stem cell properties. EMT is sufficient to induce a population with stem cell characteristics from well-differentiated epithelial cells and cancer cells. In this review, we briefly introduce the biology of EMT inducers and transcription factors in tumorigenesis and then focus on the role of these key players of the EMT in generating and maintaining cancer stem cells.

Keywords Epithelial-mesenchymal transition (EMT) · Cancer stem cell · Stemness · Tumorigenesis · Metastasis · Transcription factor · Tumor microenvironment · Extracellular matrix (ECM)

Introduction

The epithelial to mesenchymal transition (EMT) is a well-coordinated process during embryonic development and a pathological feature in tumorigenesis [1, 2]. During the EMT process, epithelial cells lose the expression of E-cadherin and other components of epithelial cell junctions, adopt a mesenchymal cell phenotype, and acquire motility as well as invasive properties [3, 4] (Fig. 1). EMT is triggered by several extracellular induction signals such as extracellular matrix (ECM) components and growth factors. Furthermore, EMT is mediated by a set of transcription factors including the bHLH factors Twist 1 and Twist 2 (also known as Dermo1), the zinc-finger proteins Snail and Slug (also known as Snai2), the zinc-finger/homeodomain proteins ZEB1 (also known as TCF8 and δEF1) and ZEB2 (also known as SIP1), as well as the forkhead factor FOXC2 [5]. The cooperation between extracellular EMT-inducing signals and transcription factors leads to stable reprogramming of epithelial cells to the mesenchymal state. Increasing evidence indicates that aberrant activation of the developmental EMT program contributes to tumor invasion, metastatic dissemination, and acquisition of therapeutic resistance [6].

Cancer stem cells (CSCs), also known as tumor initiating cells or tumorigenic cells, refer to a small subset of tumor cells that have stem-like cell properties, the ability to self-renew, form tumor spheres, differentiate into heterogeneous populations of cancer cells, and seed new tumors in a xenotransplant system. Accordingly, the most widely accepted assay to operationally identify CSCs within a population of cancer cells involves serially transplanting cell populations at limited dilutions into immunocompromised mice to seed new tumors [1, 7]. The first evidence validating the existence of a candidate CSC population occurred in human acute myeloid leukemia [8, 9]. Current data demonstrate that CSCs normally have characteristics associated with mesenchymal cells and also play a critical role in tumor initiation, growth, metastasis, and therapeutic resistance [1, 7, 10]. For example, $CD44^{+}CD24^{-}$ breast

G. Ouyang (✉) · Z. Wang · X. Fang · J. Liu
Key Laboratory of the Ministry of Education for Cell Biology and Tumor Cell Engineering, School of Life Sciences, Xiamen University, Xiamen 361005, China
e-mail: oygldz@yahoo.com.cn

C. J. Yang
College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China

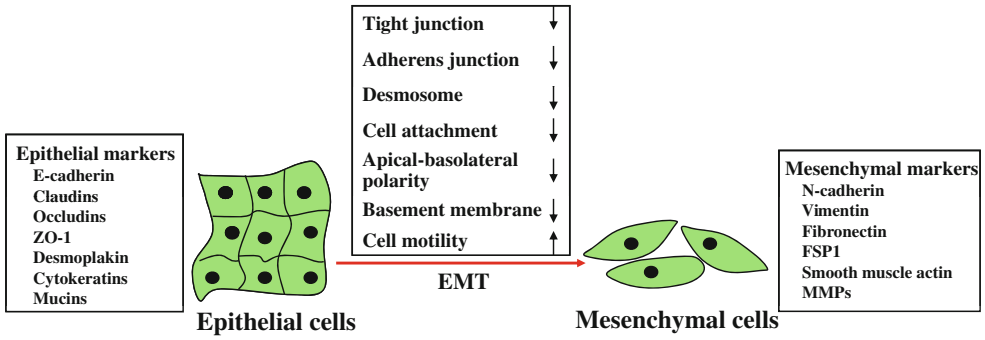

Fig. 1 Overview of molecular markers and cellular changes during the epithelial-mesenchymal transition (*EMT*). EMT is a well-coordinated event during embryonic development and a pathological feature in tumorigenesis. During the EMT process, epithelial cells undergo dramatic phenotypic changes, lose expression of E-cadherin and other components of epithelial cell junctions, adopt a mesenchymal cell phenotype, and acquire motility and invasive properties that allow them to migrate through the extracellular matrix

cancer cells are much more invasive than their non-stem-like counterparts [11], and invasive prostate cancer cells are tumor initiating cells with a stem cell-like genomic signature [12]. Moreover, many reports of radioresistance and chemoresistance in CSCs suggest that this subpopulation of cancer cells may contribute to tumor maintenance and recurrence. Glioma stem cells have been shown to be responsible for the initiation, propagation, recurrence, and therapeutic failures of gliomas [10]. $CD133^{+}$ cell subpopulations in gliomas are enriched for CSCs and show greater tumorigenic potential relative to $CD133^{-}$ glioma cells, and contribute to glioma radioresistance and the repopulation of tumors [13–15]. Poorly differentiated breast cancers display higher content of CSCs than do well-differentiated breast cancers [16]. Cancer cells with stem cell properties are enriched in residual tumors that remain after chemotherapy. Resistance to chemotherapy distinguishes CSCs from other cancer cells, and chemotherapy selectively enriches for self-renewing breast CSCs [17]. Current anti-cancer therapies mostly fail to eradicate CSCs and instead favor the expansion of the CSC pool and/or select for resistant CSC clones [10, 16]. Strategies aimed at efficiently targeting CSCs are critical for monitoring the progress of cancer treatment and for evaluating new therapeutic agents [18].

During malignant tumor progression, tumor cells migrate into and invade the surrounding tissue either as single cells or in collective clusters, thereby forming the tumor-host interface (i.e., the invasive front) [19]. $CXCR4^{+}CD133^{+}$ cells are a distinct subpopulation of highly migratory pancreatic CSCs that are located at the invasive front of pancreatic tumors, and patients with higher numbers of $CXCR4^{+}CD133^{+}$ cells show increased incidence of tumor metastasis [20]. Of note, some tumor cells in colorectal cancer, mainly at the invasive front, not only express EMT-associated genes but also maintain expression of stemness-associated genes [21]. This finding may indicate a relationship between EMT and CSCs in tumor invasion and metastasis. In particular, accumulating evidence supports that both EMT and CSCs play a critical role in tumor metastasis, therapeutic resistance, and recurrence. Interestingly, the EMT and CSC markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients [22]. Both the EMT concept and the CSC model cover distinct aspects of tumorigenesis; however, each alone can not explain the sum of the cellular events in tumor progression [21]. Therefore, there may be a direct relationship between EMT and the properties of CSCs. Brabletz et al. [21] proposed an integrated model—the migrating CSC concept that covers all aspects of human tumor progression. Mobile CSCs are located predominantly at the tumor-host interface and are derived from stationary CSCs through the acquisition of a transient EMT phenotype in addition to stemness. A recent report demonstrated that EMT is sufficient to generate a cell population with stem cell characteristics [1]. Induction of EMT in nontumorigenic, immortalized mammary epithelial cells by ectopic expression of either Twist 1 or Snai1 results in a population of stem-like cells. Moreover, the stem-like cells isolated from mouse and human normal and neoplastic mammary glands express markers associated with the EMT. E-cadherin mRNA in stem-like $CD44^{high}CD24^{low}$ cells is strongly decreased compared to the level in $CD44^{low}CD24^{high}$ cells; however, the levels of mRNAs encoding mesenchymal markers and EMT-inducing transcription factors such as N-cadherin, fibronectin, Twist 1, Snai1, ZEB2, and FOXC2 are significantly upregulated. Furthermore, inducers of EMT can increase the number of tumor-initiating cells in HER2/neu- or Ras-activated human mammary epithelial cells [1]. This study provided direct support for a potential association between EMT and a cancer stem-like cell phenotype. These findings suggest that the induction of EMT promotes cultured breast cells to adopt properties of stem cells and that EMT is sufficient to stimulate the generation of CSCs from differentiated cancer cells. Therefore, inducers of EMT may

not only play an essential role in EMT but may also be involved in reprogramming differentiated tumor cells into CSCs. Of note, several studies have documented that only minor subpopulations of $CD44^{+}CD24^{low}$ displaying stem-like properties, such as $CD44^{+}CD24^{-/low}ESA^{+}$ or $CD44^{+}CD24^{-/low}ALDH^{+}$, can be defined as CSCs [23, 24]. Here we focus on recent findings regarding the role of EMT signaling pathways in generating and maintaining CSCs (Fig. 2).

EMT signaling pathways involved in generating and maintaining CSCs

Transcription factors in EMT and CSCs

The term EMT describes a multi-step event during which epithelial cells lose numerous epithelial characteristics and assume properties that are typical of mesenchymal cells, which requires complex changes in cell architecture and behavior. E-cadherin (encoded by *CDH1*) is a central adhesion molecule located at cell-cell adhesion junctions and is essential for the formation and maintenance of the epithelial cell phenotype. Loss of E-cadherin is consistently observed at sites of EMT in embryonic development and tumorigenesis. Functional loss of E-cadherin is currently regarded as one of the hallmarks of EMT and a central event in the progression of papilloma into invasive carcinoma because the reduction of cell adhesion between cancer cells facilitates their ability to migrate individually and invade [6, 25]. The *CDH1* promoter is frequently repressed directly or indirectly by specific transcriptional repressors such as Snai1, Slug, Twist 1, ZEB1, ZEB2, FOXC2, KLF8, and E47, which disrupts the polarity of epithelial cells and maintains a mesenchymal phenotype [6, 26, 27]. Among these developmental transcription factors, the Twist, Snai1, and ZEB family members are well-investigated in EMT and CSCs (Table 1). Note that these *CDH1* repressors act as triggers of the EMT program both by inhibiting a subset of common epithelial genes and by activating mesenchymal genes [28].

