Chapter 2

Epithelial-Mesenchymal Transition in Tumor Microenvironment Induced by Hypoxia

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Additional information is available at the end of the chapter

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Abstract

A tumor microenvironment contains various noncancerous cells including adipocytes, fibroblasts, immune and inflammatory cells, neuroendocrine cells, pericytes, vascular and lymphatic endothelial cells, and the extracellular matrix that surrounds cancerous cells. In the tumor microenvironment, cancer cells interact and cross talk with non-cancerous cells and orchestrate different mechanisms of cancer such as tumorigenesis, angiogenesis, and metastasis. Moreover, the expansive nature of cancer cells and chaotic angiogenesis affect microcirculation as well as alter the oxygen concentration progressively. Hypoxia, a key player in the multistep process of cancer metastasis, is important in different regions of the tumor microenvironment. Hypoxia may transform cancer cells to become more aggressive and invasive by triggering overexpression of several hypoxia-related factors that activate epithelial-mesenchymal transition (EMT). Herein, the current knowledge of how hypoxia-driven EMT is presented in the tumor microenvironment of solid cancers is discussed.

Keywords: cancer, cancer metastasis, epithelial-mesenchymal transition, hypoxia, tumor microenvironment

1. Tumor microenvironment: current perspective

The tumor microenvironment contains a multinetwork of cells, soluble factors, extracellular matrix (ECM) components, and signaling molecules that surround and neighbor cancer cells. It mediates aberrant tissue function and modulates subsequent progression in solid cancers [1]. In this microenvironment, the main structures are the parenchyma, stroma, growth factors, lymphokines and cytokines, and inflammatory and matrix metalloproteinase enzymes

(**Figure 1**). While cancer cells are located in the parenchyma, noncancerous cells and ECM constitute the stroma. Currently, it is known that noncancerous cells have key roles in several mechanisms of carcinogenesis, tumor progression, and metastatic cascade [2]. Noncancerous cells behave differently in the tumor microenvironment than healthy tissue. However, it is still unclear how noncancerous cells and noncellular components of the tumor niche collaborate and assist cancer cells to acquire invasive and metastatic features.

It is known that chronic inflammation is an important factor in shaping the tumor microenvironment. The major inflammatory cells located in the tumor microenvironment are T lymphocytes, natural killer cells, and tumor-associated macrophages (TAMS). TAMs are important in ECM destruction/restructuring of the tumor microenvironment, tumor cell motility, and triggering angiogenesis. These cells have both tumor-progressive and tumor-suppressive effects.

In the tumor microenvironment, fibroblasts have various roles under inflammatory conditions. However, they attain new characters and called as "*carcinoma-associated fibroblasts*" after the beginning of the neoplastic process. They constitute 50–70% of the volume of many solid epithelial tumors, such as pancreas, stomach, and breast cancers [3]. In addition, they are particularly effective in carcinogenesis, tumor progression, and metastasis [4, 5]. Studies on carcinoma-associated fibroblasts demonstrated that during the chronic inflammation and wound healing, only activated fibroblasts promote tumor growth. There are hypotheses about the production of cancer-associated fibroblasts. Genetic changes in normal fibroblast or exposure to EMT may directly arise from mesenchymal stem cells [6].

Cancer stem cells (CSCs) are also tumor microenvironment-specific cells. CSCs have been intensively researched recently. Today, we know many cancer types consist CSCs in their microenvironment, which is associated with aggressive tumor biology and treatment resistance. Moreover, CSCs are responsible for immune modulation during the carcinogenesis [7].

