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REVIEW

Mechanism and regulation of epithelial–mesenchymal transition in cancer

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Irene K Guttilla Reed

Department of Biology, University of Saint Joseph, West Hartford, CT, USA

Abstract: During development and the pathogenesis of certain diseases, including cancer, the epithelial–mesenchymal transition (EMT) program is activated. It is hypothesized that EMT plays a major role in tumor invasion and the establishment of distant metastases. Metastatic disease is responsible for the vast majority of cancer-related deaths, which provides a precedent for elucidating pathways that regulate EMT. EMT is defined as the transition of cells with an epithelial phenotype into cells with a mesenchymal phenotype through a series of genetic and environmental events. This leads to the repression of epithelial-associated markers, upregulation of mesenchymal-associated markers, a loss of cell polarity and adhesion, and increased cell motility and invasiveness. EMT is a reversible and dynamic process, and can be regulated by signals from the microenvironment such as inflammation, hypoxia, and growth factors or epigenetically via microRNAs. These signals modulate key EMT-associated transcription factors and effector proteins that control cellular phenotype and regulate tumor plasticity in response to changing conditions in the microenvironment and the progressive nature of cancer. Understanding the complex regulatory networks controlling EMT can provide insight into tumor progression and metastasis.

Keywords: EMT, metastasis, microRNA, transcription factor, growth factor, tumor progression

Introduction: epithelial–mesenchymal transition and its role in cancer plasticity

Cancer is a heterogeneous disease regulated by complex mechanisms that promote both tumor initiation and progression. However, metastatic disease still causes over 90% of cancer-related deaths regardless of diverse cancer phenotypes.[1] It is hypothesized that epithelial–mesenchymal transition (EMT) initiates the metastatic cascade. EMT is a normal cellular process that regulates embryogenesis and wound healing; however, it can be exploited during tumor progression to generate an invasive cellular phenotype.[2] EMT has primarily been characterized in carcinomas as well as in some mesenchymal tumors, such as sarcomas, gastrointestinal stromal tumors, and a subtype of glioblastomas.[3] Factors involved in initiating or maintaining the EMT program have been identified in a wide variety of carcinomas including breast, prostate, colorectal, head and neck, ovarian, lung, and more recently endometrial.[4,5]

In cancer, EMT is a multistep and reversible process defined by the loss of epithelial characteristics (such as cell–cell junctions, adhesion, and apical–basal polarity) and the gain of mesenchymal characteristics (such as increased motility, invasive properties, and a spindle-like morphology).[6,7] These phenotypic changes result from

Correspondence: Irene K Guttilla Reed
Department of Biology, University of Saint Joseph, 1678 Asylum Avenue, West Hartford, CT 06117, USA
Tel +1 860 231 5768
Fax +1 860 231 6716
Email ireed@usj.edu

the rearrangement of cytoskeletal proteins (particularly F-actin) due to increased mesenchymal markers. In addition to morphological changes and increased invasive potential, cells that have undergone EMT display increased resistance to apoptosis, attenuation of cell-cycle progression, and stem cell-like properties.[6,7] Initiation of the EMT cascade leads to local invasion, followed by the dissemination of circulating tumor cells (CTCs), and eventually the establishment of distant metastases. This process is dependent on the plasticity of EMT since evidence suggests that establishment of metastases is a consequence of mesenchymal–epithelial transition (MET).[8] Clinically detectable macrometastases are largely epithelial in nature, and recent studies suggest that the downregulation of EMT-promoting factors is crucial for the establishment and proliferation of metastatic colonies.[9]

Due to the dynamic and transient nature of EMT, clinical relevance of this process in metastasis has been debated.[10,11] However, strong evidence exists for the involvement of EMT regulatory mechanisms in various stages of tumor initiation, development, and progression. Further characterizing a partial-EMT phenotype may be crucial to understand the intricacies of the EMT program in vivo. Cells may reside in a state where they express newly acquired mesenchymal markers while still possessing epithelial markers as they transition from one phenotypic state to another. Indeed, circulating mesenchymal cells have been shown to work in concert with epithelial cells to protect them from anoikis, conferring a heterogeneous cell population with representation from both transition states.[12]

The aim of this review is to summarize the prominent regulatory mechanisms involved in activation and maintenance of the EMT program in cancer progression and metastasis. EMT is a complex and multilayered process that involves the coordination of transcription factors (TFs), effector proteins, and cellular regulators such as microRNAs. Adding to the complexity is the role of the microenvironment in facilitating EMT, as this process can be initiated by myriad signals such as hypoxia, growth factors, and inflammation. Elucidating the orchestration among these pathways will provide insight into the plastic and heterogeneous nature of tumor cells, and the cellular events leading to metastasis.

