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Epithelial-Mesenchymal Transition and its Regulation in Tumor Metastasis

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Abstract

Epithelial-mesenchymal transition (EMT) plays a key role in cancer metastasis. This process is a complex, multi-functional, and tightly regulated developmental program. EMT has been extensively investigated, but the molecular regulation of its signaling pathway is highly complex. In this study, the different elements of EMT cascades that could be targeted were determined. Difficulties in translating the preclinical findings in routine clinic were also distinguished. Future research will provide insights into the activation and regulation of various EMT programs in different tumor types and at distinct stages of tumor development. These results will likely facilitate the development of early detection strategies and improve the therapeutic targeting of malignant solid tumors.

Keywords: tumor, tumor metastasis, EMT, regulation of EMT

1. Introduction

Cancer metastasis is the major cause of cancer morbidity and mortality. This process accounts for approximately 90% of cancer deaths. Epithelial-mesenchymal transition (EMT) is a characteristic of the majority of metastatic cells. EMT is a natural transdifferentiation mechanism that governs changes in cell states along the epithelial versus mesenchymal axes and confers epithelial-mesenchymal plasticity upon epithelial cells. In particular, epithelial cells are transformed from highly differentiated, polarized, and organized cells into undifferentiated, isolated, and mesenchymal-like cells with migratory and invasive properties. In this chapter, we summarize evidence supporting the widespread involvement of EMT in tumor pathogenesis and the regulation of cancer metastasis.

2. EMT: a naturally occurring transdifferentiation program

Normal adult tissues in terminally differentiated cells have been reprogrammed into pluripotent stem cells in the past 10 years [1, 2]. This process has resulted in the wide acceptance of the initial hypothesis that nearly any type of dedifferentiation or transdifferentiation is possible if the ectopic expression of a transcription factor is properly combined into adult cells. The successful experiments on reprogramming have led to the exploration of the factors that change the state of cells in nature rather than forced to ectopic gene expression.

EMT is the most important cell biology program among naturally occurring transdifferentiation programs. This process converts epithelial cells into mesenchymal derivatives, which is the reverse process of mesenchymal-epithelial transformation (MET) [3]. Accumulated evidence for the past two decades has suggested that EMT occurs during development to ensure the interconversions of cells utilized in the formation of different types of cells, thereby forming the organs of organization and complex multicellular organisms [3, 4]. This cell biological program is orchestrated by a group of transcription factors (EMT-TFs), such as the Snail, Twist, and Zeb families [4, 5].

Two other aspects of EMT are worthy to be discussed in detail. The EMT program in some epithelial tissues is apparently correlated with the residence of cells in stem cell-like states. Moreover, versions of the EMT program are adopted by cancer cells to obtain a series of processes associated with higher levels of malignancy. EMT exhibits the presence of mechanical connections between an individual and the pathogenesis of cancer. These processes prior to EMT are insignificant.

EMT governs changes in cell states along the epithelial versus mesenchymal axes and converts epithelial cells to mesenchymal cells when this program is fully executed. Weinberg described the extreme poles of the epithelial versus mesenchymal axes. Epithelial cells, frequently with polygonal shapes in monolayer culture, are polarized along their apical-basal axis and are tightly connected with one another laterally via adherens and tight junctions in vivo. These lateral ties can ensure the structural integrity of epithelial cell sheets. By contrast, full mesenchymal cells exhibit spindle-like morphology with no sign of apical-basal polarity. These cells are loosely attached to the surrounding extracellular matrix (ECM) through focal adhesions. These features can help improve motility and explain the invasion of mesenchymal cells relative to their epithelial counterparts.

The deep layer of biological contact between epithelial and mesenchymal cells is determined by the differences in their respective transcription programs. These programs also control the expression of other gene products and key structural proteins, including those involved in the maintenance of the cytoskeleton and the strengthening of cell-cell adhesion [3–5]. Thus, epithelial cells express different types of keratin to form intermediate filaments, whereas vimentin constitutes the intermediate filament protein of mesenchymal cells. The expression of cell adhesion molecules and polarized complexes in mesenchymal cells is generally inhibited. EMT is marked by the replacement of E-cadherin by N-cadherin, which leads to the formation of weak cell adhesion between adjacent cells.

EMT can be significantly and rapidly activated in epithelial cells in response to physiological signals in a cell autonomous or non-cell autonomous manner. When gastrulation is used as an example, EMT responds to the induction signal as follows. The program is activated in ectodermal epithelial cells and completely converts epithelial cells into mesoderm mesenchymal cells, such as fibroblast growth factor and Wnt signaling pathway [6]. Similarly, EMT can be rapidly activated in adult tissues; it reacts to wounding and promotes rapid wound healing. This process is necessary to reconstruct the epithelial barrier that is essential for protecting internal organs from external injury [7]. Such rapid conversion between epithelial and mesenchymal states suggests the plasticity of epithelial cells, which facilitates their response to EMT-inducing signals. In addition, this plasticity demonstrates that residence in one of these two states is maintained in a metastable manner, with complex molecular and cellular mechanisms to ensure that a cell is in one or another state for a long period.

The description of EMT as a binary that shifts cells from a fully epithelial state into a fully mesenchymal state misreads the normal actions of this program. EMT is typically only from a fully epithelial state to a partially mesenchymal state, with certain key epithelial markers retained [8, 9]. Nevertheless, obtaining even a subset of mesenchymal traits to endow cells that previously resided in a fully epithelial state with a suite of mesenchymal traits will produce far-reaching effect on their biology.

