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Epithelial-mesenchymal transition (EMT): A biological process in the development, stem cell differentiation and tumorigenesis

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Abstract

The lineage transition between epithelium and mesenchyme is a process known as epithelial-mesenchymal transition (EMT), by which polarized epithelial cells lose their adhesion property and obtain mesenchymal cell phenotypes. EMT is a biological process that is often involved in embryogenesis and diseases, such as cancer invasion and metastasis. The EMT and the reverse process, mesenchymal-epithelial transition (MET), also play important roles in stem cell differentiation and de-differentiation (or reprogramming). In this review, we will discuss current research progress of EMT in embryonic development, cellular differentiation and reprogramming, and cancer progression, all of which are representative models for researches of stem cell biology in normal and in diseases. Understanding of EMT and MET may help to identify specific markers to distinguish normal stem cells from cancer stem cells in future.

Keywords

EMT; MET; stem cells; reprogramming; cancer stem cells

1. Introduction

Pluripotent stem cells (PSCs) possess a pluripotent potential to give rise to cell types in three germ lines. PSC differentiation or reprogramming to generate induced pluripotent stem cells (iPSCs) is related to the changes of cellular morphology and surface markers, orchestrated by tremendous molecular regulating processes accompanying with the cellular mobility changes. Transition between epithelial features and mesenchymal counterparts has been documented existing in either pluripotent stem cells or differentiated cells. The epithelium is

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characterized by apical-basal polarity and tight cell-cell junctions between neighboring epithelial cells, which is associated with cell integrity and stability (Pasti and Labouesse, 2014). On the contrary, mesenchymal cells are identified as fibroblast-like, elongated, and spindle-shaped in appearance by loose cell-cell interaction without tight intracellular adhesion, resulting in more mobility to increase cell migration and anchorage distantly (Pittenger and Martin, 2004). By morphological transition, cells obtain motility to migrate into their organogenetic destinations while de-mobility enables the cells to colonize in site, which is a common mission in stem cell fate (Kavanagh et al., 2014).

The phenomenon that epithelial cells acquire mesenchymal traits, termed as epithelial-mesenchymal transition (EMT), has been observed in physiological and pathological processes, including embryogenesis (Gros and Tabin, 2014; Yoshino et al., 2014), inflammation (Correa-Costa et al., 2014), fibrosis (Grande et al., 2015; Lovisa et al., 2015; Schneider et al., 2012), wound healing (Banerjee et al., 2015), and cancer progression (Ruscetti et al., 2015; Wei et al., 2015). This morphology-related plasticity is also a common theme in carcinogenesis where cancer-initiating cells generate and invade. There are three types of EMT process, named as EMT-Type 1, 2, 3, being proposed to relate to embryo formation, tissue regeneration and cancer progression (Kalluri and Weinberg, 2009), whereas the EMT standing at the stage of embryonic development is sequentially divided as primary, secondary and tertiary segments, representing early gastrulation, mesodermal development, and endocardium morphogenesis, respectively (Thiery et al., 2009).

Loss of E-cadherin expression is considered a key event in an EMT where the cell-cell contacting modulators, cell polarity-related cytoskeleton and extracellular matrix are involved (Puisieux et al., 2014). Since first described in 1982 (Greenburg and Hay, 1982), the EMT has been documented to be regulated by various transcription factors (TFs) (Galvan et al., 2015; Wei et al., 2015; Yu et al., 2015), small non-coding RNA (Dang et al., 2015; Zhou et al., 2015), epigenetic modulators (Choi et al., 2015a; Cicchini et al., 2015), and exogenous inducers (An et al., 2015; Buczek et al., 2015; Choi et al., 2015b; Hwang-Verslues et al., 2013; Park et al., 2015; Zavadil et al., 2001). Families of the zinc-finger proteins Snails (Snai1, Snai2/Slug, Snai3/Smuc), the E-box-binding proteins Zebs (Zeb1/Tcf8, Zeb2/Sip1), the basic helix-loop-helix protein Twists (Twist1, Twist2), and the forkhead box proteins FOXCs (FOXC1, FOXC2) are major TFs that repress the expression of E-cadherin directly (Puisieux et al., 2014). These molecular regulators form an intricate network to mediate the cellular conversion in processes of development and pathologies, among which stem cells lead an important role (Taube et al., 2010).

However, in the studies of relationship between epithelial/mesenchymal lineage and stem cells, whether the EMT or its reversal process, mesenchymal-epithelial transition (MET), enables cells to obtain the pluripotency still remains controversial. Clarification of the relationship between cellular transformation and pluripotent characters will shed light on harnessing cell fate and provide a clue to determine disease status. This review mostly addresses the events in embryo development that is involve in epithelial and mesenchymal lineage transition, and discuss its relevance in stem cell differentiation and reprogramming in normal and diseases.

