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REVIEWS

Review

Epithelial-to-Mesenchymal Transition: Epigenetic Reprogramming Driving Cellular Plasticity

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Epithelial-to-mesenchymal transition (EMT) is a process in which epithelial cells lose their junctions and polarity to gain a motile mesenchymal phenotype. EMT is essential during embryogenesis and adult physiological processes like wound healing, but is aberrantly activated in pathological conditions like fibrosis and cancer. A series of transcription factors (EMT-inducing transcription factor; EMT-TF) regulate the induction of EMT by repressing the transcription of epithelial genes while activating mesenchymal genes through mechanisms still debated. The nuclear interaction of EMT-TFs with larger protein complexes involved in epigenetic genome modulation has attracted recent attention to explain functions of EMT-TFs during reprogramming and cellular differentiation. In this review, we discuss recent advances in understanding the interplay between epigenetic regulators and EMT transcription factors and how these findings could be used to establish new therapeutic approaches to tackle EMT-related diseases.

Trends

EMT is a reversible and plastic process that plays pivotal roles in multiple physiological and pathological conditions.

EMT is associated with vast epigenetic changes and its outcome is influenced by the epigenetic landscape of the cell of origin.

The expression of EMT transcription factors (EMT-TFs), the core regulators of EMT machinery, is altered upon forced epigenetic modifications.

Multiple physical interactions have been demonstrated between EMT-TFs and epigenetic modifiers, which partly can explain their roles in cell fate determinations.

Targeting the epigenetic network to block EMT in disease progression appears a novel promising strategy for EMT-related pathologies.

Epithelial-to-Mesenchymal Transition Controlling Cellular Plasticity in Development and Disease

Epithelial-to-mesenchymal transition (EMT) evolved as a process by which the middle primary germ layer, the mesoderm, was established. This allowed higher tissue complexity by enabling the generation of three **blastic layers** [1] (see Glossary). Upon EMT, epithelial cells undergo a transition, resulting in the loss of **apicobasal polarization** and acquisition of migratory and invasive capabilities (Figure 1 and Box 1). EMT is considered a cellular reprogramming, orchestrated by the activation of a series of EMT-inducing transcription factors (EMT-TFs). The phenotypic changes that occur during the EMT involve the remodeling of typical epithelial **adhesive junctions** and cytoskeleton changes in a highly dynamic and in many cases reversible way. In particular, during embryogenesis, maintenance of reversible cellular plasticity is highly important. Different waves of EMT are essential for tissue development, while the reverse process mesenchymal-to-epithelial transition (MET) is important for final developmental cellular differentiation [2].

This plastic EMT program is indispensable for various physiological and pathological processes in adult organisms. For instance, EMT has been associated with heart regeneration after injury, wound healing in the skin and in the ovarian surface epithelium, cancer progression, and **fibrosis**. The EMT–MET balance has also been demonstrated to be essential for the acquisition

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of stem cell properties. During the initiation phase of fibroblast reprogramming into induced pluripotent stem cells, MET is required to generate intermediate cells with epithelial characteristics that under the influence of the **Yamanaka reprogramming factors** convert to fully pluripotent stem cells [3,4]. In addition, forced passage of luminal mammary gland cells through an EMT program is sufficient to convert these differentiated epithelial cells into mammary stem cells, which are able to reconstitute mammary ductal trees [5]. These studies indicate that cellular plasticity controlled by EMT regulatory mechanisms are essential for the fate of adult stem cells to keep or gain multipotency/**pluripotency**. In addition, EMT has been strongly linked with cancer progression, providing benign epithelial tumor cells with features such as cancer stemness, **anoikis** resistance, migration, invasion, and therapy resistance [2].

Recently, the dogma claiming that EMT is a strict switch from an epithelial phenotype to a mesenchymal one has been questioned with studies pointing to the existence of intermediate states that transiently harbor both epithelial and mesenchymal features (intermediate/partial-EMT) [6,7]. Intermediate EMT states were shown to be important for pathological conditions, such as kidney fibrosis. In these partial EMT conditions, cells acquire mesenchymal traits while retaining renal tubular epithelial characteristics contributing to fibrogenesis [8]. Variability in the degrees of EMT has also been described in cancer progression and this plasticity likely contributes to tumor heterogeneity. Whether intermediate EMT states are defined metastable states linked with particular functions during cancer progression or just a broad spectrum of transitional states is under debate and is in need of further experimental support. All together, these observations make it increasingly clear that cellular plasticity linked with EMT/MET represents multiple transitional states between stable epithelial and mesenchymal differentiation.

Molecular Regulation of EMT

EMT is induced by an interplay of soluble growth factors such as hepatocyte growth factor, members of the transforming growth factor (TGF; e.g., TGF-β) and fibroblast growth factor families, insulin growth factor (e.g., insulin growth factor 1), epidermal growth factor as well as extracellular matrix such as collagen or hypoxic conditions. These factors activate signaling pathways leading to either expression or post-transcriptional and post-translational modification of EMT transcription factors (EMT-TFs; Figure 1) [9,10]. Three main families of EMT-TFs have been described with the SNAI (SNAI1/Snail and SNAI2/Slug), ZEB (ZEB1 and ZEB2), and TWIST (TWIST1 and TWIST2) nuclear proteins, playing pivotal roles in the orchestration of EMT [11]. These TFs have been shown to interact with a variety of proteins involved in transcriptional regulation including proteins that function in **epigenetic** modification, forming together regulatory complexes. This cooperativity drives expression of EMT responsive genes such as *CDH1,* a tumor suppressor gene that encodes E-cadherin. E-cadherin is an essential component of the adherens junctions, recurrently mutated in various metastatic epithelial malignancies [12].

These EMT-TFs were originally identified by their ability to directly repress the paradigmatic epithelial marker gene, *CDH1*. In general, upon EMT induction, all EMT-TFs undergo modification, form complexes, and repress *CDH1* by binding a central core domain in its promoter containing multiple 5′-CACCTG-3′ E-boxes [13–16]. Forced expression of any one of these EMT-TFs in epithelial cancer cell lines is sufficient to initiate a partial or complete switch from an epithelial to a mesenchymal cell morphology associated with increased motility, invasive capacity, therapy resistance, and cancer stem cell characteristics [14,17,18]. It is noteworthy that although forced expression of a single EMT-TF can provoke comparable epithelial-to-mesenchymal morphological changes, under physiological conditions they mostly act in concert. Besides *CDH1*, a plethora of other epithelial genes have been demonstrated to be directly repressed by EMT-TFs, mainly genes involved in cellular adhesion, polarity, and

Glossary

Adhesive junctions: a global term referring to the different adhesive complexes important for cell–cell and cell–extracellular matrix contacts, including (from apical to basal) tight junctions, adherens junctions, desmosomes, and gap junctions. Focal adhesions are also a type of adhesive junctions connecting the cytoskeleton of the cell to the extracellular matrix.

Anoikis: programmed cell death process initiated in cells that lose their cell–extracellular matrix interactions (refers to the Greek word for 'homelessness'). Anoikis resistance or anchorage-independent growth is an important hallmark of metastatic cancer cells.

Apicobasal polarization: refers to the type of polarization found in epithelial cells, which contain an apical and basolateral side, caused by the asymmetrical distribution of cellular components and adhesive complexes. The apical side constitutes the upper part of the cell facing the lumen of organs, while the basolateral side is crucial in maintaining cell–cell and extracellular matrix contacts. The basolateral side is characterized by different adhesive complexes ordered in a very specific way.