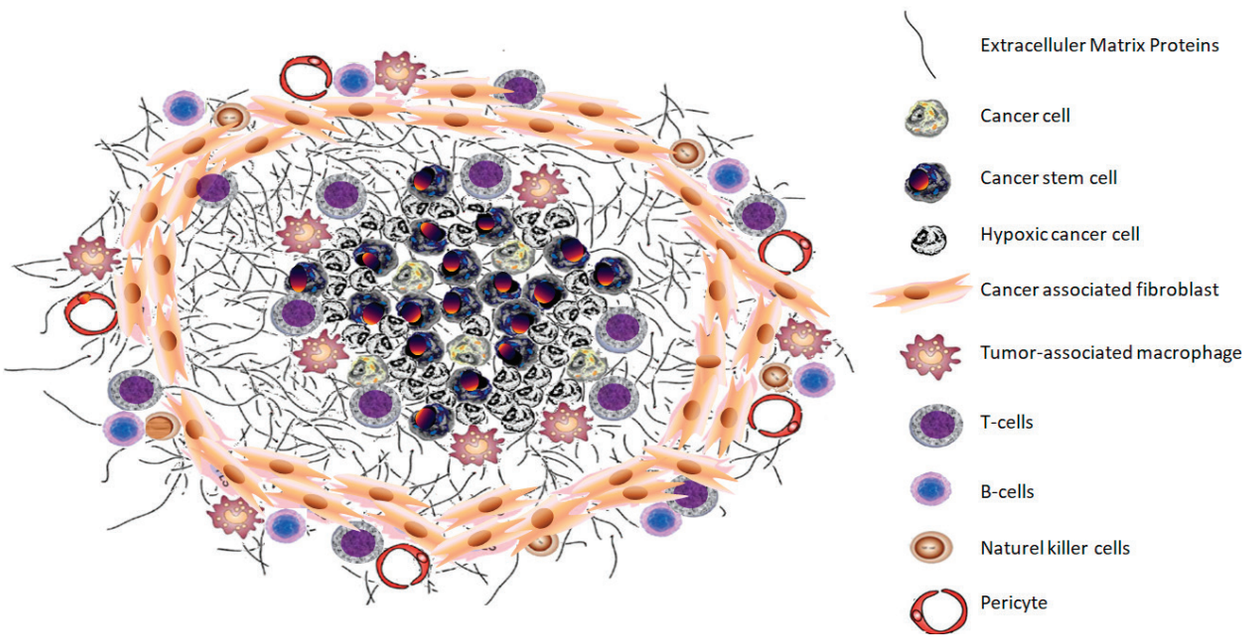

Figure 1. A schematic view of the tumor microenvironment.

In the tumor microenvironment, a unique network has been shown to be created by mainly carcinoma-associated fibroblasts and CSCs with the participant of other noncancerous cells. This network modulates and regulates different mechanisms of the neoplastic processes, such as carcinogenesis, tumor progression, angiogenesis, and metastasis.

Similar to healthy tissue, tumor tissue supplies oxygen and substances from blood and lymphatic vessels. Therefore, angiogenesis is a crucial step for tumor growth [8]. However, due to the rapid growth of tumor tissue, new blood vessel production is usually insufficient. This situation results with decrease of tissue oxygen levels termed as *hypoxia*. This new condition forces cells to acquire new and devastating behaviors such as resistance to environmental changes, invasiveness, and also metastatic phenotypes via different mechanisms. In this review, we aim to focus on the role of hypoxia and hypoxia-driven EMT in tumor microenvironment from the current perspective.

2. Hypoxia in tumor microenvironment

Hypoxia is a hallmark of tumor microenvironment. It emerges due to an inadequate blood source, which keeps proliferation cells viable. The cellular machinery uses several mechanisms in response to hypoxia. When a decrease in the level of oxygen develops, changes in numerous transcriptional regulators are altered.

2.1. Hypoxia: definition

Basically, hypoxia refers to the imbalance between the level of oxygen that the tissues require and that can be supplied. It is noteworthy that *normoxia* describes the "atmospheric" oxygen level which is approximately 20–21% (160 mmHg). However, every healthy tissue has lower and distinct oxygen levels; therefore, *physoxia* is a better terminology that defines the normal range of oxygen levels in different tissues [9]. The oxygen level of different tissues and cancers is presented in **Table 1** [10]. Therefore, hypoxic conditions often occur when the oxygen

Tissue/organ	Physoxia (% O_2)	Cancer	Hypoxia (% O_2)
Brain	4.6	Brain tumor	1.7
Breast	8.5	Breast cancer	1.5
Cervix	5.5	Cervix cancer	1.2
Kidney (cortex)	9.5	Renal cancer	1.3
Liver	4.0–7.3	Liver cancer	0.8
Lung	5.6	Lung cancer (nonsmall cancer)	2.2
Pancreas	7.5	Pancreas tumor	0.3
Rectal mucosa	3.9	Rectal cancer	1.8

Table 1. Physoxia and hypoxia of several tissues/organs and cancers.

tension (pO_2) decreases lower than 2.5 mmHg, even though tumor oxygen levels are dictated by the initial tissue and tumor microenvironment [11, 12]. Moreover, hypoxic regions are heterogeneously distributed particularly in the locally advanced tumors [13].