2. The EMT in embryo development

Fertilized oocytes are a type of homozygous stem cells that have potential to generate all tissue types in development (Lin et al., 2003). Accompanied with its implantation into uterus and differentiation process, the monoplast generates triploblastic body, which is composed of differentiated cell clusters and experiences cellular changes to satisfy the environment stress and the request for survival. Mapping the journey of morphological variety from totipotency to lineage specification will help to explore the relevance in cellular homostasis and tissue morphogenesis.

2.1. The mesenchymal transition in embryo implantation and pre-implantation

Primary EMT takes place in the cells that never undergo the cellular aspect changes, and is confined in the early embryo developmental segments, including embryo implantation and early gastrulation (Figure 1) (Acloque et al., 2009; Thiery et al., 2009). The mammalian embryogenesis is a self-organizing process that is determined not only by maternal distributed factors but also by the cellular interaction within the embryo. A zygote undergoes cellular cleavage to establish the apolar inner cell mass (ICM) and the polar trophectoderm (TE), the first two lineages that appears in the blastocyst stage localizing at inside and outside of the embryo, respectively. Differentiation of the ICM and the TE is initiated from late 8-cell stage when the cells are compacted to assemble conjunctional complex in a distinguishable apical-basal polarized layer. The ICM will give rise to the embryo and extraembryo tissue, while TE contributes to the placenta to provide the interacting signaling between fetus and maternal body (Saiz and Plusa, 2013).

The embryo implantation process involves blastocyst migration, apposition, attachment, adhesion, and invasion into the epithelial lining of the endometrium, where it is classified as an immediate reception in rodents/primates and a pre-receptive phase in other domestic animals (Bowen and Burghardt, 2000). Differentiation of TE lineage is symbolized by Cdx2 expression in outer cells, a specific gene for trophectoderm formation, which co-expresses with a pluripotent POU-family transcription factor Oct3/4 in a reciprocal repressive model (Toyooka et al., 2016). Upregulation of Cdx2 requiring to switch off Oct3/4 indicates that establishment of TE is the first differentiation event in mammalian embryogenesis (Niwa et al., 2005; Strumpf et al., 2005). The sequential superficial/central implantation in ruminant species having a prolonged pre-attachment period provides a window to look into the molecular and cellular changes during peri-attachment periods. The TE in pre-implanted bovine conceptuses was found to express epithelial cytokeratin as well as mesenchymal vimentin and N-cadherin. The EMT-related transcripts, SNALs, ZEBs and TWISTs, were upregulated in pre-implanted conceptuses of day 22, compared to those in day 17 and day 20 conceptuses (Yamakoshi et al., 2012). Loss of E-cadherin, an epithelial adhesion molecule, is associated with invasive phenotype of extravillous trophoblasts, while a reduction in N-cadherin, the mesenchymal adhesion molecule, decreased the invasive capacity of human trophoblast cells (Duzyj et al., 2015; Ng et al., 2012). Interestingly, SNAL1 and SNAL2 are expressed not in inner cells but in outer cells at 2-cell to 8-cell of blastocyst, indicating that the implantation process for noninvasive early-stage trophoblasts requires asymmetrically partial EMT to have special extracellular matrix expression as well (Bell and Watson, 2009).

The significance of the epiblast as epithelial integrity is associated with the selective counteracting mechanical stress and is unique to the early development of amniotes (Sheng, 2015). The polarity-dependent and position-dependent models are both associated with the cell fate segregation in mammal embryos (Saiz and Plusa, 2013; Sasaki, 2010). Cellular localization in murine embryos are related to the expression of transcription factors that are critical for cell differentiation (Toyooka et al., 2016). E-cadherin was showed to be important to ICM compaction and inner-outer lineage segregation. Lacking E-cadherin in embryo resulted in impaired cell adhesion, delayed compaction and disorganized cell allocation, indicating that it is rather epithelial cell-cell interaction than mesenchymal phenotype acting to anchor intracellular signaling in the embryo preimplantation stage (Bessonnard et al., 2015). Even though the development prior to the appearance of pre-gastrulation epiblast is variable in different species, a fully-epithelialized, unilaminar epiblast is a conserved model of start point in embryogenesis of all amniotes.

2.2. Primary EMT in early gastrulation

Gastrulation is a process of epithelial rearrangement resulted from cell division-mediated intercalations, which is necessary for the cellular spatial-patterning movements (Firmino et al., 2016). It is a morphogenetic process to form a three-layer organism consisting of the endoderm layer inside, the ectoderm outside, and the mesoderm in the middle, represented by internalization of the mesendoderm, convergence to the midline, and extension along the anteroposterior axis, all of which is conserved throughout evolution in various species (Thiery et al., 2009). These dramatic shape-changes require locally produced and anisotropically applied forces. Depletion of myosin regulatory light chain in the embryo was able to block force generation at gastrulation by destabilizing the myosin II (MII) hexameric complex and inhibiting MII contractility (Pfister et al., 2016). Interestingly, most subapical clusters in early mesoderm move apically and enhance in density and intensity. This phenomenon depended on MII and was correlated with the pulse actomyosin accumulation before the cells gained morphology change, indicating that contractile myosin-driven cell movement is prior to transcript-driven EMT during early gastrulation (Weng and Wieschaus, 2016).