Blastic layers: refers to the three primary germ layers formed during the gastrulation of the vertebrate/mammalian blastula. These germ layers are named endoderm, mesoderm, and ectoderm, and give rise to specific tissue types in the later stages of the embryonal development.

ceRNAs: RNAs that function as a decoy for other RNA molecules. Typically, lncRNAs can function as ceRNAs for certain miRNAs. By targeting miRNAs to their miRNA binding sites, the ceRNAs act as molecular sponges, thereby preventing the miRNAs from performing their function.

Chromatin-remodeling complexes: molecules responsible for the condensation or relaxation of chromatin, thereby repressing or activating gene expression, respectively. These complexes are composed of different functional elements combining ATPase-dependent chromatin-remodeling activity with post-translational histone-modification enzymes and

cytoskeletal (re)organization [19–21], while the expression of mesenchymal-related genes is upregulated. Based on the absence of functional E-boxes in the promoter regions of mesenchymal-induced genes and often the lack of physical binding of the EMT-TF to these gene loci, the correlated upregulation of mesenchymal marker genes was long considered as an indirect effect. Therefore, EMT-TFs were mainly described as transcriptional repressors. Recently, it was demonstrated that EMT-TFs can also act as transcriptional activators as part of a larger **transcriptional complex** together with other DNA binding factors, such as the **TEA domain (TEAD) transcription factors** [22]. In addition, passive transcriptional regulation by EMT-TFs via competition, displacement, or preventing other transcription factors that bind the same or overlapping E-box containing regulatory elements has been demonstrated in some cellular systems [23,24].

Despite numerous efforts to identify a common EMT-TF response gene set, it has become clear that the downstream effectors are strongly dependent on the cellular context and on the presence or absence of cell-specific coactivators/repressors [25,26]. The EMT-TF regulatory network is very complex and plastic as it is influenced at various levels by transcriptional regulation, mRNA stability, alternative splicing, post-translational modification that influences subcellular localization and protein stability, and the presence or absence of cell-specific cofactors [7,11,21]. Related to this, multiple **miRNA** feedback mechanisms have been found to play essential roles in balancing the expression levels of the EMT-TFs [27,28]. The complexity of the network and the variables in each cellular system result in widely diverse EMT phenotypes, as mentioned earlier as partial/intermediate EMT, making it challenging to pinpoint the essential components of the network and their roles in human disease. In the following section, we focus on the direct interplay between EMT-TFs and epigenetic enzymes.

EMT and Epigenetic Regulation

Because TF binding to genes is transient, epigenetic reprogramming provides a more stable/long-term regulation, while remaining reversible, which is critically important in the context of cellular plasticity. Several layers of epigenetic regulation have been described (Figure 1): (i) DNA methylation, (ii) histone modifications, and (iii) RNA interference. Each of these modifications involves tightly regulated specific machineries to maintain a cell/tissue-specific **epigenetic landscape**. EMT-TFs function cooperatively with epigenetic enzymes to regulate EMT downstream effector genes.

Epigenetic factors are activated in response to signals that regulate specific subsets of genes. TGF-β1, a potent EMT-inducing cytokine, leads to the activation of numerous signaling pathways such as the SMADs, phosphoinositide 3-kinase/Akt (PI3K/Akt), or mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK) pathways. Subsequently, these activated pathways lead to the phosphorylation of histones or epigenetic enzymes, affecting chromatin compaction, enzymatic activity, or epigenetic-remodeling complex recruitment to the chromatin (reviewed in [29,30]).

EMT and DNA Methylation

DNA methylation is a stable and heritable mark usually linked with gene repression and important for genomic imprinting, X-chromosome inactivation, and genome stability [31]. A methyl group is covalently attached to cytosine in a CpG-rich dinucleotide sequence (CpG island) by DNA methyltransferases (DNMTs) [31]. It is a plastic and reversible process, with active demethylation catalyzed by the ten-eleven translocation (TET) oxidases [32]. Methylated cytosines can be bound by methyl-CpG binding-domain proteins (MBDs), which recruit histone-modifying enzymes to further modulate the proximate chromatin environment.

structural proteins. Different chromatin-remodeling complexes have been described, such as NuRD, SWI/SNF, PRC2.

Epigenetic: a term that originally defined heritable gene modification independent of mutations. It now encompasses all mechanisms that lead to changes in gene regulation without affecting the DNA sequence (e.g., DNA methylation, histone modifications).

Epigenetic landscape: a general term referring to the whole of epigenetic marks present on the genome of a specific cell type making up its identity. The term was initially used by Waddington to visualize the fact that cells with the same genomic composition can give rise to different outcome depending on the conditions.

Fibrosis: a process in which fibrous connective tissue is generated in an organ as a response to damage or injury. Although physiologically important during wound healing, fibrosis can have pathological consequences when the excessive amount of connective tissue leads to organ failure.

Heterochromatin/Homochromatin: heterochromatin refers to densely packed DNA, which is less accessible to factors of transcriptional complexes leading to gene repression. By contrast, homochromatin, or euchromatin, is a more relaxed DNA state, making more accessible regulatory sequences.

Hypermethylation/Hypomethylation: during DNA methylation, the cytosine base of DNA in CpG islands is methylated. Hypomethylation refers to a state in which CpG islands, which are typically methylated, are less or unmethylated. It can both refer to a local effect or to global hypomethylation on a genome level. In any case, hypomethylation is typically linked to active gene expression. Hypermethylation refers to the contrary state in which CpG islands are more methylated locally or globally, mostly linked to gene repression.

lncRNAs: these RNAs share similar function as miRNA but are 200 bp in length. In the genome, lncRNAs are located in introns, intergenic regions, or in antisense of a protein-coding gene. lncRNA mode of action includes direct transcriptional regulation, interference with RNA processing, modulation of miRNA expression, and

During EMT, the DNA methylation landscape is altered in a consistent way. Focal **hypermethylation** of the CpG islands in the *CDH1* promoter is seen in various cancer cell lines and is associated with recruitment of DNMT1 to these sites via its interaction with the EMT-TFs. DNMT1 interacts with SNAI proteins through their SNAG domain [33,34], and with ZEBs through the SMAD-binding domain [35] (Figure 2). Most of these studies focused only on the methylation status and expression of *CDH1*. The global effect of the protein–protein interaction between EMT-TF and the methyltransferase enzymes during EMT and cellular plasticity remains elusive. However, a recent study showed that ZEB2 is essential for the cellular plasticity and multilineage differentiation of embryonic stem cells (ESCs) potentially through a mechanism that controls the cellular methylation state [36]. Under differentiating conditions, *Zeb2*-null ESCs were stuck in an epiblastlike cell fate, failed to silence their pluripotency program, and associated with retained expression of *Tet1* and a naive methylome state. Interestingly, *Tet1* knockdown in *Zeb2*-null ESCs rescued their ability to exit the pluripotency state and enter lineage commitment. These experimental data indicate that the functions of the EMT-TFs are tightly connected with changes in DNA methylation, controlling cellular plasticity.