Cancer cells respond to hypoxia in two ways through apoptosis or resistance and survival, which is driven by the exposure time. If cancer cells are able to survive, they acquire new and unique features. Hypoxic conditions affect the gene transcription, which affords the ability of the cancer to survive through invasiveness, genetic instability, and metastasis. Furthermore, treatment (radiotherapy and/or chemotherapy) resistance may emerge [14]. A hypoxic response is mediated by hypoxia-inducible factors (HIFs), which control many facets of cancer cell viability [15].

2.2. Hypoxia-inducible factors

The HIFs orchestrate the responses to hypoxia in normal and cancer cells. Recently, three subtypes of HIFs have been introduced: HIF-1, -2, and -3. They are heterodimeric complexes and mainly act to mediate cellular processes including angiogenesis, cell proliferation, and tissue remodeling in response to hypoxia. HIFs are composed of basic helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) proteins including an O_2-labile alpha subunit (HIF-1α, -2α, and -3α) and a stable beta subunit (HIF-β). They interact with hypoxia-responsive elements that contain a conserved RCGTG core sequence [16]. HIF-1α was the first introduced prototypic member of HIF family, and has been shown to regulate O_2-dependent transcriptional responses [17]. After a while from the discovery of HIF-1α, a new HIF protein, HIF-2α, was introduced by independent research groups [18–21]. Currently, it is known that HIF-1α is the first biomolecule that responds to acute hypoxia, and HIF-2α is the major regulator under chronic hypoxic conditions. This phenomenon has been referred as the "*hypoxic shift*" [22]. Holmquist-Mengelbier et al. demonstrated that HIF-1α is active for a short duration particularly under hypoxia or anoxia (O_2 level <0.1%). However, HIF-2α is active for a long duration under less severe hypoxia (O_2 level <5.0%) [23]. Furthermore, Pietras et al. reported that the activation of HIF-2α may cause aggressive and infiltrative histopathological features under normal oxygen levels, which is termed as "*pseudohypoxic phenotype*" [24–26]. Tian et al. reported a correlation between HIF-2α and vascular endothelial growth factor mRNA expression levels in the endothelium [21]. Therefore, HIF-2α overexpression may lead to an increase in chaotic vascularization in the tumor microenvironment. In 2002, HIF-3 was introduced by Makino et al. [27]. Although the functions of HIF-3 are not clear yet, Heikkila et al. indicated that HIF-3 might regulate the activity of other HIF complexes [28].

Hypoxic conditions occur heterogenically in almost all types of solid cancers, which lead to HIF protein overexpression. Under physiologic conditions, HIF-1α is constitutively expressed; however, it is degraded rapidly upon its hydroxylation by prolyl hydroxylases (PHDs) [29]. In contrast, the O_2-dependent PHD inhibition develops under hypoxia and HIF-1α protein expression is increased [30]. Under physiological conditions, HIF-1α regulates the expression of important genes that regulate numerous biological processes. In

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the tumor microenvironment, elevated HIF-α protein expression, which was induced by hypoxia or other oncogenic signals, promotes tumor growth, angiogenesis, and proliferation through the regulation of critical genes (**Figure 2**). Recent evidence has shown that HIF-1α/-2α can impact tumor development through critical oncoproteins and tumor-suppressor genes such as MYC, p53, and mTOR signaling pathway [31–35]. HIFs may also promote the immune-suppressive mechanisms that promote apoptotic resistance in the tumor microenvironment [36]. Therefore, HIF-1α overexpression due to pathological hypoxia is generally related to poor prognosis and tumor progression in solid cancer [37, 38]. Moreover, HIFs promote the progression of cancer through EMT induction. During the EMT, carcinoma cells undergo migration and invasion, leading to cancer progression and metastasis [39].