The EMT cascade and molecular players

Induction of EMT

A variety of microenvironmental cues can initiate the EMT program including growth factor signaling, hypoxia, and inflammatory pathways, and these pathways are not mutually exclusive. For example, the well-characterized EMT-inducing growth factor TGF-β (transforming growth factor beta) is directly activated by hypoxia via hypoxia-inducible factor 1 alpha (HIF1α).[13] HIF1α can also induce epigenetic regulation of EMT by transcriptionally targeting HDAC3, which cooperates with the EMT-associated TF Snail1 to mediate gene repression of epithelial-specific promoters.[14] EMT also occurs during wound healing; however, chronic inflammation at a particular site can lead to persistent EMT and fibrosis.[5] During wound healing, EMT is mediated by inflammatory immune cells and fibroblasts.[15] This process parallels EMT in cancer since tumor-associated macrophages and the tumor stroma have been shown to play a role in invasion and metastasis of breast cancer cells.[16] However, it should be noted that mesenchymal markers are also expressed in CTCs of both early and metastatic breast cancer patients regardless of hormone status and tumor grade, suggesting that EMT may also play a role in tumor initiation.[17] These upstream signals initiate EMT primarily through the direct activation or epigenetic regulation of EMT-inducing TFs,[6] which exert phenotypic effects on the cell by modulating downstream epithelial and mesenchymal effector molecules (Figure 1).

The EMT cascade

In order for tumor cells to escape from the primary site and travel to distant organs, they need to become more motile and degrade the basement membrane from the extracellular matrix. This step initiates local invasion and eventually leads to intravasation (cellular infiltration of endothelium and blood and/or lymphatic vessels), which generates CTCs (Figure 2).[9] A small subset of these CTCs may undergo extravasation and colonize micrometastases via MET (Figure 2).[9] Additional signals are required for these colonies to proliferate into macrometastases that can be clinically detected.[9] Traditionally, EMT is thought to play a role in these later stage events in tumor progression (local invasion and maintenance of CTCs); however, recent evidence suggests involvement of EMT in malignant transformation and tumor initiation.[9] Expression of the EMT-inducing TF Twist1 was detected in patients with atypical breast ductal hyperplasia, a very early-stage neoplastic disease.[18]

Induction of the EMT program is not governed by one singular cascade of molecular events. As a result, many studies have focused on characterization of various EMT markers in progressive stages of tumor development. Clusters of cells on the invasive front often display decreased levels of E-cadherin, a hallmark of the epithelial phenotype.[6]

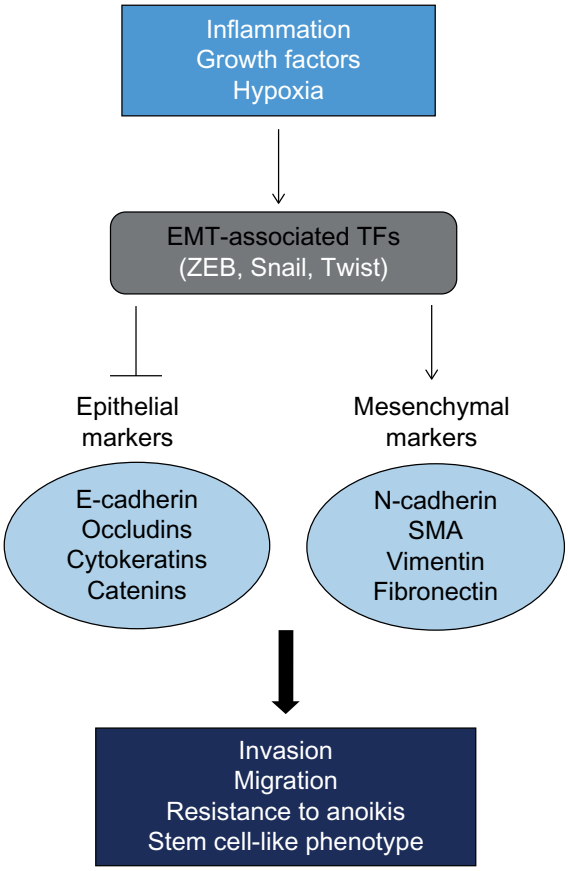

Figure 1 Overview of key molecular players in epithelial–mesenchymal transition.
Notes: EMT can be induced by a variety of mechanisms including inflammatory responses, hypoxia, and growth factors. Activation of EMT-associated transcription factors (TFs) leads to the repression of epithelial markers and activation of mesenchymal markers, which influences cellular phenotype by increasing invasion, migration, and resistance to anoikis. EMT is also associated with a stem cell-like phenotype, which enhances tumor-initiating capacity and chemoresistance.
Abbreviations: EMT, epithelial–mesenchymal transition; SMA, smooth muscle actin.

