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Review

Linking epithelial-to-mesenchymal-transition and epigenetic modifications

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ABSTRACT

Cancer, as well as other human disorders, has long been considered to result from the consequence of genetic mutations in key regulatory genes that reside in pathways controlling proliferation, cellular differentiation, DNA damage and repair. In the case of cancer, mutations are well documented to arise in key oncogenes and critically important tumor-suppressor genes as part of the disease progression process. In addition to more accepted, genetic mutations, a rapidly increasing body of evidence supports the general view that profound alterations also occur in 'epigenes', whose products serve to define the 'epigenetic landscape' of tumor cells. Aberrant changes in epigenetic mechanisms such as DNA methylation, histone modifications and expression of micro RNAs play an important role in cancer and contribute to malignant transitions. Here we review recent studies linking epigenetic mechanisms to epithelial-to-mesenchymal transition as defined in normal processes, as well as abnormal transitions that lead to oncogensis.

1. Introducing epigenetics and epithelial-to-mesenchymal transition (EMT)

Epigenetics can be defined as heritable alterations in states of gene expression that are not linked to changes in the DNA sequence [1,2]. A wealth of emerging literature suggests that the precise organization of DNA in chromatin has important functional consequences. Essentially all DNA-templated processes such as transcription, replication, repair, recombination, and segregation are influenced by the complexity of the chromatin architecture. Chromatin states, whether in the broadest terms, active or silent, establish, maintain and propagate different patterns of gene expression during normal differentiation and development. Mistakes made in establishing these chromatin states, governed by chromatin remodeling activities, lead to mis-expression or improper silencing with far-reaching implications for human biology and human disease [3–8]. The fundamental and interrelated epigenetic events involved in gene regulation, development and tumor progression are DNA methylation, histone modifications, chromatin remodeling and micro-RNA expression. Recent studies on epigenetic mechanisms in cancer have demonstrated that epigenetic alterations also play important roles in epithelial-to-mesenchymal transition (EMT).

EMT is a crucial process during normal development. Several milestones in embryogenesis, including gastrulation, neural crest formation and heart morphogenesis, rely on dynamic transitions between epithelium and mesenchyme [9]. Typically, EMT involves loss of epithelial polarity, loss of adhesive properties, and acquisition of a fibroblastoid phenotype with increased cell motility. Also during metastasis, carcinoma cells transition to a fibroblast-like phenotype with drastically reduced cell–cell contact and increased migratory abilities. A collection of compelling evidence suggests that cancer progression is initiated via an EMT. These changes result in dispersed and isolated cells which are capable of invading the surrounding stroma, intravasating into the bloodstream, and eventually repopulating at distant sites as micrometastases [10–13]. Orchestration of complex and interlinked networks is necessary for cells to transition into a mesenchymal phenotype [14–16]. Molecular mechanisms underlying EMT were not analyzed until the early 1980s, but since then a large number of molecular differences between epithelial and mesenchymal cells have been described. Much more recently, researchers have learned that cancer cells have to acquire genetic as well as epigenetic changes to undergo EMT. In this review, we discuss how multiple, and likely, interconnected, mechanisms – covalent DNA methylation, histone modifications, chromatin remodeling and miRNAs – are or might be associated with EMT and cancer progression (Fig. 1).

2. Epigenetic mechanisms in cancer cells

2.1. DNA methylation

One of the more extensively described epigenetic modification in humans is the methylation of cytosine. DNA methylation is believed to be a mechanism of stable gene silencing, which is crucial for regulating gene expression and chromatin states, in an interplay

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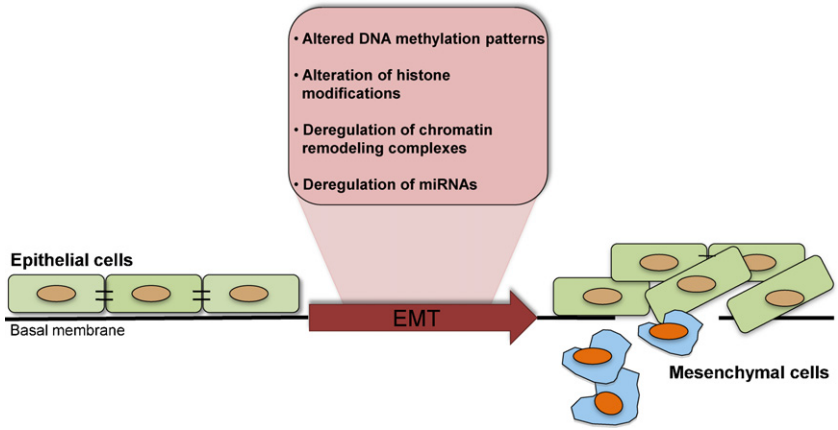

Fig. 1. Known epigenetic changes involved in epithelial-to-mesenchymal transition of tumor cells.