The establishment of the embryonic apical-basal polarity is contributed to well-defined intercellular adhesive structures. This complex process is coordinated by disruption of epithelial cell-cell junction, breakdown of cell-basement membrane interaction, and changes in cytoskeletal architecture. Decomposition of basement membrane is the first recognized step in EMT process during gastrulation, which is mediated by certain molecular families. Inhibition of Rho pathway caused disruption of cell-basement interaction and microtubule instability (Nakaya et al., 2008; Stankova et al., 2015). Epithelialization and differentiation of the apical membrane during blastoderm stages is regulated by some transmembrane signals, one of which is Crumbs homolog 2 (Crb2). Crb2-mutant murine embryo showed a disturbed epiblast polarity and impaired EMT process at the primitive streak, resulting in impaired blastoderm formation and embryonic lethality (Xiao et al., 2011).

Gastrulation lasts a short duration and thus requires rapid changes in gene expression and function. Downregulation of E-cadherin, for example, is controlled both at the

transcriptional level by Snai1, Zeb, Twist, and by P38 interacting protein (IP)-p38-MAP kinase complex (Lamouille et al., 2014). Reciprocal transcriptional repression of Snail2 and Sox3 factors mediated the mesendoderm internalization in chick embryos. Snail-expressing cells ingressed into the primitive streak, whereas Sox3-positive cells formed ectodermal derivatives outside, indicating that the relationship between Snail and Sox determines the embryonic cellular localization(Acloque et al., 2011). In addition, FGFR was known to be required for cell migration during the gastrulation by regulating several pathways, including RhoA, the non-canonical Wnt pathway, PDGF signaling, and the cell adhesion protein N-cadherin. In FGFR1-knockout mice, epiblast cells in the primitive streak were less mobile on account of sustained E-cadherin expression (Hardy et al., 2011). O-fucosyltransferase-2 (POFUT2) specifically adds O-fucose to Thrombospondin type 1 repeat (TSR) superfamily regulating diverse biological activities ranging from cell motility to inhibition of angiogenesis. Pofut2-mutant murine embryos underwent extensive mesoderm differentiation when the embryo established anterior/posterior polarity, demonstrating that O-fucosylation of TSRs is essential for restricting pattern of EMT during gastrulation (Du et al., 2010).

2.3. EMT from primary to tertiary in embryo development

During gastrulation, the majority cells undergoing EMT contribute to ingressive mesoderm which is crucial to be separated from adjacent non-ingressing neuroectoderm cells to generate anterior/posterior regionalization. During cellular transition between epithelial and mesenchymal phenotype, a hybrid epithelial-mesenchymal state might exist to enable the cells to move collectively, to identify an external signaling, and to acquire maximum cellular transition (Jolly et al., 2016). A fully-transformed mesenchymal fate is more associated with mesoderm development, while in diploblastic and some triploblastic animals the EMT process may be only partially involved in endoderm formation (Nakaya and Sheng, 2008). The neural crest delamination in ectoderm is also a consequence of EMT which is coordinated by Snails, FoxD3 and SoxE factors expression, even though the precise combination varies temporally and spatially (Theveneau and Mayor, 2012).

Snail expression in presomitic mesoderm is asymmetric at left-right pattern and induces desynchronic somitogenesis. The endogenous retinoic acid-induced asymmetrically regression of Snail prevents the cellular invasiveness and ensures bilateral synchronic mesoderm segmentation (Morales et al., 2007). The migratory cells during early-gastrulation thus acquire a condense population mediated by a transient mesenchymal-epithelial transition (MET) process to obtain an epithelial homogeneity, which is initiated and stabilized by integrating signals to control the reorganization of the ECM, cytoskeleton, and adhesion junctions (Rowton et al., 2013). These secondary epithelia undergo a secondary EMT with more restricted differentiated potential to generate different types of cells, including the notochord, somites, heart, gut, kidney, body wall and lining of the coelom (Ohta et al., 2010; Thiery et al., 2009).

