Epigenetics in Cancer Metastasis
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Abstract

Cancer has been traditionally regarded as a disease caused by genetic mutations. However, since the development of epigenetic and our better understanding of cancer itself, scientists now recognize the role of epigenetic factors in cancer. Cancer metastasis, the spread of cancer cell to secondary sites, is one of the most dangerous events in late cancer stages. With no exception, epigenetic factors also play important roles in cancer metastasis as they do in other cancer events. E-cadherin gene has been identified as the key regulator of metastasis and is under the control of a series of epigenetic factors. By summarizing results of epigenetic regulation of E-cadherin gene and other potential genes involved in metastasis, I wish to propose a model that multiple epigenetic factors work together to control metastasis. Also, some current therapies targeting epigenetic regulations are reviewed and potential therapeutic strategies are raised.

According to Global Cancer Statistics 2011, cancer is the most frequent cause of death in developed countries and the second most frequent in developing countries[1]. Among the many deleterious aspects of cancer, metastasis represents the worst prognosis. Metastasis is defined as the dissemination of tumorous cells from the primary tumor to different loci in the body and establishment of secondary tumors, of which the most crucial step is the epithelial-mesenchymal transition (EMT) event[2]. EMT was initially observed in embryogenesis process when differentiated epithelial cells lose their differentiation and gain the ability to migrate[3]. Later on, more evidence accumulated to indicates that EMT also happens during cancer metastasis[4]. Due to the devastating consequences of cancer metastasis, it has always been a hot topic in cancer research.

Although cancer has been considered as a genetic disease traditionally, there has been a growing number of evidence showing the involvement and importance of epigenetic factors in cancer, especially in metastasis[5]. Major epigenetic alterations include DNA methylation, Histone modification and MicroRNAs. While genetic alterations impact protein sequences, epigenetic alterations regulate the expression level of genes by changing chromatin organization[6]. Thus, epigenetic alterations in cancer metastasis has drawn attention from many cancer biologists in recent years[7].

In this review, I will summarize the recent progress in characterizing the epigenetic regulation of cancer metastasis. Focus will be given to DNA methylation as well as histone modification and how their alteration influences cancer metastasis or EMT. In addition, recent therapeutic strategy based on epigenetic regulation will be covered. Last but not least, I will give brief future perspectives in this field and some possible future directions.
Overview of Epigenetic Regulation

Epigenetic regulation covers a wide range of chromatin, DNA, RNA and even protein modification events. In this review, I will discuss epigenetic regulation on chromatin which includes both histone modifications and DNA methylations. As we know, the basic element of chromatin is the nucleosome, a structure formed by core DNA wrapped around a histone octamer. The histone octamer consists of 2 copies of each of the core histones, namely H2A, H2B, H3 and H4. Both the DNA and histones can be modified in various ways in vivo in order to regulate gene expression[8, 9]. Specifically, histone post-translational modifications (PTMs), including phosphorylation, methylation, acetylation and ubiquitylation, can happen on all the basic amino acid residues on histone tails[10]. For instance, lysine has 3 methylation sites, arginine has 2 sites and histidine only has 1 site. This means that lysine has the potential to be mono-methylated, di-methylated and tri-methylated which all have different effects on chromosome organization. As for DNA modification, DNA methylation is the predominant form of modification on DNA. Although in bacteria DNA methylation can happen on adenine, DNA methylation in mammalian cells is restricted to the 5th position carbon on cytosine in CpG sequence[11]. For the purpose of this review, I am going to focus the discussion on DNA methylation, histone methylation and histone acetylation in the following sections.

DNA methylation in metastasis

1. Basic DNA methylation facts

There are around 28 million CpGs in the human genome where DNA methylation can happen; 60-80% of these sites are methylated in different spatial and temporal situations, for example during embryo development and disease[12]. Three DNA methyl-transferases are in charge of DNA methylation in mammals: DNMT1, DNMT3A and DNMT3B[13]. DNMT1 only has DNA methylation maintenance activity, but DNMT3A and DNMT3B are able to generate de novo DNA methylation. In general, high density DNA methylation in the promoter region of genes will inhibit the expression of the genes.

2. DNA methylation and E-cadherin

From the description of DNA methylation, it is not hard to imagine that changes in DNA methylation state across the genome or even at one specific gene would cause a dramatic impact on cell physiology and behavior. Indeed, DNA methylation changes are observed in EMT events [14]. An important hallmark of EMT is the loss of E-cadherin protein which is encoded by CDH1 gene. E-cadherin, a trans-membrane glycoprotein for cell adhesion, is essential for epithelial structure [15]. The loss of E-cadherin results in more invasive tumors, according to experiment in mice models [16]. Interestingly, even though some cases of CDH1 gene mutation are found in patient tumor samples, epigenetic suppression of E-cadherin protein expression is more frequently observed [17]. Among these epigenetic suppression cases, DNA methylation plays an important role in a great proportion of cases [17]. The 5’ promoter region of E-cadherin gene in human
genome contains a large CpG island. This island has been shown to be abnormally DNA hyper-methylated in many cases of different types of human carcinomas, including breast cancer and prostate cancer [18]. This hypermethylation is associated with the suppression of E-cadherin expression. The same phenomenon is also observed in cancer cell line experiment [19]. However, different patterns of promoter hyper-methylation in different cell lines revealed heterogeneity among different events [19]. More importantly, the extent of E-cadherin gene promoter region DNA hyper-methylation coincides with the invasiveness of tumors in human breast cancers [20]. This finding indicates that the loss of E-cadherin protein may give the tumor cells the potential to metastasize. However, this result should be taken with caveats. First of all, the experiment was done on samples of different stages of carcinomas from different patients. As stated before, the heterogeneity of E-cadherin promoter DNA methylation pattern was not taken into account in this type of experiment. A possible route to circumvent this problem is by doing biopsies on different stages of the same tumor which may be difficult in human patients, but possible in mice.

