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Tumor Inflammatory Microenvironment in EMT and Metastasis

Tingting Yuan¹,³, Yadi Wu²,³ and Binhua P. Zhou¹,³

¹Departments of Molecular and Cellular Biochemistry, ²Molecular and Biomedical Pharmacology, ³Markey Cancer Center, University of Kentucky School of Medicine, Lexington, KY, USA

1. Introduction

Approximately 90% of cancer-related death is caused by metastasis. The increased motility and invasiveness of metastatic tumor cells is reminiscent of events that occur at the epithelial-mesenchymal transition (EMT), which is a characteristic that occurs during embryonic development, tissue remodeling, wound healing and metastasis. Interestingly, EMT is a dynamic process and mainly occurs at the edges of wounds during healing and at the invasive fronts of metastatic tumors, which suggest that EMT is influenced by stimuli that emanate from the inflammatory microenvironment. The tumor microenvironment consists of many kinds of cells including infiltrated inflammatory cells, such as neutrophils, lymphocytes, macrophages and myeloid derived suppressor cells (MDSC). These infiltrated immune cells secrete cytokines, chemokines and growth factors, such as TNF-α, TGF-β, IL-6, fibroblast growth factor (FGF) and epidermal growth factor (EGF). These growth factors contribute significantly to the invasive and metastatic traits of cancer cells by inducing EMT. Here, we discuss new insights into the molecular pathways and key regulators that link inflammatory tumor microenvironment to EMT and metastasis.

2. Cancer and immunity: Immunity’s roles in tumor suppression and promotion

One of the most challenging questions in immunology is to understand how the immune system affects cancer development and progression. In recent years, after a long eclipse, different lines of work have lead to a renaissance of the inflammation-cancer connection (Balkwill and Mantovani 2001; Coussens and Werb 2002; Mantovani, Allavena et al. 2008). It is now believed that the immune system plays a dual role in cancer: on one hand, it can function as an extrinsic tumor suppressor (Dighe, Richards et al. 1994; Kaplan, Shankaran et al. 1998; Smyth, Thia et al. 2000; Girardi, Oppenheim et al. 2001; Shankaran, Ikeda et al. 2001; Street, Trapani et al. 2002) by destroying cancer cells or inhibiting their outgrowth; on the other hand, the immune system can also promote tumor progression by establishing conditions within the tumor microenvironment that facilitate tumor outgrowth (Schreiber, Old et al.). Inflammatory responses play decisive roles at different stages of tumor
development, including initiation, promotion, malignant conversion, invasion, and metastasis (de Visser, Eichten et al. 2006; Grivennikov, Greten et al. 2010).

3. EMT and metastasis

Epithelial-mesenchymal transition (EMT) is a phenotypic conversion during embryonic development when tissue remodeling and cell migration shape the future organism, such as in embryonic development and wound healing. During EMT, epithelial cells lose the adherent junctions that keep them in contact with their neighbors. They gain a mesenchymal cell phenotype that enables them to break through the basal membrane and migrate over a long distance, a result of profound changes in their cytoskeleton architecture and gene expression profile (Kalluri and Neilson 2003). This concept was pioneered by the seminal study from Elizabeth Hay using chick primitive streak formation as a model in 1967 (Hay 1995). Hay realized that an epithelial phenotypic conversion was of crucial importance during gastrulation and cell migration in the early vertebrate embryo. She proposed that differentiated epithelial cells could undergo a dramatic "transformation" into mesenchymal cells (Greenburg and Hay 1988; Hay 1995). However, this "transformation" is reversible: mesenchymal cells can revert back to epithelial cells through a reverse process called mesenchymal-epithelial transition (MET). As a result, the term “transition” is now used.

EMT does not only occur during embryonic development or as a physiological response to injury. It is also an important element in cancer progression and other pathologies that involve organ degeneration, such as fibrosis. At the cellular level, pathological EMTs are very similar to physiological EMTs in that they are governed by similar signaling pathways, regulators, and effective molecules.