The somite epithelialization shares a common regulation way with intersomitic border formation which progresses in a rostral to caudal direction along the axis. The stimulators initiating secondary EMT remain largely unknown, one of which is postulated to be the signals emanated from the neural tube and notochord. Another proposed mechanism is that

in a passive way, ventral somitic EMT is merely due to lack of epithelial-maintaining signals when the somatic cells escape from the pro-epithelializing activity (Kalcheim, 2015). Interestingly, caudal FGF signaling was demonstrated to control the trunk neural crest cell specification and emigration, whereas rostrally generated retinoic acid (RA) activity gradient is only required to control early neural crest cell specification. This opposing gradient of FGF and RA pathway orchestrates progressive neural and limb development (Martinez-Morales et al., 2011). BMP and Wnt signaling pathways were reported to be required for proper pattern of the neural tube and somites, providing evidence that both genes are crucial for regulating mammalian anterior-posterior (A–P) axial elongation during the EMT process (Andre et al., 2015; Martinez-Morales et al., 2011). The Wnt-regulated basic helix-loop-helix (bHLH) transcription factor mesogenin 1 (Msgn1) is a major regulator controlling paraxial pre-somitic mesoderm (PSM) differentiation, by directly activating the transcriptional programs that define PSM identity, the EMT, cellular motility, and segmentation (Chalamalasetty et al., 2014). BMP family was also reported to be close related to Snails expression in the primitive streak along its A–P axis. SMADs, the downstream factors of BMP signaling pathway, also expresses on the pre-migratory cells adjacent to the primitive streak and lateral neural crest, demonstrating that BMP-SMAD signaling induces EMT via MMP-mediated basal membrane disruption in early tail bud stage, whereas Noggin inhibits BMP signaling and results in the suppression of EMT and basement membrane formation in late gastrulation (Ohta et al., 2010).

Cellular migration and differentiation of epicardium-derived progenitor cells (EPDC) is one of the major cardio-vasculogenesis events. Epicardium is a single cell layer of mesothelium lining the outer heart. In tamoxifen-induced Wt1-knockout immortalized epicardial cells (Cre+ CoMEECs), loss of Wt1 led to a robust increase in E-cadherin expression in a dose-dependent manner, indicating that Wt1 is important in regulating epicardial EMT-related development. Treatment of the Cre+ CoMEECs with tamoxifen led not only to the changes in the EMT-expressing pattern, but also to reduced cell migration. The mesodermal lineage deficiency was rescued by increased expression of Snai1, underscoring the importance of the EMT in generating differentiated cells (Martinez-Estrada et al., 2010). The key EMT-stimulator transforming growth factor (TGF)-β was reported to lead nuclear accumulation of myocardin-related transcription factors (MRTFs) in the epicardium. Mice lacking MRTFs showed a series of cardio-vasculogenic disorganization, including coronary plexus malformation, endothelial cell dysfunction and sub-epicardial hemorrhage (Trembley et al., 2015).

The EMT process was also investigated during other embryonic mesodermal lineage development. Stroma components have been demonstrated to provide important inter-epithelial signals for the formation of overlying coelomic epithelial cell sheet during nephric duct development. The mesonephros is a transient structure in which a single epithelial cell layer nephric coelomic epithelium (Neph-CE) covers multiple types of cells. Ablation of inter-epithelial crosstalk caused increased sensitivity to the EMT-induced external stress (Yoshino et al., 2014). Moreover, Wnt signaling is also related to the EMT and is critical for proper formation of the female reproductive tract. Less cellular proliferation and delayed uterine gland formation were seen in embryos with stabilization of β-catenin, accompanied

with the induction of an EMT event from pre-birth to 5 days of post-birth (Stewart et al., 2013).

An *in vitro* analysis showed that embryonic hematopoiesis regionally distributed at different embryo stages. The hematopoietic bud was dispersive in the entire epiblast during early- and mid-gastrulation but was restricted in the posterior at the headfold stage, whereas the EMT-inducers Activin A and BMP-4 enhanced hematogenic potency of anterior part (Kanatsu and Nishikawa, 1996). One of the EMT transcripts Zeb2/Sip1 was demonstrated to be essential for murine embryonic hematopoietic stem and progenitor cell differentiation and mobilization (Goossens et al., 2011). The population fitting the description of cells in the EMT process was observed in the early embryonic hematopoietic liver microenvironment but not in nonhematopoietic liver at the late gestation and the post-natal stage. Furthermore, the hematopoietic supportive capacity of the EMT cells disappeared after hepatocytic maturation, indicating that the EMT process does contribute to hematopoiesis in embryonic stage (Chagraoui et al., 2003).