with histone modifications and chromatin-associated proteins. In mammalian cells, DNA methylation is mostly found within CpG dinucleotides, which tend to form clusters known as CpG islands, and in regions of large repetitive sequences, such as retrotransposon elements and centromeric repeats [17–19]. CpG islands are mostly located at the 5′ end of genes and mark approximately 60% of human gene promoters [19,20]. DNA methylation has long been associated with gene silencing and is especially important for genomic imprinting, wherein one of the two parental alleles is hypermethylated to ensure monoallelic expression, and for inactivation of the X-chromsome in females [21,22]. Furthermore, repetitive genomic sequences are heavily methylated to maintain chromosomal integrity by preventing chromosomal rearrangements, translocations and gene disruption through the reactivation of transposable elements [5,17,23,24]. DNA-methylation is also a mechanism to control expression of germline-specific genes, like MAGE (Melanoma antigen-encoded gene) family members. It is further used to silence tissue-specific genes, such as MASPIN (mammary serine protease inhibitor, also known as SERPIN5B), in tissues were they should not be expressed [25–27]. Extensive DNA methylation changes, probably induced by cell differentiation, have recently also been described to occur at CpG island "shores", which are areas of relatively low CpG density close to CpG islands [28,29]. The recent discovery that 5-methylcytosine can be converted into 5-hydroxymethylcytosien by the 2-oxoglutarate- and Fe (II)-dependent oxygenases TET1, TET2 and TET3 indicates that there is much more complexity to the relationship between DNA methylation and gene expression than first believed [30–34]. More insight is necessary to understand the role of 5-hydroxymethylcytosine, detected in ES cells and Purkinje neurons, to get a much clearer understanding of this new modification and its potential contribution to the cancer epigenome.

Gene silencing through DNA methylation is achieved by a variety of mechanisms. For example, it can inhibit binding of transcription factors to target sites, or alternatively, function as docking sites for methyl-binding domain proteins (e.g. MBD proteins, MeCP2), which induce gene silencing through the recruitment of histone deacetylases (HDACs) [35,36]. In normal cells, CpG islands in expressed genes are often found to be unmethylated. Cancer cells, however, acquire distorted methylation patterns, which display an inversion of the pattern found in normal tissues. In fact, changes in DNA methylation pattern were the first epigenetic alterations described in cancer cells [37,38]. Cancer cells are characterized by a general loss of DNA methylation (approximately 20–60% less cytosine methylation) [39]. On the other hand, site-specific hypermethylation at CpG islands is frequently observed at the same time [5]. While the underlying mechanisms that initiate these global changes are still under investigation, recent studies indicate that changes occur very early in cancer development and may contribute to cancer initiation [40].

2.2. Covalent histone modifications

Clearly, DNA-methylation is one critical layer in a complex mechanism that is responsible for establishing chromatin states, a layer which in some organisms is well-documented to be influenced by histone modifications. The fundamental packaging element of chromatin is the nucleosome, which consists of 147 pairs of DNA wrapped around an octamer of eight globular histone proteins (two each of H2A, H2B, H3 and H4) [41,42]. The N-and C-termini of the histones protrude from the nucleosome into the nuclear milieu, were they can be highly decorated with a diverse set of post-translational modifications (PTMs) that are recognized to govern the structure and function of chromatin. These modifications include acetylation, phosphorylation, methylation, citrullination, ADP-ribosylation, and ubiquitylation [43,44]. PTMs such as these can also occur within the globular domains of the histone proteins, providing a staggering degree of complexity to the 'language' of covalent histone modifications that may contribute to a histone or epigenetic "code" that remains under active investigation (see below).

A rapidly emerging body of literature indicates that epigenetic alterations are fundamental for normal development, cell differentiation and also play an important role in abnormal human pathologies, such as cancer. Modulation of chromatin by covalent histone marks is one fundamental way of regulating DNA accessibility during processes such as gene transcription, DNA replication and DNA damage repair. For example trimethylation of lysines (K) 4, 36 or 79 on histone 3 (H3K4me3, H3K36me3, H3K79me3), and acetylation of H3K9 and H3K14 (H3K9ac, H3K14ac, and in some developmentally-regulated enhancer elements, even H3K27ac) most often correlate with transcriptional activation, whereas histone modifications like di- or trimethylation of H3K9 (H3K9me2 and H3K9me3) and trimethylation of H3K27 (H3K27me3) generally mediate gene repression [45–47]. The histone code postulates that the state of chromatin, active or repressed, depends on the combination of histone modifications, which regulate critical downstream events by providing a signalling platform to recruit "readers" or "effector" proteins [48–50]. Alterations in this "code" either through changes in the "writer" and "erasers" of these covalent marks, or the effector protein complexes that read them, are closely linked with oncogenesis [7,8,18]. For example, a wide-ranging study of histone-modifying enzymes even allowed the discrimination between cancer samples and their normal