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Epithelial-Mesenchymal Transition and Cancer Stem Cells

Gaoliang Ouyang[1,2]
[1]*State Key Laboratory of Stress Cell Biology, School of Life Sciences, Xiamen University, Xiamen 361005,*
[2]*Laboratory of Stem Cells and Tumor Metastasis, School of Life Sciences, Xiamen University, Xiamen 361005, China*

1. Introduction

The epithelial to mesenchymal transition (EMT) is a highly coordinated process and 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. The conversion of epithelial cells to mesenchymal cells is critical for the formation of the body plan and in the differentiation of multiple tissues and organs during embryonic development and involves profound phenotypic changes such as the loss of cell-cell adhesion, the loss of cell polarity, and the acquisition of migratory and invasive properties (Thiery *et al*. 2009). EMT is also involved in the physiological response to injury and in the pathological processes such as organ fibrosis. Accumulating evidence suggests that aberrant activation of the EMT developmental program contributes to tumor initiation invasion, metastatic dissemination and acquisition of therapeutic resistance (Yang, *et al*., 2004; Yang and Weinberg, 2008; Thiery *et al*., 2009; Singh and Settleman, 2010; Acloque *et al*., 2009; Kalluri and Weinberg, 2009). EMT induction can participate in cancer initiation to promote the clonal expansion of premalignant epithelial cells (Tellez *et al*., 2011). Cancer cells undergoing EMT acquire the capacity to migrate, invade the stroma and metastasise. During the process of metastasis, the EMT program enables these cancer 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 macro-metastases (Ruan *et al*., 2009a; Ruan *et al*., 2009c; Ouyang *et al*., 2010). EMT-inducing signalling pathways, such as TGF-β, Wnt, Notch and Hedgehog (Hh), along with other tumor microenvironmental cues, induce well-differentiated epithelial cells to convert into motile mesenchymal cells via the activation of multiple EMT transcription factors, including Twist1, Twist2, Snai1, Slug, ZEB1 and ZEB2. Similarities between developmental and oncogenic EMT have led to the identification of common contributing pathways, suggesting that the reactivation of developmental pathways in cancers contributes to tumor progression. For example, developmental EMT regulators including Twist1, Twist2, Snail, Slug and Six1, and Cripto, along with developmental signaling pathways including TGF-β and Wnt/β-catenin, are misexpressed in breast cancer and correlate with poor clinical outcomes.

Evidence has recently been accumulating to support the hypothesis that tumors contain a subpopulation of tumor cells called cancer stem cells (CSCs), also known as tumor initiating cells or tumorigenic cells, which exhibit stem-like cell properties to self-renew, form tumor spheres, differentiate into heterogeneous populations of cancer cells, and seed new tumors in a xenotransplant system (Dontu *et al*., 2003; Gupta and Weinberg, 2009). In addition to initiating tumors, CSCs are thought to be capable of initiating metastasis. The CSC hypothesis provides an attractive model of tumor development and progression, holding that solid tumors are hierarchically organized and sustained by a small subset of the tumor cell population with stem cell properties. Under this hypothesis, sustained metastatic growth requires the dissemination of a CSC from the primary tumor followed by its re-establishment in a secondary site. The CSC hypothesis has fundamental and important clinical implications, as the current development of cancer therapeutics is largely based on screening agents with the ability to cause bulk tumor regression in animal models or in clinical trials (Bao *et al*., 2006; Rich and Bao, 2007; Bao *et al*., 2008; Gupta and Weinberg, 2009). Strategies aimed at efficiently targeting CSCs are critical for monitoring the progress of cancer treatment and for evaluating new therapeutic agents. The elucidation of signalling pathways which regulate CSC self-renewal and survival provides potential therapeutic targets. In addition, CSC behaviors are constantly regulated both by inside regulators such as transcription factors and external signals from their niches, including neighboring stromal, immune, and non-stem tumor cells. Targeting the neighboring non-stem cancer cells, stromal cells or the paracrine factors secreted by these cells may target CSCs indirectly, and thereby contribute to long-term remissions (Polyak and Hahn, 2006).

Both the EMT and CSCs play a critical role in tumor metastasis, therapeutic resistance and recurrence; however, each alone can not explain the sum of the cellular events in tumor progression and the significance of EMT in regulating the stemness of CSCs remains unknown until very recently. Balancing these two concepts has led researchers to investigate a possible link between EMT and the CSC phenotype. Brabletz *et al*. (2005) proposed an integrated model–the migrating cancer stem cell 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. In a recent report, Mani *et al*. (2008) found that Twist1, Snai1 or TGF-β can transform nontumorigenic, immortalized human mammary epithelial cells (HMLEs) into mesenchymal-like cells and dedifferentiate *HER2/neu*-infected HMLE (HMLEN) cells into $CD44^{high}CD24^{low}$ cancer stem-like cells 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. Moreover, other EMT inducers or regulators such as ZEB1, YB-1, LBX1 and Six1 have also been shown to induce well-differentiated cells and cancer cells to form populations with stem cell-like characteristics via promoting EMT, indicating that there is a crosstalk between the EMT program and the pathways involved in regulating stemness in stem cells (Mani *et al*., 2008; Morel *et al*., 2008; Evdokimova *et al*., 2009; Illopoulos *et al*., 2009; McCoy *et al*., 2009; Polyak and Weinberg, 2009; Wellner *et al*., 2009; Yu *et al*., 2009; Ouyang *et al*., 2010; Singh and Settleman, 2010). The critical roles of TGF-β, Wnt, Notch 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. Furthermore, the expression of stemness and EMT markers in CSCs were associated with resistance to conventional anti-cancer therapies and

treatment failure, highlighting the urgency of improving tools for detecting and eliminating minimal residual disease. In this chapter, we focus on recent findings regarding the role of EMT signaling pathways in regulating the stemness of cancer stem cells.