A question lying unsolved here is, what does DNA methylation do to regulate the E-cadherin expression or what is the mechanism of this regulation? Evidence suggests that DNA methylation prevents transcription factors, like CREB and c-Myc [21], from binding to the promoter regions of genes through physical obstruction, which in turn inhibits the gene expression. Apart from this, certain proteins have been indicated as ‘DNA methylation readers’ [22]. There are three major classes of readers: MBDs, Kaiso family and UHRFs. Of these, MBDs are the most extensively studied class [23]. MBD is the general name for proteins containing a methyl CpG-binding domain which includes MeCP2, MBD1 etc. MeCP2 is able to bind to the methylated CpG island in promoter region of genes, including E-cadherin gene. This binding will further recruit HDACs to de-acetylate the histone H3 tails in the same region, which contributes to suppression of gene expression due to chromatin compaction [24]. However, the details and more functions of MBDs and other methylation readers remain to be explored. Implications from current data suggest that DNA methylation readers may act as the bridge between DNA methylation and histone modification to regulate gene expression directly.

3. Other DNA methylation targets

Not surprisingly, DNA methylation has more targets other than E-cadherin gene affecting EMT and metastasis. There are a huge amount of studies showing that DNA methylation involves in the regulation of gene expression correlated with metastasis in various cancer types. Abnormal methylation of Estrogen Receptor promoter, together with E-cadherin, has been shown to appear more frequently as the human breast cancer progress towards malignant stage [20]. Moreover, RASSF1A, WIF1 and MINT17 hyper-methylation are specifically found in breast cancer and colorectal cancer lymph node metastasis cases [25]. Interestingly, leukocyte cellular adhesion molecules, Integrins CD11b, was found up-regulated in metastatic tumors by DNA hypo-methylation [26] which might explain its role in cancer signaling [27]. Besides, Dlg5 [28], NDRG1 [29], HMX3,
IRF4, FLI1 and PPP2R5C[30] hyper-methylation are related to metastasis in individual cancer types.

One noteworthy issue is that DNA hyper-methylation of genes is reported to be far more prevalent than hypo-methylation cases in metastasis[31]. In theory, hypo-methylation of EMT promoting genes should be able to induce its expression and promote EMT. One possible explanation for this discrepancy may be that EMT is a spontaneous event which requires many suppressor genes in order to prevent it from happening.

**Histone modification in metastasis**

1. **Histone methylation and acetylation**

   Histone methylation and histone acetylation are the two most extensively studied types of histone PTMs. Most of the histone modification research focus on histone H3 tail modification. As stated above, modification can happen on all the basic amino acid residues, but lysine seems to be favored [32]. Major known modification sites include but are not limited to: H3K4 (H3 Lysine 4), H3K9, H3K27, H3K36, H3K56, H3K79, H4K20, H3R2, H3R8, H3R17, H3R26 and H4R3 [33]. Histone methylation is conducted by three families of enzymes, namely SET-domain containing proteins, DOT1-like proteins and protein arginine N-methyltransferase (PRMT) family proteins. While SET-domain containing proteins and DOT1-like proteins primarily target lysine on histone, PRMT’s function is limited to arginine methylation [34]. These methyltransferases utilize the methyl group from S-adenosylmethionine (SAM) to methylate the corresponding lysine, arginine or histidine residue on histone tails, no matter whether the histone is inside chromatin or free [35].

   As for histone acetylation, histone acetyltransferases (HAT) and histone deacetylases (HDAC) are the enzymes in charge. HATs use acetyl-CoA as the donor to transfer the acetyl group to lysine residue on histone. In general, acetyl group attached will neutralize the positive charge on lysine, which in turn weakens the electric interaction between the lysine residue and DNA negative-charged backbone[36]. Thus, histone acetylation will potentially loosen the chromatin structure and promote gene expression. However, the effect of histone methylation on gene expression varies depending on the site of methylation.

2. **Regulation and balance of histone modification**

   Because of its slow turnover rate, histone methylation was considered to be irreversible at first, until the first histone demethylase, KDM1A, was discovered [37]. This is a major turning point in the history of histone methylation research for its revelation that histone methylation is reversible and dynamic. There are two identified families of demethylases: amine oxidases and jumonji C-domain containing dioxygenases (JMJDs) and their targets are methylated lysine [37]. After several years of further investigation, we now have a more comprehensive understanding of histone methylation and acetylation regulation.