Twist 1 and Twist 2

Twist proteins are highly conserved basic helix-loop-helix (bHLH) transcription factors that play an important role in embryogenesis and tumorigenesis. Twist 1 and Twist 2 are significantly overexpressed in various human solid tumors

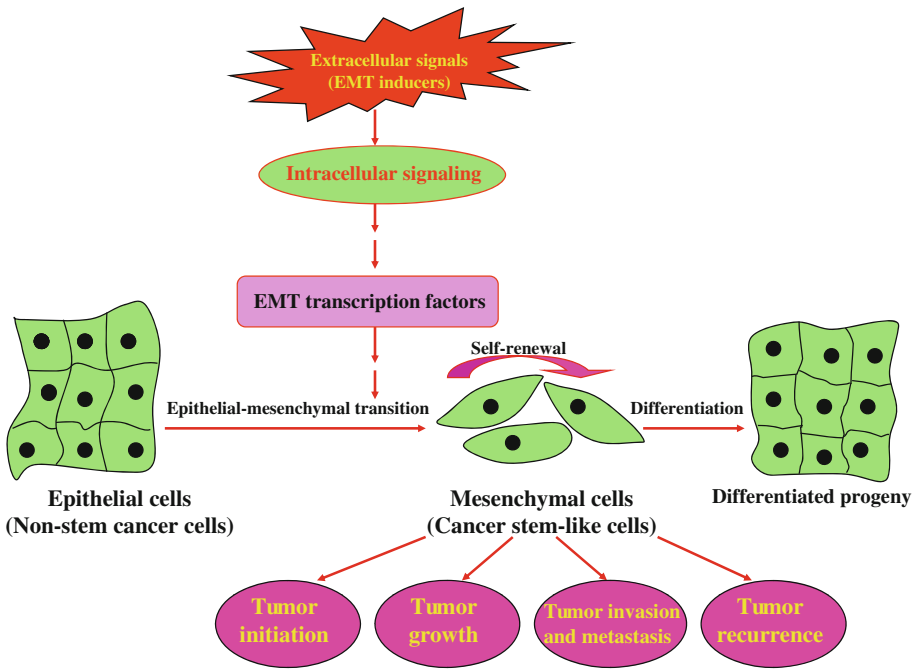

Fig. 2 Schematic diagram of the epithelial mesenchymal transition (*EMT*) generates cancer stem-like cells from epithelial cancer cells. EMT can be triggered by several extracellular inducing signals such as TGF-β, Wnt, and other tumor microenvironmental cues. Receptor-mediated signaling in response to these EMT signals activates intracellular effectors and then a set of EMT transcription factors including Twist 1, Twist 2, Snai1, Slug, ZEB1, ZEB2, Goosecoid, and FOXC2. The signaling cascades from extracellular EMT-inducing signals to EMT transcription factors result in reprogramming of epithelial nonstem cancer cells into mesenchymal cells. These mesenchymal cells take on typical properties and abilities of cancer stem cells (*CSCs*) to self-renew and differentiate into heterogeneous populations of cancer cells. CSCs not only have potent tumorigenic capacity to be involved in tumor initiation and growth but also play an essential role in tumor metastasis, therapeutic resistance, and recurrence

Table 1 Summary of transcription factors of EMT that regulate stemness of CSCs

EMT transcription factors	Functions in maintenance of stemness of CSCs
Twist	Twist 1 induces HMLEs into mesenchymal-like cells and dedifferentiates HMLEN cells into CSCs via EMT. Constitutively expressing Twist 1 in HMLER cells augments the stem-like cell pool, mammosphere formation, and tumorigenic property in vivo. Twist 1 induces a breast CSC phenotype by downregulating the expression of CD24
Snai1	Constitutively expressing Snai1 in HMLER cells augments the stem-like cell pool, mammosphere formation, and tumorigenic property in vivo. Snai1 promotes ovarian cancer cells to acquire CSC characteristics
Slug	Protects hematopoietic progenitor cells from radiation-induced apoptosis in vivo; promotes ovarian cancer cells to acquire CSC characteristics
ZEB1	ZEB1 is overexpressed in CSCs induced by LBX1 via EMT. ZEB1 promotes tumorigenesis and links activation of EMT and maintenance of stemness of CSCs by repressing stemness-inhibiting miRNAs
ZEB2	ZEB2 is overexpressed in CSCs induced by Twist 1, TGF-β, or LBX1 via EMT
Goosecoid	Promotes breast cancer cell motility and metastasis via EMT
FOXC2	FOXC2 is highly expressed in basal-like breast cancer and confers stem cell properties on epithelial cells
YB-1	Ectopic expression of YB-1 confers MCF-10A cells various stem cell properties
LBX1	Promotes cell migration and enlarges $CD44^{high}CD24^{low}$ cell population and contributes to breast cancer progression via EMT
Six1	Promotes the expansion of stem/progenitor cell population in the mouse mammary gland and subsequent mammary tumor development via EMT
HIF-1α	HIF-1α overexpression promotes EMT and metastasis by inducing Twist 1; high levels of HIFs in hypoxic tumor cells may promote cancer cells to acquire properties of CSCs
HIF-2α	HIF-2α-specific target genes are expressed at significantly higher levels in glioblastoma stem cells in comparison to nonstem cancer cells under hypoxia. HIF-2 is also required for VEGF expression in GSCs, but not nonstem cancer cells. HIF-2α might provide CSCs a growth advantage even without hypoxia in vitro and in vivo

and are involved in tumor invasion and metastasis through their ability to promote EMT [29] (our unpublished data). Twist 1 and Twist 2 mediate the growth and commitment of human mesenchymal stromal/stem cells (MSC) [30]. The levels of Twist 1 and Twist 2 are very high in freshly purified human bone marrow-derived MSCs but decrease following ex vivo expansion. Overexpression of Twist 1 and Twist 2 in human MSC cultures upregulates the level of the MSC marker, STRO-1, and the early osteogenic transcription factors, Runx2 and Msx2. Therefore, Twist 1 and Twist 2 are potential mediators of MSC self-renewal and lineage commitment. Also these proteins may act to regulate critical transcription factors and osteo/chondrogenic inductive factors that are important in early events to determine cell fate decisions in human MSC populations [30]. In a recent report, Mani and colleagues found that Twist 1 can transform nontumorigenic, immortalized human mammary epithelial cells (HMLEs) into mesenchymal-like cells and dedifferentiate *HER2/neu*-infected HMLE (HMLEN) cells into $CD44^{high}CD24^{low}$ CSCs via EMT. The resulting populations that have undergone an EMT and display mesenchymal morphology and stem cell markers can efficiently form mammospheres, soft agar colonies, and tumors [1]. Vesuna et al. [31] demonstrated that Twist 1 is directly involved in generating a breast CSC phenotype through downregulating the expression of CD24.

Snai1 and Slug

The Snail family members Snai1 and Slug are highly conserved zinc-finger transcription repressors implicated in embryonic development and tumorigenesis. Both Snai1 and Slug can be upregulated by the TGF-β, Wnt, FGF, HGF, and ER signaling pathways. Snai1-induced EMT accelerates tumor metastasis through enhanced invasion and the induction of multiple immunosuppression. Inhibition of Snai1-induced EMT can simultaneously suppress both tumor metastasis and immunosuppression in cancer patients [32]. In mouse xenografts, activated TrkB promotes highly invasive and metastatic tumors by activating a Twist 1-Snai1 axis that is critically involved in EMT-like transformation, tumorigenesis, and metastasis. The knockdown of Snai1 blocks EMT-like transformation and anoikis suppression induced by TrkB but fails to inhibit tumor growth in contrast to Twist 1 depletion. Instead, Snai1 RNAi specifically inhibits lung metastases [33].

The well-established roles of Snai1 and Slug in EMT during embryogenesis and tumor progression indicate that they may also be involved in generating and maintaining the stemness of CSCs. Slug can protect hematopoietic progenitor cells from radiation-induced apoptosis in vivo [34]. A recent report demonstrated that Snai1 and Slug are critical for ovarian cancer cells to acquire stem cell characteristics, and upregulation of Snai1 and Slug in ovarian

cancer cells is associated with increased cell survival and acquisition of radioresistance and chemoresistance [35]. Furthermore, Mani and colleagues found that Snai1 can generate cells with properties of stem cells via EMT induction like Twist 1 does [1]. When EMT is transiently induced in HMLEN cells through the ectopic expression of Snai1 or Twist 1, the cells undergo an EMT and form more colonies in soft agar suspension culture but fail to form tumors more efficiently than untreated cells in vivo. However, constitutively expressing either Snai1 or Twist 1 in *H-Ras*^{V12}-infected HMLE (HMLER) cells augments the stem-like cell pool, mammosphere formation, and tumorigenic property in vivo. This study also demonstrated that the long-term maintenance of the EMT/stem cell state may depend on continuous EMT-inducing signals [1].

