Chapter 4
Epigenetic Mechanisms of Cancer Metastasis

Jing Liang and Yongfeng Shang

Abstract Metastasis refers to the process that cancer cells leave their primary tumor mass, break into blood and lymphatic vessels, and travel to distant organ sites throughout the body where they may establish new colonies. Metastasis is responsible for 90 % of cancer mortality. Increasing evidence suggest that epigenetic mechanisms, such as DNA methylation and histone modifications, play an important role in mediating the invasion-metastasis cascade. Targeting deregulated epigenetic modification enzymes by small-molecule inhibitors is a promising therapeutic strategy for the treatment of metastatic cancers.

Keywords Epithelial-mesenchymal transition (EMT) • Cancer metastasis • DNA methylation • Histone modification • Epigenetic therapy

Abbreviations

DNMT	DNA methyltransferase
ECM	Extracellular matrix
EMT	Epithelial-mesenchymal transition

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HATs	Histone acetyltransferases
HDACi	HDAC inhibitors
HDACs	Histone deacetylases
MET	Mesenchymal-epithelial transition
MMPs	Matrix metalloproteinases
PTMs	Post-translational modifications

Cancer is characterized by uncontrolled growth and spread of abnormal cells. Primary tumors do not necessarily cause obvious discomfort to the patient, simply because some organs are well expansible while maintaining their normal functions. A primary tumor in the breast will not cause much trouble to the patient's overall physiological function, unless breastfeeding is needed. Only 10 % of deaths from cancer are due to primary tumors, while metastasis is responsible for the remaining 90 % of cancer mortality. Metastasis refers to the process that cancer cells leave their primary tumor mass, break into blood and lymphatic vessels, and travel to distant organ sites throughout the body where they may establish new colonies. Metastases of breast cancer can be found in the brain, liver, bones, and lungs. Insidious growth of metastatic cancer cells in these sites is life-threatening because the physiological functions of such vital organs are greatly compromised.

While the molecular mechanisms underlying tumorigenesis have been studied in great detail, our understanding of cancer metastasis is limited, partly due to the difficulty to set up the in vitro and in vivo experimental models for this process. Currently, cancer metastasis is considered to be a cascade that consists of a series of interrelated steps, including (i) detachment of tumor cells from the primary tumor; (ii) invasion into the surrounding tissues; (iii) intravasation into the blood or lymphatic vessels; (iv) dissemination in the blood stream or the lymphatic system and, finally, (v) extravasation and colonization at a secondary site (Fig. 4.1) [1, 2]. While certain genetic lesions provide primary tumor cells good opportunity for successful metastasis, the interplay between tumor cells and the surrounding stroma eventually endow tumor cells the ability to fulfill these daunting tasks. Epigenetic mechanisms, including DNA methylation, histone modification, nucleosome remodeling, and RNA-mediated targeting, alter gene expression profile via changes in the chromatin states. The epigenome of cells is dynamic in response to extra- and intracellular signals, while under certain circumstances it can also be transmitted to the next generation of cells to maintain cell identity. Increasing evidence suggest that epigenetic mechanisms play important roles in tumor progression and metastasis. In this chapter, we first describe our current understanding of the major molecular events governing the invasion-metastasis cascade, and then discuss how epigenetic factors, particularly DNA methylation and histone modifications, contribute to the metastasis process.