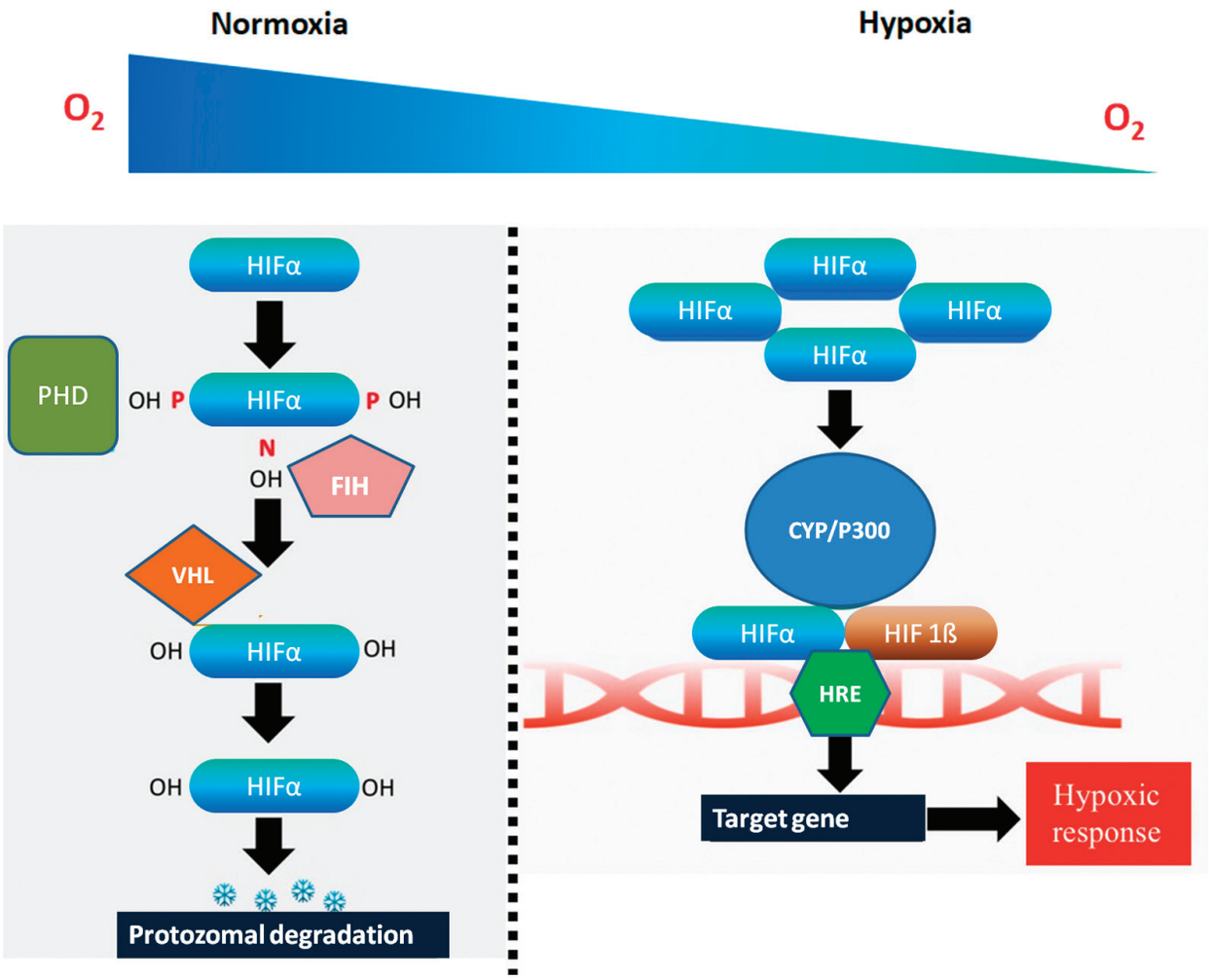

Figure 2. Regulation of HIF in normoxia and hypoxia. During normoxia, PHD enzymes and FIH take role in hydroxylation of HIF-α. Hydroxylation of HIF-α by PHDs creates a binding site for the Von Hippel-Lindau (VHL), HIF-α-VHL interaction leads to proteasomal degradation. Under hypoxic conditions, PHDs and FIH are inhibited due to lack of oxygen. Inhibition of PHDs and FIH lead to HIF-α stabilization and dimerization with its transcriptional partner HIF-1β. HIF-α-HIF-1β interaction leads to translocation to the nucleus and binding to consensus hypoxia-responsive elements (HRE) within the promoters or enhancers of HIF target genes.

3. EMT in cancer: an overview

3.1. Epithelial-mesenchymal transition: definition

Epithelial and mesenchymal cells have various functional characteristics. The epithelium is a thin layer which consists of the collection of cells with similar features that have been associated with one to another by cell-to-cell junctions such as tight junctions, adherens junctions, desmosomes, and gap junctions. Epithelium layer is polarized because apical side and the basal side have different properties that are referred as *apicobasal polarity*. Of note, cell-to-cell junctions consist of cadherins; however, cell-to-basal lamina or ECM junctions consist *laminin*. Moreover, actin is another cell-to-cell adhesion complex which has strong apicobasal polarity. All of these junctions provide immobility to the epithelium. On the other hand, mesenchymal cells do not have these features and only have focal points that adhere to their neighbor mesenchymal cells. Similar to epithelial cells, adhesions between mesenchymal cells can involve cadherin for cell-to-cell junctions and integrins for adhesion to ECM. However, they do not have junctions for basal lamina. In addition, interstitial collagen and fibronectin are important for the ECM adhesion of the mesenchymal cell. They do not have the same ECM molecules associated with the apical-basolateral surface (**Figure 3**).