Similarly, high-grade tumors associated with a poor prognosis often display molecular signatures associated with the EMT program; however, these signatures can vary between tumor type, and do not always correlate with disease-free survival.[19–22] Nevertheless, Snail1/2 and Twist-induced EMT has been associated with an increased population of CTCs that maintain mesenchymal markers and characteristics.[23,24] While the functional relevance of the expression of mesenchymal markers such as vimentin is unclear, recent evidence suggests that maintenance of the mesenchymal phenotype may protect CTCs from natural killer cells, increasing the probability that they will survive circulation and continue onto extravasation.[25]

The final step in metastasis is colonization, which is dependent on both genetic and environmental cues. Metastatic lesions are commonly epithelial in nature, suggesting that the reversion from a mesenchymal phenotype to an epithelial phenotype (MET) is favorable for establishing a metastatic niche. Tsai et al demonstrated that although Twist1 activation was required for the early steps of metastasis (invasion and intravasation), loss of this EMT-inducing signal was essential for proliferation and colonization.[23] However, the exact mechanisms governing this phenomenon remain unclear. One explanation is the ability of Twist1 to downregulate E-cadherin,[26] since the restoration of E-cadherin can induce MET in several systems.[27] Alternatively, microRNAs have also been shown to regulate MET by targeting (and repressing) EMT TFs such as ZEB1, ZEB2, and Snail2, leading to an increase in the expression of epithelial markers.[28] Interestingly, MET and E-cadherin-mediated cell–cell adhesion are crucial for reprogramming fibroblasts into induced-pluripotent stem cells,[29,30] which eludes to the parallel between EMT and a stem cell-like phenotype in both normal and neoplastic cells.[31] Though it has been demonstrated that EMT generates CTCs, it is plausible that this process could also be responsible for local recurrence if these cells do not invade and migrate but retain cancer stem cell-like (tumor-initiating) properties.

Molecular players

EMT is characterized by a loss of epithelial markers and a gain of mesenchymal markers (Figure 1). In concert, these effector proteins modulate cytoskeletal rearrangement leading to changes in cellular adhesion and motility. Epithelial markers include cell junction proteins (ie, E-cadherin and claudins), tight junction proteins (ie, ZO and occludins), cytokeratins, and catenins. E-cadherin in particular is a hallmark of EMT, and interacts with intercellular adhesion networks to maintain cell polarity, differentiation, migration, and signaling in proliferation pathways. The downregulation of E-cadherin is sufficient to induce EMT in some, but not in all experimental cancer models.[8,32] E-cadherin loss can be due to inactivating gene mutations,[33] however, most often downregulation is due to epigenetic or transcriptional silencing.[3,34,35] A study by Onder et al suggests that even though loss of E-cadherin protein expression is sufficient for metastasis, active β-catenin is necessary for invasion of cells in culture and an experimental metastasis model.[36] Together, these findings suggest that even though E-cadherin is an integral player in maintaining the epithelial phenotype, it is likely that additional factors act in concert with the loss of this protein to induce a full EMT.

Upregulation of mesenchymal markers such as vimentin, fibronectin, N-cadherin, and smooth muscle actin leads to the detachment of tumor cells, proteolytic digestion of the

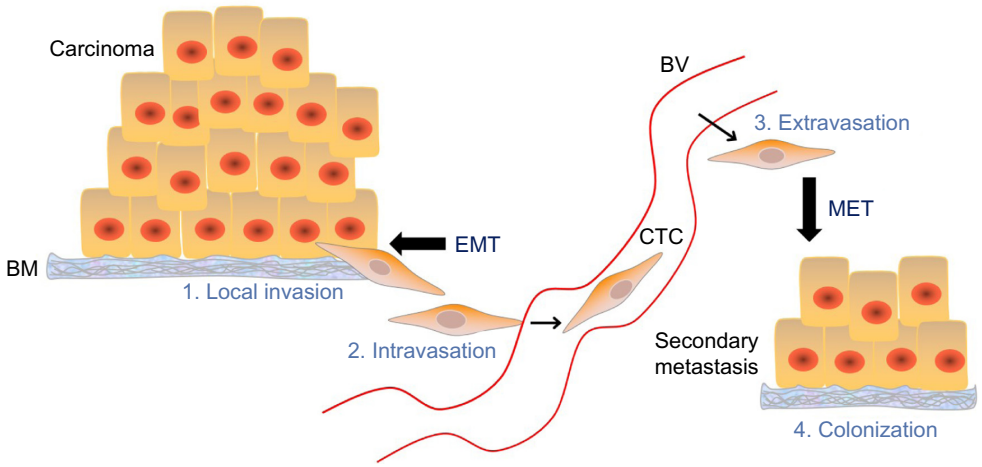

Figure 2 The metastatic cascade.
Notes: Cells from epithelial tumors (carcinomas) can undergo EMT to generate mesenchymal cells with more motile and invasive properties (local invasion [1]). These invasive cells enter the bloodstream or lymphatic system and become circulating tumor cells (intravasation [2]), which can migrate into distant organ tissues with the appropriate cellular signals (extravasation [3]). Cues from the microenvironment then induce an MET to establish secondary micrometastases (colonization [4]).
Abbreviations: BM, basement membrane; CTC, circulating tumor cell; BV, blood vessel; EMT, epithelial–mesenchymal transition; MET, mesenchymal–epithelial transition.