ZEB1 and ZEB2

ZEB1 and ZEB2, two members of the ZEB family, are implicated in the malignancy of various human tumors and are important regulators in EMT and contribute to the drug resistance and stemness of CSCs [28]. Interestingly, ZEB1 can promote tumorigenesis and link the activation of EMT with the maintenance of CSC stemness by repressing stemness-inhibiting microRNAs (miRNAs), which reinforces the direct relationship between EMT and the stemness of CSCs [36].

In addition to Twist, Snail, and ZEB family members, other developmental transcription factors such as Goosecoid and FOXC2 have also emerged as key factors that regulate E-cadherin and promote EMT during embryonic development and tumorigenesis. Furthermore, these transcription factors may play a critical role in the stemness maintenance of CSCs via EMT (Table 1). Goosecoid, a conserved transcription factor, is overexpressed in human breast tumors and can elicit an EMT to promote cell motility and significantly enhance the ability of breast cancer cells to form pulmonary metastases in mice [37]. FOXC2 is associated with aggressive basal-like breast cancer and also confers stem cell properties on epithelial cells. FOXC2 specifically promotes mesenchymal differentiation via EMT and may serve as a critical mediator to orchestrate the mesenchymal component of the EMT program [1, 38].

TGF-β signaling in EMT and CSCs

TGF-β signaling pathway is a key player in metazoan biology and has a dual role in tumorigenesis as a tumor suppressor in early stage tumors or as a promoter of tumor progression and metastasis [39, 40]. TGF-β induces EMT through a Smad-dependent transcriptional pathway and a MAPK-dependent pathway [4, 39]. In the Smad-dependent pathway, the binding of TGF-β to its receptors results in the phosphorylation of the receptor-related Smad (R-Smad) proteins. The phosphorylated R-Smads then form heteromeric complexes with Smad4 and translocate into the nucleus to promote the transcription of target genes associated with EMT. TGF-β employs HMGA2 (high-mobility group A2) to induce the expression of Snai1, Slug, and Twist 1 to promote EMT [41]. Independent of Smad activity, TGFBR2 can directly phosphorylate the cell polarity protein, Par6, to promote the dissolution of cell junction complexes [41, 42]. In addition, TGF-β signaling also cross-talks with other EMT-inducing pathways, and both of these pathways act in concert to trigger EMT programs [43].

TGF-β family members and their signaling pathways also play a key role in the self-renewal and maintenance of stem cells in their undifferentiated state. A recent report about the role of TGF-β-induced EMT in human breast cancer demonstrated that the TGF-β pathway is specifically activated in $CD44^+$ breast cancer cells [44]. The specific activation of TGF-β signaling in $CD44^+$ breast cancer cells is due to the restricted expression of TGFBR2 in these cells and its epigenetic silencing in $CD24^+$ cells. TGFBR inhibitor treatment specifically induces $CD44^+$ cancer cells to undergo a mesenchymal-to-epithelial transition (MET) [44]. $CD44^{high}CD24^{low}$ cells isolated from HMLEs display a mesenchymal morphology and express characteristic mesenchymal markers, such as increased expression of N-cadherin and vimentin [1]. After treatment with TGF-β1, HMLEs adopt the $CD44^{high}CD24^{low}$ expression profile. The $CD44^{high}CD24^{low}$ subpopulations also display many characteristics of stem cells including self-renewal, tumorigenic and metastasis capability, and the ability to differentiate into myoepithelial or luminal epithelial cells. In addition, treatment of HMLER with TGF-β accelerates the emergence of $CD44^+CD24^{-/low}$ cells from $CD44^{low}CD24^+$ nontumorigenic mammary epithelial cells via the activation of the Ras/MAPK signaling pathway [45]. In MCF-10A cells, the knockdown of Akt1 promotes TGF-β-induced EMT and a stem cell-like phenotype [46].

TGF-β may exert a similar effect on generating and maintaining the stem cell-like pool of other tumors. TGF-β is highly expressed in high-grade gliomas, and elevated TGF-β activity confers poor prognosis in glioma patients. Recently, TGF-β and LIF have been reported to induce the capacity to self-renew and prevent the differentiation of glioma-initiating cells (GICs) isolated from patient-derived glioma tissues [47]. TGF-β increases GIC self-renewal through the Smad-dependent induction of LIF and the subsequent activation of the JAK-STAT pathway. The induction of GIC self-renewal by TGF-β and LIF promotes oncogenesis in vivo [47]. TGF-β-FOXO signaling is shown

to be essential in the maintenance of leukemia-initiating cells in chronic myeloid leukemia (CML) [48].

Wnt signaling in EMT and CSCs

Wnt/β-catenin signaling pathway plays a key role in EMT and the maintenance of stemness properties of stem cells. In epithelial cells, β-catenin-E-cadherin complexes locate at adhesion junctions. Translocation of β-catenin from adhesion junctions to the nucleus might result in the loss of E-cadherin and, subsequently, the EMT. Consistent with its role in embryonic development, many β-catenin target genes are involved in promoting stemness [21]. Aberrant nuclear expression of β-catenin might confer cancer cells with these two capabilities, EMT and stemness, which promote malignant tumor progression. GSK3β is an endogenous inhibitor of Snail and can phosphorylate Snai1. GSK3β downregulation by the FGF-dependent PI3-K/Akt pathway directly results in the activation of the Snai1-EMT signaling cascade. Therefore, inhibition of GSK3β function by Wnt and other pathways can promote Snai1 stability and nuclear import to induce EMT [49, 50]. In patients with a CML blast crisis, a β-catenin mutation may confer self-renewal properties on granulocyte-macrophage progenitors [51]. In skin cancer, β-catenin signaling is essential to maintain the stemness properties of CSCs. Ablation of the β-catenin gene results in the loss of CSCs and a complete tumor regression [52, 53]. A recent paper demonstrated that inhibiting the Wnt pathway through LRP6 decreases the ability of cancer cells to self-renew and seed tumors in vivo [54]. Moreover, inhibition of Wnt signaling blocks tumor formation by promoting epithelial differentiation and repressing the EMT transcription factors, Twist 1 and Slug. These data provide a molecular link between the Wnt pathway and CSCs self-renewal, EMT, and metastasis in basal-like breast cancer [54].

Notch signaling in EMT and CSCs

As an ancient cell signaling system, the Notch pathway regulates cell fate specification, stem cell maintenance, and the initiation of differentiation in embryonic and postnatal tissues. Also, this pathway contributes to EMT and to cancer stem-like cell characteristics in tumorigenesis. The Notch signaling pathway is essential for both nonneoplastic neural stem cells and embryonal brain tumors. The upregulation of Notch signaling is a hallmark of $CD133^+$ CSCs in embryonal brain tumors, and blocking the Notch pathway by pharmacologic inhibitors of γ-secretase results in a depletion of $CD133^+$ stem-like cells in these tumors [55]. The activation of Notch signaling is associated with chemo-resistance and EMT phenotypes in gemcitabine-resistant pancreatic cancer cells [56].

Hedgehog signaling in EMT and CSCs

Hedgehog (Hh) signaling is essential for embryonic pattern formation, hematopoiesis, and also plays an important role in tumorigenesis and stem cell maintenance [57–59]. Hh signaling components such as *Ptch*, *Gli1*, and *Gli2* are highly expressed in normal and malignant human breast stem/progenitor cells. Activation of Hh signaling increases mammosphere-initiating cell number and mammosphere size; these effects are mediated by the polycomb gene, *Bmi-1* [60]. Hh signaling is also activated in Bcr-Abl-positive leukemic stem cells (LSCs) by the upregulation of Smo. Loss of Smo in Bcr-Abl-positive hematopoiesis effectively inhibits the development of Bcr-Abl-positive leukemias in mice and abrogates the ability of the disease to re-transplant, indicating that the expansion of the Bcr-Abl-positive LSC pool is dependent on Hh signaling activation [59]. Another paper also revealed that the loss of Smo impairs hematopoietic stem cell renewal, lowers the propagation of Bcr-Abl-positive chronic myelogenous leukemia (CML), and decreases the growth of imatinib-resistant mouse and human CML [58]. However, a conditional Smo deletion or overactivation has no significant effects on adult HSC self-renewal and function, and the Hh signaling pathway is dispensable for adult HSC function [61]. These results confirm recent findings that pharmacological Smo inhibition may only affect short-term repopulating HSCs in regular hematopoiesis; however, long-term repopulating HSCs and the long-term regeneration of hematopoiesis are not affected [59]. In addition, medulloblastomas arising from Patched-1-deficient or Patched-mutant mice contain $CD15^+$ CSCs [62, 63]. A recent report directly demonstrated a key and essential role of Hh signaling in regulating the stemness of CSCs via EMT. Stem cells of human colon carcinomas at all stages acquire a high Hh-Gli signature coincident with the development of metastases. The growth of colon cancer xenografts, their recurrence and metastases require active Hh-Gli. Moreover, the self-renewal of colon CSCs in vivo relies on Hh-Gli activity, which induces a robust EMT [64].

MicroRNAs: new regulators of EMT and CSCs

The deregulation of miRNAs correlates with various human cancers and is involved in the initiation and progression of human cancers [65]. Recently, miRNAs have also been identified as a new class of EMT regulators due to their regulation of EMT-inducing transcription factors, such as Twist 1, Snai1, ZEB1, and ZEB2 [66].