In 1953, Abercrombie and Heaysman observed that the migration of epithelial cells slows down and they realign when contact each other by forming adhesive junctions [40, 41]. Conversely, mesenchymal cells, particularly fibroblasts, reorient their direction and move away by generating lamellipodia. This process is termed contact inhibition of locomotion. Thereafter, they demonstrated that any defect in contact inhibition of locomotion contributes to the development of invasive and aggressive characters of cancer cells [42–44]. Today, it is known that contact inhibition of locomotion is important in EMT.

EMT is a complex course where epithelial cells are transformed into mesenchymal cells. It is coordinated by several different influential factors that lead to behavioral changes in epithelial cells. Concisely, epithelial cells lose core properties including the apicobasal polarity, cell adhesion, and increase mesenchymal cell properties during the transition [45, 46].

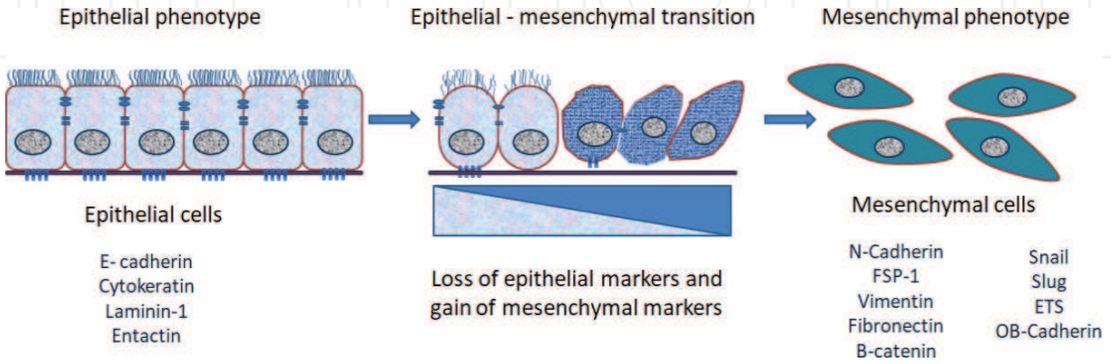

Figure 3. A schema for epithelial-mesenchymal transition. Loss of epithelial markers and gain of mesenchymal markers during the transition from epithelial phenotype to mesenchymal phenotype.

EMT is a naturally occurring transdifferentiation process and is critical during embryonic development and organogenesis. This phenomenon also occurs during wound healing, tissue regeneration, organ fibrosis, and carcinogenesis. In addition, a post-EMT behavior of a part of cells may include reverse transition, which is referred as the *mesenchymal-epithelial transition* [47].

The majority of tumors originate from epithelial tissues of lung, colon, breast, pancreas, prostate, bladder, ovary kidney, liver, and head and neck. Currently, EMT is important in cancer progression and metastasis. Epithelial cells may acquire several abilities such as motility, invasion, and malignant features via EMT [48]. Moreover, it is known that inflammation is the key inducer of EMT in cancer progression. Inflammation may trigger a number of signaling pathways involved in carcinogenesis. However, the specific signals that are induced during the pathologic EMT in epithelial cancers remain unclear [49].

3.2. EMT in physiology and diseases

The mechanisms under the induction and progression of EMT vary dramatically, even though motile cells with mesenchymal phenotype develop consequently. EMT is classified into three different subtypes: type-1, -2, and -3. Type-1 EMT (physiologic EMT) is related to implantation, embryogenesis, and organ development. It is impacted by remodeling and diversification of tissue during morphogenesis. Type-1 EMT is not related with inflammation, fibrosis, and systemic dissemination and generally occurs transiently. Type-2 EMT impacts tissue regeneration and fibrosis, and the process depends on continued inflammation in adults. It continues until the underlying injuries or infections are resolved/repaired. Type-2 EMT may produce mesenchymal cells that are activated. Most notably are the myofibroblasts that produce extreme levels of collagen-rich ECM. Type-3 EMT happens in the context of tumor growth/cancer progression and the tumors transform to a mesenchymal phenotype. The type-3 EMT induction is assisted by genomic changes by cancer cells. It may produce cells that have aggressive properties, which promote movement into the bloodstream in order to spread to other organs.