basement membrane, and the generation of CTCs (Figure 1). One of the most well-characterized mesenchymal effector proteins is vimentin, a type III intermediate filament that plays a role in cell attachment and migration by controlling cytoskeletal dynamics.[37] Vimentin is highly expressed in high-grade ductal carcinomas and is a predictive biomarker for lymph-node metastasis and poor prognosis in colorectal cancer.[20,38,39] Though it is unclear whether vimentin expression is sufficient to induce a mesenchymal phenotype, it has been shown to correlate with increased Snail2 (Slug) expression.[40] A related factor, Snail1, has also been shown to increase matrix metalloproteinase-2 (MMP-2) expression by binding to its promoter in squamous cell carcinoma.[41] MMPs are proteolytic enzymes involved in remodeling the extracellular matrix and dismantling the epithelial membrane, and several MMPs have been implicated in the EMT cascade including MMP-2, MMP-3, and MMP-9.[42] Additional genes such as ETS1, FLT1, VSIG, stremelysin-3, FOXC2, HOXB7, and EMT-inducing TFs such as Twist, ZEB1/2, Snail1/2 are also expressed at the invasive front.[42] Many of these proteins are downstream effector molecules of various signaling pathways, including Wnt/β-catenin signaling, Notch signaling, and the Sonic Hedgehog pathway.[42]

Transcriptional regulators of EMT

The EMT program can be regulated by a multitude of TFs; however, three families are primarily responsible for the onset and maintenance of EMT. Members of the Snail, ZEB, and Twist families of TFs are integral to the regulation of embryonic development, and a comprehensive review has shown that these factors are elevated in a wide variety of invasive tumors.[43] ZEB, Snail, and Twist TFs facilitate EMT by downregulating epithelial genes (such as E-cadherin) and upregulating mesenchymal genes.[43] Though these TFs are not typically expressed in normal epithelium, introduction of ZEB, Snail, Twist, or the homeobox TF Goosecoid induces EMT in human mammary epithelial cells.[6] Recent studies have also shown that the majority of these TFs can regulate other hallmarks of cancer such as angiogenesis induction, apoptosis/anoikis resistance, and immortalization in addition to invasion and metastasis.[43]

Snail1 and Snail2 (also known as Slug) are zinc finger TFs that can bind directly to the promoter of E-cadherin. Though these two factors do have overlapping functions, they are capable of interacting with different target genes and can be activated via independent mechanisms. For instance, Snail2 also inhibits epithelial desmosomal markers such as desmoplakin and desmoglein.[44] In addition to the E-cadherin repressing abilities of Snail1, this factor also enhances invasive and angiogenic properties in vitro and in vivo, presumably through the activation of vimentin, fibronectin, and MMPs.[45,46] These TFs also interact with several signaling pathways involved in the induction and maintenance of EMT including Wnt/β-catenin, serine/threonine receptor signaling, and the PI3K/AKT/mTOR axis.[47] Both Snail1 and Snail2 are overexpressed in a variety of cancers including breast, lung, ovarian, pancreatic, colorectal, and esophageal,[43] and Snail1 is also associated with breast cancer recurrence.[48] Moody et al showed that Snail1 was sufficient to promote mammary

tumor recurrence in an inducible HER2/Neu mouse model, and high levels of Snail1 predicted decreased relapse-free survival in breast cancer patients.[48] Interestingly, the recurrent tumors generated displayed a mesenchymal as opposed to an epithelial phenotype, suggesting that the mechanisms for recurrence and metastasis are independent. It is possible that in addition to generating CTCs, EMT may also be responsible for local recurrence due to the failure of mesenchymal cells to migrate and invade surrounding tissue. Indeed, tumor cells surviving after hormonal and chemotherapeutic treatments in breast cancer patients displayed an EMT gene signature and tumor-initiating properties.[49]