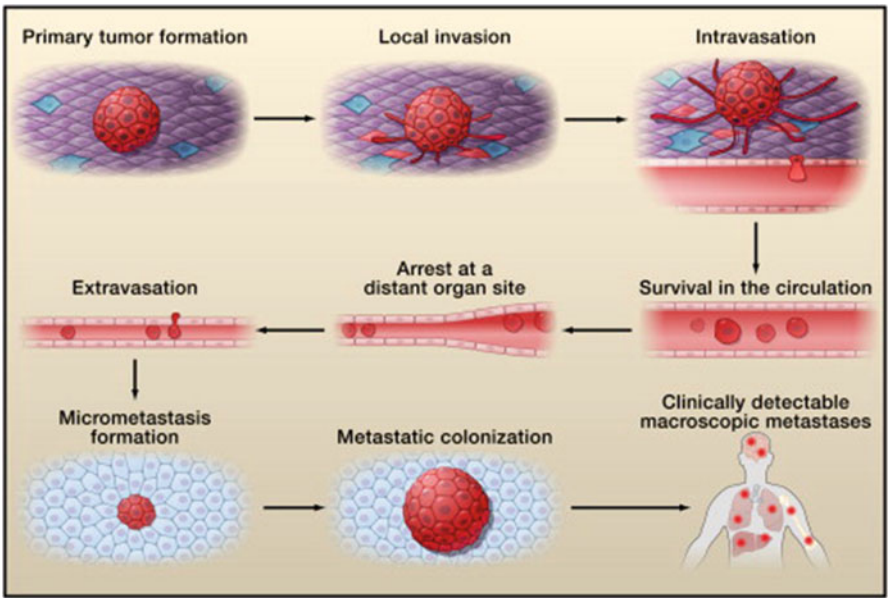

Fig. 4.1 The invasion-metastasis cascade. During metastatic progression, tumor cells exit their primary sites of growth, invade into the extracellular matrix, and enter into the blood or lymphatic vessels (local invasion, intravasation). Circulating cancer cells translocate systemically (survival in the circulation, arrest at a distant organ site, extravasation), and adapt to survive and thrive in the foreign microenvironments of distant tissues (micrometastasis formation, metastatic colonization). Carcinoma cells are depicted in *red* (From S. Valastyan and R.A. Weinberg. Tumor Metastasis: Molecular insights and evolving paradigms. *Cell* 147: 275–292, 2011. Reprinted with kind permission from Elsevier Limited)

4.1 The Epithelial-Mesenchymal Transition Enables Carcinoma Cells to Become Invasive and Represents the Initial Step of Metastasis

Malignant cells arise from epithelial tissues form carcinomas, which contribute to the majority of life-threatening cancers. Early stage carcinoma cells retain the typical epithelial property, which includes lateral tight connection by specialized junction structures, aligned apical-basal polarity through association with the basement membrane, and lack of cell motility. Later on, many of these cancer cells acquire the ability to invade the nearby stroma, travel throughout the body via the lymphatic or hematogenous circulation, and form disseminated colonies in distant organs. To understand how epigenetic mechanisms play a role in cancer metastasis, we need to dissect the process into sequential biological steps, with the notion that these steps are interrelated to each other in reality.