2. EMT signaling pathways in regulating stemness of CSCs

During the EMT process, epithelial cells undergo specific series of events and 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. The functional loss of E-cadherin expression is considered a one of the hallmarks of EMT and a crucial 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 (Thiery *et al*., 2009; Yilmaz and Christofori, 2009). *E-cadherin* promoter is repressed directly or indirectly by specific developmental transcription factors such as Twist1, Snai1, Slug, ZEB1, ZEB2, FOXC2, KLF8 and E47, which disrupts the polarity of epithelial cells and maintains a mesenchymal phenotype (Kang and Massague, 2004; Yang and Weinberg, 2008; Thiery *et al*., 2009). Knockdown of E-cadherin by shRNA triggered EMT and resulted in acquisition of a mesenchymal phenotype and increased CSC activity in HMLER breast cancer cells (Gupta *et al*., 2009).

2.1 EMT signaling from the microenvironment in regulating stemness of CSCs

Tumor development is a continuous reciprocal interaction between tumor cells and their surrounding microenvironment, in which stromal cells and the extracellular matrix (ECM) play a decisive role in tumorigenesis (Bissell and Radisky, 2001; Hanahan and Weinberg, 2011). Tumor microenvironment not only provides support for initiation and growth of the primary tumor, but also facilitates tumor metastatic dissemination to distant organ as an active participant. Tumor cells can only thrive in an aberrant microenvironment composed of altered ECM and various non-transformed 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 (Hanahan and Weinberg, 2000; Bissell and Radisky, 2001; Bhowmick *et al*., 2004; Bissell and Labarge, 2005; McAllister *et al*., 2008; Hanahan and Weinberg, 2011). In adult tissues, normal stem cells reside within highly defined anatomical niches that provide both cell-intrinsic and cell-extrinsic factors to maintain stem cells in undifferentiated states to self-renew or give rise to the full repertoire specialized cells in the tissues. Like their normal stem cells, CSCs have the ability both to self-renew and to differentiate to specialized cells with limited proliferation potential. Accumulating evidence has emerged that factors derived from the tumor microenvironment serve to regulate the stemness of CSCs. CSC niche can be considered as the tumor microenvironment surrounding CSCs that contributes to maintain the stemness of CSCs. CSCs may reside in and constantly affected by their aberrant niches, where cell-cell and cell-matrix interactions can provide unregulated external 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. Recent work has begun to address the importance of the tumor microenvironment in regulating the EMT during tumorigenesis and also found that the emergence of CSCs occurs in part as a result of EMT, for example, through cues from tumor microenvironment components.

TGF-β signaling. TGF-β is a multifunctional cytokine that plays critical roles in tumor suppression and tumor progression, cell differentiation and tissue morphogenesis, and extracellular matrix production through activation of Smad and non-Smad signaling pathways. Current data show that TGF-β signaling pathway has a dual role in tumorigenesis as a tumor suppressor in early stage tumors or as a promoter of tumor progression and metastasis (Derynck *et al*., 2001; Massague, 2008). In the Smad-dependent pathway, TGF-β ligands bind to heterotetrameric complexes of receptors with serine-threonine kinase activity leading to an increase in their ability to phosphorylate the receptor-related Smad (R-Smad) proteins. The phosphorylated Smad2 and Smad3 then form heteromeric complexes with Smad4 and translocate into the nucleus to regulate the transcription of target genes. The amplitude and duration of Smad2/3-based signaling transpires through their physical interaction with a plethora of transcription factors, and with a variety of transcriptional co-activators and co-repressors in a gene- and cell-specific manner. Currently, TGF-β is recognized as a master regulator of EMT, during embryogenesis and tissue morphogenesis (i.e., type 1 EMT), wound healing and tissue fibrosis (i.e., type 2 EMT), and tumor invasion and metastasis (i.e., type 3 EMT). Multiple transcription factors, including ZEB1, ZEB2, and Snai1, are induced by TGF-β-Smad signaling and play important roles in TGF-β-induced EMT. TGF-β employs HMGA2 (high-mobility group A2) to induce the expression of Twist1, Snai1 and Slug to promote EMT (Thuault *et al*., 2006). Non-Smad signaling activated by TGF-β also plays important roles in induction of EMT. Independent of Smad activity, TGFBR2 can directly phosphorylate the cell polarity protein, Par6, to promote the dissolution of cell junction complexes (Ozdamar *et al*., 2005; Thuault *et al*., 2006). In addition, TGF-β signaling also cross-talks with other signaling pathways to act in concert to trigger EMT programs. Of these, Ras and Wnt signaling pathways synergize with TGF-β signaling, and play a critical role in the induction of EMT (Polyak and Weinberg, 2009; Vincent *et al*., 2009).

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 (Shipitsin *et al*., 2007). 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) (Shipitsin *et al*., 2007). $CD44^{high}/CD24^{low}$ cells isolated from HMLEs display a mesenchymal phenotype (Mani *et al*., 2008). 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 $CD44^{low}CD24^+$ non-tumorigenic mammary epithelial cells via the activation of the Ras/MAPK signaling pathway (Morel *et al*., 2008). In MCF-10A cells, the knockdown of Akt1 promotes TGF-β-induced EMT and a stem cell-like phenotype (Iliopoulos *et al*., 2009). Recently, the activating transcription factor 3 (ATF3) is induced by TGF-β in the MCF10CA1a breast cancer cells and plays an integral role for TGF-β to upregulate its target genes *Snail*, *Slug* and *Twist1*, and to enhance cell motility. Interestingly, ATF3 increases the expression of the TGF-β itself, forming a positive-feedback loop for TGF-β signaling. Moreover, ectopic expression of ATF3 promotes EMT and increases

$CD24^{low}$-$CD44^{high}$ population of cells, mammosphere formation and tumorigenesis (Yin *et al*., 2010).

TGF-β may exert a similar effect on regulating the stem cell-like pool of other tumors. TGF-β is highly expressed in high-grade gliomas and upregulated TGF-β activity confers poor prognosis in glioma patients. 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 (Penuelas *et al*., 2009). 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 tumorigenesis *in vivo* (Penuelas *et al*., 2009). TGF-β-FOXO signaling is shown to be essential in the maintenance of leukemia-initiating cells in chronic myeloid leukemia (CML) (Naka *et al*., 2010).