ZEB1 and ZEB2 (also known as SIP1) are also zinc finger TFs that directly bind to and repress E-cadherin, and induce invasion and metastasis in vitro and in animal models.[50] These TFs can be activated by multiple EMT-inducing signals including TGF-β, hypoxia, and inflammatory cytokines.[51] Expression and repression of ZEB factors results in a rapid EMT and MET, respectively. These factors are also regulated by the miR-200 family of microRNAs, and reciprocally repress the expression of miR-200.[52] This double negative feedback loop between ZEB factors and miR-200 further exemplifies the dynamic nature of EMT control, and suggests a mechanism for plasticity depending on external signals. Recent studies have also shown that hypoxia in glioblastoma cells induced ZEB1 and fibronectin (but not Snail1, Snail2, or Twist) and conferred a mesenchymal phenotype, and the invasive phenotype of these cells was inhibited by siRNA targeting ZEB1.[53] This effect is directly mediated by HIF1α, which binds to the ZEB1 promoter.[54] In addition to repressing E-cadherin, ZEB2 can induce several mesenchymal genes, and specifically transcriptionally upregulates vimentin.[55]

Twist1 and Twist2 are basic helix–loop–helix TFs that do not bind to the promoter of E-cadherin but decrease its transcription indirectly.[26] Twist1 is strongly associated with the metastatic cascade, and is upregulated in cells that can invade, intravasate, extravasate, and metastasize, but is not required for primary tumor formation.[26] Inhibition of Twist1 significantly decreased the formation of metastatic nodules in a mouse mammary carcinoma model, and elevated Twist1 expression was strongly associated with invasive lobular breast carcinomas in patients (in contrast to ductal carcinomas or normal tissue).[26] Furthermore, the loss of FOXF2, which directly represses Twist1, promotes metastasis in triple negative breast cancer.[56] The expression of Twist can also induce EMT in Madin-Darby canine kidney and human mammary epithelial cells, leading to decreased E-cadherin and α, β, and γ-catenin expression, and elevated vimentin, N-cadherin, and smooth muscle actin expression coupled with increased invasion in vitro.[26]

Though members of the ZEB, Snail, and Twist families of TFs are considered master regulators of EMT, little is known about the hierarchy of these factors since they are often studied individually or in limited groups.[6] However, redundancy and crosstalk are prevalent within this transcriptional network. For example, the expression of both ZEB1 and ZEB2 is regulated by Snail1 in certain contexts, and Snail2 activates ZEB1 by directly binding to its promoter.[57,58] Snail1 also increases the stability of Twist1, which then leads to the activation of Snail2.[59] These interactions are likely context dependent and suggest spatiotemporal regulation of these factors. Further elucidation of these pathways will help to establish functional relationships among these factors in the context of EMT, and may identify specific regulatory mechanisms driving the invasion and metastasis of cancer subtypes.

Role of microRNAs in EMT

MicroRNAs are small, 21–24 nucleotide single-stranded RNA molecules that negatively regulate target messenger RNA (mRNA) transcripts by direct sequence interaction and subsequent alteration of mRNA stability or translation.[60] The mode of microRNA-directed mRNA silencing appears to be tissue specific, and many microRNAs work in conjunction to fine tune protein expression on a global level.[61] MicroRNAs are involved in the regulation of many normal biological processes, such as development, differentiation, and stem cell maintenance.[60] However, dysregulation of microRNAs has been shown to contribute to many diseases, including cancer.[60] Various microRNAs have been shown to play a role in the regulation of EMT either through repression of EMT-inducing TFs or genes involved in the maintenance of the epithelial phenotype (Table 1). These pathways are further complicated by reciprocal feedback loops between microRNAs and TFs, or the induction/repression of microRNAs by EMT regulators such as TGF-β, hypoxia, and p53 (Table 2).

The miR-200 and ZEB1/2 feedback loop

The miR-200 microRNA family consists of miR-200a, miR-200b, miR-200c, miR-141, and miR-429.[62] These microRNAs work in concert to repress EMT by targeting ZEB1 and ZEB2, which are direct repressors of E-cadherin. ZEB1 and ZEB2 also transcriptionally repress miR-200c in a double negative feedback loop, facilitating the maintenance of a mesenchymal state (Figure 3).[63] Other targets of the miR-200 family