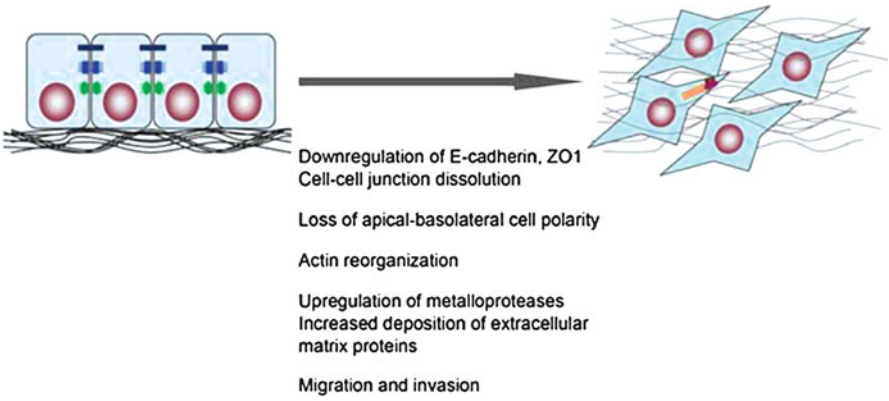

Fig. 4.2 Epithelial-mesenchymal transition. Epithelial-mesenchymal transition (EMT) occurs when epithelial cells lose their epithelial cell characteristics, including dissolution of cell-cell junctions, i.e. tight junctions (*black*), adherens junctions (*blue*) and desmosomes (*green*), and loss of apical-basolateral polarity, and acquire a mesenchymal phenotype, characterized by actin reorganization and stress fiber formation (*red*), migration and invasion (From J. Xu, S. Lamouille and R. Derynck. TGF-[beta]-induced epithelial to mesenchymal transition. *Cell Res* 19: 156–172, 2009. Reprinted with permission from Nature Publishing Group)

As long as tumor cells are separated from the surrounding tissues by an intact basement membrane, they are not truly "malignant". Once in situ carcinoma cells breach the basement membrane and invade into the stroma, the initial step of metastasis is manifested. Arise from epithelial tissues, original carcinoma cells has poor motility, and the lateral cell-cell connection and the apical-basal polarity do not allow these cells to invade through the underlying extracellular matrix (ECM). To initiate the first step of metastasis, carcinoma cells must transform someway to shed these epithelial properties that suppress invasion, and acquire mesenchymal phenotypes such as better motility, loose cell-cell contacts, and affinity to the stroma. The epithelial-mesenchymal transition (EMT) is now generally accepted to be the critical initial step of cancer metastasis (Fig. 4.2).

EMT was recognized as a feature of embryogenesis in the early 1980s. One example of EMT in embryonic development is the formation of the mesoderm, the precursor of mesenchymal tissues, during gastrulation in early embryogenesis. At this stage, ectoderm cells, which are located at outer side of the embryo and arrayed in an epithelial cell layer, migrate inward toward the center of the embryo to form the mesoderm, where fibroblasts and hematopoietic cells originate. EMT can also be witnessed in wound healing, in which process the epithelial cells at the edge of a wound acquire mesenchymal phenotype and become motile and invasive to fill in the gap in the epithelium created by the wounding. Therefore, EMT is not a unique nature of carcinoma cells; rather it is the activation of a reprogramming behavior that is usually confined to early embryogenesis and wound healing in adult.

Fundamental alterations of gene expression lead to reconstituted cellular machinery, and ultimately result in the phenotypic changes of EMT cells. Among all the molecules that influence epithelial versus mesenchymal phenotypes, the epithelial specific E-cadherin, encoded by *CDH1* gene, plays the dominant role. E-cadherin is a transmembrane glycoprotein, and the cytoplasmic domains of E-cadherin are tethered to the actin fibers of the cytoskeleton via a complex of α- and β-catenins. E-cadherin molecules are displayed on adjacent epithelial cells and tether the apposed plasma membranes to one another. Loss of E-cadherin is consistently observed at the sites of EMT. Epithelial originated cells acquire a mesenchymal morphology and increased motility when the expression of E-cadherin is suppressed, whereas re-expression of this protein in invasive cancer cells strongly suppressed their metastatic dissemination. Besides E-cadherin, loss of expression of other epithelial specific proteins, such as α-, β-, and γ-catenins, and gain of expression of mesenchymal markers, such as fibronectin, vimentin, N-cadherin, is constantly observed during EMT [3, 4].