The miR-200 family of miRNAs (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) is regarded as both an important marker for epithelial cells and a powerful master

regulator of EMT [67]. miR-200 suppresses the expression of ZEB1 and ZEB2 to favor an epithelial phenotype [67–69]. In addition, let-7, miR-335, miR-205, miR-206, miR-126, miR-146a, and miR-101 have also been identified as metastasis suppressor miRNAs [17, 68, 70, 71]. Conversely, miRNAs such as miR-155, miR-10b, miR-21, miR-373, and miR-520c are involved in promoting tumor invasion and metastasis by regulating EMT [72–75].

Interestingly, various miRNAs are also involved in regulating the stemness of embryonic stem cells, adult stem cells, or CSCs. miRNAs are crucial for normal embryonic stem cell self-renewal and cellular differentiation [76]. Recent reports demonstrated that a subset of the miR-290 cluster in the mouse and the miR-371 cluster in humans are direct regulators of the cell cycle in ES cells [77]. A subset of the miR-290 cluster, including miR-291-3p, miR-294, and miR-295, increased the efficiency of reprogramming by *Oct4*, *Sox2*, and *Klf4*, but not by these factors plus *cMyc* [77]. A recent report demonstrated that the level of miR-145 is low in self-renewing hESCs but is much higher during differentiation. Furthermore, the pluripotency factors *OCT4*, *SOX2*, and *KLF4* are direct targets of miR-145. miR-145 upregulation is sufficient to inhibit hESC self-renewal and induce lineage-restricted differentiation of hESCs [78].

Multiple members of the let-7 family of miRNAs are often inhibited in human cancers. A recent paper showed that let-7 is reduced in breast CSCs and can negatively regulate the stemness of breast CSCs and tumorigenesis by silencing H-Ras and HMGA2, regulators of self-renewal or differentiation of breast CSCs, respectively. Ectopic overexpression let-7 in breast CSCs reduces proliferation, mammosphere formation, and the proportion of undifferentiated cells in vitro. Also, in NOD/SCID mice, tumor formation and metastasis is reduced when let-7 is overexpressed [17]. These findings indicate that a low level of let-7 is required to maintain CSCs, and let-7 may link EMT with CSCs. Interestingly, a recent paper demonstrated that miR-200c is differentially expressed between human breast CSCs and nontumorigenic cancer cells. miR-200c can target *Bmi*, a known regulator of stem cell self-renewal, and strongly inhibits the ability of normal breast stem cells to form mammary ducts and tumor formation driven by human breast CSCs [79]. In addition, the stem cell factors, Sox2 and KLF4, are also targets of miR-200c. ZEB1 links EMT-activation with the maintenance of stemness of CSCs by suppressing stemness-inhibiting miRNAs such as miR-200c and miR-203 [36]. Therefore, miR-200c is an important regulator of multiple stem cell functions that control both EMT and self-renewal and/or proliferation in normal and neoplastic stem cells.

These data demonstrate that miRNAs are involved in EMT and in the induction, maintenance, and differentiation of ESCs, adult stem cells, and CSCs. Furthermore, miRNAs may contribute to the generation and maintenance of CSCs from cancer cells via EMT. Therefore, the miRNA signatures of CSCs likely represent a new layer of regulatory control over cell fate decisions of CSCs by EMT. It is highly probable that the same set of specific miRNAs may contribute to the maintenance of the CSC phenotype and to the invasion and metastasis of tumor cells through the regulation of EMT [80].

New players of EMT in modulating stemness of CSCs

YB-1

Y-box binding protein-1 (YB-1), a member of the cold-shock domain (CSD) protein superfamily, regulates gene expression through both transcriptional and translational mechanisms and is involved in various biological activities such as tumorigenesis. YB-1 is overexpressed in $\sim$75% of human breast cancers, and high YB-1 levels provoke remarkably diverse breast carcinomas through the induction of genetic instability [81, 82]. Increased expression of YB-1 in premalignant mammary epithelial cells with elevated Ras-ERK signaling inhibits proliferation, disrupts mammary morphogenesis, and induces EMT and promotes invasive properties and cell dissemination [83]. YB-1 regulates EMT by directly promoting the cap-independent translation of mRNAs encoding Snai1, LEF-1, ZEB2, and other transcription factors involved in EMT and by suppressing cap-dependent translation of growth-related genes. Furthermore, premalignant MCF-10AT human mammary epithelial cells ectopically expressing YB-1 appear to obtain various stem cell properties such as low proliferation rates, upregulation of the stem cell markers p63, CD44, and downregulation of CD24 [83]. Therefore, MCF-10AT cells with ectopic upregulated YB-1 may acquire CSC phenotypes by inducing EMT.

LBX1

Ladybird homeobox 1 (LBX1) is a developmentally regulated homeobox transcription factor implicated in normal myogenesis and neurogenesis. A recent report demonstrated that LBX1 is overexpressed in the unfavorable ER/PR/HER2 triple-negative basal-like subtype of human breast cancer [84]. Moreover, LBX1 is a potent activator of EMT and can regulate the expression of the known EMT inducers TGF-β2, Snai1, ZEB1, and ZEB2. In mammary epithelial cells, LBX1 induces EMT, enhances cell migration, enlarges the $CD44^{high}CD24^{low}$ progenitor cell population, and cooperates with activated H-Ras to cause tumorigenesis [84]. These data indicate that LBX1 is an important regulator of EMT and stemness of breast CSCs and contributes to breast cancer progression.

Six1

The Six1 homeoprotein is a critical transcription factor involved in the development of numerous organs and is frequently overexpressed in various cancers. Six1 has been shown to play an important role in the expansion of the precursor cell population during embryogenesis. Overexpression of Six1 in immortalized mammary epithelial cells induces malignant transformation [85] and facilitates breast cancer cells to undergo EMT and metastasis by increasing TGF-β signaling [86]. Six1 also promotes the expansion of the stem/progenitor cell population in the mouse mammary gland and subsequent mammary tumor development via EMT [87]. Therefore, overexpression of Six1 is sufficient to induce highly aggressive and invasive mammary tumors with EMT and CSC features.

GATA-3

The transcription factor GATA-3 is critical for cell survival, terminal cell differentiation, and cell-type specific functions in multiple organs. GATA-3 is a key regulator of mammary gland formation, is specifically expressed in both ductal and alveolar luminal epithelial cells, and promotes the differentiation of mammary stem cells along the luminal lineage through a network of transcriptional regulation [88, 89]. Also, the expansion of GATA-3-negative stem-like cells exhibits significantly enhanced motility [90]. Loss of GATA-3 results in a high level of $p18^{Ink4c}$ in luminal progenitor cells, which prevents them from entering an active cell cycle and differentiating [88]. However, the role of GATA-3 in EMT needs to be further characterized.

PTEN

The PTEN tumor suppressor protein is a critical regulator in embryonic development, tumorigenesis, and stem cell self-renewal [91, 92]. In chick embryos, overexpression of PTEN strongly inhibits the EMT of mesoderm cells ingressing through the anterior and middle primitive streak, but it does not affect the EMT of cells located in the posterior streak [93]. PTEN negatively regulates neural stem cell self-renewal, and conditional *Pten* deletion in the fetal central nervous system enhances the self-renewal and frequency of neural stem cells [94]. A conditional deletion of *Pten* in a subpopulation of adult neural stem cells in the subependymal zone (SEZ) results in persistently increased neural stem cell self-renewal without signs of exhaustion and enhances constitutive neurogenesis [95]. Loss of PTEN is sufficient to enlarge the pool of self-renewing neural stem cells and promotes their escape from the homeostatic mechanisms of proliferation control by modulating G_0-G_1 cell cycle entry; the ultimate result is tumorigenesis [91]. PTEN can negatively regulate PI3-K/mTOR signaling, which positively regulates STAT3 signaling [96, 97]. Knockdown Akt1 in human mammary epithelial cell MCF-10A promotes TGF-β-induced EMT and a stem cell-like phenotype [46]. Interestingly, *Pten* deletion leads to the generation of transplantable leukemia-initiating cells whereas normal hematopoietic stem cells are unable to maintain themselves without *Pten*. Rapamycin, the inhibitor of mTOR, not only blocks the generation or maintenance of leukemia-initiating cells, but also restores normal HSC function [96]. Activation of the PTEN/mTOR/STAT3 pathway has also been shown to be required for the survival and maintenance of breast cancer stem-like cells, indicating that pro-survival signaling pathways are critical for CSC maintenance [98].

Microenvironment in regulating EMT and stemness of CSCs

It is widely recognized that tumor microenvironment actively contributes to tumor initiation, progression, metastasis, and recurrence, and influences the response to conventional anti-tumor treatments [99]. Tumor cells can only thrive in an aberrant microenvironment composed of altered ECM and various nontransformed neighbor cells. Cross-talk between cancer epithelial cells and their neighboring stromal cells is known to be critical to the growth and progression of tumors [100–104]. A major question that arises is what elements in the tumor microenvironment foster the selection and dominance of the CSC subpopulation. CSCs may reside in aberrant niches, where cell-cell and cell-matrix interactions can provide unregulated signals to support and maintain the undifferentiated phenotype of CSCs. CSCs may remain dormant in their aberrant niches until they are activated by the altered signals in the microenvironment. Targeting the neighboring nonstem cancer cells, stromal cells, or the paracrine factors secreted by these cells may target CSCs indirectly and thereby contribute to long-term remissions [105]. Recent work has begun to address the importance of the tumor microenvironment in regulating the EMT during tumorigenesis and also in the maintenance of the stemness of CSCs. The contextual signals in tumor microenvironment might influence the probability of interconversion between the CSC and nonCSC compartments [7].