Wnt signaling. Among many embryonic signaling pathways, Wnt pathway is one of critical pathways involved in regulating the stemness of CSCs and in the acquisition of EMT characterics during tumorigenesis. Wnt signals are transduced to the canonical pathway for cell fate determination, and to the noncanonical pathway for control of cell movement and tissue polarity. In the absence of active Wnt ligands, β-catenin is complexed with scaffold proteins Axin and APC, and phosphorylated by GSK-3β and CKIα. Phosphorylated β-catenin is then ubiquitinated and undergoes proteasome-mediated degradation. Canonical Wnt signals are transduced through membrane Frizzled (FZD) receptors and LRP5/LRP6 co-receptor to the β-catenin signaling cascade. In the presence of active Wnt signaling, Wnt ligands bind to FZD and LRP, resulting in the phosphorylation of LRP6 by GSK-3β in its cytoplasmic region, leading to the recruitment of Dishevelled (Dvl) and Axin. β-catenin is then released from phosphorylation by GSK-3β and degradation by proteosome. The accumulated β-catenin translocates to the nucleus and regulates the expression of target genes. Noncanonical Wnt signals are transduced through FZD receptors and ROR2/RYK co-receptors to the Dishevelled-dependent or the Ca^{2+}-dependent signaling cascades. The inappropriate expression of the Wnt ligand and Wnt binding proteins and the inappropriate activation of the Wnt signaling have been found in a variety of human cancers. 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 (Brabletz *et al*., 2005). Aberrant nuclear expression of β-catenin might confer cancer cells with these two capabilities, EMT and stemness, which promote malignant tumor progression. GSK-3β is an endogenous inhibitor of Snail and can phosphorylate Snai1. GSK-3β down-regulation by the FGF-dependent PI3-K/Akt pathway directly results in the activation of the Snai1-EMT signaling cascade. Therefore, inhibition of GSK-3β function by Wnt and other pathways can promote Snai1 stability and nuclear import to induce EMT (Zhou *et al*., 2004; Bachelder *et al*., 2005). In patients with a CML blast crisis, a β-catenin mutation may confer self-renewal properties on granulocyte-macrophage progenitors (Jamieson *et al*., 2004). 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 (Reya and Clevers, 2005; Malanchi *et al*., 2008). Inhibiting of Wnt pathway through LRP6 decreases the ability of cancer cells to self-renew and seed tumors *in vivo* (DiMeo *et al*, 2009). Moreover, inhibition of Wnt signaling blocks tumor formation by promoting epithelial differentiation and repressing the EMT transcription factors, Twist1 and Slug. These data indicate that Wnt

pathway is involved in CSC self-renewal, EMT and metastasis in basal-like breast cancer (DiMeo *et al*, 2009).

Notch signaling. Notch signaling is important for development and tissue homeostasis and regulates cell fate specification through local cell interactions in invertebrate and vertebrate organisms. For example, Notch activity promotes EMT during cardiac development via transcriptional induction of Snai1 and induces EMT in immortalized endothelial cells *in vitro* (Luika *et al*., 2004). Notch pathway is also activated in many human cancers and contributes to EMT and to cancer stem-like cell characteristics in tumorigenesis. Notch signaling pathway is essential for both nonneoplastic neural stem cells and embryonal brain tumors. The activation 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 (Fan *et al*., 2006). Notch signaling is associated with chemo-resistance and EMT phenotypes in gemcitabine-resistant pancreatic cancer cells (Wang *et al*., 2009). Recently, miR-200 members has been shown to target Notch pathway components, such as Jagged1 (Jag1) and the mastermind-like co-activators Maml2 and Maml3, thereby mediating enhanced Notch activation by ZEB1 (Brabletz *et al*., 2011).

Hedgehog signaling. As an ancient cell signaling system, the Hedgehog (Hh) signaling is an important developmental pathway. In the absence of Hh ligands Shh, Ihh or Dhh, Hh receptor Ptch inhibits a second transmembrane protein Smo. This repression is relived when Hh ligands bind to Ptch. Subsequently Smo causes activation of Hh pathway targets via the Gli family of transcription factors (Gli1, Gli2, and Gli3). Hh signaling is essential for embryonic pattern formation, hematopoiesis, and also plays an important role in tumorigenesis and stem cell maintenance (Trowbridge *et al*., 2006; Dierks *et al*., 2008; Zhao *et al*., 2009). 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* (Liu *et al*., 2006). 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 (Dierks *et al*., 2008). 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 (Zhao *et al*., 2009). However, a conditional Smo deletion or over-activation has no significant effects on adult HSC self-renewal and function, and the Hh signaling pathway is dispensable for adult HSC function (Gao *et al*., 2009). 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 (Dierks *et al*., 2008). In addition, medulloblastomas arising from Patched-1-deficient or Patched-mutant mice contain $CD15^+$ CSCs (Read *et al*., 2009; Ward *et al*., 2009). Hh/Wnt feedback is involved in regenerative proliferation of epithelial stem cells in bladder (Shin *et al*., 2011). 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 (Varnat *et al*., 2009).

Extracellular matrix proteins. The extracellular matrix 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 (Erkan *et al*., 2007; Ruan *et al*., 2009a). 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 (Kirkland, 2009), indicating that Type I collagen may be involved in generating and maintaining human colorectal CSCs via EMT.