4.2 The Interplay Between Cancer Cells and the Stroma Provide Signals for the Epithelial-Mesenchymal Transition

EMT is an induced, reversible process, as carcinoma cells often revert back to a more epithelial phenotype once these cells reach the distant organs, where EMT-inducing signals are no longer existed. Therefore, the metastases resemble the phenotype of the primary carcinomas. This process is often called mesenchymal-epithelial transition (MET). EMT can be induced or regulated by various growth and differentiation factors, including TGF-β (transforming growth factor-β), TNF-α (tumor necrosis factor- α), EGF (epidermal growth factor), HGF (hepatocyte growth factor), and IGF-1 (insulin-like growth factor-1). These heterotypic signals are mainly released by the stroma of primary carcinomas, and cells located at the outer edges of the neoplasm sense these signal and undergo EMT. Among these factors, the major inducer of EMT is TGF-β, which can also produced by cancer cells themselves to generate a positive feedback loop for EMT induction. Culturing EpRas tumor cells in the presence of TGF-β, these epithelial, cobblestone-like cells change to an elongated fibroblastic phenotype. The levels of tumor-associated TGF-β (often TGF-β1) were frequently found to be correlated with increasing degrees of tumor invasiveness. High levels of TGF-β in the blood are often predictive of poor prognosis for the cancer patient. EMT-inducing growth factors activate different intracellular signaling cascades in carcinoma cells, such as NF-κB and Wnt pathways, leading to the alteration of gene expression profiles that favor mesenchymal phenotypes.

In response to various inductive signals, EMT-inducing transcription factors can serve as major signaling mediators of the EMT program to promote metastasis. Ectopic expression of these transcription factors enables epithelial cells undergo

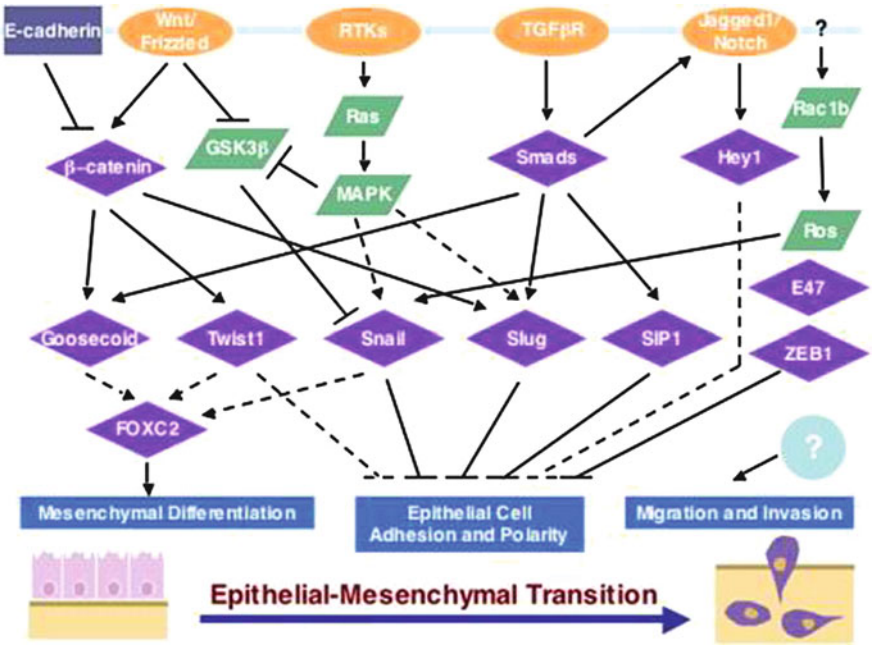

Fig. 4.3 Signaling pathways and transcription factors that regulate the epithelial-mesenchymal transition in carcinoma cells. In cancer cells, the TGF-β signaling pathway induces multiple EMT-inducing transcription factors, including Slug, SIP1, and Goosecoid, via activation of Smads. The Wnt pathway and loss of E-cadherin from adherens junctions activate β-catenin, which in turn induces several EMT-inducing transcription factors as well, such as Slug, Twist1, and Goosecoid. Multiple tyrosine kinase receptor (TKR) pathways, including FGFR, EGFR, PDGFR, and HGFR, can induce the expression of Snail and Slug through the Ras-MAPK pathway. Among all the EMT-inducing transcription factors, Snail, Slug, SIP1, and E47 directly suppress E-cadherin transcription, while Twist1, Goosecoid, and FOXC2 seem to function indirectly. FOXC2 is induced in tumor cells expressing Twist1, Snail, and Goosecoid and mediates mesenchymal differentiation. *Solid lines* indicate direct transcriptional or posttranscriptional regulations. *Dashed lines* indicate indirect regulation (From J. Yang and R.A. Weinberg. Epithelial-mesenchymal transition: At the crossroads of development and tumor metastasis. *Dev Cell* 14: 818–829, 2008. Reprinted with kind permission from Elsevier Limited)