Not only do EMT-TFs regulate the methylation state of certain genes, their expression is also regulated by DNA methylation (Figure 2). The methylation status of *TWIST1/2* promoters is inversely correlated with the expression levels of *TWIST1/2* in colorectal cancer. Low-grade budding cases that are negative/low for the TWIST1/2 protein show strong hypermethylation of their promoters. Inversely, no *TWIST* promoter CpG methylation was detected in TWIST1/2-positive high-grade budding cases and associated with other adverse features like lymphatic vessel invasion, lymph node metastasis, and overall survival. These data suggest that *TWIST* promoter methylation could serve as an alternative prognostic marker for colorectal cancer patients [37]. Similarly, *ZEB2* expression has been shown to be regulated by promoter methylation in pancreatic cancers and hepatocellular carcinomas [38,39]. Furthermore, the expression of *SNAI1* and *SNAI2* is inversely correlated with methylation of their first introns [40]. In addition to the EMT-TFs themselves, components of their transcriptional regulatory circuits, such as miRNA expression (e.g., miRNA-200) [41–43], have been shown to be controlled by DNA methylation (Figure 2).

EMT and Histone Modification

The nucleosome, the fundamental repeating unit of chromatin, consists of DNA coiled around an octamer of histone proteins (H2A, H2B, H3, and H4). Besides their role as a structural necessity for packing the large amount of genetic information, nucleosomes also pose a natural barrier for polymerases that require DNA access. The properties of nucleosomes, their dynamics, and the resulting level of chromatin compaction strongly affect transcription and gene expression.

Many types of post-transcriptional modifications (e.g., methylation, acetylation, ubiquitinylation, phosphorylation, and sumoylation) have been identified on lysine (K) and arginine (R) residues of the N-terminal histone tails; these modifications affect gene expression by either directly altering the histone–DNA interaction or indirectly via differential recruitment of **chromatin-remodeling complexes** or the RNA polymerase complex [44] (Figure 1).

As such, the chromatin landscape is highly altered during EMT in which epithelial genes shift from an active state (marked by histone acetylation: H3Kac; and methylation: H3K4me3) to intermediate **poised states** (marked by histone methylation: H3K27me3 and H3K4me3) and finally be stably repressed (marked by histone methylation: H3K27me3, H3K9me3; and DNA methylation) and vice versa for the activation of mesenchymal genes (Figure 1). This entire epigenetic sequence is not necessary to lock gene transcription but it will certainly contribute to the stability of repression and will subsequently influence EMT transition.

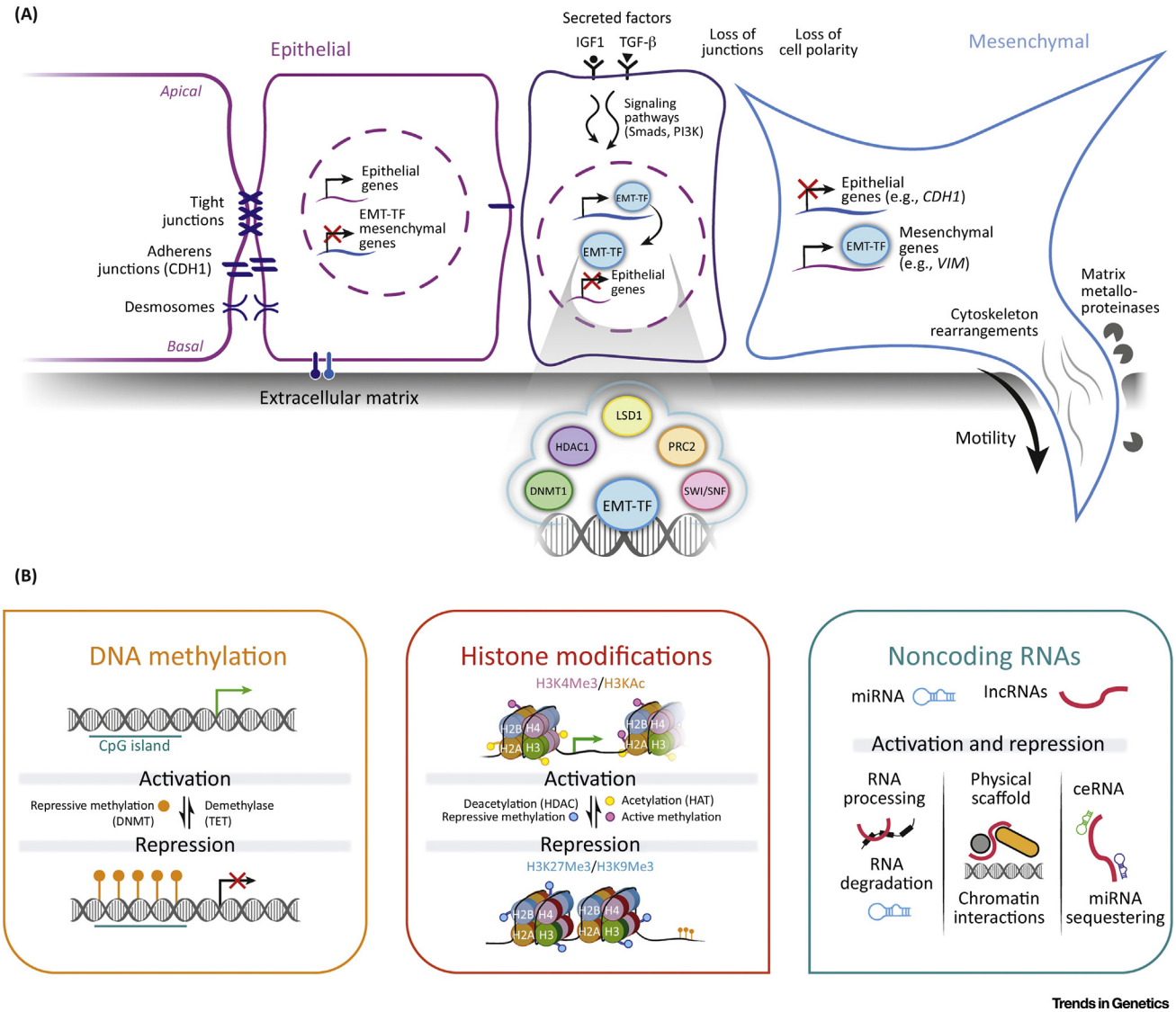
acting as a scaffold molecule for the formation of protein complexes.
miRNAs: small noncoding RNAs of size 21–25 bp important in gene repression by binding to a specific set of mRNA through their 6-bp seed region leading to mRNA degradation, translational blockage, or deadenylation.
Pluripotency: pluripotent cells have the potency to differentiate into cells of all three germ layers and can therefore give rise to all cells of the adult body. ESCs are a typical example of 'naïve' pluripotent cells. However, the term is also used to refer to 'induced' pluripotent cells generated from differentiated cells.
Poised state: also called bivalent state, it represents an inactive chromatin state simultaneously marked by either an active mark (H3K4me3) or a repressive mark (H3K27me3). Physiologically, poised chromatin is found mostly near promoter regions of genes associated with pluripotency and development in stem cells and can rapidly be activated/repressed upon stimulation.
Stroma: refers to the supportive connective tissue framework of a biological organ. The term 'stromal cells' is typically used to refer to all cell types of an organ that are important for the structural integrity and proper functioning of an organ, thereby making a matrix in which the cells important for the biological function of the organ (epithelial cells/parenchyma) are embedded.
TEAD transcription factors: TEAD transcription factors (also called transcriptional enhancer factors) contain a conserved TEA DNA binding domain. Near the C terminus, these transcription factors typically interact with other cofactors, such as YAP (Hippo pathway), to form transactivating or repressing complexes, regulating gene expression. TEAD factors play a crucial role in several developmental processes, but have also been linked to cancer.
Transcriptional complex: to initiate transcription successfully, a transcriptional complex is formed at the transcription start site, not only including RNA polymerase, but also a lot of extra factors [transcription factors, (co)activators, mediators, etc.]. The interplay between all these factors generates a specific protein complex and DNA conformation, which enables transcription.