Table 1 Select microRNAs that regulate EMT in cancer

MicroRNA	Target(s)	Effect on EMT	Cancer type
miR-9	E-cadherin	Pro-EMT	Breast[86]
miR-10b	HOXD10	Pro-metastatic	Breast[87]
miR-15a/16-1	AP4	Anti-EMT	Colorectal[73]
miR-21	PTEN,[88,89] PDCD4,[90] TIAM1[91]	Pro-EMT; pro-metastatic	Colorectal,[90,91] skin,[88] breast[89]
miR-27	APC	Pro-EMT	Gastric[92]
miR-30a	Snail1	Anti-EMT	Lung[93]
miR-31	ZEB1, HDAC2,[94] CDK2,[94] SATB2,[95] TIAM1[91]	Either anti-EMT or pro-EMT depending on cancer type	Liver,[94] colorectal[91,95]
miR-34	Snail1, BMI-1, CD33, CD133	Anti-EMT	Colorectal[72]
miR-93	TGF-βR2	Anti-EMT	Breast[96]
miR-100	SMARCA5	Pro-EMT	Breast[97]
miR-101	ZEB1,[98] EZH2,[99,100] HMGA2[101]	Anti-EMT	Prostate,[99] liver,[98,100] pancreatic[101]
miR-106b	Twist1	Anti-EMT	Endometrial[102]
miR-124	Snail2	Anti-EMT	Prostate[103]
miR-138	ZEB2,[104] Vimentin,[104] EZH2,[104] Twist2[105]	Anti-EMT	Skin,[104] colorectal[105]
miR-139	ZEB1, ZEB2	Anti-EMT	Liver[106]
miR-153	ZEB2, SET7	Anti-EMT	Ovarian[107]
miR-155	RhoA	Pro-EMT	Breast[108]
miR-192	ZEB2	Anti-EMT	Liver[109]
miR-194	BMI-1	Anti-EMT	Endometrial[110]
miR-200	ZEB1,[62] ZEB2,[62,111] BMI-1,[63,65] moesin,[64] fibronectin,[64] TrkB[64]	Anti-EMT	Breast,[62,64,65] pancreatic,[63] endometrial,[64] gastric[111]
miR-203	BMI-1,[63] Snail1[71]	Anti-EMT	Pancreatic,[63] breast[71]
miR-204	Snail2,[112] Sirt1[113]	Anti-EMT	Liver,[112] bone[113]
miR-205	ZEB1, ZEB2	Anti-EMT	Breast[62]
miR-221/222	ERα,[81] p27(Kip1), p57, ATXN1,[114] TRPS1[115]	Pro-EMT	Breast[81,114,115]
miR-300	Twist1	Anti-EMT	Head and neck, breast[116]
miR-338-3p	ZEB2, MACC1	Anti-EMT	Gastric[117]
miR-506	Vimentin,[118] Snail2[118,119]	Anti-EMT	Breast,[118] gastric[119]

Abbreviation: EMT, epithelial–mesenchymal transition.

include moesin, fibronectin, and TrkB which are involved in cytoskeletal reorganization, cell motility, and resistance to anoikis, respectively, and restoration of miR-200c suppresses anoikis resistance in breast and endometrial cancer cell lines.[64] Downregulation of miR-200 family members has been observed in invasive breast cancers that lack E-cadherin and present with a mesenchymal phenotype.[62] MiR-200c also suppresses stem cell function and tumor initiating capacity by targeting BMI-1, which plays a role in the maintenance of EMT by targeting the PI3K/Akt inhibitor PTEN, promoting the activation of Snail.[65]

Table 2 Regulators of EMT-associated microRNAs

miR-10b	Upregulated by Twist1[87]
miR-21	Upregulated by TGF-β[120]
miR-31	Upregulated by TGF-β[120]
miR-34	Upregulated by p53, inhibited by Snail1 and ZEB1,[72]
miR-103/107	Upregulated by hypoxia[121]
miR-124	Downregulated by TGF-α[103]
miR-155	Regulated by TGF-β/Smad pathway[108]
miR-181a	Upregulated by TGF-β in metastatic cells[122]
miR-200c	Inhibited by ZEB1,[63] upregulated by p53[123]
miR-203	Inhibited by ZEB1[63] and Snail1[71]
miR-210	Upregulated by hypoxia[124]
miR-221/222	Upregulated by hypoxia[115]
miR-506	Downregulated by NFκB[118]

Abbreviations: EMT, epithelial–mesenchymal transition; TGF, transforming growth factor.

Interestingly, recent studies have shown that the miR-200 family members can be overexpressed in certain cancer types such as endometrial, pancreatic, and ovarian, and higher expression levels are associated with a poor prognosis.[66] Though this seems contradictory to the maintenance of a mesenchymal state by a miR-200/ZEB feedback loop, re-expression of miR-200 may be required for the induction of MET and colonization. Indeed, metastatic mouse 4T1 cells displayed high levels of miR-200 as did the metastatic lesions they formed, and the overexpression of miR-200 in cells that were previously unable to colonize led to the formation of lung metastases.[67] This observation is further supported by miR-200 regulation of Sec23a, which mediates the secretion of metastasis-suppressing proteins.[68] A recent study by Madhavan et al demonstrated that expression of miR-200 family members, especially miR-200b, could be utilized as a