EMT and acquire mesenchymal phenotype (Fig. 4.3). Examples of these EMT-inducing transcription factors include Twist, Snail, Slug, ZEB2/ZFXH1B/SIP1, and Goosecoid. These proteins are often highly expressed during embryogenesis, and their reactivation allows carcinoma cells to initiate the EMT program. Accumulating evidence associates these transcription factors with various malignancies, especially those with highly invasive behavior and poor prognosis. These transcription factors can regulate a panel of downstream target genes that collaboratively program an EMT process. For example, the zinc-finger transcription factors Snail, Slug, ZEB1 and ZEB2 are capable of directly repressing the transcription of E-cadherin and several polarity factors, including Crumbs3 and Lgl2 [5, 6].

4.3 Disseminated Cancer Cells Found Metastases in Distant Organs

Once primary tumor cells undergo EMT, they acquire abilities for further invasion. Before they enter the general circulation, EMT-transformed tumor cells first need to pave the way in the ECM by either dissolving the stroma or pushing aside any cells that stand in their path. Among the molecular events that govern this process, the best understood is the secretion of matrix metalloproteinases (MMPs), which is a class of secreted proteases that can degrade specific components of the ECM. MMPs are secreted by tumor-recruited stroma cells, such as macrophages, mast cells, and fibroblasts. Dissolving of the ECM by MMPs creates spaces for the tumor cells to move. MMPs also mobilize and activate some growth factors that have been tethered in the inactive form to the ECM or to the cell surface.

Motile tumor cells adhere to the vessels of blood or lymphatic systems in the stroma and enter the general circulation, a process that is often referred to intravasation. Before these cells reach their destination, they may experience much ordeal such as mechanical shearing forces of the blood stream and attack from immune cells in the circulation. The molecular mechanisms that govern these events are largely unknown. Theoretically, metastatic cancer cells can disseminate all over the body, but several organs, such as the lung, bone, liver, and brain are clearly more prone for metastases to grow. In addition, subtypes of tumor cells from different tissue origin have their own preference of metastatic organs. The vast majority of cells that end up forming small micrometastases (<2 mm diameter) never succeed in growing into macrometastases (>2 mm diameter) that are clinically relevant for the patients' health. In this sense, colonization is extremely inefficient, and often considered to be the rate-limiting step in the entire invasion-metastasis cascade. Although the detailed molecular mechanisms are yet to be clarified, it is important to understand that it is the complex interplay between metastatic cancer cells and the microenvironment of a foreign tissue that essentially determines whether colonization will be successful and metastatic diseases will ever develop. Dispersed cancer cells will only survive and found colonies in the environment with appropriate chemokines, trophic factors, and mitogens, while seeding cancer cells also release heterotypic signals to reshape the landed tissues. In this adaptation process, gene expression reprogramming in the metastatic cancer cells is inevitable.

Analogous to oncogenes and tumor suppressor genes that regulate tumorigenesis, there are many genes that function positively or negatively in the regulation of metastasis. Metastasis promoting regulators can be growth factors, growth factor receptors, transcription factors, or key components in signal transduction pathways. When genes encode these molecules are ectopically expressed in epithelial cells, they are able to elicit metastasis-prone phenotypic changes, such as increased cell motility and decreased cell polarity. On the other hand, metastasis suppressor genes encode proteins that specifically inhibit invasion and metastasis without affecting the growth of primary tumors. To date, over 20 metastasis suppressor genes have been identified, with the best characterized is *CDH1*, which encodes the

epithelial-specific E-cadherin. Although the molecular functions of many metastasis suppressor genes are yet to be clarified, altered expression of these genes, whether due to genetic defects or epigenetic regulations, can significantly affect the overall invasion-metastasis process [7].