Each of these histone modifications is catalyzed by specific enzymes that can be classified as (i) epigenetic writers, which mark amino acid residues on histone tails, (ii) epigenetic readers that bind these epigenetic marks, or (iii) epigenetic erasers, which catalyze the removal of epigenetic marks (Box 2). Many of these enzymes have been shown to regulate EMT-TFs expression (Table 1) and directly or indirectly interact with them (Table 2).

Yamanaka reprogramming factors: a set of four transcription factors (Oct4, Sox2, cMyc, and Klf4) that have been demonstrated in 2006 by Shinya Yamanaka's laboratory to be sufficient to reprogram adult fibroblasts into induced pluripotent stem cells.

The Lysine-Specific Demethylase, KDM1A/LSD1

During TGF-β-induced EMT of nontransformed hepatocytes, a clear change in nuclear morphology has been observed, with nuclear enlargement and a transition to an 'open',



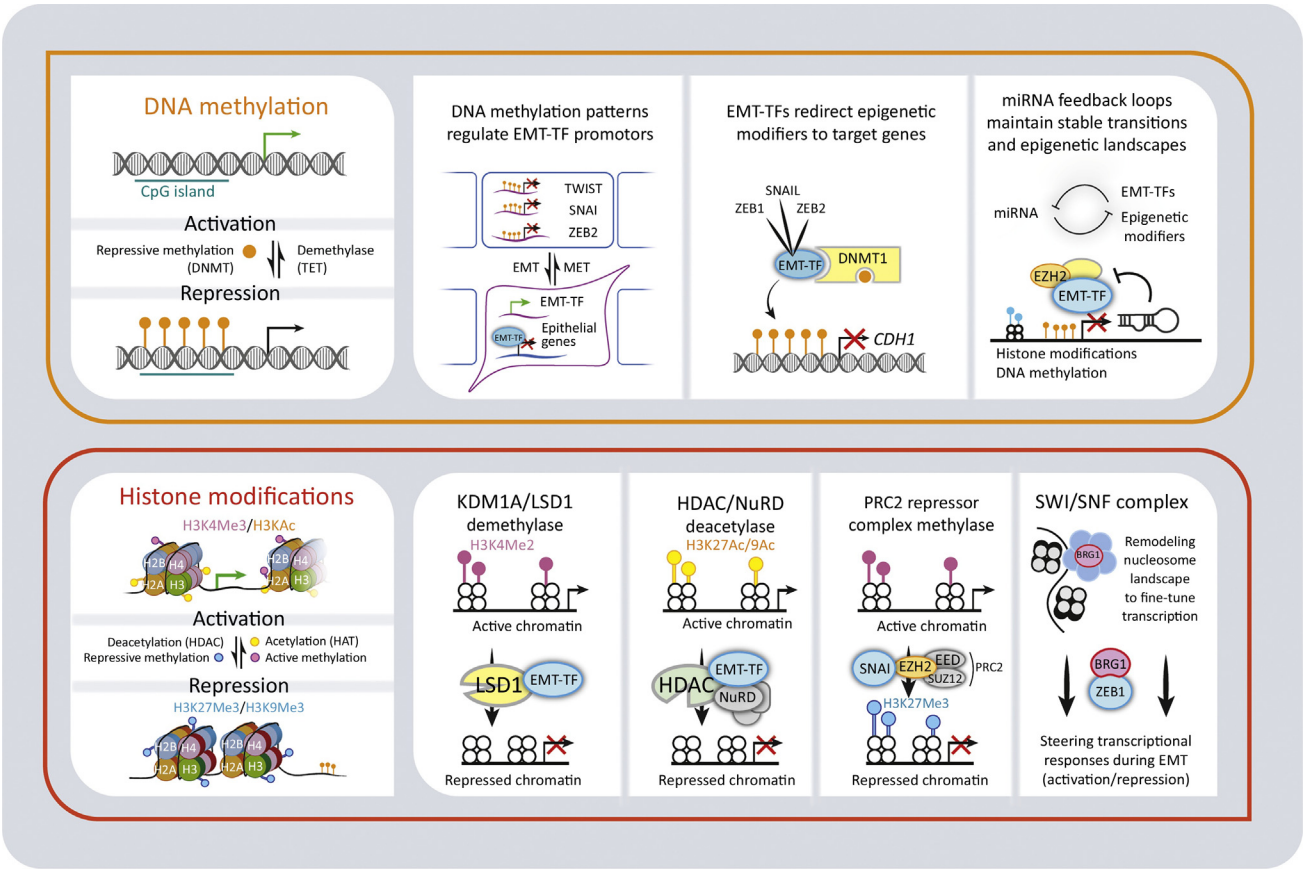
Trends in Genetics

Figure 1. Epithelial-to-Mesenchymal Transition and Epigenetic Mechanisms Involved. (A) Epithelial cells are characterized by an apicobasal polarization with structured junctions. Mesenchymal cells lack polarization, have acquired an elongated spindle-cell morphology, and have strong migratory capacity. During epithelial-to-mesenchymal transition (EMT), epithelial cells lose epithelial gene expression (including *CDH1*), which disrupts intercellular junctions, while gaining mesenchymal gene expression (including *VIM*) inducing actin cytoskeleton rearrangements, secretion of matrix metalloproteinases, and enhanced motility. (B) Representation of the three major epigenetic mechanisms implicated in EMT regulation including (i) DNA methylation, (ii) histone modifications, and (iii) regulation by noncoding RNAs. CDH1, cadherin1/E-cadherin; ceRNA, competitive endogenous RNAs; DNMT, DNA methyltransferase; HAT, histone acetylase; HDAC, histone deacetylase; IGF1, insulin growth factor 1; lncRNA, long noncoding RNA; LSD1, lysine-specific demethylase 1A; miRNA, micro RNA; PRC2, Polycomb repressive complex 2; SWI/SNF, SWItch/sucrose nonfermentable; TET, ten-eleven translocation; TGF-β, transforming growth factor beta; VIM, vimentin.