From a clinical perspective, metastasis is the most critical aspect of tumorigenesis: we have already addressed that more than 90% of cancer mortality is caused by metastasis. Aberrant control of epithelial proliferation and angiogenesis underlie the initiation and growth of primary carcinomas (Hanahan and Weinberg 2011). However, additional steps must be completed before a metastatic tumor is successfully established. The spread of malignant cells consists of a series of steps, all of which are thought to be important for metastatic outgrowth in different organs. Basically, these steps include local invasion toward and entry into blood vasculature (intravasation), survival within the circulation system, arrest in distant capillary beds or “homing” to distal organs, exit from blood vasculature (extravasation), and eventual outgrowth and re-establishment of malignant growths in secondary locations (Woodhouse, Chuaqui et al. 1997; Chambers, Naumov et al. 2001; Fidler 2003; Hanahan and Weinberg 2011).

3.1 Classification of EMT into three different subtypes

Based on recent intensive study in this field, EMT can be divided into three subtypes, which have different biological functional consequences (Kalluri 2009; Kalluri and Weinberg 2009; Zeisberg and Neilson 2009). Type 1 EMT occurs during implantation, embryo formation, gastrulation, and neural crest migration, which describes the transition of epithelial cells to generate diverse mesenchymal cell types. These primary mesenchymal cells can revert back to form secondary epithelia in mesodermal and endodermal organs through MET. Type 2 EMT occurs during wound healing, tissue regeneration and organ fibrosis, which is usually
associated with injury and chronic inflammation. Type 2 EMT ceases once inflammation is attenuated, but if inflammation persists, this type of EMT will eventually lead to tissue fibrosis and organ destruction. Unlike Type 1 EMT, these mesenchymal cells have no potential to undergo MET and turn back to epithelial cells. Type 3 EMT occurs during tumor progression, which describes how neoplastic cells at the invasive front of primary tumors undergo a transition to acquire increased motility and invasive ability, enabling them to invade and metastasize through the blood stream or lymph node system, eventually generating life-threatening metastatic lesions at distant organs.

Because studies of EMT often involve various model systems ranging from different epithelial cell types to assorted stimulations, it is important to use validated biomarkers to examine the phenotypic conversion during all three classes of EMT. Common biomarkers include cell-surface and extracellular molecules, cytoskeletal proteins and specific transcription factors. For example, down-regulation of E-cadherin is a hallmark of EMT, and loss of E-cadherin expression facilitates the induction of EMT (Huber, Kraut et al. 2005). E-cadherin is a cell-cell adhesion molecule that participates in homotypic, calcium-dependent interactions to form epithelial adherent junctions (Cowin, Rowlands et al. 2005; Junghans, Haas et al. 2005). In addition, E-cadherin repressors, such as Snail, Slug, Twist and ZEB1/2, are commonly used as EMT markers. Snail is the first described E-cadherin repressor and is also the common downstream target of various signaling pathways that lead to EMT. Vimentin, an intermediate filament mainly expressed in fibroblasts, endothelial cells and hematopoietic cells, is also commonly used as an indicator for Type 3 EMT, since expression of vimentin in tumor cells correlates with their invasiveness and metastatic potential. Furthermore, differential expression of integrin is also used as a biomarker of EMT, since integrins modulate the interaction of cells with extracellular matrix (ECM). For example, increased expression of α5 integrin is commonly found in Type 2 and Type 3 EMT (Qian, Zhang et al. 2005; Davidson, Marsden et al. 2006; White, Blanchette et al. 2007).

3.2 Type1 EMT in the formation of mesoderm and neural crest

EMT is crucially important to tissue morphogenetic events during embryonic development, such as the mesoderm formation, neural crest formation, heart valve development, and secondary palate formation. Without EMT, development cannot proceed through the blastula stage. Mesoderm formation and neural crest development represent the major EMT programs that occur during early embryonic development; the resulting mesenchymal and neural crest cells act as progenitors and further differentiate into various cell types via MET. For example, gastrulation EMT produces the mesoderm, giving rise to muscle, bone and connective tissues, whereas neural crest delamination EMT gives rise to glial and neuronal cells, adrenal glandular tissues, pigment-containing cells of the epidermis and skeletal and connective tissues. The heart valve development and secondary palate formation occur in relatively well-differentiated epithelial cells that are destined to become defined mesenchymal cells types.