Other microenvironment cues. In addition to TGF-β, Wnt, Notch, and Hh which play 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 (Huber *et al*., 2005; Yang and Weinberg, 2008). 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 from tumor microenvironment and their receptors regulate the stemness of CSCs via EMT remains to be established. Interleukin-6 (IL-6) is a tumor microenvironment-derived extracellular signaling factor capable of inducing EMT (Sullivan *et al*., 2009). 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, Twist1 and Snai1 via the activation of STAT3 (Sullivan *et al*., 2009). Moreover, IL-6 can induce malignant properties in mammospheres from human ductal breast carcinoma and normal mammary gland (Sansone *et al*., 2009). 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 (Ancrile *et al*., 2007). Recently, IL-6 signaling has also been shown to contribute to glioma malignancy by promoting glioma stem cell (GSC) growth and survival (Wang *et al*., 2009). 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 (Wang *et al*., 2009). A recent report revealed that carcinoma-derived IL-6 is involved in activation of cancer-associated fibroblasts. Reciprocal activation of prostate cancer cells and cancer-associated fibroblasts stimulates epithelial-mesenchymal transition and cancer stemness (Giannoni *et al*., 2010).

2.2 EMT transcription factors in regulating stemness of CSCs

E-cadherin 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. Transcription factors such as Twist1, Snai1, Slug, ZEB1, ZEB2, FOXC2, KLF8 and E47, can repress the *E-cadherin* promoter directly or indirectly (Kang and Massague, 2004; Yang and Weinberg, 2008; Thiery *et al*., 2009). These transcription factors play critical roles in mediating type 1 EMT during embryogenesis and tissue morphogenesis; however, their aberrant activation of EMT developmental programs during tumorigenesis is considered a hallmark of disease progression and metastasis initiation. Among these developmental transcription factors, the Twist, Snai1 and ZEB family members are well-investigated in EMT and CSCs.

Twist1 and Twist2. Twist proteins are highly conserved basic helix-loop-helix (bHLH) transcription factors that play an important role in embryogenesis and tumorigenesis. Twist1 and Twist2 are significantly over-expressed in various human solid tumors and are involved in tumor invasion and metastasis through their ability to promote EMT (Ansieau *et al*., 2008). Twist1 and Twist2 mediate the growth and commitment of human mesenchymal stromal/stem cells (MSC) (Isenmann *et al*., 2009). The levels of Twist1 and Twist2 are very high in freshly purified human bone marrow-derived MSCs but decrease following ex vivo expansion. Over-expression of Twist1 and Twist2 in human MSC cultures up-regulates the level of the MSC marker, STRO-1, and the early osteogenic transcription factors, Runx2 and Msx2. Therefore, Twist1 and Twist2 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 (Isenmann *et al*., 2009).

In a recent report, Mani *et al*. (2008) found that Twist1 can transform nontumorigenic, immortalized human mammary epithelial cells (HMLEs) into mesenchymal-like cells and dedifferentiate *HER2/neu*-infected HMLE (HMLEN) cells into $CD44^{high}CD24^{low}$ cancer stem cells via EMT. Induction of EMT in nontumorigenic, immortalized mammary epithelial cells by ectopic expression of either Twist1 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 an EMT. Compare to the level in $CD44^{low}/CD24^{high}$ cells, the expression of *E-cadherin* mRNA in stem-like $CD44^{high}/CD24^{low}$ cells is strongly decreased (~150-fold), while the levels of mRNAs encoding mesenchymal markers and EMT-inducing transcription factors are significantly upregulated, specifically *N-cadherin* (~200-fold), *Fibronectin* (~60-fold), *Twist1* (~198-fold), *Snai1* (~9-fold), *ZEB2* (~30-fold), and *FOXC2* (~16-fold). Furthermore, Twist1 can considerably increase the number of tumor-initiating cells in HER2/neu- or Ras-activated human mammary epithelial cells. 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. This study provided direct support for a potential association between EMT and cancer stem-like cell phenotype. Vesuna *et al*. (2009) further demonstrated that Twist1 is directly involved in generating a breast CSC phenotype through down-regulation the expression of CD24. Twist2, like Twist1, overrides oncogene-induced premature senescence by promoting EMT in human epithelial cells (Ansieau *et al*., 2008).

Twist2 has been shown to be overexpressed in several types of human cancers, but the expression pattern of Twist2 is different from that of Twist1 in these cancers, suggesting that

Twist1 and Twist2 may have overlapping but distinct roles in different set of tumors. Twist2 is involved in $p12^{CDK2\text{-}Ap1}$-induced EMT of hamster cheek pouch carcinoma-I cells (Tsuji *et al*., 2008). Our recent studies have suggested a role of Twist2 in regulating EMT and CSC stemness in human breast cancer progression (Fang *et al*., 2011). Twist2 is a potent inducer of EMT in human mammary epithelial cells and breast cancer cells. Ectopic expression of Twist2 in mammary epithelial cells and breast cancer cells increases the size and number of their $CD44^{high}/CD24^{low}$ stem-like cell subpopulations, promotes the expression of stem cell markers and increases the self-renewal capabilities of stem-like cells. Moreover, exogenous expression of Twist2 leads to constitutive activation of STAT3 and down-regulation of *E-cadherin* (Fang *et al*., 2011). In addition, we also showed that the Twist2-driven EMT plays critical roles in ovarian cancer progression by promoting a cancer stem cell phenotype to augment tumor metastasis and therapeutic resistance (Mao *et al*., our unpublished data). Therefore, Twist2 may contribute to breast and ovarian cancer progression by activating the EMT program and enhancing the self-renewal of cancer stem-like cells.

Snai1 and Slug. The Snail family is highly conserved zinc-finger transcription repressor and plays a pivotal role in embryonic development and tumorigenesis. Both Snai1 and Slug can be activated by the TGF-β, Wnt, FGF, HGF and ER signaling pathways and Snai1 is specifically activated at the tumor-stroma interface. Snai1 has a critical role in EMT both during embryonic development and in tumor progression by inhibiting junction components such as E-cadherin, claudins, occludin and desmoplakin (Vincent, *et al*., 2009). 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 (Kudo-Saito *et al*., 2009). Casas *et al*. (2010) reported that direct induction of Slug is essential for Twist1 to induce EMT and that Twist1 and Slug act together to promote EMT and tumor metastasis. In addition, Snai1 is a cofactor for Smad3/4 and these transcription factors form a transcriptional repressor complex to inhibit *CAR*, *occluding* and *E-cadherin* transcription during TGF-β-induced EMT in mammary epithelial cells (Vincent, *et al*., 2009).