3. Epithelial or mesenchymal specificity of pluripotent stem cells

Stem cells are a class of undifferentiated or poorly differentiated cells, which are able to self-renew and to produce well-differentiated daughter cells. A variety of markers, such as Oct4, Nanog, alkaline phosphatase (ALP), stage specific embryonic antigen (SSEA), CD133, and aldehyde dehydrogenase (ALDH), have been indicated to relate to cellular pluripotency (Hess et al., 2006; Li et al., 2001; Thomson et al., 1998; Tondreau et al., 2005). Stem cells can be further classified as totipotent, pluripotent, and multi/uni-potent by their differentiation potential. Among them, embryonic stem cells (ESCs), which are generated from ICM in embryo blastocyst, are a totipotent *in vitro* model able to mimic embryo development (Henderson et al., 2002; Thomson et al., 1998). Reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) provides an unprecedented opportunity to generate a new patient-specific cell source (Guha et al., 2013; Inoue et al., 2014; Maekawa et al., 2011; Takahashi et al., 2007; Takahashi and Yamanaka, 2006). ESCs and iPSCs, joint named pluripotent stem cells (PSCs), share similar gene expression profiles and development potential and thus provide a novel cellular tool for regenerative medicine, disease modeling, and preclinical drug screening (Halevy and Urbach, 2014; Liang and Zhang, 2013). As for the widely recognized differentiating potential of stem cells, determination of the epithelial and mesenchymal specificity during stem cell differentiation and reprogramming will provide more evidences to the relationship between E-M characteristics and pluripotency.

3.1. EMT and PSC differentiation

ESCs are derived from inner cell mass (ICM) in the blastocyst stage, providing the possibility that undifferentiated ESC cells dictate the feature of ICMs to form an accessible and tractable differentiating model to investigate the lineage selection at gastrulation (Slawny and O'Shea, 2013). An *in vitro* EMT process has been investigated during ESCs and iPSCs differentiation into three germ layers, which are regulated by special transcripts and modulators.

In murine ESCs, E-cadherin positively express on the intercellular membrane accompanied by pluripotent markers (Nanog, Oct4, Sox2 and alkaline phosphatase). Depletion of Snail did not impact the expression of pluripotent markers, Phospho-histone H3 and TUNEL experiment, indicating that Snail is dispensable for pluripotency maintenance, cell cycle and cell survival regulation. Undifferentiated ESCs retained the epithelial phenotype while the differentiation procedure was showed as an endogenous burst of Wnt signaling-mediated Snail expression, which regulates neuroectodermal and the consequent lineage fate defining mesoderm commitment (Lin et al., 2014). Another EMT-transcript, Zeb2, was indicated to modulate the cell-fate decision between neuroectoderm and mesoderm in the duration from undifferentiated ESCs to primary germ layer differentiation (Chng et al., 2010). Moreover, miR-200a was demonstrated to exert an opposite function to Snail to stall ESC differentiation. In consideration of repressive actions of miR-200 family on the EMT by targeting the transcription factors Zeb1 and Zeb2, this sequential orchestration of EMT-related transcripts and miRNAs plays an important role in regulating ESC-derived early lineage selection (Gill et al., 2011).

3.2. MET in somatic reprogramming

Reprogramming refers to re-establishment of epigenetic marks and induction of pluripotent stem cells by transfer of nuclear contents into oocytes (Noggle et al., 2011; Yamada et al., 2014), by fusion with ES cells (Ambrosi et al., 2007; Lluis et al., 2008), and by transfection of defined factors into adult mature cells (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). Among them, somatic reprogrammed iPSCs, which share similar plasticity to ESCs (Bilic and Izpisua Belmonte, 2012) and thus undergo the same trend of EMT during differentiation (Li and Niyibizi, 2012), have captured more and more attention because of their potential to differentiate into patient-specific cells. Whether somatic reprogramming fits the opposite process of EMT is an attractive question, and the clarification of this question will shed light on controlling the efficiency of iPSC reprogramming.

The reprogramming of somatic cells into iPSCs requires defined factors, such as Sox2, Oct4, c-myc and Klf4 (called as Yamanaka factors). It was demonstrated that Sox2, Oct4, and c-myc suppress TGF-β signaling while Klf4 activates multiple epithelial genes. During reprogramming, the cells aggregated into a distinguished epithelial-like colony with well-defined intercellular junctions. A pan-cytokeratin containing E-cadherin was upregulated after introducing Yamanaka factors. Expression of mesenchymal marker Vimentin was repressed concomitantly to the emergence of iPSC colony (Hoffding and Hyttel, 2015). Meanwhile, a reduction of the mesenchymal-like marker fibronectin (Fn) and Snail was observed, demonstrating that reprograming to iPSCs experiences the reverse process of EMT, the MET (Li et al., 2010). The morphology change of fibroblasts to iPSCs resembled an artificial rewinding of mesenchyme to epithelial-like epiblast. A more detailed lineage dissection found that a short period of TGF-β treatment enhanced reprogramming process, whereas a 12-day of full-term TGF-β treatment blocked reprogramming, indicating that optimal de-differentiation requires a time-sensitive trend that the efficiency of reprogramming is increased by a sequential early-EMT and delayed-MET in order (Liu et al., 2013).