3. EMT and cancer pathogenesis

Nearly 80% of malignant tumors are derived from epithelial tissues, which produce common cancers, such as tumors of the lung, colon, breast, pancreas, prostate, bladder, ovary, kidney, and liver. The epithelial states of the corresponding normal cells of origin in each determined case sustain the expression of cytokeratin and E-cadherin, which are signs of the epithelial states of early tumor tissues. In addition, tumor cells in early tumors remain the key biological phenotypes of epithelial cells, such as a lack of motility and capability to form a continuous cell sheet. These qualities exhibit a sharp contrast with those of advanced cancer cells, which are products of a complex succession process that is frequently referred to as “tumor progression.” Highly invasive tumor cells present mesenchymal features, such as motility and invasion, and the latter is associated with metastasis [10–12]. The acquisition of these malignant features at the mechanism level can be explained by activating EMT, which is previously dormant in tumor cells during tumor progression.

The acquisition of mesenchymal features in breast cancer is positively correlated with tumor progression and an aggressive subtype of the disease [13, 14]. Investigating a large body of loss-of-function and gain-of-function in xenograft tumor models is a direct means to describe the link between the activation of EMT and the degree of malignancy of a tumor. The consumption of EMT-TFS, such as Twist, Snail, and Zinc finger E-box binding homeobox 1 (ZEB1), in both human and mouse breast cancer cell lines significantly inhibits the metastatic dissemination, whether the site is the primary site of tumor formation (e.g., mammary fat pad mass) or after experimental introduction of cancer cells into venous circulation (i.e., tail-vein

injection). By contrast, the ectopic activation of EMT can enhance metastatic dissemination of orthotopically implanted human breast cancer cells by forcing the expression of EMT-TFS [15–17].

EMT in patients with breast cancer does not only promote systemic dissemination but also function as a major factor of drug resistance and disease recurrence [18, 19]. A similar phenomenon has been observed in a mouse model of Her2-induced tumors in which Snail EMT-TF can be autoactivated in recurrent malignancy *in vivo* and make the tumors highly mesenchymal phenotype [20]. The results evidently show an association between EMT activation and tumor relapse. Moreover, the link between EMT activation and enhanced tumorigenicity has been verified in various human cancer cell lines [21]. The inhibition of epithelial-mesenchymal plasticity suppresses the valid transition of carcinoma cells from a weakly tumorigenic into a highly tumorigenic state by blocking the activation of Zeb1 EMT-TF; that is, a state where tumor-initiating cells demonstrate their improved capabilities [22].

Recent studies have associated EMT with the acquisition of immunosuppressive capabilities in various types of cancers. The expression of Snail EMT-TF in melanoma can simultaneously inhibit the differentiation of cytotoxic T cells and induce the immune inhibition of the formation of regulatory T cells; the latter effect is mediated through the production of platelets [23]. Meanwhile, EMT in breast cancer cells enhances the resistance of tumor cells to cytotoxic T cell-mediated lysis, which at least partially induces autophagy [24, 25]. The activation of EMT-TF ZEB1 in lung cancer cells has been linked to the upregulation of programmed death ligand 1 (PD-L1), which is an immunosuppressive molecule that can block tumor-infiltrating lymphocyte attack [26].

EMT activation has a pleiotropic function in driving cancer progression, and an increasing number of reports confirm that invasive cancer is related to various types of aggressive carcinoma cells. Thus, we believe that all carcinomas essentially develop traits that are related with malignancy through the activation of an EMT program in their constituent neoplastic cells. However, the EMT program associated with common cancers has not yet been determined because many clinical pathologists doubt the existence of this program and its role in the production of high-grade cancer [27]. Such reluctance primarily originates from the fact that all markers of clinical biopsy are difficult to score. Although scoring will be possible, cancer cells that underwent EMT are also difficult to distinguish from normal host tissues and adjacent tumor stromal cells. Many fibroblasts and myofibroblasts self-express EMT-related markers. Clear evidence of this program during tumor development can be obtained from cell detection that co-express both mesenchymal and epithelial traits with certain retained epithelial markers inherited from their fully epithelial precursors because cancer cells frequently experience the only part of the EMT program. Recent analysis suggests that all subtypes of invasive breast cancer tumor cells exhibit both epithelial and mesenchymal characteristics as shown by the *in situ* hybridization in human breast cancer specimens of pooled epithelial and mesenchymal markers [28]. In addition, a part of the circulating tumor cells is isolated from the peripheral blood of patients with advanced prostate and breast cancers, and they also co-express both epithelial and mesenchymal markers [28–30].

4. EMT and tumor metastasis

Through the blood or lymphatic system, tumor cells can migrate far from the primary site and then settle and grow in a remote site to complete tumor metastasis. This process plays a key role in tumor disease, malignance level, and death of over 90% of tumor patients. Several questionnaires have shown the low efficiency of tumor metastasis. The majority of tumor cells can fall off into the blood or lymphatic system, such that only a small portion can form micro metastases, and even fewer can achieve actual metastases and present specific organ affinity. Moreover, the metastatic times of different tumors are not the same [31]. Thus, understanding this process is significant, and the importance of EMT should be discussed from different aspects.