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* (Inoue *et al*., 2002). 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 (Kurrey *et al*., 2009). Furthermore, Mani and colleagues (2008) found that Snai1 can generate cells with properties of stem cells via EMT induction like Twist1. When EMT is transiently induced in HMLEN cells through the ectopic expression of Snai1, 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 Snai1 in $H\text{-}Ras^{V12}$-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 (Mani *et al*., 2008).

ZEB1 and ZEB2. The ZEB family proteins, ZEB1 and ZEB2, 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 (Peinado *et al*., 2007). 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 (Wellner *et al*., 2009).

Bmi-1. Bmi-1, a member of the polycomb-repressive complex 1 (PRC1), is commonly deregulated in various tumors and plays an important role in maintaining self-renewal in normal and malignant human mammary stem cells (Dimri *et al*., 2002; Liu *et al*., 2005; Liu *et al*., 2006). Bmi-1 inhibits PTEN and induces EMT in human nasopharyngeal epithelial cells and is also involved in the regulation of self-renewal and differentiation of stem cells (Song *et al*., 2009b). A recent report showed that *Bmi-1* can be regulated by Twist1 directly. Bmi-1-containing PRC directly represses *E-cadherin* expression. Bmi-1 and Twist1 are mutually essential to promote EMT and tumor-initiating capability of human head and neck squamous cell carcinoma cells (Yang *et al*., 2010). We also showed that Bmi-1 is involved in Twist2-induced EMT of mammary epithelial cells and breast cancer cells and cancer stem cell self-renewal (Fang *et al*., 2011). The current findings highlight the critical role of the polycomb group protens in regulating EMT and the stemness of CSCs.

LBX1. Ladybird homeobox 1 (LBX1) is a well established homeobox regulator implicated in normal myogenesis and neurogenesis. Recent work has shown that LBX1 is over-expressed in the unfavorable ER/PR/HER2 triple-negative basal-like subtype of human breast cancer (Yu *et a*l., 2009). Moreover, LBX1 is a potent activator of EMT and can regulate the expression of the known EMT inducers TGF-β2, Snai1, ZEB1 and ZEB2. LBX1 induces EMT, enhances cell migration, enlarges the $CD44^{high}/CD24^{low}$ progenitor cell population in mammary epithelial cells, and cooperates with activated H-Ras to cause tumorigenesis and correlates with the basal subtype of human breast cancer (Yu *et a*l., 2009). These results suggest that LBX1 is an important developmental regulator of oncogenic EMT and stemness of breast cancer stem cells and contributes to breast cancer aggressiveness.

Six1. Six1, one of member of Six family of homeodomain proteins, is involved in the expansion of the precursor cell population during embryogenesis. In addition to the role of the Six family members in epithelial plasticity during muscle and kidney development, Six1 is frequently overexpressed in various cancers and has been shown to play an important role in inducing features of EMT in both a mammary carcinoma cell line and in mammary tumors derived from mammary specific Six1 overexpressing transgenic mice (McCoy *et al*., 2009; Micalizzi *et al*., 2009). Overexpression of Six1 in immortalized mammary epithelial cells induces malignant transformation and facilitates mammary carcinoma cells to undergo EMT and metastasis by increasing TGF-β signaling (Coletta *et al*., 2008; Micalizzi *et al*., 2009). Six1 also promotes the expansion of the stem/progenitor cell population in the mouse mammary gland and subsequent mammary tumor development via EMT (McCoy *et al*., 2009. Therefore, over-expression of Six1 in breast cancer induce highly aggressive and invasive mammary tumors with EMT and cancer stem cell features.

YB-1. Mammalian Y-box binding protein-1 (YB-1) is a member of the cold-shock domain (CSD) protein superfamily. Targeted disruption of YB-1 in mice causes severe developmental defects and embryonic lethality. YB-1 is involved in tumorigenesis and exhibits both pro-oncogenic role and tumor-suppressive functions by regulating gene expression through transcriptional and translational ways. YB-1 is over-expressed in ~75% of human breast cancers and high YB-1 levels provoke remarkably diverse breast carcinomas through the induction of genetic instability (Bargou *et al*., 1997; Bergmann *et al*., 2005). 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 (Evdokimova *et al*., 2009). 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 (Evdokimova *et al*., 2009). Therefore, MCF-10AT cells with ectopic upregulated YB-1 may acquire cancer stem cell phenotypes by inducing EMT.

Hypoxia-inducible factors (HIFs). Intratumoral hypoxia occurs when tumor cells are located greater than the distance from functional blood vessels for adequate diffusion of oxygen as a result of rapid tumor cell growth and abnormal blood vessels. 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 (Ruan *et al*., 2009c). 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*. 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 (Covello *et al*., 2006). In glioblastomas, CSCs differentially respond to hypoxia with a distinct induction of HIF-2α (Li *et al*., 2009). HIF-2α-specific target genes such as *Oct4*, *Glut1* and *SerpinB9* are expressed at significantly higher levels in GSCs compared to matched non-stem cancer cells under hypoxic treatment. HIF-2α is also required for VEGF expression in GSCs, but not in non-stem 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* (Li *et al*., 2009).

Hypoxia can also induce EMTs in tumors through the upregulation of HIF-1α, Snai1, Twist1, ZEB1, ZEB2, lysyl oxidase (LOX) and by activating Wnt and Notch pathways (Erler *et al*., 2006; Pouyssegur *et al*., 2006; Yang *et al*., 2008). Twist1 has a critical role in EMT and metastatic phenotypes induced by hypoxia or over-expression of HIF-1α. In primary tumors of head and neck cancer patients, co-expression of HIF-1α, Twist1 and Snai1 correlates with metastasis and a poor prognosis (Yang *et al*., 2008). 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 Twist1 in hypoxia-mediated EMT and invasion (Pouyssegur *et al*., 2006; Yang *et al*., 2008). Jagged2 is upregulated in bone marrow stroma under hypoxia and promotes the growth of cancer stem-like cells by activating their Notch signaling. Hypoxia-induced Jagged2 activation in both tumor invasive front and normal bone stroma has a critical role in breast cancer metastasis and self-renewal of cancer stem-like cells (Xing *et al*., 2011). 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 (Keith and Simon, 2007). Hypoxic microenvironment may be not only a critical niche favorable for expansion and stemness maintenance of CSCs in solid tumors, and 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 (Keith and Simon, 2007; Li *et al*., 2009).