3.2.1. Type-1 EMT

Type-1 EMT is the exchange from epithelial cells to mesenchymal cells in the embryonic phase events such as implantation, embryogenesis, and organ development. After early embryogenic stages, fertilized egg implantation to the endometrium is associated with an EMT [50, 51]. This is the first step of type-1 EMT that is accompanied by embryonic morphogenesis. At the gastrulation stage, EMT continues with the generation of three germ layers and a primitive streak is made in the epiblast layer [52]. The formation of the primitive streak is the most important part of gastrulation. Primitive streak leads to three germ layers. Thereafter, all tissues are generated during organogenesis by cell migration and differentiation. The EMT coordinates almost every stage of this process [53].

At gastrulation level, the EMT is mainly orchestrated by Wnt signaling [54]. Of note, the TGF-β superfamily, including Nodal and Vg1, and FGF receptors are in close relation to Wnt signaling. Moreover, different signaling modalities through BMPs, c-Myb, and msh homeobox 1 (Msx-1) play roles in the regulation of type-1 EMT [55].

3.2.2. Type-2 EMT

Type-2 EMT is the transition of epithelial cells to mesenchymal cells, which occurs during wound healing and fibrosis due to inflammation. It is orchestrated by fibroblasts and inflammatory cells, which release multiple inflammatory molecules, signals, and ECM components such as collagens, laminins, elastin, tenascin, and other matrix molecules. A variety of studies demonstrated an association between EMT and progressive organ fibrosis such as kidney and lung disease [56, 57].

Inflammatory cells and fibroblasts produce proteins such as FSP1, S100 cytoskeletal proteins, α-SMA, and collagen I that develop during the development of organ fibrosis [57]. These proteins have been used as a biomarker for fibrosis of organs, which are undergoing an EMT associated with chronic inflammation. However, epithelial markers, including cytokeratin and E-cadherin, continue to be expressed until they gain a complete fibroblastic phenotype [58]. Rastaldi et al. evaluated the EMT in human renal biopsies of 133 patients with kidney fibrosis. The EMT was detected in the fibrotic kidney based on the staining for cytokeratin, vimentin, α-SMA, and zona occludens 1 (ZO-1) [59]. Kidney fibrosis has been associated with multiple inflammatory cells that induce EMT with various growth factors such as TGF-β, EGF, and FGF-2 [60]. As the role of TGF-β has been determined in kidney fibrosis, several researchers focused on the inhibition of TGF-β using BMP-7 [61]. Morrissey et al. demonstrated that BMP-7 provided the reversal of EMT and repaired tubular structural damage and repopulation of healthy tubular epithelial cells of mice with kidney fibrosis [62].

3.2.3. Type-3 EMT

Type-3 EMT is the transmission of epithelial cells to mesenchymal cells in cancer progression, also known as the "*oncogenic epithelial-mesenchymal transition*." Due to its complexity, oncogenic EMT is more complex than physiologic EMT. The role of type-3 EMT has been demonstrated in different cancer cells. For example, breast and prostate cancer cells can be classified as epithelial predominated or mesenchymal predominated [63, 64]. Zajchowski et al. studied different molecules to predict invasiveness of breast cancer by using gene array method and showed that epithelial proteins are related to noninvasiveness, whereas mesenchymal proteins are related to invasiveness [65]. Currently, several *in vitro*/*in vivo* studies demonstrated that mesenchymal status leads to an invasive phenotype, motility, and metastasis in cancers.

In solid tumors, loss of E-cadherin [66], cadherin transformation [67], adhesion loss, changes in apicobasal polarity, and tissue architecture modifications have been demonstrated in EMT. In addition, vimentin, N-cadherin, fibronectin, that are the mesenchymal markers, are highly expressed during the EMT [48]. In carcinogenesis and tumor progression, the loss of E-cadherin and increase in the N-cadherin, which is referred as "*cadherin switch*," are the most significant indicators of the EMT. Currently, it is known that cadherin switch breaks down cell-to-cell junctions and controls the contact inhibition of locomotion. Moreover, it may modulate signal transduction in metastatic cascade [68]. The association between tumor progression and cadherin switch has been demonstrated in prostate cancer, urothelial bladder carcinoma, and malignant melanoma [67, 69, 70].

In literature, there are evidences that support the idea of "*high levels of mesenchymal markers are often related to aggressive tumor behavior and poor prognosis*." In cervix cancers, the correlation between lymph node metastasis and vimentin positivity was also determined [71]. Nevertheless, this correlation has been reported in a small number of cancer types. Therefore, it is hard to mention that vimentin is a definitive predictor of aggressiveness for all cancer types. Ahmad et al. suggested another biomarker for metastatic breast cancer: *stromelysin-3*. Stromelysin-3 is a matrix metalloproteinase and marker for mesenchymal cells. Breast carcinoma cells that undergo EMT are able to express stromelysin-3, which may partly explain the increased metastatic propensity detected in these tumors [72].