EMT is a characteristic of most metastatic cells [32]. In particular, highly differentiated epithelial cells are converted into undifferentiated and isolated ectomesenchymal cells with migration and invasive properties. Migration is one of the four basic steps in tumor cell metastasis. Various substances, such as secretion factors, growth factors, and ECM components, can stimulate the migration of tumor cells. Cell migration stimulated by these substances can be divided into random and directed migrations. Thus, tumor cells can migrate and metastasize. The migration capability of tumor cells is related to their potential to metastasize. The density of the negative charges that separate from the surface of the tumor cells increases, which enhances electrostatic repulsion between cells. This process facilitates the removal of tumor cells into a free state from the tumor tissue. The adhesion of molecules on the surface of tumor cells is mediated by cell adhesion molecules, namely selectins, integrins, Ig superfamily, and cadherin [33]. Intercellular adhesion capability decreases in the same type, which leads to the detachment of tumor cells from the primary tumor and the abnormal intercellular adhesion contact with the implantation of tumor cells in the vascular wall. Invasion is the important biological characteristic of malignant tumors. Every organization or organ has its own structure. Tumor cells that invade an organ should respond to environmental stress, such as the lack of oxygen and nutrients, low pH, active oxygen free radicals, and inflammation regulatory factor. When the invasive capability is strong, the degree of tumor malignancy will be high. Epithelial cells lose the characteristics of the epithelial cells of ectomesenchymal cells, and the phenotype EMT process is the molecular basis of cancer stem cell invasion and metastasis. In addition, during tumor development, many tumor cells exhibit changes in good plasticity through morphology and phenotype transformation, such as collective amoeboid transition (CAT) and mesenchymal-to-amoeboid transition (MAT) [34]. EMT is a transient dynamic process that is influenced by the microenvironment. Furthermore, in vitro studies are necessary to build an improved genetic mouse model and reliable marker for EMT to realize real-time monitoring of the body, understand the mechanism of tumor evolution and EMT, establish new EMT signs, and to explore the role of transcription factors in the induction of EMT [35]. The loss of epithelial cell polarity during EMT reduces contact between the environment and stromal cells. This process enhances cell migration and mobility, which results in mesenchymal phenotypes. Moreover, changes in cell phenotype, coupled with an alteration in the expression levels of E-cadherin, vimentin, N-cadherin, and α -SMA, among others, in particular, a drop in E-cadherin level, can reduce the adhesion of cells, which facilitates the

invasion and metastasis of cells. The loss of E-cadherin expression has been considered the most notable feature of EMT [36]. EMT presents increased opportunities for cell metastasis during tumorigenesis, probably because of its loose cell characteristics. EMT can promote the transfer of various tumor cells. Tumor metastasis includes several steps, such as attacks that are the precondition for cell transfer. EMT plays an important role during tumor invasion. Non-invasive tumors are turned into highly invasive tumors when the E-cadherin protein of tumor cells is cut. Experiments have established EMT marks in some tumor cells in metastases.

Snail, Twist, and ZEB1 transcription factors closely linked with EMT can enhance invasion and promote the degradation of E-cadherin. EMT is the interaction between tumor cells and adjacent tumor-associated stromal cells caused by the induction of the transcription factor in the tumor cells. The activation of tumor EMT typically occurs during signal swaps between tumor cells and adjacent stromal cells. The progression of primary tumor cells can raise each model into the surrounding stroma. The recruitment of cells form a “reactive” matrix, induce the release of EMT signals, and start tumor cells by activating the EMT transcription factors [37]. **Figure 1** shows the tumor metastasis and EMT.