4. Distinct story of relationship between EMT/MET and pluripotency in cancers

Compared to PSCs, adult stem cells exist in post-natal tissues or organs that have restricted differentiation potential. These adult stem cells are capable of self-renewal and producing more mature functional offspring of limited number of cell types (Visvader and Clevers, 2016; Wang et al., 2016). The cells residing in tumors having limited potential of differentiation and self-renewal are termed as tumor-initiating cells (TICs) or cancer stem cells (CSCs) (Ponti et al., 2005; Singh et al., 2004; Wang et al., 2006). This small population is documented to be selected by cellular surface antigens (CD133, CD34, CD44, ABCB5) (Jin et al., 2006; Ricci-Vitiani et al., 2007; Schatton et al., 2008; Trempus et al., 2007), pluripotent markers (SSEA-1, Oct-4) (Levings et al., 2009; Son et al., 2009), Hoechst 33342 dye-efflux capability (Harris et al., 2008; Ho et al., 2007) and Aldehyde Dehydrogenase (ALDH) (Carpentino et al., 2009; Visus et al., 2011), and was indicated to be responsible for tumorigenesis (O'Brien et al., 2007). The transition between epithelial and mesenchymal counterparts exists in various tumors and contributes to tumor metastasis and chemoresistance (Fischer et al., 2015; Zheng et al., 2015). However, whether the direction of EMT or MET relates to the emergence of CSCs remains controversial in different CSC-purifying methods and in different tissues (Brabletz, 2012).

4.1. The EMT is related to mesenchymal-like CSC initiation

In 2008, Mani et al. reported the association between the EMT and the gain of epithelial stem cell property (Mani et al., 2008). In their study, the neoplastic mammary stem cells were determined by $CD44^{high}/CD24^{low}$ phenotype and the capability to form a mammosphere. Ectopic expression of Snail or Twist in human mammary epithelial cells (HMLEs) or exposing HMLEs to TGF-β stimulation resulted in the acquisition of $CD44^{high}/CD24^{low}$ population in the appearance of mesenchymal growth pattern, with the ability to form mammosphere and to generate the $CD44^{low}/CD24^{high}$ non-mammosphere-forming cells *in vitro*, indicating that the cells that undergo an EMT are associated with CSC acquisition (Mani et al., 2008). A distinct localization of normal gland-reconstituting mammary stem cells and breast CSCs were investigated by the same group afterwards, and found that the former is in basal layer and the latter is originated in the luminal layer, where Snail, but not Slug serves as the key regulator in CSC-initiation (Ye et al., 2015). The tumorigenicity can be also determined by an *in vitro* sphere-forming assay. The feedback loop of TF-Zeb with suppression of stemness-inhibitor miRNAs was investigated to be linked with increased sphere-forming capability in pancreatic CD44+/CD24+ cancer stem cells (Wellner et al., 2009). The downstream effector of tumor suppressor and the cyclin-dependent kinase inhibitor $p21^{CIP1/WAF1}$ was showed to be associated with the mammary neoplastic EMT. Expression of Sox2, Klf4, and Oct-4 was increased in the Ras-$p21^{CIP1-/-}$ and c-Myc-$p21^{CIP1-/-}$ tumors, elucidating that loss of $p21^{CIP1/WAF1}$ can induce a Ha-Ras- and c-Myc-dependent EMT and an enrichment of $CD44^{high}/CD24^{low}$ phenotype with an embryonic stem-like gene expression pattern in immortal human mammary MCF10A-c-Myc cells (Liu et al., 2009).

The CSC markers are sub-assorted to the cellular function (Hoechst 33342 dye-effluxing assay), nuclear transcription factor (Oct-4), and cell surface antigens (CD44, CD133, CD34, SSEA, ABCB5, ALDH). Since there is no unified CSC-specific marker in tissues and organs, the CSCs purified by different methods might exhibit distinct characters. Among them, CD44 is also used as a mesenchymal marker to specify mesenchymal stroma cells. The cells derived from the EMT process behave similarly to mesenchymal stem cells (MSCs) with the potential to differentiate into multiple lineages, share many properties that are typical for MSCs, including CD44(+), CD24(−), CD45(−), EMT-TFs, mesenchyme forkhead 1 (FOXC2), and CD140b (Battula et al., 2010). This CD44+/CD24− mesenchymal phenotype with more adherent protein expression and more mobile ability may enhance the cells to form aggregates *in vitro*, one of which meets the requirement of CSC's features in culture, and enable the cells to exhibit more metastatic capability of migration, invasion and chemoresistance (Kawamura et al., 1991). Knockdown of CD44 induced MET and subsequently resulted in impaired migration and invasion of cancer cells both *in vitro* and *in vivo* (Gao et al., 2015).