4.4 Promoter Methylation is an Important Epigenetic Mechanism to Regulate the Expression of Metastasis Related Genes

The long journey of invasion-metastasis consists of multiple steps that involve the interplay between primary tumor cells and the surrounding cells in the stroma, blood, and the distant foreign tissues. From the initial EMT to the final colonization, cancer cells constantly transduce heterotypic extracellular signals into changes of gene expression and cell behavior to better adapt themselves to metastasis. Unlike genetic defects such as gene amplification, deletion, or mutations, epigenetic regulations, such as chemical modifications of DNA sequences or histone proteins, are more flexible in response to various extracellular stimuli. In the mean time, certain epigenetic modifications can also be inherited by the next generation of cells to maintain relatively stable cell characteristics. In recent years, it is increasingly realized that epigenetic mechanisms play a profound role in the regulation of cancer metastasis. Epigenetics is commonly used to describe chromatin-based events that regulate DNA-templated processes. Direct DNA methylation at cytosine on CpG sequences, post-translational modifications (PTMs) of histones, the presence of histone variants, remodeling of nucleosomes, and non-coding RNA mediated targeting are the major epigenetic pathways that regulate many important biological processes [8]. In this chapter, we mainly focus on how DNA methylation and histone modifications play a role in the invasion-metastasis cascade.

Promoter cytosine methylation in CpG dinucleotides inactivates gene expression and can profoundly affect the metastasis cascade. DNA methylation is catalyzed by DNA methyltransferase (DNMT) enzymes. DNMT-1 is responsible for DNA methylation maintenance through its action on semimethylated CpG substrates. DNMT-3A and -3B are the *de novo* methyltransferases to newly methylate cytosine during early embryogenesis [9]. Promoter hypermethylation of certain metastatic suppressor genes is constantly observed in various invasive malignancies. Hypermethylation of the *CDH1* promoter in cancers leads to the loss of E-cadherin expression during cancer progression. A large CpG island in the 5′ proximal promoter region of the *CDH1* gene shows aberrant DNA methylation in many different human carcinomas and correlates with reduced E-cadherin protein expression. Exposure of cancer cells with demethylating agent 5′-aza-2′-deoxycytidine (5Aza-dC) reactivates E-cadherin expression in many cancer cell lines, leading to increased cell aggregation and reduced cell motility and invasiveness [6]. Kisspeptin (KISS-1) gene has been identified as a metastasis suppressor gene in various human