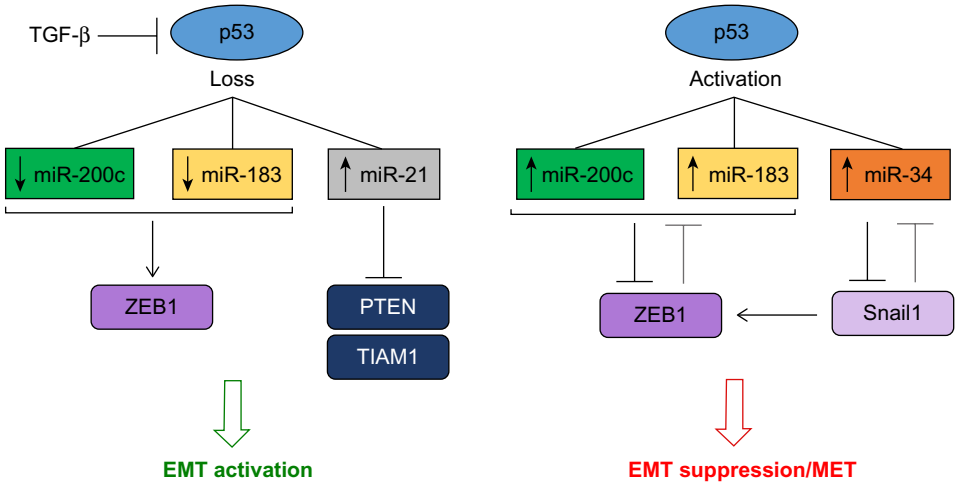

Figure 3 The interaction of p53 and microRNAs controls regulatory networks that modulate EMT.
Notes: Activation of p53 targets several microRNAs, including miR-200c, miR-183, and miR-34. Upregulation of these microRNAs suppresses EMT-inducing transcription factors such as ZEB and Snail, leading to suppression of EMT or activation of MET pathways. These transcription factors can also downregulate their targeting microRNAs in double negative feedback loops. Loss of p53 results in downregulation of miR-200c and miR-183, as well as upregulation of miR-21 which targets PTEN and the pro-metastatic gene T-cell lymphoma invasion and metastasis 1 (TIAM1). Therefore, loss of p53 through TGF-β induction, mutations, or epigenetic silencing can lead to the activation of the EMT program.
Abbreviations: EMT, epithelial–mesenchymal transition; MET, mesenchymal–epithelial transition; TGF-β, transforming growth factor beta.

prognostic marker for CTCs. Circulating miR-200b levels predicted over 80% of CTC-positive patients.[69] These data suggest that the miR-200/ZEB feedback loop acts as a fine tuning mechanism to switch cells between epithelial and mesenchymal states depending on the microenvironment. While downregulation of miR-200 may be initially required for local invasion, it appears that this microRNA needs to be reactivated in order for cells to successfully colonize and generate metastases in other tissues.

Regulation of the miR-200 family has primarily focused on the role of ZEB1/2; however, other factors have recently emerged that may modulate this microRNA in the context of EMT. Members of the miR-200 family are regulated by p53, and loss of p53 function is associated with the induction of EMT and the associated stem cell-like phenotype (Figure 3).[6] Growth factors such as TGF-β and platelet-derived growth factor (PDGF)-D also repress miR-200 via indirect or direct mechanisms (ie, methylation of the miR-200 promoter in the case of TGF-β).[70] Stable overexpression of PDGF-D in prostate cancer cells as well as treatment with pure active PDGF-D resulted in decreased levels of miR-200 and the induction of EMT; however, it is unclear whether this growth factor acts directly on miR-200 transcription.[28]

Additional regulatory feedback loops

Following the discovery of the miR-200/ZEB feedback loop, other microRNA/TF relationships were also uncovered. Two microRNAs, miR-34 and miR-203, directly target and inhibit Snail1, and conversely Snail inhibits the transcription of these microRNAs.[71,72] Like ZEB, Snail is also associated with stemness markers, and regulates the expression BMI-1, CD33, and CD133 in colorectal cancer cells. Activation of p53 can also regulate miR-34 expression, and the loss of miR-34 is required for TGF-β-induced EMT.[72] Considering that TGF-β negatively regulates p53, these recent studies provide a mechanism for EMT activation via p53 loss as a result of TGF-β signaling and the silencing of miR-34, allowing for the expression of EMT-promoting TFs such as Snail1 (Figure 3). p53 also induces the expression of miR-15a/16-1, which represses the EMT-inducing TF AP4 in colorectal cancer.[73] The interaction of AP4 with other characterized TFs such ZEB, Snail, and Twist (if any) is currently unknown, however should be explored further to elucidate the p53-microRNA-EMT cascade. These complex networks bring into question the tissue specificity of these relationships, as well as the relevance of these regulatory axes during normal development.