4.2. The EMT is related to epithelial CSC differentiation

Other studies of the relationship between the lineage transition and the tumor initiating capability indicated that induction of EMT in breast cancer cells is not associated with enhancing tumor-initiating capacity, but instead, conferred a CD44+/CD24− phenotype and the malignant properties, including cell proliferation, migration, and resistance to chemotherapy and radiation. On the other hand, MET does not lead to inhibition or loss of the tumor-initiating capacity, but markedly attenuated other malignant properties (Xie et al., 2014). Repression of the EMT inducer Prrx1 reverted the cells to the epithelial phenotype concomitant with the acquisition of the stem cell properties (Ocana et al., 2012).

Hoechst 33342 is a vital dye used the first time in 1996 to stain a small population in bone marrow which expresses multipotential hematopoietic stem cells and ATP-binding cassette transporter G (ABCG) family able to efflux Hoechst 33342 fluorescence dye (Goodell et al., 1996). This population was named as side population (SP) and had been applied as one of the representative methods to purify various tissue pluripotent cells and in cancers (Boesch et al., 2014; Goodell et al., 1997; Hirschmann-Jax et al., 2004; Jackson et al., 1999; Jonker et al., 2005). Unlike mesenchymal specificity of CD44, Hoechst 33342-effluxing SP cells may exhibit the characters associating with their lineage origins. We found that SP cells in ovarian cancers exhibited an epithelial phenotype and showed a distinct pluripotent gene profile with reduced expression of cell adhesion molecules. Cultivation of SP cells into non-SP cells resulted in decreased expression of Oct-4 and Nanog with more mesenchymal phenotype and increased invasive potential (Jiang et al., 2012). SP cells were able to be depleted by treatment of TGF-β-directed EMT which was associated with down-regulation of ABCG2 expression, while removal of TGF-β restored SP abundance with an epithelial phenotype (Yin et al., 2008). However, heterogeneity still exists in side population, and their relevance to EMT need to be further investigated. A stem-purifying method that is not associated with epithelial or mesenchymal in nature may facilitate to distinguish this difference.

5. Summary

The transition between epithelial and mesenchymal lineages is a common process in embryonic development and cancer progression, both of which encounter the developmental processes including cellular invasion into the basal membrane, migration to the distant site, seeding in the proper circumstance and growing to form aggregates, tissues, organs and metastatic sites.

Embryogenesis is a complicated process whose details are far from clarification. The embryogenic EMT enables cells to move to the accurately positioned environment for further development. The sequential transformations involved in embryogenesis are served as development-oriented events which are able to generate further differentiated cells. The *ex vivo* cellular model which mimics pluripotent embryo in nature, ESCs or iPSCs, are demonstrated to mainly experience EMT in their differentiation and MET in their de-differentiation. Even though a short period of EMT was seen in early reprogramming of iPSCs, it is much like the short period of MET in embryogenesis to enable the cells to acquire a unified status for next step differentiation, to generate tight junction between the reprogramming cells, and surrounding feeders to facilitate the de-differentiation (Figure 1).

The mature body is grown from a fertilized egg, the primary homozygous stem cell, in a vital life, while the cancers are recently documented to be initiated from a small population of cells having stem cell-like properties. The normal stem cells and CSCs share some properties but, in some ways, behave differently. The accurate description of EMT and MET in CSCs is remained controversial, and much is caused by the heterogeneity in cancers and the inconsistency of CSC feature in different tissues (Table 1). The CSCs isolated by epithelial or mesenchymal markers will be bound to show their corresponding lineage characteristics, resulting in the uncertainty in the studies of relationship between EMT/MET and differentiation/de-differentiation. Finding a unified CSC-isolating method independent of lineage markers will help to accurately clarify this question in the future.