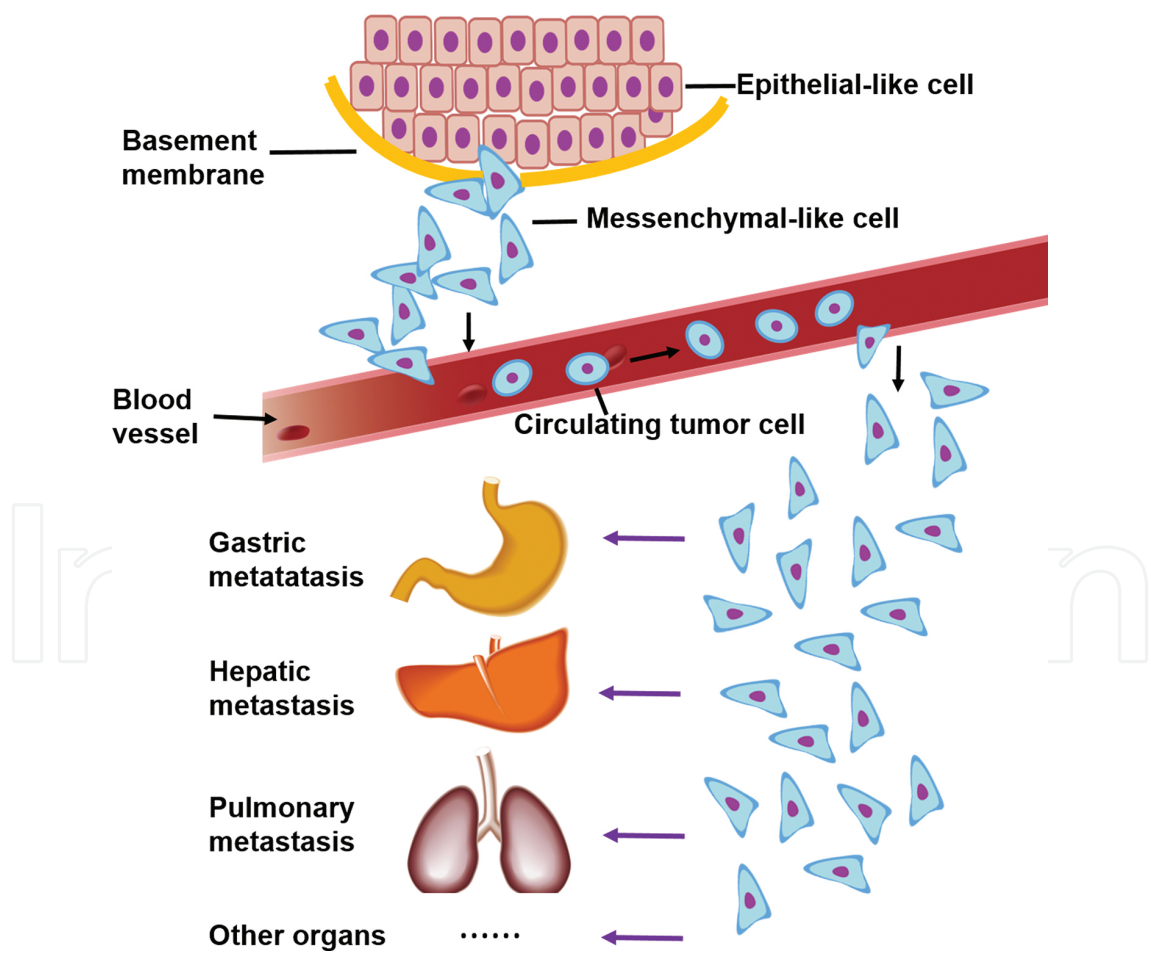


Figure 1. In EMT processes, tumor cells change from epithelial-like cells to mesenchymal-like cells and get the ability to metastasis.

5. EMT-activating transcription factors in cancer

Many transcription factors can induce EMT. Molecular reprogramming during an EMT is caused by three groups of transcription factors, namely the Snail, Twist, and ZEB families [38].

The Snail family includes Snail1, Snail2 (Slug), and Snail3 (Smuc). These factors regulate epithelial and mesenchymal markers [39, 40]. Snail1 induces signal to initiate EMT [41, 42]. These factors inhibit other epithelial markers that affect E-cadherin and bind to the E-cadherin promoter to inhibit its transcription. The Snail factors activate the expression of mesenchymal-like and pro-invasive genes that promote cell migration [43].

Snail factors are absent in normal epithelial cells. Snail1 is expressed higher than Snail2 and Snail3. An upregulated nuclear Snail1 expression is associated with tumor progression and can be found in the cytoplasm of several carcinomas. Snail1 staining is found among fibroblast-like cells, endothelial cells at the peritumoral stroma, and inflammation of colorectal carcinomas [44]. Snail1 promotes the recurrence of Her2/neu-induced breast tumors in mice, and its mesenchymal-like characteristics are exhibited in recurrent human carcinomas [45]. Therefore, recurrent breast carcinomas are induced by Snail1 spontaneously. High level of Snail1 is an independent predictor for reduced relapse-free survival in breast cancer patients. This factor is considered an independent prognostic factor for worst evolution and poor survival in many carcinomas [43].

Twist factor induces EMT by influencing other EMT-ATFs. Twist1 represses E-cadherin by inducing Snail1 or Snail2 and then binding to its promoter [46–48]. The knockdown of Twist1 in breast cancer cells represses the metastasis in xenograft models, but does not influence the formation of primary tumors [49]. Twist1 induces N-cadherin by driving its transcription and the mechanisms of post-transcription [50, 51]. Twist1 promotes the expression of mesenchymal markers without eliciting an N-cadherin/E-cadherin switch in glioblastoma cells [52]. In cell motility, the excessive expression of Twist1 upregulates the expression of cytoskeletal and ECM genes.

Twist1 and Twist2 are upregulated at the invasive front of carcinomas in cancer and stromal cells [53–55]. These factors are absent in normal epithelium but are induced in many human carcinomas, such as those of the digestive tract, liver, breast, ovary endometrium, and prostate [43]. Twist factors are upregulated in the cytoplasm and nuclei of cancer cells. Twist factors are independent prognostic factors for increased tumor recurrence, tumor aggressiveness, and the low survival rate of patients [49, 53, 56]. Twist and Snail factors play distinct but collaborative roles among EMT-ATFs.

The ZEB family includes zinc finger/homeodomain proteins, namely ZEB1 and ZEB2. The expression of ZEB factors drives an EMT by activating mesenchymal properties and repressing epithelial markers [43].

ZEB1 and ZEB2 bind to E-box sequences in the E-cadherin promoter but recruit different sets of co-repressors, namely SWI/SNF and CtBP for ZEB1 and NuRD and CtBP for ZEB2. ZEB proteins bind and repress the promoters of epithelial markers, such as R- and P-cadherins, gap

junctions (connexins 26 and 31), cell polarity markers (Crumbs3, Pals1-associated tight junction protein, and lethal giant larvae homologue 2), desmosomes (plakophilin 3, desmoplakin), and components of tight junctions (claudin 7, occludin, junctional adhesion molecule 1, and zonula occludens protein 3). ZEB proteins activate mesenchymal markers, such as N-cadherin and vimentin [43]. ZEB1 and ZEB2 repress epithelial splicing regulatory proteins-1 and 2, the overexpression of which inhibits EMT [57].