Small molecules that modulate EMT

TGF-β

TGF-β is a well-characterized growth factor that induces EMT in a variety of cellular contexts, including cancer. Stimulation of TGF-β production can occur via hypoxia, autocrine (produced by the tumor itself), and/or paracrine (produced by the tumor stroma) mechanisms.[42] TGF-β exhibits a dualistic nature

during tumor progression, acting as a tumor suppressor in early stages of tumorigenesis by inhibiting cell-cycle progression or apoptosis, and an oncogenic growth factor later in tumor development during metastatic events.[42] TGF-β binds to its receptor (TGF-βRII) and activates multiple pathways, including Ras-MAPK and Smad-dependent signaling pathways. Activation of the Ras-MAPK pathway induces expression of Snail1 and Snail2, leading to the repression of E-cadherin.[42] In brief, Smad signaling results in nuclear translocation of an activated Smad2/3 and Smad4 complex, which induces the transcription of key EMT target genes such as ZEB1.[42] TGF-β can also exert gene expression changes through activation of the Notch and Wnt pathways, which lead to EMT induction via degradation of intracellular catenins, Snail1 and Snail2 activation, and expression of mesenchymal markers such as vimentin.[42] TGF-β-induced EMT has also been implicated in chemoresistance, specifically of platinum therapies in ovarian cancer and tamoxifen in breast cancer.[74]

Other growth factors

In addition to TGF-β, a variety of other growth factors have been shown to play a role in the induction or maintenance of EMT by regulating TFs or effector proteins. Relevant factors include epidermal growth factor (EGF), fibroblast growth factor, hepatocyte growth factor, PDGF, and insulin-like growth factor. Many of these growth factors are present in normal epithelium and contribute to the differentiation and maintenance of the epithelial phenotype. However, aberrant signaling of these factors in cancer can lead to activation of the EMT cascade. For example, EGF signaling can disrupt desmosome and adherins junctions, which can lead to the dissociation of tumor cells and increased motility via upregulation of MMP-2 and MMP-9.[75] Both EGF and fibroblast growth factor can activate Snail through independent pathways; however, mesenchymal cells can also attenuate their dependence on growth factors such as EGF by upregulating other growth factor receptors such as PDGFR.[76] In prostate cancer cells, high levels of PDGF-D resulted in loss of E-cadherin and zonula occludins as well as a gain of vimentin expression and rapid tumor growth in immunodeficient mice.[77] Insulin-like growth factor can also act on EMT effector molecules such as β-catenin by sequestering and degrading E-cadherin, leading to the nuclear relocation of β-catenin and activation of target genes.[78]

Interleukins

Inflammatory cytokines also modulate EMT, presumably due to their role in wound healing and tumorigenesis. In breast cancer, IL-6 has been shown to induce EMT in epithelial-type cancer cell lines, via either ectopic expression or exposure in cell culture media.[79,80] Likewise, we and others have found that mammosphere culture conditions induce EMT and expression of IL-6 was concomitantly upregulated.[80,81] Exposure of breast cancer cells to IL-6 also generates breast cancer stem cells (characterized by a $CD44^{+}/CD24^{-/low}$ phenotype), which is consistent with activation of the EMT program. Some interleukins, such as IL-17, also act directly on EMT-inducing TFs such as ZEB1.[82] Exposure of lung cancer cells to IL-17 not only upregulated gene expression of ZEB1, but also facilitated its translocation to the nucleus.[82] The EMT-inducing effects of both IL-6 and another cytokine, IL-32, were dependent on Stat3 activation, which contributes to increased motile and invasive properties.[83,84]

Conclusion and closing remarks

The EMT program plays a significant role in tumor progression and metastasis, and is modulated by a wide variety of regulatory mechanisms including TFs, microRNAs, and growth factors. EMT should be viewed as a dynamic and reversible process that is dependent on the primary tumor and metastatic microenvironment. Complex regulatory mechanisms modulate the plasticity of EMT, allowing for this program to be fine-tuned in response to the cellular environment. This plasticity also poses challenges due to the adaptive nature of cancer cells as well as the role of EMT in normal cellular processes such as wound healing and inflammation. Drugs that promote an epithelial shift may consequently induce MET and colonization of metastases. Even if localized treatments could be administered, the mechanisms controlling the switch between an epithelial and mesenchymal state are complex. It has been proposed that the epithelial differentiation program is a default pathway for cells in a mesenchymal state, and that the absence of signals that sustain the mesenchymal phenotype may force cells to revert back to an epithelial state.[85] This hypothesis brings into question whether the best approach to modulate EMT is through targeting epithelial or mesenchymal effector molecules. Activation of the EMT program also increases stem-like characteristics and consequently chemoresistance in cell populations, and it is unclear whether cells in a permanent mesenchymal state are synonymous with cancer stem-like cells, or if these are distinct populations. Further elucidating EMT (and MET) promoting pathways in specific cancer types and clinical samples will provide a stronger framework for tissue specificity and the role of the microenvironment in modulating the EMT cascade.

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