The formation of mesoderm from the primitive ectoderm during gastrulation is the classic example of EMT. Gastrulation, observed in all metazoans, is accompanied by drastic morphogenetic changes from a single epithelial layer (the epiblast) into three embryonic germ layers, the ectoderm, mesoderm, and endoderm, to form a complex three-dimensional multilayered embryo (Shook and Keller 2003). In chicken and mouse embryo, Wnt and TGF-
β signaling provide the initial induction signals for EMT, while the FGF signal is necessary to maintain the EMT regulatory network during mesoderm formation. All these signaling events activate the expression of Snail, which represses the expression of E-cadherin and other tight junction components (such as claudins, occludins, and Crumbs) and promotes cell migration. In Snail knock-out mice the cells that form are unable to migrate, although mesoderm specification is not affected.

Neural crest formation is another example of Type 1 EMT in embryogenesis where premigratory neural crest cells form at the border of the neural plate and non-neural ectoderm as a result of signals emanating from these two tissues. Interestingly, similar signaling pathways operating during EMT at gastrulation are used in the neural crest formation. Indeed, a combination of Wnt, FGF, and TGF-β (mainly BMP) induce the expression of Snail, Sox and forkhead box D3 transcription factors (Villanueva, Glavic et al. 2002). In addition, experimental evidence shows that Notch signalling pathway plays an important role in neural crest formation through induction of Slug in frog and chick embryo (Nieto 2002). The combination of these transcription factors generates the full spectrum of phenotypic changes associated with EMT and primes the precursor cells to become migratory neural crest cells. These neural crest cells are equipped with the ability to migrate over extraordinarily long distances in the embryo, prior to their reaggregation via MET for further differentiation.

3.3 Type 2 EMT in tissue and organ fibrosis

3.3.1 Implications of EMT in fibrosis

Re-epithelization, tissue regeneration and organ fibrosis constitute Type 2 EMT. Organ fibrosis is mediated by inflammatory cells and fibroblasts, which deposit collagens, elastin, tenacin and other matrix molecules. Fibrosis-associated Type 2 EMT specifically occurs in kidney, liver, lung and intestine (Zeisberg, Tarnavski et al. 2007). A series of typical experiments has shown that EMT is an important process during tissue injury that leads to organ fibrosis. In terms of EMT proteomes, fibroblast-specific protein 1 (FSP1, also known S100A4 and MTS-1), α-SMA (smooth muscle actin) and collagen I are reliable markers to characterize the mesenchymal products generated by EMTs in the development of fibrosis in various organs. TGF-β1, as the major pro-fibrotic cytokine, induces many of the central processes involved in fibrosis, including differentiation of fibroblast to myofibroblast, ECM deposition and EMT. TGF-β not only contributes to pulmonary and hepatic fibrosis, but also plays a key role in cardiac fibrosis (Gressner, Weiskirchen et al. 2002; Willis and Borok 2007; Zeisberg, Tarnavski et al. 2007). TGF-β induces EMT via both a Smad2/3-dependent pathway and a MAPK-dependent pathway. The relevance of TGF-β-induced EMT for progression of organ fibrosis was recently further elucidated using BMP-7 as an intracellular competitor of TGF-β signaling in mouse models of kidney, liver, biliair tract, lung and intestinal fibrosis (Zeisberg, Bottiglio et al. 2003; Zeisberg, Hanai et al. 2003). The function of TGF-β in fibrosis is highlighted by the finding that Smad3-/- mice are resistant to the induction of several fibrotic diseases (Flanders 2004). TGF-β levels are also over-produced and are associated with functional impairment in patients with fibrotic pulmonary diseases such as idiopathic pulmonary fibrosis (Salez, Gosset et al. 1998). Clinical studies have also demonstrated the correlation between fibrosis and EMT (Rastaldi, Ferrario et al. 2002). Using immunohistostchemistry and in situ hybridization, an EMT was demonstrated with the
expression of several markers of tubular phenotype transition, such as cytokeratin, vimentin, α-SMA and zona occludens (ZO-1) in 133 kidney biopsies (Rastaldi, Ferrario et al. 2002). Similarly, an expression pattern of EMT was found in areas of fibrosis in the colon in patients with Crohn’s disease (Bataille, Rohrmeier et al. 2008).