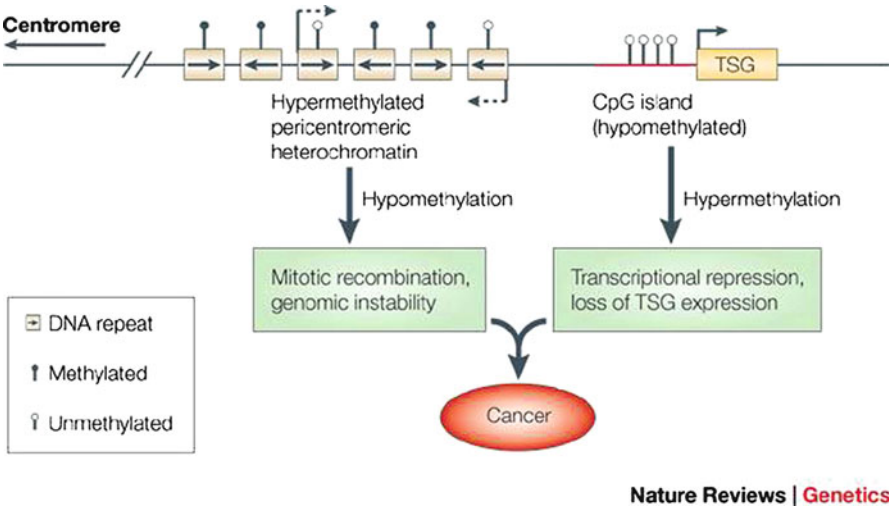

Fig. 4.4 DNA methylation and cancer. In normal cells, the repeat-rich, pericentromeric heterochromatin is hypermethylated and is transcriptionally silent. Actively transcribed tumour suppressor gene (*TSG*) is associated with a hypomethylated CpG island (indicated in *red*). In tumour cells, repeat-rich heterochromatin becomes hypomethylated and this contributes to genomic instability through increased mitotic recombination events. De novo methylation of CpG islands in the TSG or the metastasis suppressor gene can result in the transcriptional silencing of these genes, leading to unrestrained growth and spreading of the cancer cells (From K.D. Robertson. DNA methylation and human disease. *Nat Rev Genet* 6: 597–610, 2005. Reprinted with permission from Nature Publishing Group)

malignancies. KISS-1encodes a number of peptides (kp-54, kp-14, kp-13, kp-10), which are endogenous ligands to a G protein-coupled receptor called GPR54. The molecular basis of anti-metastatic activity of KISS-1 is not fully understood. Some evidences suggest that kisspeptin/GPR54 system negatively regulates MMP-9, a member of matrix metalloproteinases that degrade ECM. Secretion of KISS1 has also been demonstrated to be necessary to maintain dormancy in disseminated cancer cells, thus blocking metastatic colonization. Inactivation of KISS-1 gene by promoter hypermethylation has been observed in many invasive cancers such as melanoma, bladder cancer, and gastric carcinoma [10].

Genome-wide hypomethylation is a common feature of many malignant cells (Fig. 4.4). Hypomethylation of regulatory DNA sequences leads to overactivation of some oncogenes or EMT-inducing transcription factors, and general hypomethylation in heterochromatin regions is more deleterious as it results in genomic instability and subsequent multiple genetic defects [11]. Within a breast tumor mass, heterotypic cancer cells can often be sorted to two groups based on their phenotype and specific cell surface markers, namely stem cell-like, more invasive $CD44^{+}$ $CD24^{-}$cells and epithelial, more differentiated $CD44^{-}CD24^{+}$ cells. Promoter hypomethylation and the resultant overexpression of several EMT-inducing transcription factors have been observed in invasive $CD44^{+}$ $CD24^{-}$cells compare to their differentiated $CD44^{-}CD24^{+}$ counterparts [12].

4.5 Complex Post-Translational Histone Modifications Coordinately Regulate the Expression of Metastasis-Related Genes

In most cases, the role of promoter DNA methylation in gene regulation is relatively straightforward, in that hypermethylation inhibits gene expression whereas hypomethylation activates gene expression. By contrast, the influence of post-translational histone modifications on gene expression is far more complex and context-dependent. In eukaryotic cells, DNA is packaged into chromatin. The nucleosome is the fundamental unit of chromatin and it is composed of an octamer of the four core histones (H3, H4, H2A, H2B) around which 147 base pairs of DNA are wrapped. The core histones are predominantly globular except for their N-terminal "tails", which are unstructured and protrude from the globular core. Various post-translational chemical modifications, such as acetylation, phosphorylation, poly ADP-ribosylation, ubiquitination, and methylation, particularly those on the N-terminal tails, can alone or in combination affect gene transcription through direct remodeling of chromatin structure or recruitment of regulatory non-histone proteins (Fig. 4.5). For one particular histone modification, there are "writers" to catalyze the addition and "erasers" responsible for the removal of the mark. In addition, "readers" are those factors that bind to the specific modifications and respond to the "histone code" information conveyed by upstream signaling cascades. Aberrant histone modifications at either the candidate loci or genome-wide level, often caused by the deregulation of the writer/eraser/reader interplay, plays a significant role in mediating the invasion-metastasis cascade [13–15].