Extracellular matrix proteins

The extracellular matrix (ECM) is a complex and dynamic structural network that is composed of structural proteins, proteoglycans, latent or active growth factors, and matricellular proteins. Cancer cell attachment and invasion of

the ECM are crucial events leading to the initial disengagement from neighbor cells. Cancer cells can modify the composition of the adjacent stroma by secreting their own ECM proteins and by using the ECM proteins secreted by their neighbor stromal cells to create a permissive and supportive microenvironment for their survival, growth, and invasion [106, 107].

Type I collagen is highly expressed at the invasive front of human colorectal cancer. Type I collagen can decrease E-cadherin and β-catenin at cell-cell junctions and promote EMT on human colorectal carcinoma cells. Moreover, Type I collagen promotes a stem cell-like phenotype with an increased clonogenicity and expression of stem cell markers CD133 and Bmi-1 [108], indicating that type I collagen may be involved in generating and maintaining human colorectal CSCs via EMT.

Nonstructural ECM proteins have been described as important inducers of EMT. In addition to the TGF-β family which plays a critical role in inducing EMT and regulating the stemness of CSCs, several other autocrine and paracrine growth factors such as FGFs, IGF, HGF, EGF family members and PDGF, together with their receptors, are also involved in regulating the EMT program in development and tumorigenesis [2, 26]. These data suggest that these autocrine- or paracrine-mediated EMT may be associated with the maintenance of self-renewal in cancer stem-like cells. However, whether these secreted growth factors and their receptors regulate the stemness of CSCs via EMT remains to be established.

Matricellular proteins are another class of nonstructural extracellular matrix proteins that function as adaptors and modulators of interactions between cells and their extracellular microenvironment; they include SPARC (osteonectin), osteopontin (OPN), tenascins, CCNs, periostin, βig-h3, and thrombospondins. These ECM proteins are structurally diverse but regulate similar biological functions during embryonic development, tissue injury, and tumorigenesis [106]. Our previous studies revealed that periostin and OPN are overexpressed in various cancers, especially in their metastatic tissues [106, 109–111]. Recently, the majority of these matricellular proteins have been shown to promote EMT. The well-established roles of matricellular proteins in EMT during embryogenesis and tumor metastasis reflect their possible participation in regulating the stemness of normal and CSCs. CCN3 has been identified as a regulator of human hematopoietic stem or progenitor cells [112]. OPN is required for systemic endocrine instigation of indolent tumor growth [103]. Also, OPN is a key component of the hematopoietic stem cell niche and negatively regulates stem cell pool size [113, 114]. Recently, thrombin-cleaved OPN has been shown to regulate hematopoietic stem and progenitor cell functions through interactions with $\alpha 9\beta 1$ and $\alpha 4\beta 1$ integrins [115]. *Periostin*, *tenascin C*, and *thrombospondin 4* are upregulated in human mammary stem/progenitor cells grown as mammospheres [116]. *SPARC*, *βig-h3*, and *thrombospondin 1* are also differentially expressed between $CD44^+$ or $PROCR^+$ and $CD24^+$ cells [44]. We have also found that periostin and OPN are involved in regulating the stemness of CSCs (our unpublished data). Further work will be necessary to characterize the role of these matricellular proteins and other extracellular matrix proteins during malignant progression and the mechanisms that underlie the expansion of CSCs during tumor progression.

IL-6

Interleukin-6 (IL-6) is a tumor microenvironment-derived extracellular signaling factor capable of inducing EMT [117]. IL-6 is overexpressed in human breast tumors as well as breast cancer patient sera and is associated with a poor prognosis in breast cancer. IL-6 is secreted by cancer cells and/or stromal cells and induces MCF-7 breast cancer cells to undergo EMT characterized by impaired E-cadherin expression and induction of Vimentin, N-cadherin, Twist 1, and Snai1 via the activation of STAT3 [117]. Moreover, IL-6 can induce malignant properties in mammospheres from human ductal breast carcinoma and normal mammary gland [118]. Furthermore, oncogenic Ras induces the secretion of IL-6 in different cell types. Knockdown of IL-6, genetic ablation of IL-6, or treatment with a neutralizing IL-6 antibody can thwart Ras-mediated tumorigenesis [119]. Recently, IL-6 signaling has also been shown to contribute to glioma malignancy by promoting glioma stem cell (GSC) growth and survival [120]. GSCs preferentially express IL-6 receptors IL-6Rα and gp130. Knockdown IL-6Rα or IL-6 ligand expression in GSCs significantly decreases growth and neurosphere formation but promotes apoptosis. Furthermore, STAT3 is a downstream mediator of pro-survival IL-6 signals in GSCs. The levels of IL6 ligand and receptor are enhanced in gliomas and are associated with poor survival of glioma patients. Inhibiting IL-6Rα or IL-6 expression in GSCs promotes the survival of mice bearing intracranial human glioma xenografts [120].

Hypoxia and hypoxia-inducible factors

As one of the most pervasive microenvironmental stresses, hypoxia is now considered a common feature of solid tumors and promotes tumor angiogenesis, invasion, and metastasis [121]. Hypoxia is also involved in regulating the stemness of stem cells. Low oxygen tensions promote the maintenance of pluripotency in hESCs and prevent differentiation. Interestingly, the subpopulation of brain tumor cells expressing a stem cell marker is enlarged by hypoxia

in vitro. Hypoxia-inducible factors (HIF)-2α can regulate stem cell function and differentiation through the activation of *Oct-4*, which in turn contributes to the tumor promoting activity of HIF-2α [122]. In glioblastomas, CSCs differentially respond to hypoxia with a distinct induction of HIF-2α [123]. HIF-2α-specific target genes such as *Oct4*, *Glut1*, and *SerpinB9* are expressed at significantly higher levels in GSCs compared to matched nonstem cancer cells under hypoxic treatment. HIF-2α is also required for VEGF expression in GSCs but not in nonstem cancer cells. Thus, HIF-2α-mediated upregulation of these genes may provide CSCs with advantages in proliferation, survival, angiogenesis, metabolism, and escape from immune surveillance. Furthermore, targeting HIFs in GSCs inhibits self-renewal, proliferation, and survival in vitro, and suppresses tumor initiation potential of GSCs in vivo [123].

Hypoxia can also induce EMTs in tumors through the upregulation of HIF-1α, Snai1, Twist 1, ZEB1, ZEB2, and lysyl oxidase (LOX) and by activating Wnt and Notch pathways [124–126]. Twist 1 has a critical role in EMT and metastatic phenotypes induced by hypoxia or overexpression of HIF-1α. In primary tumors of head and neck cancer patients, co-expression of HIF-1α, Twist 1, and Snai1 correlates with metastasis and a poor prognosis [126]. Hypoxia can inhibit the expression of E-cadherin via the activation of the LOX-Snai1 pathway to promote tumor invasion and metastasis, indicating that LOX may cooperate with Snai1 and Twist 1 in hypoxia-mediated EMT and invasion [125, 126]. Therefore, high levels of HIFs in hypoxic tumor cells may promote cancer cells to acquire the properties of CSCs including self renewal and multi-potency by activating Oct4, c-Myc, Notch, Snai1, and other critical signaling pathways [127]. Hypoxic microenvironment may not only be a critical niche favorable for expansion and stemness maintenance of CSCs in solid tumors, but also a breeding ground for generating CSCs from differentiated tumor cells by promoting EMT, and a critical microenvironmental condition that is associated with radioresistance, chemotherapy resistance, and a poor clinical prognosis of solid tumors [123, 127].

Concluding remarks

Tumor invasion and metastases are the most common causes of morbidity and mortality induced by cancer. During tumor metastasis, disseminated cancer cells from primary tumors are associated with a loss of epithelial differentiation and the acquisition of a mesenchymal phenotype. Furthermore, these cancer cells appear to require the capability to self-renew in order to spawn macroscopic metastases. To give rise to the outgrowth of metastatic tumors in a new organ environment, cancer cells have to overcome various types of stresses that may lead to cell death such as loss of adhesion, nutrient depletion, and hypoxia [109]. The majority of disseminated cells are destroyed in the process of tumor metastasis; however, only a small number of cancer cells are able to survive and initiate the formation of micrometastases at the secondary sites, and even a smaller subpopulation of these micrometastases can develop into macrometastases [109]. Current evidence supports that the overwhelming majority of cells that shed from a primary tumor and disseminate to distant secondary sites lack the capability to self-renew and their ability to form macroscopic metastasis in the new microenvironment is compromised from the outset. Either CSCs or their more differentiated progeny may have the capability to form micrometastases; however, only CSCs have the capacity to self-renew and to give rise to clinically relevant macrometastases. Therefore, metastasis is a relatively inefficient process [1, 109]. The discovery that EMT generates cells with properties of self-renewing stem cells may be beneficial to explain this major problem in tumor metastasis. EMT has been regarded as a critical step in tumor invasion and metastasis. During the metastatic process, the EMT program enables these cancer stem-like cells to disseminate from a primary tumor and also promotes their self-renewal capability to ensure generation of the critical tumor mass required for progression from micro- to macrometastases. Therefore, metastatic cancer cells may come not only from CSCs pre-existing as a subpopulation of the primary tumor, but may also be derived from primary epithelial cells that underwent EMT and acquired mesenchymal and metastatic behavior.