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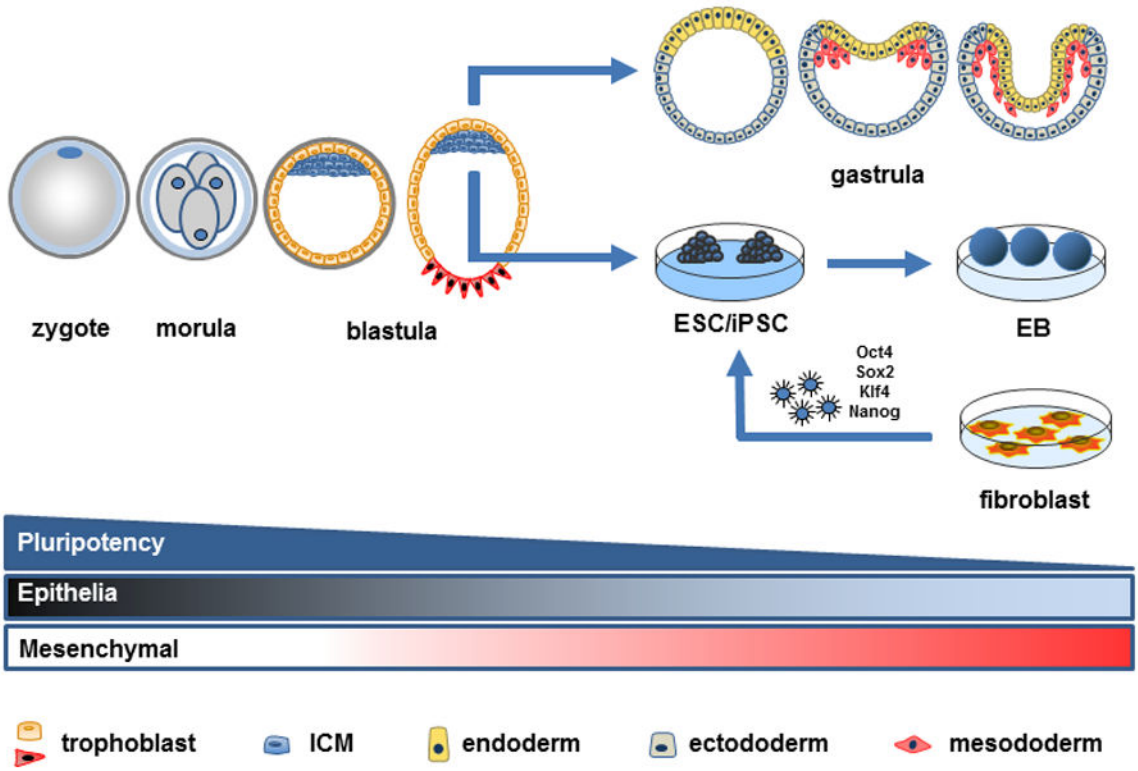

Figure 1. Schematic relationship between embryonic differentiation and E-M transition

Embryonic differentiation is triggered after the oocyte is fertilized. Primary epithelial (blue)-mesenchymal (red) transition takes place in the cells that never undergo the cellular aspect changes, which is confined in the early embryo developmental segments, including embryo implantation and early gastrulation. During gastrulation, the cells undergoing EMT contribute to ingressive mesoderm formation. Differentiation of ESC/iPSCs, an *ex vivo* embryonic cellular model, generates mesodermal characterized cells inside of embryonic body (EBs), whereas reprogramming of fibroblast to iPSCs undergoes the counterpart of EMT, the MET. The pluripotency and cellular epithelial/mesenchymal characterization is showed along with *in vivo* and *ex vivo* embryonic differentiation flow.

Table 1

Distinct relationship between E/M lineage and CSC features

Lineage relationship	Origin	Models	Inducer	Stem cell features		Biological function of E-M transition	Reference
				CSC marker	Other feature		
Mesenchymal lineage relatedCSC initiation	Breast	HMLEs	TGF-β stimulationEctopic expression of Snail or Twist	CD44+/CD24−	Mammosphere-forming cells	CSC initiation	(Mani et al., 2008)
	Pancreas	pancreatic cancer cells	Zeb1-Mir200 loop	CD44+/CD24+	Sphere-forming capacity	Self-renewal, drug resistance	(Wellner et al., 2009)
	Breast	MCF10A	loss of $p21^{CIP1/WAF1}$	CD44+/CD24−	Expression of Sox2, Klf4 and Oct-4	Enrichment of CD44+/CD24− phenotype	(Liu et al., 2009)
	Breast	HMECs	Ectopic expressing Twist, Snail, or TGF-β1 stimulation	CD44+/CD24−	CD45(−), EMT-TFs, FOXC2(+), and CD140b(+)	Derive cells similar to MSCs	(Battula et al., 2010)
	Breast	Slug+ or Snail+ knock-in mice	Slug+ or Snail+ knock-in	CD44+/CD24−	Epcam(−)	A distinct localization of normal mammary stem cells and breast CSCs	(Ye et al., 2015)
Epithelial lineage related CSC initiation	Breast and other	Chicken embryo, zebrafish, human cancer cells, MDCK cells	Prrx1 knock-in or knock out	CD44+/CD24−		MET concomitant with the acquisition of the stem cell properties	(Ocana et al., 2012)
	Ovarian	Ovarian cancer cells	TGF-β stimulation	SP cells	Expression of Oct-4, Nanog, sphere-forming capacity	Enhanced differentiation of SP cells into non-SP cells	(Jiang et al., 2012)
	Breast	MCF7 cells	TGF-β stimulation	SP cells	In vivo tumorigenicity	Depletion of SP cells by transcriptional repression of the ABCG2 gene	(Yin et al., 2008)

CSCs: cancer stem cells, HMLEs or HMECs: human mammary epithelial cells, SP: side population, MSCs: mesenchymal stem cells, MDCK cells: Madin-Darby Canine Kidney