Recently, the genetic and biochemical properties that underlie acquirement of cancer cell invasiveness and metastasis are the major areas of intensive research. Xue and colleagues demonstrated that cancer cells departing HER-2/neu expressed a GFP transgene that was facilitated by FSP-1. Moreover, the low rate of metastasis was detected in FSP-1 null mice [73]. This research provided important evidences for the mechanism of metastasis related to EMT. In addition, Yang et al. reported that tumor cells were able to behave like mesenchymal cells and express mesenchymal markers [74]. Besides the evidences about EMT in the metastatic process, some studies have also shown data on reverse EMT. They suggest that the reversibility of EMT is observed during embryonic development and also during the tumor growth at metastatic side. Tumor cells try to undergo not only growth but also cell differentiation to resemble the originating epithelium. Brabletz et al. demonstrated the similarity of epithelial nature between primary tumor side and metastatic tissue for colorectal cancers [75]. It indicates that the induction of an EMT is likely to be central and crucial for the metastatic cascade and implicates EMT during the colonization process.

3.3. Molecular mechanisms and pathways of EMT

In pathologic or physiologic events, the EMT is triggered and controlled by different signaling pathways. Several transcription factors have been described for the regulation of EMT. Tumor growth factor-β signaling appears to be one of the most important pathways. It generally acts as an epithelial cell proliferation suppressor. However, it may also positively affect the tumor progression and metastasis [76, 77]. TGF-β can induce the EMT via two signaling pathways. The first pathway involves Smad proteins that regulate the action of tumor growth factor-β by affecting ALK-5 receptors. Smad proteins mediate signaling pathway effects on motility of cells [61, 78]. Inhibitory Smad can induce autocrine production of TGF-β, thereby, reinforcing epithelial-mesenchymal transition [79]. Recently, β-catenin and LEF found to be relevant with Smad in PDGF-induced EMT [80]. Currently, it is known that TGF-β/Smad/LEF/PDGF axis has important effects on EMT during cancer progression. The second mechanism for TGF-β-induced EMT is MAPK-dependent pathway [81].

Several studies have demonstrated the association between reduced cancer cell E-cadherin levels and activation of EMT [82, 83]. Eger et al. showed that the cFos oncogene induction in mouse mammary epithelial cells induced the EMT by decreasing E-cadherin [84]. The movement of β-catenin from the cytoplasm to nucleus causes acquisition of mesenchymal phenotype by affecting E-cadherin expression. Nuclear buildup of β-catenin has been shown

to reduce E-cadherin expression and acquisition of invasive phenotype [85]. Scarpa et al. described the E-cadherin loss as an activation and contact-dependent cell polarity process via Rac signaling [86]. Currently, it is known that reduced E-cadherin levels are highly correlated with poor prognosis and decrease in survival in various cancers such as hepatocellular carcinoma, nonsmall cell lung, oral, esophageal, gastric, cervix and breast cancer, and bone and soft tissue sarcoma [87–95].

3.4. EMT in cancer metastasis: guilty or innocent?

Cancer metastasis is a complex multistep process with sequential molecular and cellular events that promote the transformation of cells, intravasation, survival and ultimately extravasation, implantation, growth, and colonization in a new and foreign tissue environment. As mentioned above, several evidences support that EMT has a major role in cancer metastasis. The EMT signifies the first step of the metastatic cascade. During EMT, cancer cells are able to invade adjacent cell layers following the loss of cell-to-cell adhesion and acquiring motility. Principally, the result of cellular motility is similar to the extensive cell migration and tissue reorganization that occurs during the embryogenesis and organogenesis; however, subsequent steps have different and complex events.

After a journey in the bloodstream, cancer cells that can escape from the immune system, extravasate from the circulation in order to implant and proliferate at the target organ, "*seed and soil theory*." Thereby, a colony of the primary tumor can regrow by inducing angiogenesis in a foreign and apparently "hostile" background. This process is induced by not only genetic/epigenetic factors but also by the nonneoplastic stromal cells [96]. *In vivo* studies demonstrated that this development is generally supplemented with partial or complete EMT. Therefore, the induction of EMT results in the acquisition of metastatic properties in different carcinoma cell lines. Main indicators for the acquisition of mesenchymal properties are the high level of mesenchyme-specific proteins [46]. In contrast to many studies, Tarin et al. reported that the acquirement of mesenchymal markers during tumor progression reflects genomic instability. Therefore, they advocated that EMT does not occur in carcinogenesis [97]. However, synchronized and complex gene-expression patterns are required to provide tumor cells with the mesenchymal properties. Moreover, genomic instability may have more important role in the regulation of EMT. For instance, SNAI1 regulates expression of EMT-associated genes in colorectal carcinoma [98].