Box 1. Epithelial-to-Mesenchymal Transition

Half a century ago, Betty Hay was the first to use the term 'EMT' to describe how epithelial cells are transformed into mesenchymal cells during the early stages of embryogenesis. During this well-organized multistep process, polarized epithelial cells lose the connection with the basement membrane that underlies the epithelial layer by either proteolytic degradation of the extracellular matrix (ECM) or loss of the integrin-mediated ECM–cell anchoring. At the same time, transitioning cells lose their polarization and detach from the epithelial sheet by loss of epithelial cell–cell contacts, including the E-cadherin containing adherens junctions. During next steps, the actin cytoskeleton is drastically reorganized to allow the cell to change its shape, essential for motility and invasion properties. At the same time, and to prevent loss of epithelial integrity, the surrounding epithelial cells close the gap. Finally, EMT is completed by *de novo* expression of mesenchymal markers such as Vimentin and the process was originally defined as a transformation between two cellular states, but due to the plastic nature and the description of multiple intermediate states, 'transformation' has given way to 'transition' and more recently 'plasticity'.

hypochromatic chromatin structure with scattered punctuate nucleoli. This nuclear reorganization was correlated with a global reduction of the **heterochromatin** mark, H3K9me2, and depended largely on the expression of the histone-modifying enzyme KDM1A/LSD1 [45]. LSD1 is a flavin-containing amino oxidase that specifically catalyzes the demethylation of mono-methylated and di-methylated lysines on histone 3. In ESCs, LSD1 regulates the balance



Trends in Genetics

Figure 2. Enzyme Network of Epigenetic Regulation of Epithelial-to-Mesenchymal Transition (EMT). The most relevant enzymatic systems known to modify DNA and/or histones are depicted. EMT-TFs are able to recruit the methyltransferase DNMT1, resulting in DNA methylation and gene repression. A large number of histone-modification enzymes have been identified in complex with EMT-TFs, affecting chromatin compaction and locus accessibility during gene regulation. BRG1, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4 (SMARCA4); DNMT, DNA methyltransferase; EED, embryonic endoderm development; EMT-TFs, EMT-inducing transcription factors; EZH2, enhancer of zeste homolog 2; HAT, histone acetylase; HDAC, histone deacetylase; KDM1A/LSD1, lysine (K)-specific demethylase 1A; miRNA, micro RNA; NuRD, nucleosome-remodeling and deacetylase; PRC2, Polycomb repressive complex 2; SUZ12, suppressor of zest 12; SWI/SNF, SWItch/sucrose nonfermentable.

Box 2. Major Epigenetic Complex Related to EMT

Chromatin-remodeling enzymes are often found in complexes functioning as either transcriptional repressors or activators. In the following section, we describe the main ones related to EMT.

NuRD Complex

The NuRD complex is mainly considered a transcriptional repressor. It is an evolutionarily conserved multisubunit protein complex that combines ATP-dependent chromatin-remodeling activity (Mi-2α/β) with histone deacetylase activity (HDAC1/2). Other nonenzymatic subunits include methyl-CpG-binding domain protein (MBD2/3) and some essential structural proteins (Rbbp4/7, p66a/b, and MTA1/2/3) with yet unknown roles in the complex [120]. These components form the main core of the Mi2/NuRD complex, but often many other epigenetic factors, including LSD1, are found to be part of the complex [121]. The composition of the NuRD complex varies depending on cell context and in response to paracrine signals. For example, the different members of the MTA family can direct the Mi-2/NuRD complex to perform unique functions within a given cell or tissue *in vivo*. It has been reported that metastasis-associated proteins (MTA) are upregulated in many cancers and that their elevated expression is correlated with oncogenic transformation and tumor progression [122].

PRC1 Complex

PRC1 complex is a major regulator of histone ubiquitination, composed of Ring1A/1B E3 ligases, PCGF activity enhancer (e.g., PCGF4/BMI1), CBX chromodomain, and HPH unit [79]. Each of the subunits have several paralogs forming different PRC1 complexes. However, the impact of such diversity on PRC1 function is not clear. The monoubiquitination of lysine 119 on histone 2A by Ring1 ligase prevents H3K4 methylation and increases chromatin compaction, leading to transcriptional repression important for embryonic development and lineage commitment. PRC1 is recruited to the chromatin on H3K27 trimethylated residues mediated by PRC2 through the CBX unit or independently of histone marks by interacting with other complex (e.g., REST) or TF (e.g., RUNX1) [79].

PRC2 Complex

The core of the PRC2 complex is composed of three proteins: EED and Suz12, which are essential for complex stability and regulation of the enzymatic activity, and the EZH1/2 histone methyltransferase unit that dimethylates and trimethylates H3K27 residues. In stem cells, PRC2 is essential for self-renewal and pluripotency by repressing genes that control cell fate decision, lineage commitment, and specification [71,123–131]. In malignancy, PRC2 members are often overexpressed, and their expression is correlated with cancer stem cell properties and metastasis [68,69,132–134]. Global PRC2 binding to DNA via RbAp48/46 and EED is promoted, but it is not known how PRC2 is recruited to specific loci to regulate self-renewal and differentiation. There have been reports of several other PRC2-associated proteins influencing PRC2 recruitment to DNA and its activity, including AEBP2 [135], Polycomb-like protein (Pcl) [136], and JARID2 [137].

SWI/SNF Chromatin-Remodeling Complex

SWI/SNF, originally identified in yeast, is a nucleosome-remodeling complex that ATP-dependently destabilizes histone–DNA interactions in nucleosomes. The primary function of this destabilization is to aid gene expression by opening up the transcription factor-binding sites. In mammalian cells, SWI/SNF is composed of one of two mutually exclusive catalytic ATPases, Brahma (BRM/SMARCA2) and Brahma-related gene 1 (BRG1/SMARCA4), and eight to 10 BRG1/BRM-associated factors. In general, normal SWI/SNF function requires all subunits and acts as a tumor suppressor [138–140]. Mutations affecting the complex have been found in approximately 20% of human malignancies [141,142].

between self-renewal and differentiation. Increased *LSD1* expression has been documented in various cancer types [46], and *LSD1* overexpression is correlated with inhibition of *CDH1* expression and induction of various mesenchymal marker genes, associated with enhanced cell migration and invasion [47–50]. In cancer cell lines with endogenous silenced *CDH1* expression, depletion of *LSD1* resulted in partial de-repression and elevated H3K4me2 levels at the *CDH1* promoter [47].

Because LSD1 alone cannot bind DNA, it is believed that the EMT-TFs recruit LSD1-containing chromatin-remodeling complexes to their target promoters (Figure 2). SNAI proteins directly interact with LSD1 via their SNAG domain. More recently, it was shown that ZEB proteins too

Table 1. Epigenetic Regulation of EMT-TFs and the Impact on EMT[a]