EMT-inducing signaling pathways including TGF-β, Wnt, Notch, Hh, and other tumor microenvironmental cues induce the well-differentiated epithelial cells to convert into motile mesenchymal cells via the activation of multiple EMT transcription factors such as Twist 1, Twist 2, Snai1, Slug, ZEB1 and ZEB2. The critical roles of TGF-β, Wnt, Notch, Hh, and other signaling pathways in promoting EMT and the stemness maintenance of stem cells adds to a growing body of evidence that cancer cells often reactivate latent developmental programs to regulate the multistep process in tumorigenesis. Indeed, normal embryogenesis and tumorigenesis share many of the same basic processes and molecular pathways. CSCs and their normal tissue stem cell counterparts share many phenotypic and functional properties including capacity to self-renew. TGF-β, Wnt, Notch, and Hh pathways have all been implicated in normal and neoplastic stem cell self-renewal. Therefore, the knowledge gained from the multifaceted players of EMT during development may provide useful information to uncover the roles of these EMT regulators in generating and maintaining CSCs in tumorigenesis and metastasis and offer

new avenues of therapeutic intervention with the potential to go beyond traditional anticancer approaches.

Acknowledgments We apologize to those authors whose work was not cited due to the limited space. We would like to thank Shideng Bao (Stem Cell Biology and Regenerative Medicine, Lerner Research Institute, Cleveland Clinic) and reviewers for critical comments. This work was supported by grants from the National Nature Science Foundation of China (nos. 30570935, 30871242), the Science Planning Program of Fujian Province (2009J1010), a Berkeley Scholar Fellowship to G.O., and the National Basic Research Program of China (no. 2010CB732402) to C.J.Y. and G.O.

References

1. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133:704–715
2. Huber MA, Kraut N, Beug H (2005) Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin Cell Biol 17:548–558
3. Acloque H, Adams MS, Fishwick K, Bronner-Fraser M, Nieto MA (2009) Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. J Clin Invest 119:1438–1449
4. Kalluri R, Weinberg RA (2009) The basics of epithelial-mesenchymal transition. J Clin Invest 119:1420–1428
5. Thiery JP, Sleeman JP (2006) Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 7:131–142
6. Thiery JP, Acloque H, Huang RY, Nieto MA (2009) Epithelial-mesenchymal transitions in development and disease. Cell 139:871–890
7. Gupta PB, Chaffer CL, Weinberg RA (2009) Cancer stem cells: mirage or reality? Nat Med 15:1010–1012
8. Bonnet D, Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 3:730–737
9. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE (1994) A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 367:645–648
10. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444:756–760
11. Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, Pilotti S, Pierotti MA, Daidone MG (2005) Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer Res 65:5506–5511
12. Klarmann GJ, Hurt EM, Mathews LA, Zhang X, Duhagon MA, Mistree T, Thomas SB, Farrar WL (2009) Invasive prostate cancer cells are tumor initiating cells that have a stem cell-like genomic signature. Clin Exp Metastasis 26:433–446
13. Bao S, Wu Q, Li Z, Sathornsumetee S, Wang H, McLendon RE, Hjelmeland AB, Rich JN (2008) Targeting cancer stem cells through L1CAM suppresses glioma growth. Cancer Res 68:6043–6048
14. Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, Shi Q, McLendon RE, Bigner DD, Rich JN (2006) Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Res 66:7843–7848
15. Rich JN, Bao S (2007) Chemotherapy and cancer stem cells. Cell Stem Cell 1:353–355
16. Pece S, Tosoni D, Confalonieri S, Mazzarol G, Vecchi M, Ronzoni S, Bernard L, Viale G, Pelicci PG, Di Fiore PP (2010) Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell 140:62–73
17. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, Song E (2007) let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell 131:1109–1123
18. Gupta PB, Onder TT, Jiang G, Tao K, Kuperwasser C, Weinberg RA, Lander ES (2009) Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell 138:645–659
19. Christofori G (2006) New signals from the invasive front. Nature 441:444–450
20. Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ, Heeschen C (2007) Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 1:313–323
21. Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T (2005) Opinion: migrating cancer stem cells—an integrated concept of malignant tumour progression. Nat Rev Cancer 5:744–749
22. Aktas B, Tewes M, Fehm T, Hauch S, Kimmig R, Kasimir-Bauer S (2009) Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. Breast Cancer Res 11:R46
23. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 100:3983–3988
24. Charafe-Jauffret E, Ginestier C, Iovino F, Tarpin C, Diebel M, Esterni B, Houvenaeghel G, Extra JM, Bertucci F, Jacquemier J, Xerri L, Dontu G, Stassi G, Xiao Y, Barsky SH, Birnbaum D, Viens P, Wicha MS (2010) Aldehyde dehydrogenase 1-positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer. Clin Cancer Res 16:45–55
25. Yilmaz M, Christofori G (2009) EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev 28:15–33
26. Yang J, Weinberg RA (2008) Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 14:818–829
27. Kang Y, Massague J (2004) Epithelial-mesenchymal transitions: twist in development and metastasis. Cell 118:277–279
28. Peinado H, Olmeda D, Cano A (2007) Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat Rev Cancer 7:415–428
29. Ansieau S, Bastid J, Doreau A, Morel AP, Bouchet BP, Thomas C, Fauvet F, Puisieux I, Doglioni C, Piccinin S, Maestro R, Voeltzel T, Selmi A, Valsesia-Wittmann S, Caron de Fromentel C, Puisieux A (2008) Induction of EMT by twist proteins as a collateral effect of tumor-promoting inactivation of premature senescence. Cancer Cell 14:79–89
30. Isenmann S, Arthur A, Zannettino AC, Turner JL, Shi S, Glackin CA, Gronthos S (2009) TWIST family of basic helix-loop-helix transcription factors mediate human mesenchymal stem cell growth and commitment. Stem Cells 27:2457–2468
31. Vesuna F, Lisok A, Kimble B, Raman V (2009) Twist modulates breast cancer stem cells by transcriptional regulation of CD24 expression. Neoplasia 11:1318–1328
32. Kudo-Saito C, Shirako H, Takeuchi T, Kawakami Y (2009) Cancer metastasis is accelerated through immunosuppression during Snail-induced EMT of cancer cells. Cancer Cell 15:195–206
33. Smit MA, Geiger TR, Song JY, Gitelman I, Peeper DS (2009) A Twist-Snail axis critical for TrkB-induced epithelial-mesenchymal transition-like transformation, anoikis resistance, and metastasis. Mol Cell Biol 29:3722–3737