In addition to Twist, Snail and ZEB family members and the transcription factors mentioned above, 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. 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 (Hartwell *et al*. 2006). 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 (Mani *et al*., 2007; Mani *et al*., 2008).

2.3 Other players of EMT in regulating stemness of CSCs

microRNAs. microRNAs (miRNAs) are a newly discovered endogenous class of small non-coding RNAs of 18-25 nucleotide in length that modulate gene expression as negative regulators at the post-transcriptional level by specifically binding and cleaving target mRNAs or inhibiting their translation. Current reports demonstrated that the deregulation of miRNAs correlates with various human cancers and is involved in the initiation and progression of human cancers (Ruan *et al*., 2009b). Recently, miRNAs have also been identified as a new class of EMT regulators due to their regulation of EMT-inducing transcription factors, such as Twist1, Snai1, ZEB1 and ZEB2 (Ma and Weinberg, 2008).

The miR-200 family of miRNAs (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) is both an important marker for epithelial cells and a powerful master regulator of EMT in embryonic development and tumorigenesis (Park *et al*., 2008). miR-200 suppresses the expression of ZEB1 and ZEB2 to favor an epithelial phenotype and inhibit EMT (Gregory *et al*., 2008; Korpal *et al*., 2008; Park *et al*., 2008). Moreover, let-7, miR-335, miR-205, miR-206, miR-126, miR-146a and miR-101 have also been reported as metastasis suppressors (Yu *et al*., 2007; Gregory *et al*., 2008; Tavazoie *et al*., 2008; Varambally *et al*., 2008). Conversely, miRNAs such as miR-155, miR-10b, miR-21, miR-373 and miR-520c are involved in promoting tumor invasion and metastasis via regulating EMT (Ma *et al*., 2007; Huang *et al*., 2008; Kong *et al*., 2008; Yan *et al*., 2008). For example, TGF-β stimulation of normal mammary epithelial cells elicits their increased expression of miR-155 via a Smad4-dependent pathway. Once expressed, miR-155 participates in EMT by inhibiting RhoA expression, leading to the dissolution of tight junctions (Kong *et al*., 2008).

Recently, 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 (Marson *et al*., 2008). 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 (Judson *et al*., 2009). 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 *c-Myc* (Judson *et al*., 2009). 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 (Xu *et al*., 2009).

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 over-expression 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 over-expressed (Yu *et al*., 2007). 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 (Shimono *et al*., 2009). Iliopoulos *et al*. (2010) demonstrated that downregulation of miR-200 lead to increased expression of Suz12, a subunit of the polycomb repressor complex 2, increased binding of Suz12 to the *E-cadherin* promoter, and upregulated H3-K27 trimethylation and polycomb-mediated inhibition of *E-cadhrein* expression. The interactions between the miR-200 family are required for CSC formation. Xia *et al.* (2010) reported that miR-200a not only regulates EMT by targeting ZEB2 but also stem-like transition via differentially and specifically by β-catenin signaling in nasopharyngeal carcinoma cells. This finding demonstrates for the first time the function of miR-200a in shifting nasopharyngeal carcinoma cell states via a reversible process coined as epithelial-mesenchymal to stem-like transition through differential and specific mechanisms. 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 (Wellner *et al*., 2009). Induction of EMT can be controlled by miR-200 family whose abundance depends on the balance between Akt1 and Akt2 rather than on the overall activity of Akt (Iliopoulos *et al*., 2009). A recent report showed that EMT induction is epigenetically driven, initially by chromatin remodeling through H3K27me3 enrichment and later by ensuing DNA methylation to sustain silencing of miR-200b, miR-200c, and miR-205 (Tellez *et al*., 2011). These current data highlight the central role of miRNAs in regulating EMT and self-renewal and/or proliferation of normal and neoplastic stem cells. The miRNA signatures of CSCs likely represent a new layer of regulatory control over cell fate decisions of CSCs via EMT.

p53. The tumor suppressor p53 is known to function as transcription factor. Recently, p53 has been shown to exhibit a role in regulating both EMT and EMT-associated stem cell properties through transcriptional activation of miR-200c (Chang *et al*., 2011). Loss of p53 in human mammary epithelial cells decreases the expression of miR-200c and activates the EMT program, accompanied by an increased mammary stem cell population. Moreover, loss of p53 correlates with a down-regulated level of miR-200c, but an increased expression of EMT and stemness markers, and development of a high tumor grade in a cohort of breast tumors. Therefore, the p53-miR-200c pathway most likely accounts for regulating the EMT-associated cancer stem cell population (Chang *et al*., 2011).

3. Concluding remarks

EMT is regarded as a critical step in tumor invasion and metastasis. 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-renewal in order to spawn macroscopic metastases. 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 (Bao *et al*., 2004). Current evidence supports that metastasis is a relatively inefficient process and 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. The discovery that EMT generates cells with properties of self-renewing stem cells has linked EMT with both tumor metastasis and acquisition of stem-like cell properties, indicating that cancer cells undergo an EMT are capable of metastasizing through their acquired invasiveness and, following dissemination, through their acquired self-renewal potential, which enables them to spawn the large cell populations that constitute macroscopic metastases (Taube *et al*., 2010).