Enzymes	Activity	Marks	Epigenetic complex	Regulated EMT-TFs	Regulatory effect	Cell type	Refs
Histone methyl transferases (HTMs) and histone demethylases (HDMs)							
KDM6B/JMJD3	HDM	H3K27m2/3		SNAI1, SNAI2, TWIST1	Positive (TGF-β induced)	Breast, MDCK	[143]
				SNAI1, ZEB1	Negative (vitamin D induced)	Colorectal cancer	[144]
MMSET/WHSC1	HMT	H3K36m2		TWIST	Positive	Prostate cancer	[145]
DOT1L	HMT	H3K79	DOTL1-cMyc-p300		Positive	Breast cancer	[146]
UTX	HDM	H3K27m2/3	UTX-LSD1-HDAC1-DNMT1	ZEB1, ZEB2, SNAI1	Negative		
LSD1	HDM	H3K4m2	UTX-LSD1-HDAC1-DNMT1		Negative		
EED	HMT	H3K27	PRC2	miR200	Positive (TGF-β induced)	Lung and colon cancer	[68]
JARID2/PRC2	HMT		JARID2-EZH2	miR200	Positive (TGF-β induced)	Lung and colon cancer	[69]
EZH2	HMT		PRC2	SNAI1, ZEB1, ZEB2	Negative (TGF-β induced)	Ovarian cancer	[147]
KDM5A/RBP2/JARID1A	HDM	H3K4m2/3		SNAI1, TWIST1	Positive	Lung and renal cancer	[148,149]
KDM5B/JARID1B	HDM			miR200	Positive	Lung cancer	[150]
G9A	HMT	H3K9m1/2	NuRD (GATA3/MTA3/G9A)	ZEB2	Negative	Breast cancer	[57]
PHF8	HDM	H3K9		SNAI1, ZEB1	Positive (TGF-β induced)		[151]
KDM4B	HDM	H3K9me3		ZEB1	Positive (TGF-β induced)	Pancreatic cancer	[152]
MLL4	HMT	H3K4me2	UTX-MLL4-cMyc	ZEB1, ZEB2, SNAI1	Positive	Breast cancer	[153,154]
PRMT1	HMT	H4R3me2as		ZEB1	Positive	Breast cancer	[155]
JMJD2A/KDM4A	HDM	H3K9me3		SNAI2	Positive	Neural crest	[156]
Histone acetyl transferases (HATs) and histone deacetylases (HDACs)							
SIRT1	HDAC	H3K9Ac		ZEB1, ZEB2, SNAI1, SNAI2	Positive	Prostate cancer	[157]
P300	HAT	H3K	DOTL1-cMyc-p300	ZEB1, ZEB2, SNAI1	Positive	Breast cancer	[146]
CBP	HAT		MTDH-CBP	TWIST1	Positive		[158]
HDAC1	HDAC		DNMT1-cMyc-HDAC1	ZEB1, ZEB2, SNAI1	Negative		[146]
			UTX-LSD1-HDAC1-DNMT1	ZEB1, ZEB2, SNAI1	Negative		[154]
HDAC type I	HDAC		SMAD pathway	SNAI1, SNAI2, ZEB	Positive (TGF-β induced)		[60,159,160]
Histone ubiquitin ligases (UbLs) and deubiquitinase (dUb)							
BMI-1	UbL		PI3K/Akt pathway	SNAI1	Positive	B lymphoma	[75]
Nonhistone post-translational modifications							
P/CAF	HAT	ZEB1-Ac	P300-P/CAF	ZEB1	Negative		[130]

[a]In this table are listed enzymes affecting epigenetic regulation of EMT. They are grouped according to their enzymatic activity (columns 'Activity' and 'Marks') and involvement in the regulation of EMT-TFs (column 'Regulated EMT-TF'). In addition, their impact on EMT-TF activity (column 'Regulatory Effect') and the tissue in which the epigenetic regulation has been described (column 'Cell Type'). When available, the epigenetic complex that is regulating the EMT-TF is supplemented (column 'Epigenetic Complex').

Table 2. Interactions between Epigenetic-Remodeling Enzymes and EMT-TFs[a]

Enzymes	Complex	Interactor	Domain of interaction	Mark	Gene targeted	Cell type	Refs
Histone methyl transferases (HTMs) and histone demethylases (HDMs)							
LSD1	LSD1-CoREST-CtBP	ZEB1	?	H3K4me2	Gh – repression	Pituitary development	[52]
		ZEB2	?		CD11b – repression	T-ALL	[53]
		SNAI1	SNAG domain		CDH1 – repression	HEK293, Breast and Colon cancer	[161]
		SNAI2	SNAG domain		CDH1, desmoplakin, occludin – repression	HEK293, K562, HCT116	[51]
SUV39H1		SNAI1	SNAG domain	H3K9me3	CDH1 – repression	HEK293, breast cancer	[162]
SET8		TWIST	?	H4K20me1	CDH2 – activation CDH1 – repression	Breast cancer	[163]
		ZEB1	?		Vimentin – activation CDH1 – repression	Prostate cancer	[164]
EZH2/SUZ12	PRC2	SNAI1	HOTAIR; SNAG domain	H3K27me3	HNF4a, HNF1a, CDH1, PTEN – repression	Hepatocyte TGF-β treated	[72,89]
EZH2/EED		SNAI2	?		CDH1 – repression	Xenopus embryo	[71]
EZH2		TWIST1	?		P16 and P14 (Ink4A/Arf locus) – repression	BMSC senescence	[165]
G9A	NuRD	SNAI1	Zinc-finger domain	H3K9me1/2	CDH1 – repression	HNSCC; breast cancer	[166,167]
	NuRD (G9A-MTA1)	ZEB2	?			Breast cancer	[57]
	NuRD/CtBP-G9A-MTA1/2	ZEB1	?			HeLa cells	[168]
PRMT5		SNAI1	Through AJUBA	H4R3	CDH1 – repression	HEK293T, P19 embryonic stem cells	[169]
Histone acetyl transferases (HATs) and histone deacetylases (HDACs)							
SIRT1		ZEB1	Stabilized by MPP8	H4K16Ac/H3K9Ac	CDH1 – repression	Prostate cancer	[157,170]
P300	P300-P/CAF	ZEB1	N terminal	H3/H4	TGF-β-dependent genes – activation	*Xenopus* embryos	[25]
mSIN3A	mSIN3A-HDAC1/2	SNAI1	SNAG domain	H3/H4	CDH1 – repression	MDCK, HEK293T, and keratinocyte	[58]
mSIN3A	mSIN3A-HDAC-PHD12	SNAI2	?	H3K	CDH1 and Cad6b – repression	Neural crest	[171]
HDAC1	CtBP-HDAC1	SNAI2	Through CtBP	H3/H4	BRCA2 – repression	Breast cancer	[172]
HDAC1/2		ZEB1	Through CtBP?	H3K	CDH1 – repression	Pancreatic cancer	[173]
Histone ubiquitin ligases (UbLs) and deubiquitinase (dUb)							
BMI-1		TWIST1	?	H2AK119Ub1	CDH1 and p16INK4a – repression	HNSCC	[75]
	BMI-1-PRC1	SNAI1	?		PTEN and CDH1 – repression	Nasopharyngeal epithelial cells	[77]
RING1A/B		SNAI1	Zinc-finger domain		CDH1 – repression	Pancreatic cancer	[76]
Other							
BRG1	SWI/SNF	ZEB1	N terminal		CDH1 – repression	Colon cancer	[61]

[a]In this table are listed the epigenetic-remodeling enzymes, grouped according to their enzymatic activity (column 'Mark') and interaction with EMT-TFs (column 'Interactor'). Genes targeted by the nuclear complexes and the specific impact on gene regulation are shown (column 'Gene Targeted') along with the cell type or the cell model the study(ies) characterized (column 'Cell Type'). When available, the EMT-TF interacting domain (column 'Domain of Interaction') and the complex characterized in the paper (column 'Complex') are specified.

can interact with LSD1. Blocking functionally the ZEB-LSD1 interaction or inhibiting LSD1 partially prevents EMT, showing the importance of this complex for EMT induction [51,52] (Table 2). The observation that multiple/most EMT-TFs interact with this demethylase suggests a common downstream regulatory mechanism, which opens new targeting horizons. Although the lysine demethylase activity of LSD1 appears essential, it is only one piece of the puzzle. LSD1 often is part of larger chromatin-remodeling complexes, such as the nucleosome-remodeling and the deacetylase (NuRD) complex.