34. Inoue A, Seidel MG, Wu W, Kamizono S, Ferrando AA, Bronson RT, Iwasaki H, Akashi K, Morimoto A, Hitzler JK, Pestina TI, Jackson CW, Tanaka R, Chong MJ, McKinnon PJ, Inukai T, Grosveld GC, Look AT (2002) Slug, a highly conserved zinc finger transcriptional repressor, protects hematopoietic progenitor cells from radiation-induced apoptosis in vivo. Cancer Cell 2:279–288
35. Kurrey NK, Jalgaonkar SP, Joglekar AV, Ghanate AD, Chaskar PD, Doiphode RY, Bapat SA (2009) Snail and Slug mediate radio- and chemo-resistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. Stem Cells 27:2059–2068
36. Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, Waldvogel B, Vannier C, Darling D, zur Hausen A, Brunton VG, Morton J, Sansom O, Schüler J, Stemmler MP, Herzberger C, Hopt U, Keck T, Brabletz S, Brabletz T (2009) The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat Cell Biol 11:1487–1495
37. Hartwell KA, Muir B, Reinhardt F, Carpenter AE, Sgroi DC, Weinberg RA (2006) The Spemann organizer gene, Goosecoid, promotes tumor metastasis. Proc Natl Acad Sci USA 103:18969–18974
38. Mani SA, Yang J, Brooks M, Schwaninger G, Zhou A, Miura N, Kutok JL, Hartwell K, Richardson AL, Weinberg RA (2007) Mesenchyme Forkhead 1 (FOXC2) plays a key role in metastasis and is associated with aggressive basal-like breast cancers. Proc Natl Acad Sci USA 104:10069–10074
39. Massague J (2008) TGFbeta in Cancer. Cell 134:215–230
40. Derynck R, Akhurst RJ, Balmain A (2001) TGF-beta signaling in tumor suppression and cancer progression. Nat Genet 29:117–129
41. Thuault S, Valcourt U, Petersen M, Manfioletti G, Heldin CH, Moustakas A (2006) Transforming growth factor-beta employs HMGA2 to elicit epithelial-mesenchymal transition. J Cell Biol 174:175–183
42. Ozdamar B, Bose R, Barrios-Rodiles M, Wang HR, Zhang Y, Wrana JL (2005) Regulation of the polarity protein Par6 by TGFbeta receptors controls epithelial cell plasticity. Science 307:1603–1609
43. Polyak K, Weinberg RA (2009) Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer 9:265–273
44. Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, Nikolskaya T, Serebryiskaya T, Beroukhim R, Hu M, Halushka MK, Sukumar S, Parker LM, Anderson KS, Harris LN, Garber JE, Richardson AL, Schnitt SJ, Nikolsky Y, Gelman RS, Polyak K (2007) Molecular definition of breast tumor heterogeneity. Cancer Cell 11:259–273
45. Morel AP, Lievre M, Thomas C, Hinkal G, Ansieau S, Puisieux A (2008) Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLoS One 3:e2888
46. Iliopoulos D, Polytarchou C, Hatziapostolou M, Kottakis F, Maroulakou IG, Struhl K, Tsichlis PN (2009) MicroRNAs differentially regulated by Akt isoforms control EMT and stem cell renewal in cancer cells. Sci Signal 2:ra62. doi:10.1126/scisignal.2000356
47. Penuelas S, Anido J, Prieto-Sanchez RM, Folch G, Barba I, Cuartas I, García-Dorado D, Poca MA, Sahuquillo J, Baselga J, Seoane J (2009) TGF-beta increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. Cancer Cell 15:315–327
48. Naka K, Hoshii T, Muraguchi T, Tadokoro Y, Ooshio T, Kondo Y, Nakao S, Motoyama N, Hirao A (2010) TGF-beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. Nature 463:676–680
49. Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M, Hung MC (2004) Dual regulation of Snail by GSK-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat Cell Biol 6:931–940
50. Bachelder RE, Yoon SO, Franci C, de Herreros AG, Mercurio AM (2005) Glycogen synthase kinase-3 is an endogenous inhibitor of Snail transcription: implications for the epithelial-mesenchymal transition. J Cell Biol 168:29–33
51. Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, Gotlib J, Li K, Manz MG, Keating A, Sawyers CL, Weissman IL (2004) Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N Engl J Med 351:657–667
52. Reya T, Clevers H (2005) Wnt signalling in stem cells and cancer. Nature 434:843–850
53. Malanchi I, Peinado H, Kassen D, Hussenet T, Metzger D, Chambon P, Huber M, Hohl D, Cano A, Birchmeier W, Huelsken J (2008) Cutaneous cancer stem cell maintenance is dependent on beta-catenin signalling. Nature 452:650–653
54. DiMeo TA, Anderson K, Phadke P, Fan C, Perou CM, Naber S, Kuperwasser C (2009) A novel lung metastasis signature links Wnt signaling with cancer cell self-renewal and epithelial-mesenchymal transition in basal-like breast cancer. Cancer Res 69:5364–5373
55. Fan X, Matsui W, Khaki L, Stearns D, Chun J, Li YM, Eberhart CG (2006) Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. Cancer Res 66:7445–7452
56. Wang Z, Li Y, Kong D, Banerjee S, Ahmad A, Azmi AS, Ali S, Abbruzzese JL, Gallick GE, Sarkar FH (2009) Acquisition of epithelial-mesenchymal transition phenotype of gemcitabine-resistant pancreatic cancer cells is linked with activation of the notch signaling pathway. Cancer Res 69:2400–2407
57. Trowbridge JJ, Scott MP, Bhatia M (2006) Hedgehog modulates cell cycle regulators in stem cells to control hematopoietic regeneration. Proc Natl Acad Sci USA 103:14134–14139
58. Zhao C, Chen A, Jamieson CH, Fereshteh M, Abrahamsson A, Blum J, Kwon HY, Kim J, Chute JP, Rizzieri D, Munchhof M, VanArsdale T, Beachy PA, Reya T (2009) Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. Nature 458:776–779
59. Dierks C, Beigi R, Guo GR, Zirlik K, Stegert MR, Manley P, Trussell C, Schmitt-Graeff A, Landwerlin K, Veelken H, Warmuth M (2008) Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. Cancer Cell 14:238–249
60. Liu S, Dontu G, Mantle ID, Patel S, Ahn NS, Jackson KW, Suri P, Wicha MS (2006) Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. Cancer Res 66:6063–6071
61. Gao J, Graves S, Koch U, Liu S, Jankovic V, Buonamici S, El Andaloussi A, Nimer SD, Kee BL, Taichman R, Radtke F, Aifantis I (2009) Hedgehog signaling is dispensable for adult hematopoietic stem cell function. Cell Stem Cell 4:548–558
62. Ward RJ, Lee L, Graham K, Satkunendran T, Yoshikawa K, Ling E, Harper L, Austin R, Nieuwenhuis E, Clarke ID, Hui CC, Dirks PB (2009) Multipotent CD15+ cancer stem cells in patched-1-deficient mouse medulloblastoma. Cancer Res 69:4682–4690
63. Read TA, Fogarty MP, Markant SL, McLendon RE, Wei Z, Ellison DW, Febbo PG, Wechsler-Reya RJ (2009) Identification of CD15 as a marker for tumor-propagating cells in a mouse model of medulloblastoma. Cancer Cell 15:135–147
64. Varnat F, Duquet A, Malerba M, Zbinden M, Mas C, Gervaz P, Ruiz i Altaba A (2009) Human colon cancer epithelial cells harbour active HEDGEHOG-GLI signalling that is essential for

tumour growth, recurrence, metastasis and stem cell survival and expansion. EMBO Mol Med 1:338–351
65. Ruan K, Fang X, Ouyang G (2009) MicroRNAs: novel regulators in the hallmarks of human cancer. Cancer Lett 285:116–126
66. Ma L, Weinberg RA (2008) Micromanagers of malignancy: role of microRNAs in regulating metastasis. Trends Genet 24:448–456
67. Park SM, Gaur AB, Lengyel E, Peter ME (2008) The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev 22:894–907
68. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ (2008) The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 10:593–601
69. Korpal M, Lee ES, Hu G, Kang Y (2008) The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. J Biol Chem 283:14910–14914
70. Tavazoie SF, Alarcon C, Oskarsson T, Padua D, Wang Q, Bos PD, Gerald WL, Massagué J (2008) Endogenous human microRNAs that suppress breast cancer metastasis. Nature 451:147–152
71. Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, Laxman B, Cao X, Jing X, Ramnarayanan K, Brenner JC, Yu J, Kim JH, Han B, Tan P, Kumar-Sinha C, Lonigro RJ, Palanisamy N, Maher CA, Chinnaiyan AM (2008) Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science 322:1695–1699
72. Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, Zeng YX, Shao JY (2008) MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA 14:2348–2360
73. Huang Q, Gumireddy K, Schrier M, le Sage C, Nagel R, Nair S, Egan DA, Li A, Huang G, Klein-Szanto AJ, Gimotty PA, Katsaros D, Coukos G, Zhang L, Puré E, Agami R (2008) The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat Cell Biol 10:202–210
74. Kong W, Yang H, He L, Zhao JJ, Coppola D, Dalton WS, Cheng JQ (2008) MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. Mol Cell Biol 28:6773–6784
75. Ma L, Teruya-Feldstein J, Weinberg RA (2007) Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 449:682–688
76. Marson A, Levine SS, Cole MF, Frampton GM, Brambrink T, Johnstone S, Guenther MG, Johnston WK, Wernig M, Newman J, Calabrese JM, Dennis LM, Volkert TL, Gupta S, Love J, Hannett N, Sharp PA, Bartel DP, Jaenisch R, Young RA (2008) Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. Cell 134:521–533
77. Judson RL, Babiarz JE, Venere M, Blelloch R (2009) Embryonic stem cell-specific microRNAs promote induced pluripotency. Nat Biotechnol 27:459–461
78. Xu N, Papagiannakopoulos T, Pan G, Thomson JA, Kosik KS (2009) MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. Cell 137:647–658
79. Shimono Y, Zabala M, Cho RW, Lobo N, Dalerba P, Qian D, Diehn M, Liu H, Panula SP, Chiao E, Dirbas FM, Somlo G, Pera RA, Lao K, Clarke MF (2009) Downregulation of miRNA-200c links breast cancer stem cells with normal stem cells. Cell 138:592–603
80. Nicoloso MS, Spizzo R, Shimizu M, Rossi S, Calin GA (2009) MicroRNAs–the micro steering wheel of tumour metastases. Nat Rev Cancer 9:293–302
81. Bargou RC, Jurchott K, Wagener C, Bergmann S, Metzner S, Bommert K, Mapara MY, Winzer KJ, Dietel M, Dörken B, Royer HD (1997) Nuclear localization and increased levels of transcription factor YB-1 in primary human breast cancers are associated with intrinsic MDR1 gene expression. Nat Med 3:447–450
82. Bergmann S, Royer-Pokora B, Fietze E, Jurchott K, Hildebrandt B, Trost D, Leenders F, Claude JC, Theuring F, Bargou R, Dietel M, Royer HD (2005) YB-1 provokes breast cancer through the induction of chromosomal instability that emerges from mitotic failure and centrosome amplification. Cancer Res 65:4078–4087
83. Evdokimova V, Tognon C, Ng T, Ruzanov P, Melnyk N, Fink D, Sorokin A, Ovchinnikov LP, Davicioni E, Triche TJ, Sorensen PH (2009) Translational activation of snail1 and other developmentally regulated transcription factors by YB-1 promotes an epithelial-mesenchymal transition. Cancer Cell 15:402–415
84. Yu M, Smolen GA, Zhang J, Wittner B, Schott BJ, Brachtel E, Ramaswamy S, Maheswaran S, Haber DA (2009) A developmentally regulated inducer of EMT, LBX1, contributes to breast cancer progression. Genes Dev 23:1737–1742
85. Coletta RD, Christensen KL, Micalizzi DS, Jedlicka P, Varella-Garcia M, Ford HL (2008) Six1 overexpression in mammary cells induces genomic instability and is sufficient for malignant transformation. Cancer Res 68:2204–2213
86. Micalizzi DS, Christensen KL, Jedlicka P, Coletta RD, Baron AE, Harrell JC, Horwitz KB, Billheimer D, Heichman KA, Welm AL, Schiemann WP, Ford HL (2009) The Six1 homeoprotein induces human mammary carcinoma cells to undergo epithelial-mesenchymal transition and metastasis in mice through increasing TGF-beta signaling. J Clin Invest 119:2678–2690
87. McCoy EL, Iwanaga R, Jedlicka P, Abbey NS, Chodosh LA, Heichman KA, Welm AL, Ford HL (2009) Six1 expands the mouse mammary epithelial stem/progenitor cell pool and induces mammary tumors that undergo epithelial-mesenchymal transition. J Clin Invest 119:2663–2677
88. Pei XH, Bai F, Smith MD, Usary J, Fan C, Pai SY, Ho IC, Perou CM, Xiong Y (2009) CDK inhibitor p18(INK4c) is a downstream target of GATA3 and restrains mammary luminal progenitor cell proliferation and tumorigenesis. Cancer Cell 15:389–401
89. Asselin-Labat ML, Sutherland KD, Barker H, Thomas R, Shackleton M, Forrest NC, Hartley L, Robb L, Grosveld FG, van der Wees J, Lindeman GJ, Visvader JE (2007) Gata-3 is an essential regulator of mammary-gland morphogenesis and luminal-cell differentiation. Nat Cell Biol 9:201–209
90. Kouros-Mehr H, Bechis SK, Slorach EM, Littlepage LE, Egeblad M, Ewald AJ, Pai SY, Ho IC, Werb Z (2008) GATA-3 links tumor differentiation and dissemination in a luminal breast cancer model. Cancer Cell 13:141–152
91. Groszer M, Erickson R, Scripture-Adams DD, Dougherty JD, Le Belle J, Zack JA, Geschwind DH, Liu X, Kornblum HI, Wu H (2006) PTEN negatively regulates neural stem cell self-renewal by modulating G0–G1 cell cycle entry. Proc Natl Acad Sci USA 103:111–116
92. Rossi DJ, Weissman IL (2006) Pten, tumorigenesis, and stem cell self-renewal. Cell 125:229–231
93. Leslie NR, Yang X, Downes CP, Weijer CJ (2007) PtdIns(3, 4, 5)P(3)-dependent and -independent roles for PTEN in the control of cell migration. Curr Biol 17:115–125