EMT occurs in a variety of distinct physiological and pathological settings, including normal embryogenesis, tissue morphogenesis, tissue remodeling and repair and fibrosis, and cancer progression. A number of developmental signaling pathways have been shown to play a role in EMT such as TGF-β, Wnt, Notch, Hh and other microenvironmental cues. These EMT-inducing signaling pathways promote the well-differentiated epithelial cells to convert into motile mesenchymal cells via the activation of multiple EMT transcription factors such as Twist1, Twist2, Snai1, Slug, ZEB1 and ZEB2. Each of these factors is capable, on its own, of inducing an EMT in various normal and cancer cell lines. However, the overlapping and unique contributions of each inducer to the EMT program have not been adequately explored. 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. Therefore, the knowledge gained from the multifaceted players of EMT during development and from the acquisition of CSC traits with the EMT transdifferentiation program may provide useful information to uncover the roles of these EMT players in tumorigenesis and metastasis, and offer new avenues of therapeutic intervention with the potential to go beyond traditional anti-cancer approaches.

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5. References

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

Ancrile B, Lim KH, Counter CM (2007) Oncogenic Ras-induced secretion of IL6 is required for tumorigenesis. Genes Dev 21:1714-1719

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

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

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

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

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

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

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 Bhowmick NA, Neilson EG, Moses HL (2004) Stromal fibroblasts in cancer initiation and progression. Nature 432: 332-337

Bissell MJ, Labarge MA (2005) Context, tissue plasticity, and cancer: are tumor stem cells also regulated by the microenvironment? Cancer Cell 7: 17-23

Bissell MJ, Radisky D (2001) Putting tumors in context. Nat Rev Cancer 1: 46-54

Brabletz S, Bajdak K, Meidhof S, Burk U, Niedermann G, Firat E, Wellner U, Dimmler A, Faller G, Schubert J, Brabletz T (2011) The ZEB1/miR-200 feedback loop controls Notch signalling in cancer cells. EMBO J 30: 770-782

Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T (2005) Opinion: migrating cancer stem cells - an integrated concept of malignant tumor progression. Nat Rev Cancer 5:744-749

Casas E, Kim J, Bendesky A, Ohno-Machado L, Wolfe CJ, Yang J (2011) Snail2 is an essential mediator of Twist1-induced epithelial mesenchymal transition and metastasis. Cancer Res 71: 245-254

Chang CJ, Chao CH, Xia W, Yang JY, Xiong Y, Li CW, Yu WH, Rehman SK, Hsu JL, Lee HH, Liu M, Chen CT, Yu D, Hung MC (2011) p53 regulates epithelial-mesenchymal transition and stem cell properties through modulating miRNAs. Nat Cell Biol 13: 317-323

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

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

Derynck R, Akhurst RJ, Balmain A (2001) TGF-beta signaling in tumor suppression and cancer progression. Nat Genet 29: 117-129

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

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

Dimri GP, Martinez JL, Jacobs JJ, Keblusek P, Itahana K, Van Lohuizen M, Campisi J, Wazer DE, Band V (2002) The Bmi-1 oncogene induces telomerase activity and immortalizes human mammary epithelial cells. Cancer Res 62: 4736-4745

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

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

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

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

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

Fang X, Cai Y, Liu J, Wang Z, Wu Q, Zhang Z, Yang CJ, Yuan L, Ouyang G (2011) Twist2 contributes to breast cancer progression by promoting an epithelial-mesenchymal transition and cancer stem-like cell self-renewal. Oncogene, in press

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

Giannoni E, Bianchini F, Masieri L, Serni S, Torre E, Calorini L, Chiarugi P (2010) Reciprocal activation of prostate cancer cells and cancer-associated fibroblasts stimulates epithelial-mesenchymal transition and cancer stemness. Cancer Res 70: 6945-6956

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

Gupta PB, Chaffer CL, Weinberg RA (2009) Cancer stem cells: mirage or reality? Nat Med 15: 1010-1012

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

Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100: 57-70

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646-674

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 U S A 103: 18969-18974

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 tumor invasion and metastasis. Nat Cell Biol 10: 202-210

Huber MA, Kraut N, Beug H (2005) Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin Cell Biol 17: 548-558

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.

Iliopoulos D, Lindahl-Allen M, Polytarchou C, Hirsch HA, Tsichlis PN, Struhl K (2010) Loss of miR-200 inhibition of Suz12 leads to polycomb-mediated repression required for the formation and maintenance of cancer stem cells. Mol Cell 39: 761-772

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

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

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

Judson RL, Babiarz JE, Venere M, Blelloch R (2009) Embryonic stem cell-specific microRNAs promote induced pluripotency. Nat Biotechnol 27: 459-461

Kalluri R, Weinberg RA (2009) The basics of epithelial-mesenchymal transition. J Clin Invest 119: 1420-1428

Kang Y, Massague J (2004) Epithelial-mesenchymal transitions: twist in development and metastasis. Cell 118: 277-279

Keith B, Simon MC (2007) Hypoxia-inducible factors, stem cells, and cancer. Cell 129: 465-472

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

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

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

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

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

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

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

Liu S, Dontu G, Wicha MS (2005) Mammary stem cells, self-renewal pathways, and carcinogenesis. Breast Cancer Res 7: 86-95

Ma L, Teruya-Feldstein J, Weinberg RA (2007) Tumor invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 449: 682-688

Ma L, Weinberg RA (2008) Micromanagers of malignancy: role of microRNAs in regulating metastasis. Trends Genet 24: 448-456

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

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

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 U S A 104: 10069-10074

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

Massague J (2008) TGFbeta in Cancer. Cell 134: 215-230

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

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

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

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

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

Ouyang G, Wang Z, Fang X, Liu J, Yang CJ (2010) Molecular signaling of the epithelial to mesenchymal transition in generating and maintaining cancer stem cells. Cell Mol Life Sci 67: 2605-2618

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

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

Peinado H, Olmeda D, Cano A (2007) Snail, Zeb and bHLH factors in tumor progression: an alliance against the epithelial phenotype? Nat Rev Cancer 7: 415-428

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

Polyak K, Hahn WC (2006) Roots and stems: stem cells in cancer. Nat Med 12: 296-300