   Among all the histone modification sites, H3K4 methylation and H3K9 acetylation are shown to be expression promoting, while H3K27 and H3K9 methylation are repressive[38]. Although histone acetylation can have a direct effect on the physical interaction between histone and DNA, the effect of
Histone methylation seems to require additional media proteins to regulation gene expression. These media proteins typically have certain methyl-binding domains, for instance PHD fingers, PWWP domains etc., and directly bind to methylated histone[39]. How binding of these proteins affects the chromatin structure and gene expression is still a question under investigation.

HMTs and HATs do not conduct the modifications randomly, but rather under strict control. Specific DNA sequences will recruit HMTs or HATs to modify histones by interaction with huge protein complexes. The most well characterized recruiting sequences are the Polycomb group response elements (PREs) and Trithorax group response elements (TREs). PREs will recruit Polycomb repressive complex 2 (PRC2) to tri-methylate H3K27 and repress gene expression in that region. On the contrary, TREs will recruit TRX to methylate H3K4 and activate gene expression on site [40]. The traditional view of the relationship of DNA methylation and histone modification is that DNA methylation acts as the upstream regulator of histone modification. Nevertheless, recent evidence also shows that histone modification may also influence DNA methylation[41] putting this question into thicker mist.

3. Histone modification and metastasis genes

Like DNA methylation, histone modification is also found to be a crucial factor in EMT or metastasis. HNF3, a transcription factor collaborating with acetylation co-activator protein p300, has the ability to promote E-cadherin expression and inhibit metastasis[42] in cancer cell lines. On the contrary, E-cadherin is also under control of de-acetylation because Snail1, a well known transcription regulator, binds to the E-box of the E-cadherin promoter and recruits HDACs to de-acetylate the promoter [43]. Even more regulations are placed on E-cadherin. PRC2 was also found associated with silencing E-cadherin gene by H3K27 tri-methylation during tumor progression [44]. Chaotic as it seems, there is a potential complex but strict regulatory network to regulate E-cadherin expression. My hypothesis is: the expression of E-cadherin is under control of all the epigenetic factors, DNA methylation, histone methylation and histone acetylation (Figure 1). These factors compete with each other to gain the control and in certain stage of the cancer, some signal triggers the DNA methylation and suppressive histone methylation to dominate and E-cadherin expression is inhibited. This inhibition leads to the EMT event and probably metastasis of cancer cells. Unfortunately, much more work needs to be done to prove this notion. More genes regulated by histone modification in metastasis remain to be identified, but current research are mostly focusing on putative target, E-cadherin.

Epigenetic therapeutic strategy and methods for metastasis

1. Therapeutic targets

Based on the discussion above about epigenetic regulation of metastasis, there are several potential therapeutic targets: inhibition of DNMT activity or reversal of DNA methylation, inhibition of histone modification and preventing ‘readers’ of epigenetic modifications from binding. In fact, the first two targets have already been verified to be effective.
2. Current epigenetic therapy

5-azacytidine (5-azaC) is a DNA methylation inhibitor, which is first identified as differentiation regulator[45]. Its derivative, 5-aza-2’-deoxycytidine (5-aza-dC), shows effect to rescue expression of E-cadherin in cancer cell lines and prevent EMT from happening[46]. These effects are downstream of DNMT activity inhibition which is non-specific and broad spectrum. Indeed, there are some problems in this treatment; for instance, the MCF-7 breast cancer cell line shows more metastasis upon treatment with 5-aza-dC[47].

HDAC inhibitor is another heated research topic. These inhibitors include butyrate, TSA and SAHA. Butyrate has been shown to promote E-cadherin expression in breast cancer cell lines and induce cell-cell adhesion formation [48]. Similar effect was found in TSA and SAHA [49]. All of these inhibitors are in distinct clinical trial phases.

These wide-spectrum drugs seem to have significant effect on metastasis, but questions should be considered. First of all, are these drugs safe, in terms of not causing some oncogenes to be over-expressed upon treatment? Second, are these drugs potent enough to prevent all the metastasis event, or what is the prevention percentage? Finally, do these drugs cause additional effect to somatic cells leading to severe side-effects? Until these questions are solved, we cannot be satisfied with what we have.

Future Prospective

Research to identify epigenetically regulated genes other than E-cadherin is essential. Identifying more metastasis genes would create more therapeutic targets. If we are able to target two or more genes in metastasis in treatment, the synergy could promote a much higher therapeutic efficiency. The recent development of next-generation sequencing provides a powerful tool to identify new genes being epigenetically regulated in metastasis.

Target-specific epigenetic therapy is still an unknown field. My intent is to eliminate side-effects and unknown effect of wide-spectrum drug treatment by using target-specific treatment. Since the DNA sequence of E-cadherin promoter is available, scientists might be able to produce a molecule to target the methylated promoter region. If a de-methylase is attached to this molecule, DNA hyper-methylation of E-cadherin promoter could be reversed. This approach can be applied to other genes potentially.
Reference


18. Graff, J.R., et al., E-cadherin expression is silenced by DNA hypermethylation in human breast