94. Groszer M, Erickson R, Scripture-Adams DD, Lesche R, Trumpp A, Zack JA, Kornblum HI, Liu X, Wu H (2001) Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene in vivo. Science 294:2186–2189
95. Gregorian C, Nakashima J, Le Belle J, Ohab J, Kim R, Liu A, Smith KB, Groszer M, Garcia AD, Sofroniew MV, Carmichael ST, Kornblum HI, Liu X, Wu H (2009) Pten deletion in adult neural stem/progenitor cells enhances constitutive neurogenesis. J Neurosci 29:1874–1886
96. Yilmaz OH, Valdez R, Theisen BK, Guo W, Ferguson DO, Wu H, Morrison SJ (2006) Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. Nature 441:475–482
97. Zhang J, Grindley JC, Yin T, Jayasinghe S, He XC, Ross JT, Haug JS, Rupp D, Porter-Westpfahl KS, Wiedemann LM, Wu H, Li L (2006) PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. Nature 441:518–522
98. Zhou J, Wulfkuhle J, Zhang H, Gu P, Yang Y, Deng J, Margolick JB, Liotta LA, Petricoin E 3rd, Zhang Y (2007) Activation of the PTEN/mTOR/STAT3 pathway in breast cancer stem-like cells is required for viability and maintenance. Proc Natl Acad Sci USA 104:16158–16163
99. Hu M, Polyak K (2008) Microenvironmental regulation of cancer development. Curr Opin Genet Dev 18:27–34
100. Bissell MJ, Labarge MA (2005) Context, tissue plasticity, and cancer: are tumor stem cells also regulated by the microenvironment? Cancer Cell 7:17–23
101. Bissell MJ, Radisky D (2001) Putting tumours in context. Nat Rev Cancer 1:46–54
102. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100:57–70
103. McAllister SS, Gifford AM, Greiner AL, Kelleher SP, Saelzler MP, Ince TA, Reinhardt F, Harris LN, Hylander BL, Repasky EA, Weinberg RA (2008) Systemic endocrine instigation of indolent tumor growth requires osteopontin. Cell 133:994–1005
104. Bhowmick NA, Neilson EG, Moses HL (2004) Stromal fibroblasts in cancer initiation and progression. Nature 432:332–337
105. Polyak K, Hahn WC (2006) Roots and stems: stem cells in cancer. Nat Med 12:296–300
106. Ruan K, Bao S, Ouyang G (2009) The multifaceted role of periostin in tumorigenesis. Cell Mol Life Sci 66:2219–2230
107. Erkan M, Kleeff J, Gorbachevski A, Reiser C, Mitkus T, Esposito I, Giese T, Büchler MW, Giese NA, Friess H (2007) Periostin creates a tumor-supportive microenvironment in the pancreas by sustaining fibrogenic stellate cell activity. Gastroenterology 132:1447–1464
108. Kirkland SC (2009) Type I collagen inhibits differentiation and promotes a stem cell-like phenotype in human colorectal carcinoma cells. Br J Cancer 101:320–326
109. Bao S, Ouyang G, Bai X, Huang Z, Ma C, Liu M, Shao R, Anderson RM, Rich JN, Wang XF (2004) Periostin potently promotes metastatic growth of colon cancer by augmenting cell survival via the Akt/PKB pathway. Cancer Cell 5:329–339
110. Song G, Ouyang G, Mao Y, Ming Y, Bao S, Hu T (2009) Osteopontin promotes gastric cancer metastasis by augmenting cell survival and invasion through Akt-mediated HIF-1alpha upregulation and MMP9 activation. J Cell Mol Med 13:1706–1718
111. Song G, Cai QF, Mao YB, Ming YL, Bao SD, Ouyang GL (2008) Osteopontin promotes ovarian cancer progression and cell survival and increases HIF-1alpha expression through the PI3-K/Akt pathway. Cancer Sci 99:1901–1907
112. Gupta R, Hong D, Iborra F, Sarno S, Enver T (2007) NOV (CCN3) functions as a regulator of human hematopoietic stem or progenitor cells. Science 316:590–593
113. Nilsson SK, Johnston HM, Whitty GA, Williams B, Webb RJ, Denhardt DT, Bertoncello I, Bendall LJ, Simmons PJ, Haylock DN (2005) Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. Blood 106:1232–1239
114. Stier S, Ko Y, Forkert R, Lutz C, Neuhaus T, Grünewald E, Cheng T, Dombkowski D, Calvi LM, Rittling SR, Scadden DT (2005) Osteopontin is a hematopoietic stem cell niche component that negatively regulates stem cell pool size. J Exp Med 201:1781–1791
115. Grassinger J, Haylock DN, Storan MJ, Haines GO, Williams B, Whitty GA, Vinson AR, Be CL, Li S, Sørensen ES, Tam PP, Denhardt DT, Sheppard D, Choong PF, Nilsson SK (2009) Thrombin-cleaved osteopontin regulates hemopoietic stem and progenitor cell functions through interactions with alpha9beta1 and alpha4beta1 integrins. Blood 114:49–59
116. Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ, Wicha MS (2003) In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. Genes Dev 17:1253–1270
117. Sullivan NJ, Sasser AK, Axel AE, Vesuna F, Raman V, Ramirez N, Oberyszyn TM, Hall BM (2009) Interleukin-6 induces an epithelial-mesenchymal transition phenotype in human breast cancer cells. Oncogene 28:2940–2947
118. Sansone P, Storci G, Tavolari S, Guarnieri T, Giovannini C, Taffurelli M, Ceccarelli C, Santini D, Paterini P, Marcu KB, Chieco P, Bonafè M (2007) IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland. J Clin Invest 117:3988–4002
119. Ancrile B, Lim KH, Counter CM (2007) Oncogenic Ras-induced secretion of IL6 is required for tumorigenesis. Genes Dev 21:1714–1719
120. Wang H, Lathia JD, Wu Q, Wang J, Li Z, Heddleston JM, Eyler CE, Elderbroom J, Gallagher J, Schuschu J, MacSwords J, Cao Y, McLendon RE, Wang XF, Hjelmeland AB, Rich J (2009) Targeting interleukin 6 signaling suppresses glioma stem cell survival and tumor growth. Stem Cells 27:2393–2404
121. Ruan K, Song G, Ouyang G (2009) Role of hypoxia in the hallmarks of human cancer. J Cell Biochem 107:1053–1062
122. Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu CJ, Labosky PA, Simon MC, Keith B (2006) HIF-2alpha regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev 20:557–570
123. Li Z, Bao S, Wu Q, Wang H, Eyler C, Sathornsumetee S, Shi Q, Cao Y, Lathia J, McLendon RE, Hjelmeland AB, Rich JN (2009) Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. Cancer Cell 15:501–513
124. Erler JT, Bennewith KL, Nicolau M, Dornhofer N, Kong C, Le QT, Chi JT, Jeffrey SS, Giaccia AJ (2006) Lysyl oxidase is essential for hypoxia-induced metastasis. Nature 440:1222–1226
125. Pouyssegur J, Dayan F, Mazure NM (2006) Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature 441:437–443
126. Yang MH, Wu MZ, Chiou SH, Chen PM, Chang SY, Liu CJ, Teng SC, Wu KJ (2008) Direct regulation of TWIST by HIF-1alpha promotes metastasis. Nat Cell Biol 10:295–305
127. Keith B, Simon MC (2007) Hypoxia-inducible factors, stem cells, and cancer. Cell 129:465–472