Polyak K, Weinberg RA (2009) Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer 9: 265-273

Pouyssegur J, Dayan F, Mazure NM (2006) Hypoxia signalling in cancer and approaches to enforce tumor regression. Nature 441: 437-443

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

Reya T, Clevers H (2005) Wnt signalling in stem cells and cancer. Nature 434: 843-850

Rich JN, Bao S (2007) Chemotherapy and cancer stem cells. Cell Stem Cell 1: 353-355

Ruan K, Bao S, Ouyang G (2009a) The multifaceted role of periostin in tumorigenesis. Cell Mol Life Sci 66: 2219-2230

Ruan K, Fang X, Ouyang G (2009b) MicroRNAs: novel regulators in the hallmarks of human cancer. Cancer Lett 285: 116-126

Ruan K, Song G, Ouyang G (2009c) Role of hypoxia in the hallmarks of human cancer. J Cell Biochem 107: 1053-1062

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

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

Shin K, Lee J, Guo N, Kim J, Lim A, Qu L, Mysorekar IU, Beachy PA (2011) Hedgehog/Wnt feedback supports regenerative proliferation of epithelial stem cells in bladder. Nature 472: 110-114

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

Singh A, Settleman J (2010) EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene 29: 4741-4751

Song LB, Li J, Liao WT, Feng Y, Yu CP, Hu LJ, Kong QL, Xu LH, Zhang X, Liu WL, Li MZ, Zhang L, Kang TB, Fu LW, Huang WL, Xia YF, Tsao SW, Li M, Band V, Band H, Shi QH, Zeng YX, Zeng MS (2009) The polycomb group protein Bmi-1 represses the tumor suppressor PTEN and induces epithelial-mesenchymal transition in human nasopharyngeal epithelial cells. J Clin Invest 119: 3626-3636

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

Taube JH, Herschkowitz JI, Komurov K, Zhou AY, Gupta S, Yang J, Hartwell K, Onder TT, Gupta PB, Evans KW, Hollier BG, Ram PT, Lander ES, Rosen JM, Weinberg RA, Mani SA (2010) Core epithelial-to-mesenchymal transition interactome gene-expression signature is associated with claudin-low and metaplastic breast cancer subtypes. Proc Natl Acad Sci USA 107: 15449-15454

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

Tellez CS, Juri DE, Do K, Bernauer AM, Thomas CL, Damiani LA, Tessema M, Leng S, Belinsky SA (2011) EMT and stem cell-like properties associated with miR-205 and miR-200 epigenetic silencing are early manifestations during carcinogen-induced transformation of human lung epithelial cells. Cancer Res 71: 3087-3097

Thiery JP, Acloque H, Huang RY, Nieto MA (2009) Epithelial-mesenchymal transitions in development and disease. Cell 139: 871-890

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

Timmerman LA, Grego-Bessa J, Raya A, Bertrán E, Pérez-Pomares JM, Díez J, Aranda S, Palomo S, McCormick F, Izpisúa-Belmonte JC, de la Pompa JL (2004) Notch promotes epithelial-mesenchymal transition during cardiac development and oncogenic transformation. Genes Dev 18: 99-115

Trowbridge JJ, Scott MP, Bhatia M (2006) Hedgehog modulates cell cycle regulators in stem cells to control hematopoietic regeneration. Proc Natl Acad Sci U S A 103: 14134-14139

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 tumor growth, recurrence, metastasis and stem cell survival and expansion. EMBO Mol Med 1: 338-351

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

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

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

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

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

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

Xia H, Cheung WK, Sze J, Lu G, Jiang S, Yao H, Bian XW, Poon WS, Kung HF, Lin MC (2010) miR-200a regulates epithelial-mesenchymal to stem-like transition via ZEB2 and beta-catenin signaling. J Biol Chem 285: 36995-37004

Xing F, Okuda H, Watabe M, Kobayashi A, Pai SK, Liu W, Pandey PR, Fukuda K, Hirota S, Sugai T, Wakabayshi G, Koeda K, Kashiwaba M, Suzuki K, Chiba T, Endo M, Mo YY, Watabe K (2011) Hypoxia-induced Jagged2 promotes breast cancer metastasis and self-renewal of cancer stem-like cells. Oncogene Epub ahead of print, PMID: 21499308

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

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

Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA (2004) Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 117: 927-939

Yang J, Weinberg RA (2008) Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 14: 818-829

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

Yang MH, Hsu DS, Wang HW, Wang HJ, Lan HY, Yang WH, Huang CH, Kao SY, Tzeng CH, Tai SK, Chang SY, Lee OK, Wu KJ (2010) Bmi1 is essential in Twist1-induced epithelial-mesenchymal transition. Nat Cell Biol 12: 982-992

Yilmaz M, Christofori G (2009) EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev 28: 15-33

Yin X, Wolford CC, Chang YS, McConoughey SJ, Ramsey SA, Aderem A, Hai T (2009) ATF3, an adaptive-response gene, enhances TGF{beta} signaling and cancer-initiating cell features in breast cancer cells. J Cell Sci 123: 3558-3565

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

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

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

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

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Over the last thirty years, the foremost inspiration for research on metastasis, cancer recurrence, and increased resistance to chemo- and radiotherapy has been the notion of cancer stem cells.The twenty-eight chapters assembled in Cancer Stem Cells - The Cutting Edge summarize the work of cancer researchers and oncologists at leading universities and hospitals around the world on every aspect of cancer stem cells, from theory and models to specific applications (glioma), from laboratory research on signal pathways to clinical trials of bio-therapies using a host of devices, from solutions to laboratory problems to speculation on cancersâ€™ stem cellsâ€™ evolution. Cancer stem cells may or may not be a subset of slowly dividing cancer cells that both disseminate cancers and defy oncotoxic drugs and radiation directed at rapidly dividing bulk cancer cells, but research on cancer stem cells has paid dividends for cancer prevention, detection, targeted treatment, and improved prognosis.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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