Mi-2/Nucleosome-Remodeling and the Deacetylase (NuRD) Complex

The NuRD complex is composed of a multisubunit protein complex steering transcriptional repression (Box 2). EMT-TFs of the ZEB and TWIST families bind and recruit the NuRD complex to their target promoters [53,54], including the *CDH1* promoter, which is essential for cancer cell migration and invasion [55] (Table 2 and Figure 2). This interaction was found to be pivotal for the role of ZEB2 in Schwann cell differentiation, myelination, and nerve repair [56]. Moreover, amino-terminal ZEB2 truncations/mutations mitigating the ZEB2–NuRD interaction have been identified in an atypical Mowat–Wilson syndrome patient, demonstrating the importance of this interaction for the ZEB2 functions [54,56]. Mowat–Wilson syndrome is a rare genetic disorder in which some of the main body dysfunctions can be explained by disrupted EMT. More recently, a negative reciprocal feedback regulatory loop between GATA3/NuRD(MTA3) and ZEB2/NuRD(MTA1) was identified, dictating the fate of mammary epithelial cells giving, respectively, an epithelial or mesenchymal phenotype depending on the epigenetic complex. During breast cancer progression, GATA3 and MTA3 are downregulated, which balances the equilibrium to ZEB2/NuRD(MTA1) that contributes to metastasis formation of breast cancer [57] (Table 2).

The Sin3A Repressor Complex

Another histone deacetylase 1 and 2 (HDAC1/2)-containing complex that can regulate H3 deacetylation at the *CDH1* promoter is the SIN3A repressor complex. The SNAG domain of SNAI1 is essential for recruitment of this SIN3A/HDAC corepressor complex to the *CDH1* promoter [58] (Table 2). Pretreatment of cancer cells with the HDAC Class I and II inhibitor Trichostatin A (TSA) attenuates both Snail-1-mediated downregulation of epithelial markers and upregulation of mesenchymal markers. TSA was also sufficient to almost completely reverse the SNAI1-mediated EMT program within 24 h [59,60] (Table 1).

SWItch/Sucrose Nonfermentable Complex

SWItch/sucrose nonfermentable (SWI/SNF) is a nucleosome-remodeling complex that ATP-dependently destabilizes histone–DNA interactions in nucleosomes. The primary function of this destabilization is to aid gene expression by opening up the transcription factor-binding sites. BRG1, a protein part of the SWI/SNF chromatin-remodeling complex (Box 2), interacts with ZEB1 to regulate *CDH1* levels (Figure 2). Blocking the interaction between ZEB1 and BRG1 induces expression of E-cadherin and downregulation of the mesenchymal marker vimentin. ZEB1 and BRG1 colocalize in E-cadherin-negative cells from cancer lines and in the **stroma** of normal colon [61] (Table 2). The Wnt signaling pathway could be essential for the SWI/SNF effect on EMT as WNT5A promotes EMT through SMARCD3 (BRG1-associated factor 60c) and the ZEB1–BRG1 interaction correlates with nuclear beta-catenin [61,62].

The Polycomb Repressive Complex 1 and 2 (PRC)

Both Polycomb repressive complex 1 (PRC1) and Polycomb repressive complex 2 (PRC2) are transcriptional repressive complexes working in cooperation and are known important EMT regulators (Box 2) [63–65]. PRC2 regulates *ZEB1/2* expression by inhibiting the miR-200 family expression after TGF-β induction. PRC1 has been reported to induce EMT in hypoxic conditions upon TWIST1 upregulation by hypoxia-inducible factor-1 alpha (Table 1) [66–70].

Both TWIST and SNAI family proteins interact with PRC1/2. The interaction with TWIST is not fully characterized, but SNAI interacts through its SNAG domain with the PRC2 subunit, histone–lysine *N*-methyltransferase enzyme, enhancer of zeste homolog 2 (EZH2; Table 2 and Figure 2). In neural crest initiation, the SNAI2–EZH2 interaction is essential for the regulation of a subset of neural crest genes, including *CDH1*, and for proper neural crest cell migration [71]. In addition, the PRC2 complex was demonstrated to be essential in cancer cells for the SNAI1/2-mediated repression of *CDH1* [72,73]. It has recently been suggested that ZEB1 could recruit PRC2 indirectly through the C-terminal binding protein CtBP complex [74].

In addition, TWIST1 and SNAI interact, respectively, with BMI-1 [75] or RING1 B [76], which are elements of the PRC1 E3 ligase core. These interactions recruit PRC1 to the tumor suppressor gene *PTEN* [72] and the *CDH1* promoter [76] to induce EMT [65,77] (Table 1). Moreover, BMI-1 expression is regulated by EMT-TFs, directly by TWIST1 [75] or indirectly by ZEB1 through miR-200 repression [78].

During EMT induction and gene regulation, PRC1 recruitment might be a later event in the epigenetic cascade as it is recruited through H3K27me3 and H3K9me3 marks [79], respectively, regulated by PRC2 and SUV39H1/2, both interacting with EMT-TFs (Table 2). In that regard, PRC1 recruitment to the chromatin could be independent of EMT-TFs interaction and the lack of PRC2 or SUV39H1/2 could impair PRC1 function.

EMT and Noncoding RNA

Although the noncoding genome was initially thought to be junk DNA, it is now widely accepted that it is indispensable for regulating gene expression [80]. Two types of regulatory RNA molecules with proven important roles in EMT include miRNAs and **long noncoding RNAs** (**lncRNA**) consisting of transcripts longer than 200 bp (Figure 1).

miRNAs

Multiple miRNAs control EMT by either directly affecting the expression of EMT-TFs or their associated co-factors, including the aforementioned epigenetic modifiers and chromatin-remodeling complexes such as SUZ12 (PRC2 component), DNMTs, and SIRT deacetylase [6]. In turn, these enzymes epigenetically regulate miRNA expression by reduction of repressive marks, enrichment of permissive marks, and DNA methylation. The majority of described miRNAs strengthen the epithelial phenotype and counteract EMT by directly reducing the expression of EMT-TFs or other invasion-associated factors. Nevertheless, miRNAs such as miR-544a and miR-21 are potent inducers of EMT by targeting epithelial differentiation markers [81,82].

Recently, a core miRNA signature upon EMT induction has been put forward [83]. Members of the miR200 family are key central nodes within this miRNA network and are indispensable for maintenance of the epithelial phenotype by targeting *ZEB1* and *ZEB2* mRNA [84]. A similar intricate association has been found for miR34, acting in a double-negative feedback loop with SNAI1 [85]. These different loops work together to establish the epithelial phenotype, thereby making up the core of the EMT regulatory network [83].

Long Noncoding RNAs

Similar to miRNAs, some lncRNAs directly regulate the expression of EMT-TFs. For example, lncRNAs ZEB1 antisense 1 (ZEB1-AS1) and ZEB2 antisense 1 (ZEB2-AS1) promote ZEB1 and ZEB2 expression, leading to increased metastasis and poor prognosis in several cancer types [86,87]. ZEB2-AS1 does so by preventing the splicing of an intron in the 5′-untranslated region of the ZEB2 gene, which contains an internal ribosome entry site necessary for activating ZEB2 expression. Notably, this lncRNA is under the direct transcriptional control

of SNAI1 and is therefore an essential player in reinforcement of the EMT transcriptional circuit [87].