3.3.2 Re-epithelialization of wounded skin

Re-epithelialization recapitulates several aspects of EMT. Re-epithelialization requires epithelial cells at the edge of wounded tissue to loosen their cell–cell and cell–ECM contacts and assume a migratory phenotype, reminiscent of EMT. Slug has a crucial role in wound-healing, which is expressed in keratinocytes at the boundary of wounds. Importantly, epithelial cell outgrowth from skin explants was markedly reduced in Slug knockout mice, whereas overexpression of Slug in cultured human keratinocytes result in increased cell spreading and desmosomal disruption (Savagner, Kusewitt et al. 2005). Arnoux et al further found that EGF can activate Erk5, which specifically enhances Slug promoter activity and controls wound healing in keratinocyte-derived HaCaT cells in vitro (Arnoux, Nassour et al. 2008). However, it should be noted that not all features of EMT are seen. First, the migrating keratinocytes remain part of a cohesive cell sheet since they retain some intercellular junction. Second, the epithelial cells do not actually become mesenchymal (i.e., interstitial) cells. They retain epithelial characteristics. Once wound closure is complete, the involved epithelial cells revert to their tissue-specific, differentiated state.

3.4 Type 3 EMT in cancer metastasis

Cancer metastasis is believed to consist of four distinct steps: invasion, intravasation, extravasation and metastatic colonization (Chambers, Groom et al. 2002; Pantel and Brakenhoff 2004). During invasion, tumor cells lose cell-cell adhesion, gain mobility and leave the site of the primary tumor to invade adjacent tissues. In intravasation, tumor cells penetrate through the endothelial barrier and enter systemic circulation through blood and lymphatic vessels. In extravasation, cells that survive anchorage-independent growth conditions in the bloodstream attach to vessels at distant sites and leave the bloodstream. Finally, in metastatic colonization, tumor cells form macrometastases in the new host environment (Chambers, Groom et al. 2002; Pantel and Brakenhoff 2004). All of these steps, from initial breakdown of tissue structure through increased invasiveness, and ultimately distribution and colonization throughout the body, are characteristics of the developmental process at EMT/MET. The similarity of genetic controls and biochemical mechanisms underlying the acquisition of the invasive phenotype, and the subsequent systemic spread of the cancer cells, highlights that tumor cells usurp this developmental pathway for their metastatic dissemination. We will further discuss this type of EMT, with more detail on how it is regulated by different signaling pathways and molecular in various tumor microenvironments.

3.5 Molecular regulation of EMT

The hallmark of EMT is the loss of E-cadherin expression, an important caretaker of the epithelial phenotype. Loss of E-cadherin expression is often correlated with the tumor grade and stage, because it results in disruption of the cell-cell adhesion and an increase in nuclear
β-catenin (Cowin, Rowlands et al. 2005; Junghans, Haas et al. 2005). Several transcription factors have been implicated in the regulation of EMT, including zinc finger proteins of the Snail/Slug family, the basic helix-loop-helix factor Twist, E12/E47, Goosecoid, ÐEF1/ZEB1 and SIP1 (Nieto 2002; Yang, Mani et al. 2004; Hartwell, Muir et al. 2006). These factors act as a molecular switch of EMT program by repressing a subset of common genes that encode cadherins, claudins, integrins, mucins, plakophilin, occludin and ZO1 to induce EMT. For example, Snail expression is associated with E-cadherin repression in metastasis; it also correlates with tumor recurrence and poor prognosis in various cancers (Elloul, Elstrand et al. 2005; Moody, Perez et al. 2005; Bruyere, Namdarian et al. 2009). In addition, extensive crosstalk among these transcription factors forms a signaling network that is responsible for establishing and maintaining mesenchymal cell phenotypes. Furthermore, some of these transcription factors, including Snail, play an important part in overcoming oncogene-induced senescence (Ansieau, Bastid et al. 2008), inhibiting tumor immunosuppression (Kudo-Saito, Shirako et al. 2009) and generating tumorigenic cancer stem cells (Mani, Guo et al. 2008). These transcription factors communicate and respond to extracellular signals such as growth factors, cytokines and hypoxia from their microenvironment to induce EMT.

Many signaling pathways trigger EMT in both embryonic development and in normal and transformed cell lines. The signaling pathways include those triggered by different members of the TGF-β superfamily, Wnts, Notch, EGF, FGF and many others (Fig. 1).