ZEB1 inhibits epithelial phenotype, although this factor is found in isolated fibroblasts and immune cells in the interstitial matrix. This factor is not expressed in normal epithelium and well-differentiated carcinomas that express E-cadherin [58, 59]. ZEB1 is highly expressed in invading dedifferentiated cancer cells of many tumors, such as colorectal, breast, liver, endometrial, lung, prostate, and pancreatic carcinomas. ZEB1 and ZEB2 are expressed by stromal cells in epithelial tissues and organs of normal E-cadherin-positive epithelial cells [60]. ZEB-dependent paracrine signaling from the stroma can cooperate in E-cadherin repression in other parts of the tumor [61].

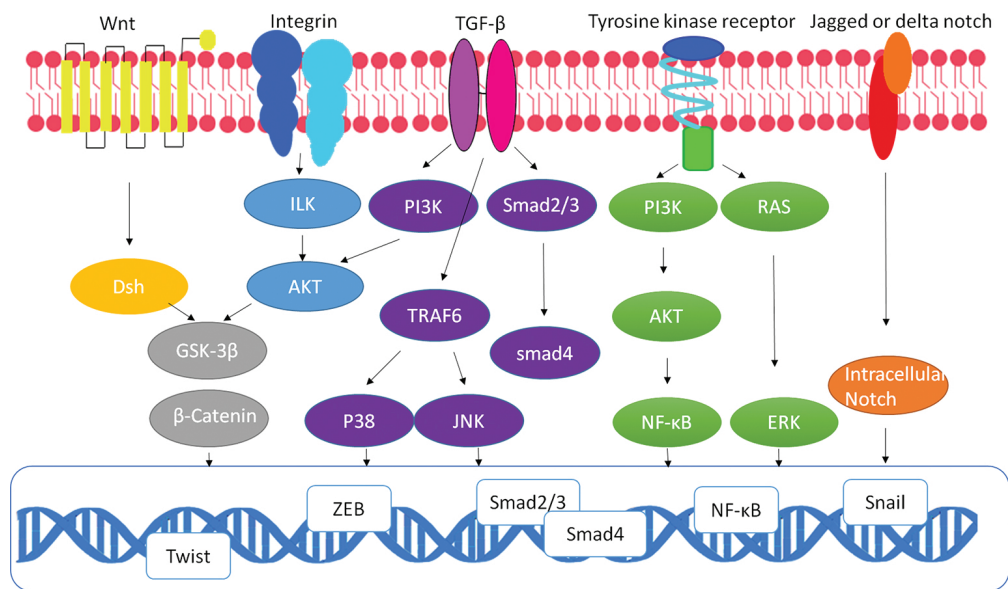


Figure 2. The main signaling pathways involved in EMT.

Other transcription factors also induce EMT and tumor invasiveness. The homeobox factor goosecoid induces EMT by activating mesenchymal genes and repressing epithelial markers [62]. TGF-β induces goosecoid in breast epithelial cells, and goosecoid is overexpressed in ductal breast carcinomas and atypical ductal hyperplasia [62]. **Figure 2** shows the main signaling pathways involved in EMT.

6. EMT in a clinical perspective

EMT significantly affects metastasis in cancers [63]. This process has attracted increasing attention because metastasis is vital in cancer recurrence and in death caused by cancer [64,

65]. In addition, understanding this process is important to determine medical diagnosis and treatment approach. Notably, sufficient information on biomarkers can lead to accurate forecast and precise therapeutic methods for metastases. Hence, proper diagnostic and therapeutic treatment for patients with early-stage cancer can promote good prognosis and prolong survival time to further improve the quality of life of patients [66, 67]. Furthermore, TBLR1, Sam68, SNAI1, Twist 2, etc. have been diagnosed markers or prognostic factors. This information is clinically important to predict survival and provide promising therapeutic targets for patients with early stage cancer [68, 69].

Sulforaphane or salinomycin treatment changes the stemness properties of cancer stem cells (CSCs), which may have been caused by the regulation of the expression of a special gene or protein. Sulforaphane downregulates Twist-1 and vimentin. By contrast, salinomycin treatment does not only significantly reduce vimentin level but also induce and upregulate E-cadherin expression in special cancer cell lines. Various reports have indicated a key role of E-cadherin in EMT. Thus, studying these cadherins can be a promising strategy [70, 71].

Epithelial cancer cells tend to differentiate to acquire invasive and stem cell-like properties. Thus, EMT regulators may function as therapeutic targets of cancer progression and recurrence. EMT-TFs, such as Twist, Snail, and ZEB, will be regarded as potent therapeutic targets for pharmacologic inhibition. Traditionally, EMT regulators are nearly impossible to target; however, evidence suggests that molecular links can offer the possibility for targeting regulation as a therapeutic intervention of EMT among metabolic adaptation, epigenetic alteration, and EMT [4, 72–74].