Multiple lncRNAs contribute to the epigenetic regulation of EMT by physically interacting with the silencing complex PRC2 and targeting it to specific cancer genes and regulators. Several lncRNAs function in this way, including HOTAIR, EBIC, H19, MALAT1, SPRY4-IT1, and UBC (reviewed in [88]). A recent study demonstrated how the lncRNA HOTAIR serves as a physical scaffold between SNAI1 and EZH2, forming a tripartite complex necessary for SNAI1 repressive activity [89]. Interestingly, HOTAIR also mediates miR-34a repression through stabilization of a JARID2–PRC2 complex, allowing SNAI1 expression, and thus further fueling EMT [90]. In a similar manner, MEG3, another lncRNA, interacts with JARID2–PRC2 to repress *CDH1* and the miR-200 family members [91].

Another property of lncRNAs is their ability to function as **competitive endogenous RNAs (ceRNAs)**. Through competitive binding of miRNAs, ceRNAs have a suggested function as sponges preventing their target mRNAs from degradation [92]. For example, H19 and lncRNA-ATB can sequester miR200 family members away from their target mRNAs, thereby leading to de-repression of ZEB family members and consequently activating EMT. Likewise, lncRNAs such as UCA1 and ROR have oncogenic properties by modulating pivotal tumor-suppressive pathways [93].

Preventing EMT via Epigenetic Drugs As an Anticancer Therapy

Reverting or preventing EMT would not only restrain invasion and metastasis but at the same time also inhibit cancer stem cell properties and chemoresistance. Therefore, EMT-TFs have become a target of prime interest for designing novel or improved anticancer therapies [94]. However, targeting EMT-TFs themselves may be, in theory, an effective approach, but technically challenging. Therefore, targeting essential cofactors or druggable essential upstream/downstream effectors may have more potential than the EMT-TFs. Some preclinical studies focused on small molecules inhibiting signaling pathways activating EMT, such as MAPK/MEK (PD0325901), PI3K/Akt (HS-173), Src (saracatinib), and TGF-β pathway (LY2157299/galunisertib) inhibitors, which showed promising effect forcing MET and resensitizing cancer cells to chemotherapies [95–99].

Because of the reversibility of the epigenetic marks and the enzymatic nature of the regulators, one could propose to target the specific chromatin-regulating enzymes to reverse the EMT process efficiently. In addition, with the observation that EMT-related gain of cancer stem cell properties and chemoresistance are associated with vast epigenetic changes, several clinical trials are ongoing to combine classical chemotherapy with epigenetic therapies. For instance, activity of DNMTs can be blocked by nucleoside analogs such as 5-azacytidine or 5-aza-2′-deoxycytidine (Decitabine), leading to **hypomethylation** and gene de-repression [100–102]. 5-Aza treatment can prevent EMT *in vitro* [103–105], and similar results have been documented with HDAC inhibitors. However, broad-spectrum drugs, targeting signaling pathways of epigenetic-remodeling enzymes, can be a double-edged sword. While the inhibition of HDAC or DNMTs represses EMT and tumor growth in some models, other models showed the opposite with induction of EMT-TF expression leading to EMT [106–110].

Most of the HDAC inhibitors target multiple HDAC isoforms. Therefore, possible improvements may come from a next generation of more selective HDAC inhibitors that can minimize the observed side effects and context-dependent variable results [111]. For instance, HDAC6 has been demonstrated to be an essential mediator of TGF-β1-induced EMT [112–114]. Multiple selective HDAC6 inhibitors are currently being tested in clinical trials. An example of such specific effects is a Class I HDAC inhibitor mocetinostat that, in contrast to other HDAC

inhibitors, interferes with ZEB1 expression and function, reversing drug resistance by sensitizing tumor cells to chemotherapy [115].

Especially promising are the novel KDM1A/LSD1 inhibitors as anticancer drugs, since most EMT-TFs interact with this demethylase (Table 2). As a proof of concept, the upregulation of ZEB2 levels sensitizes leukemia cells toward LSD1 inhibition [53]. Pretreatment of cancer cells with an LSD1/2 inhibitor prevented SNAI1-mediated downregulation of epithelial and upregulation of mesenchymal marker genes [59]. Multiple novel potent LSD1 inhibitors are being tested in clinical trials [116,117]. In addition, PRC2 inhibitors are tested with the latest ZLD1039, an EZH2 inhibitor that showed a strong effect on breast tumor growth and metastasis formation [118].

An alternative way would be to disrupt specific interactions between EMT-TFs and an epigenetic enzyme. For the well-described interaction between LSD1 and SNAI1, using TAT-SNAG, a cell-permeable peptide corresponding to the SNAG domain, partially prevented EMT, giving strength to this strategy [51]. However, even if some interactions are well described, the majority of them are not known or it is unclear whether it is a direct or indirect interaction, or if post-translational modification is necessary, making it more difficult to design an efficient disrupting peptide/small compound.

Finally, due to the central role of miRNA and lncRNA in the repression of EMT-TF (e.g., miR-200 family) or activity of epigenetic-regulating enzymes (e.g., HOTAIR, H19), modulating miR/lncR expression (e.g., 5-azacytidine) or re-introducing them could be another way. Strategies to treat patients with miR are under investigation, especially to improve miRNA stability and specificity to tumors, but preclinical models already show encouraging results (reviewed in [119]).

Concluding Remarks and Future Perspectives

As discussed in this review, EMT-TFs and chromatin-remodeling enzymes are intimately connected through physical interaction and/or via direct gene regulation. Nevertheless, up to now definitive proof is missing that these interactions play pivotal roles in the physiological and pathological EMT processes *in vivo*. Most of the aforementioned results were obtained under *in vitro* settings, using mostly transformed cancer cell lines, often focusing on only one EMT-TFs or one epigenetic mark/regulator. The high complexity of the EMT regulatory network, variability of the cellular context, and/or cellular microenvironment and tissue heterogeneity *in vivo* are some of the hurdles to overcome to clearly define the exact roles of epigenetics during *in vivo* EMT (see Outstanding Questions). Extending our knowledge of the EMT-TF epigenetic network would help us to propose new ways to treat more efficiently and specifically EMT-driven diseases.

Outstanding Questions

EMT and different chromatin-remodeling enzymes are tightly connected, supported by an exponential number of studies increasing the complexity of the EMT-epigenetic network. Targeting EMT is a promising option for EMT-related disease, and blocking the EMT-epigenetic network is a seductive approach, but still many basic questions need to be answered:

While many interactions are well described, some seem indirect and so far not well characterized. Which EMT-TF domains are involved in direct interactions with epigenetic enzymes? Which proteins are essential for the indirect interactions? Does the disruption between EMT-TFs and epigenetic regulators reverse EMT-related properties?

Epigenetic enzymes are often mutated in cancer, resulting in their increased activity. Are mutations in epigenetic enzymes a trigger for EMT? Could it affect EMT-TF interactions, explaining discrepancy between different cancer subsets?

How functionally dependent are EMT-TFs on the epigenetic network and vice versa? Which specific pathways, if any, are involved in specific repressive or activating EMT-TFs functions?

Regarding the number of different models and approaches used to study the EMT epigenetic network, how relevant are the described interactions in the complete cellular epigenetic network?

How are epigenetic regulatory mechanisms contributing to the spectrum of partial or even metastable intermediate EMT states?

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