![Fig. 1. Overview of the Molecular regulation of EMT.](www.intechopen.com)

TGF-β is a primary inducer of EMT. It not only contributes to EMT during embryonic development, but also induces EMT during tumor progression in vivo (Zavadil and Bottinger 2005). Overexpression of Smad2 and Smad3 result in increased EMT in a mammary epithelial
model (Valcourt, Kowanetz et al. 2005). Knockout of Smad3 blocks TGF-β-induced EMT in primary tubular epithelial cells; the reduction of Smad2 and Smad3 function is associated with the decreased metastatic potential of breast cancer cell lines in a xenograft model (Zavadil, Cermak et al. 2004). It is interesting that SMAD3 and SMAD4 interact and form a complex with Snail, targeting the promoters of CAR (a tight-junction protein) and E-cadherin during TGF-β-inducing EMT in breast epithelial cells (Vincent, Neve et al. 2009). Bos et al identified that TGF-β primed cancer cells for lung metastasis through angiopoietin-like 4 via Smad signaling pathway (Bos, Zhang et al. 2009). In contrast, inhibition of TGF-β or TGF-β receptor reduces the invasive and metastatic activities of cancer cells. TGF-β can also downregulate various epithelial molecules, including E-cadherin, ZO-1 and several specific keratins; it also upregulates certain mesenchymal proteins such as fibronectin, fibroblast specific protein 1, α-smooth muscle actin and vimentin. In addition, TGF-β cooperates with numerous kinases such as RAS, MAPK, and p38MAP, to promote EMT (Zavadil and Bottinger 2005; Buijs, Henriquez et al. 2007). More specifically, p38 MAPK and RhoA mediate an autocrine TGF-β-induced EMT in NMuMG mouse mammary epithelial cells (Bhowmick, Ghiassi et al. 2001). ECM molecules, such as integrin β1 and Fibulin-5, augment TGF-β-induced EMT in a MAPK-dependent mechanism (Bhowmick, Ghiassi et al. 2001; Lee, Albig et al. 2008). Constitutive activation of Raf enhances the function of TGF-β in inducing EMT via MAPK in MDCK cells (Janda, Lehmann et al. 2002). TGF-β also induces EMT through changes in the expression of certain cell polarity molecules. For example, TGF-β can induce phosphorylation of Par6, which in turn stimulates binding of Par6 to E3 ligase Smurf1. The Par6-Smurf1 complex then mediates the localized ubiquitination of RhoA to disrupt tight junctions during EMT (Ozdamar, Bose et al. 2005). TGF-β can also downregulate Par3 expression to destroy cell polarity (Wang, Nie et al. 2008). It is interesting to note that Abl can inhibit TGF-β-mediated EMT in normal and metastatic mammary epithelial cells (MECs) (Allington, Galliher-Beckley et al. 2009). Furthermore, TGF-β can cooperate with other oncogenic pathways, such as Notch, Wnt/β-catenin and NF-κB, to maintain the mesenchymal phenotype of invasive/metastatic tumor cells (Nawshad, Lagamba et al. 2005; Zavadil and Bottinger 2005; Neth, Ries et al. 2007). The Wnt/β-catenin pathway has a particularly tight link with EMT (Li, Hively et al. 2000). On one hand, β-catenin is an essential component of adherent junctions, where it provides the link between E-cadherin and α-catenin and modulates cell-cell adhesion and cell migration. On the other hand, β-catenin also functions as a transcription cofactor with T cell factor (TCF). Nuclear translocation of β-catenin can activate expression of Slug, thus inducing EMT. Expression of β-catenin in oocyte induces a premature EMT in the epiblast, concomitant with Snail transcription. Interestingly, Snail is a highly unstable protein and is dually regulated by protein stability and cellular location. We showed that GSK-3β binds and phosphorylates Snail at two consensus motifs to dually regulate the function of this protein: phosphorylation at the first motif regulates its ubiquitination mediated by β-Trcp, and phosphorylation at the second motif controls its subcellular localization (Zhou, Deng et al. 2004). Thus, Wnt can suppress the activity of GSK-3β, and it stabilizes the protein level of Snail and β-catenin to induce EMT and cancer metastasis (Yook, Li et al. 2005; Yook, Li et al. 2006). Meanwhile, Snail can functionally interact with β-catenin to increase Wnt-dependent target gene expression, promoting EMT (Stemmer, de Craene et al. 2008). Increasing evidence indicates that Wnt signaling is strongly associated with human basal-like breast cancer. Inhibiting Wnt signaling through LRP6 reduces the capacity of cancer cells to self-renew and colonize in vivo. It also results in the re-expression of breast epithelial markers