EMT significantly affects epigenome restructuring. Thus, epigenetic therapies can be used to realize the pharmacologic inhibition of EMT, which makes EMT sensitive to chemotherapy. In recent years, potential EMT inhibitors function as an effective chemotherapeutic sensitive agent, such as HDAC enzymes LBH589 [75]. In addition, histone demethylase LSD1 epigenetic modifiers offer important information on the survival of EMT by reducing invasiveness or inhibiting the transfer function. Thus, inhibiting LSD1 [76] may lead to improved survival because of the inhibition of invasiveness and metastasis [76].

Although epigenetic therapies exhibit considerable potential for clinical applications, the clinical application of epigenetic drugs remains ambiguous because of its unclear mechanism. The mechanism of epigenetic drugs should be investigated further. Therefore, the enhancement of specificity may solve the potential problem on the treatment of particular epigenetic targets in the future [74].

Drugs are likely to become anti-cancer drugs in clinical applications. Further study on the clinical trials of cancer has shown that metformin arouses attention as a promising anti-cancer agent [77, 78]. Metformin involves systemic effects, such as reducing insulin levels and acting on one-carbon metabolism. This metabolism will be explored with epigenetic alterations based on the connection between metabolism and the epigenetic state of cells [79].

Decitabine, which is a DNA methyltransferase (DNMT) inhibitor, suppresses the migration capacities of SDH-mutant cells. This inhibition is evidently displayed by succinate abnormal accumulation in epigenetic dysregulation and the resultant EMT. Mutant enzymes to directly

explore the inhibition approach for SDH- and FH-associated cancers are worthy to be explored [80].

7. Epigenetic modification and EMT

EMT is a comprehensive reprogramming during tumor development. This process involves metabolism, epigenetics, and differentiation. In a specific tumor microenvironment, EMT-dedifferentiated cells escape the primary tumor after they acquire migration and invasion capabilities, invade the surrounding tissues, enter into the blood or lymphatic vessels, and settle in distant organs. EMT converts differentiated epithelial cancer cells to an undifferentiated state, thereby expressing stem cell markers and acquiring stem cell-like functions. This process is reversible, and mesenchymal cells can differentiate into epithelial phenotypes. Thus, an important process is developed in the macroscopic metastases in different organs.

DNA methylation, histone modification, and microRNA are the three types of epigenetic modification. Many studies have shown that epigenetic modifications play a key role in tumor metastasis [81]. The downregulation of E-cadherin (a cell adhesion molecule) expression during EMT is an important feature. Therefore, the precise regulation of E-cadherin expression via epigenetic modifications is extremely important to the occurrence of EMT. Several EMT-related transcription factors are recruited as E-cadherin gene promoter, which inhibits transcription [4]. Studies have shown that E-cadherin can be inhibited by the synergy of various histone modification enzymes. The E-cadherin gene promoter is inhibited to different extents to silence E-cadherin expression [82].

The characteristics of EMT are reversible in the type of stem cells and malignant features. Tumor stem cells are unique undifferentiated cells, rather than increasing diffusion, compared with most differentiated epithelial cells. The diffusion of anabolic needs is related to the maintenance of an undifferentiated state, which may be metabolic alterations of the links between EMT and tumor [83]. A change in cell metabolism is an important sign of cancer. The best metabolic phenotype in tumor cell is characterized by the Warburg effect, which proves that ATP is not the only metabolite of tumor cells [84]. Further studies have shown that aerobic glycolysis can better satisfy the basic needs of cell division, known as the post-Warburg model. This process is not only associated with cancer and normal cell proliferation, but also inhibits mitotic cell differentiation. In the same inducers, bupar and inactivated tumor suppressor genes, even oxygen glycolysis capability increased in the EMT, can be attributed to cell undifferentiated state. The appropriate energy level is sufficient for biosynthesis precursor, balancing normal state, and maintaining an undifferentiated state.

Epigenetic modifications are complex, dynamic, and connected with the extracellular environment and nuclear transcription. Energy availability is extremely important. Energy-rich substances, such as carbohydrates and fats, in the human body translate into ATP, along with a large number of metabolites, such as glycolysis and fatty acid oxidation. These metabolites can also drive epigenetic modifications in gene expression. A change in the intermediate metabolites of EMT may not be simple. Metabolic reprogramming plays a role in the energy

crisis causation of cancer cells. This process determines the epigenetic state by modifying the undifferentiated state of the chromatin structure. Reprogrammed genes and the change in gene expression influence EMT markers and metabolic enzymes to overcome the local restriction to obtain energy in the distant tissues and organs. The microenvironment is significant for the EMT metastasis potential of cancer cells in reprogramming metabolism, epigenetics, and differentiation. Hepatic, epidermal, and fibroblast growth factors activate and maintain the EMT process. The cancer microenvironment growth factor activity generally regulates the interaction between metabolism and EMT to coordinate cell differentiation and metabolism.

The transfer process is the key to the reversibility of EMT based on our previous study, in which EMT regulation is mainly at the transcription level [4]. However, numerous molecular mechanisms cooperate to change the behavior of tumor cells. In particular, the role of the transcription regulation of EMT in the regulation and control of gene expression of the transcription of alternative splicing is extremely important. Spliceosome assembly has experienced gradual, composition, and structure changes required for normal maintenance. The correlation of alternative splicing tumor progression becomes apparent. In fact, all major cell biology deregulations of cancer are related to the changes in the alternative splicing of a specific gene profile. Modifying the splicing expression and activities supervised by SR and hnRNP provides the main source of changes in the stitching program observed in cancer cells [85].