A significant evidence for EMT during the metastatic process was presented by Yang et al. They reported that cancer cells were able to behave like mesenchymal cells and express α-SMA, FSP1, desmin, and vimentin [74]. Studies that include functional manipulations on EMT process also provide evidences. For instance, depletion of FSP1/S100A4-positive cells in tumors suppresses metastasis [73].

In adenomatous polyposis coli (APC) and β-catenin mutation-positive colorectal cancers, β-catenin levels are predominantly observed in tumor cells localized at invasion. Moreover, tumor cells with nuclear β-catenin seem to have undergone EMT [99]. Regardless of numerous studies, the major problem for the demonstration of the role of EMT in the metastatic cascade is the detection of cancer cells that have undergone EMT in primary human tumors.

The markers of EMT indicate epithelial phenotype or mesenchymal phenotype not the EMT in cancer metastasis. Therefore, *in vivo* studies with more sensitive indicators are required for understanding the role of EMT in cancer metastasis.

4. A new insight into the mechanisms of hypoxia-induced EMT

Hypoxia is a common situation in tumor microenvironment affecting cancer cell behavior, including progression and metastasis. Currently, it is clearly known that exposure to hypoxic conditions results in HIF-1α overexpression. As mentioned previously, overexpression of HIF-1a is related with promoting EMT for cancer cells. Additionally, it has been demonstrated that hypoxia-induced EMT includes the loss of cell adhesion and cell polarity. It has been observed that hypoxic conditions decrease the E-cadherin expression, but increase N-cadherin expression, a mesenchymal marker [100].

Azab et al. previously demonstrated that multiple myeloma cancer cells cultured in hypoxic conditions and injected into mice were able to spread to the new bone marrow faster than the cells cultured under normoxic conditions [101]. The hypoxia-induced EMT is mainly driven by stabilization and activation of HIF1α. It is controlled by epigenetic changes that result in a loss of tumor-suppressor functions and gain of oncogene functions (Ras, Raf, Src, mTOR, and Myc). Besides hypoxic conditions, the HIF pathway is also regulated by hypoxia-independent manner [102, 103]. Hypoxia-independent HIF-α stabilization and activation happens in response to cytokines, lipopolysaccharide (LPS), and growth factors in EMT mediated by PI3K/AKT/mTOR,29,30 MAPK,41 and NFκB pathways [104–106].

HIF-1α regulates hundreds of genes, and not only controls malignant and metastatic cancer cells but is also resistant to treatments. Thus, inhibition of hypoxia-induced EMT or HIF-1α may be promising as an anticancer therapy. Currently, there are many researches ongoing in this field. Besides targeting HIF-1α, another strategy is to block metastasis and target genes downstream of HIF-1α. Kaneko et al. have researched the hypoxia-induced EMT in oral cavity squamous cell carcinoma and showed that hypoxia-induced EMT in oral cavity cancer was improved by GSK3-β phosphorylation via PI3 K/Akt signaling [107]. Jiao and Nan showed that hypoxia-induced EMT and chemoresistance were supplemented with HIF-1α expression and Akt activation. Moreover, they demonstrated that PI3K/Akt and HIF-1α inhibition improved the therapeutic efficacy of hypoxic chemotherapy [108]. Lo Dico et al. reported that miR-675-5p promotes glioma growth through HIF-1α stabilization. Subsequently, they examined miR-675-5p specifically in colon cancer metastasis and demonstrated overexpression contributes to tumor progression through HIF-1α-induced EMT [109, 110].

5. Conclusion

Hypoxia is a hallmark of cells in the tumor microenvironment and has a major role in the carcinogenesis and metastasis processes. Hypoxia controls many crucial events such as tumor neovascularization, metabolism, cell survival, and cell death. Furthermore, hypoxia causes

EMT and CSC-like properties including resistance to treatment. Each step of the cancer adaptive process is regulated by HIF, NFκB, PI3K, and MAPK pathways. Understanding the impact of hypoxia and clarifying the hypoxia-induced responses and signaling modalities may pave the way to achieve important steps against cancer via hypoxia/HIF-targeted treatments.

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