Malignant EMT plays a key role during transfer, and the alternative splicing program affects the cell phenotype, including protein, cell adhesion, and cytoskeleton dynamics, influences tumor microenvironment, and controls tumor metastasis formation. Cancer gene mutations initiate processes, and epigenetic changes will be necessary to promote cancer. The change in the epigenome improves the transfer of cells.

In general, cells, which are affected by external environment signals, satisfy the internal requirements of nutrient and energy metabolism, as well as cooperate actively to promote the occurrence of EMT. Thus, tumor metabolic adaptations and EMT are different mechanisms for the same target cells to survive and grow. A close link and high correlation exist between metabolic reprogramming EMT and the similarity rule and fly mechanism.

8. Regulatory role of microRNAs in EMT

MicroRNAs (miRNAs) are expressed endogenously as small, non-coding RNAs that regulate various biological processes by modulating gene expression at the post-transcriptional level [86]. MiRNAs play an important role in controlling tumor growth and progression. Some miRNAs function as oncogenes and tumor suppressors. Moreover, miRNAs are also master regulators of EMT and dynamically regulate balance between EMT and MET.

The miR-200 family consists of five miRNA sequences, namely miR-200a, miR-200b, miR-200c, miR-141, and miR-429. Aggregated miRNAs that are expressed as two independent polycistronic pri-miRNA transcripts are as follows: miR-200a, miR-200b, and miR-429 (chromosome 1); and miR-200c and miR-141 (chromosome 12) [87].

The autocrine TGF- β /ZEB/miR-200 signaling regulatory networks that control epithelial and mesenchymal states change in the cells. ZEB1/2 and TGF- β exhibit strong correlation, and negative correlations are detected between TGF- β and miR-200, as well as between ZEB1/2 and miR-200, in invasive ductal carcinomas [88]. ZEB1/2 can induce EMT by inhibiting various epithelial genes [89].

Other miRNAs can directly target EMT transcription factors. MiR-205 in mammary cells maintains epithelial differentiation [90–92]. MiR-29b in prostate cancer inhibits metastasis by regulating the EMT signal. MiR-148a in hepatocellular carcinoma (HCC) cells can negatively regulate Met/Snail signaling and prevent EMT and metastasis [93]. Snail and miR-34 form another double feedback loop. EMT is induced by TGF- β , and an increase in Snail expression can be inhibited by miR-34. A novel miR-203/SNAI1 feedback loop has also been reported in breast cancer. These double feedback loops can enhance the balance between EMT activation and control of the two states of the cell (epithelial and mesenchymal). A novel EMT network that integrates the negative feedback loops, miR-203/Snai1, and miR-200/ZEB has been proposed recently as a control epithelial cell plasticity switches during differentiation and cancer [94]. The expression of miR-10b in metastatic breast cancer cells is shown to be induced by the transcription factor Twist, which binds directly to the putative promoter of miR-10b. Twist-induced miR-10b inhibits the translation of mRNA encoding homeobox D10, which results in an increased expression of RHOC, which is a well-characterized prometastatic gene [95].

Many miRNAs interfere with EMT by targeting the structures of cell components [96–98]. MiR-155 is the direct transcriptional target of TGF- β /Smad 4 signaling [99]. MiR-155 ectopic expression can reduce the RhoA (Ras homolog gene families, member A) protein, a small GTPase protein, to modulate the formation of tight junctions in the formation of stress fibers of the actin cytoskeleton during expression and destruction [100]. The activation of miR-31 in establishing metastases results in the regression of metastasis and the enhancement of the survival of patients. In addition, the induction of miR-31 can reduce the metastatic potential of cancer cells by targeting the RhoA [101]. The upregulation of miR-9 and direct repression of E-cadherin-1 (CDH1) in human breast cancer cells are involved in the regulation of cell-cell adhesion, migration, and the epithelial cell proliferation mechanism of calcium-dependent protein. CDH1 repression results in increased cell motility and invasion [102].

9. Inhibitors of EMT

Numerous tumor EMT inhibitors have been found in this study, and many of which are difficult to apply clinically because of stability and targeting issues. TGF- β is an important factor in regulating EMT.

Various EMT inhibitors have been found in the TGF- β signaling pathway. Thyroid transcription factor-1 is a protein encoded by the NKX2-1 gene in normal tissues that can inhibit the secretion of TGF- β , which increases the expression of E-cadherin in lung cancer cells.

MMPs are other key factors in the induction of EMT. The inhibitors of MMPs play an important role in blocking EMT development in tumor cells. Several MMP inhibitors, which have been tested in clinical trials, can prevent EMT in cell experiments in vitro and inhibit tumor progression in in vivo animal experiments. Orlistat Plymouth, one of the MMP inhibitors, has a relatively significant effect on non-small cell lung cancer, colorectal cancer, and glioma, both in in vitro cell experiments and in vivo animal experiments. Conducting combination chemotherapy with marimastat, captopril, and Fragmin exerts a certain effect on the treatment of advanced kidney cancer. The human body itself can also synthesize a special kind of MMP inhibitor, a tissue inhibitor of metalloproteinase (tissue inhibitors of metalloproteinases, TIMPs). TIMPs, which are produced using genetic engineering technology, can also be used as targeted drugs to treat tumors caused by the imbalance among MMPs and inhibit the progression of EMT in tumor cells.

Certain phytochemicals or food substances exhibit anti-cancer [103, 104] and anti-EMT properties [105]. For example, AIMs (anthocyanidins) and Morusin (a prenylated flavonoid), which are isolated from the fruits of *Vitis coignetiae* Pulliat (known as meoru in Korea) and the root bark of *Morus australis* (Moraceae), respectively, demonstrate anti-cancer activities by inhibiting EMT through the suppression of nuclear factor (NF)- κ B activity [106–108].

AIMs can inhibit NF- κ B in a dose-dependent manner, and MMP-9 (EMT marker) can be regulated by NF- κ B preferentially [109]. I κ B α phosphorylation and GSK-3 activity can also be suppressed by increasing the levels of AIMs [110]. Moreover, AIM downregulates mesenchymal markers, such as Vim1, N-cadherin, and SNAI, as well as upregulates epithelial markers, such as E-cadherin [110]. The morphological changes induced by TNF- α [110] are also inhibited by AIM. The suppression of the migratory and invasive properties of cervical cells by AIM has also been reported. Cervical cancer contains a heterogeneous population of cells called CSCs. CSCs are cells with chemotherapy- and radiotherapy-resistant properties and are involved in tumor recurrence, metastasis, and high mortality [111, 112]. Morusin, however, are reported to be cytotoxic to several cancer cell lines, including cervical cells. Morusin can inhibit migration and proliferation by inhibiting tumor sphere formation through the inhibition of the NF- κ B pathway.

Recent studies in our laboratory have found several EMT inhibitors, including tetracycline and some natural products. The gelatinase inhibitor doxycycline is the prototypical anti-tumor antibiotic. We have investigated the effects of doxycycline on the migration, invasion, and metastasis of human lung cancer cell lines and in a mouse model. We have also measured the effect of doxycycline on the transcription of EMT markers and used immunohistochemistry to determine whether EMT reversal is associated with doxycycline inhibition. Doxycycline dose-dependently inhibits the proliferation, migration, and invasion of NCI-H446 human small cell lung cancer cells. It also suppresses tumor growth from NCI-H446 and A549 lung cancer cell xenografts without altering body weight, inhibits Lewis lung carcinoma cell migration, and prolongs survival. The activities of the transcription factors Twist1/2, SNAI1/2, AP1, NF- κ B, and Stat3 are suppressed by doxycycline, which reverses EMT and inhibits signal transduction, thereby suppressing tumor growth and metastasis. Our data demonstrate functional targeting of transcription factors by doxycycline to reverse EMT and suppress tumor proliferation and

metastasis. Thus, doxycycline selectively targets malignant tumors and reduces their meta-static potential with less cytotoxicity in lung cancer patients.

Apigenin is a naturally occurring compound with anti-inflammatory, antioxidant, and anticancer properties. We have investigated the effects of apigenin on migration and metastasis in experimental HCC cell lines in vitro and in vivo. Apigenin dose-dependently inhibits the proliferation, migration, and invasion of PLC and Bel-7402 human HCC cells. It also suppresses tumor growth in PLC cell xenografts without altering body weight, thereby prolonging survival. Apigenin reduces Snail and NF- κ B expression, reversed increases in EMT marker levels, increases cellular adhesion, regulates actin polymerization and cell migration, and inhibits invasion and migration of HCC cells. Therefore, apigenin may inhibit EMT by inhibiting the NF- κ B/Snail pathway in human HCC. **Table 1** shows some EMT inhibitors found in the last 3 years.

Molecules	Tumor types	Pathway	References
Cyclin G2	Ovarian cancer	Wnt/ β -catenin	Bernaudo et al. [113]
FOXO3a	Prostate cancer	Wnt/ β -catenin	Liu et al. [114]
sFRP4	Head and neck squamous cell carcinoma	Wnt/ β -catenin	Warrier et al. [115]
sophocarpine	Hepatocellular carcinoma	AKT/GSK-3 β / β -catenin	Zhang et al. [116]
Bisdemethoxycurcumin	Lung cancer	TGF- β	Xu et al. [117]
Ski	Lung cancer	TGF- β	Yang et al. [118]
GRP78	Colon cancer	TGF- β	Zhang et al. [119]
Thymoquinone	Breast cancer	TGF- β	Rajput et al. [120]
BMP-7	Cholangiocarcinoma	TGF- β	Duangkumpha et al. [121]
NDRG2	Colon cancer	TGF- β	Shen et al. [122]
Myrtucommulone-A and thymoquinone	Bladder cancer and breast cancer	PI3K	Iskender et al. [123]
ING5	Breast cancer	PI3K	Zhao et al. [124]
α -Mangostin	Pancreatic cancer	PI3K	Xu et al. [125]
NVP-BEZ235	Ovarian cancer	PI3K	Lin et al. [126]
IL-320	Colon cancer	STAT3	Bak et al. [127]
Luteolin	Pancreatic cancer	STAT3	Huang et al. [128]
FTY720	Cholangiocarcinoma	STAT3	Lu et al. [129]
NDRG2	Breast cancer	STAT3	Kim et al. [130]

Table 1. Some EMT inhibitors.

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