4.5.1 Histone Acetylation

Histone lysine acetylation neutralizes lysine's positive charge and consequently weakens the electrostatic interaction between histones and negatively charged DNA, leading to an "open" chromatin conformation in favor of transcriptional activation. In addition, acetylated lysine residues may serve as a "docking" site for non-histone proteins, which can carry their enzymatic activities and further modify chromatin. Histone acetylation is catalyzed by histone acetyltransferases (HATs) and is removed by histone deacetylases (HDACs). Usually, the catalytic activity of HATs and HDACs is not restricted to one particular lysine residue. Most of the identified human HATs, which mainly include three families, GNAT, MYST, and CBP/p300, function as transcriptional co-activators, and are recruited to chromatin by interacting with sequence-specific DNA-binding proteins [16]. For example, nuclear protein p300 acetylates multiple lysine residues on H3, H4, H2A, and H2B. p300 binds to transcription factor HNF3, and these two factors coordinately activate the transcription of *CDH1* gene, which encodes the metastasis suppressor E-cadherin. Ectopic expression of p300 in certain cancer cells can restore E-cadherin expression and repress their metastatic potential [17].

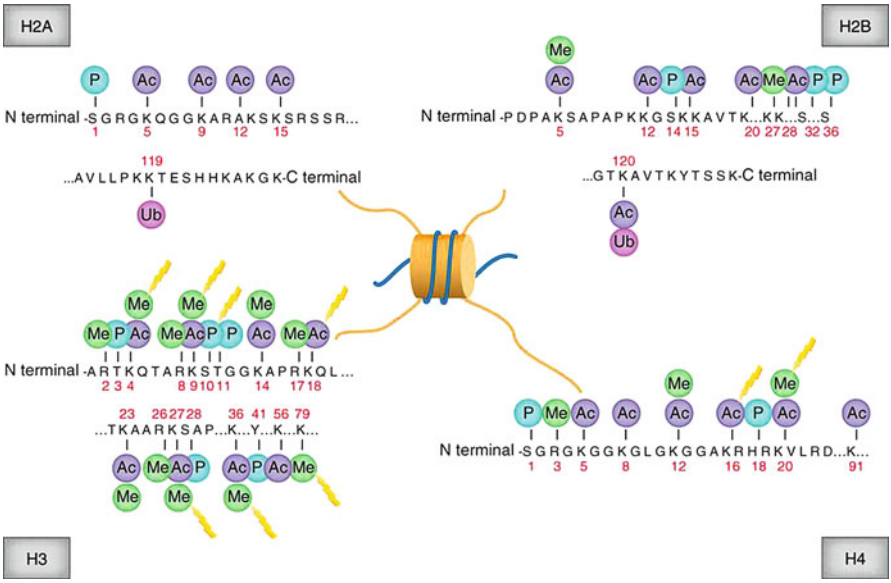

Fig. 4.5 Histone modification patterns in normal and cancer cells. Histones can undergo diverse post-translational modifications, especially on their protruding N-terminal tails. In the right combination and translated by the appropriate effectors, these modifications contribute to establishing the global and local condensed or decondensed chromatin states that eventually determine gene expression. This figure depicts the main modifications of the four core histones in normal cells (type and position in the amino acid sequence). Histone modifications typically associated with cancer have also been highlighted. *Ac* acetylation, *Me* methylation, *P* phosphorylation, *Ub* ubiquitination (From M. Rodríguez-Paredes and M. Esteller. Cancer epigenetics reaches mainstream oncology. *Nat Med* 17: 330–339, 2011. Reprinted with permission from Nature Publishing Group)

4.5.2 Histone Deacetylation

Deacetylation on lysine residues of histone proteins by HDACs are believed to cause chromosomal condensation and gene repression. There are three distinct families of histone deacetylases: the class I and class II histone deacetylases and the class III NAD-dependant enzymes of the Sir family. Class I HDACs include HDAC1, HDAC2, HDAC3, and HDAC8, and they are mainly localized to the nucleus. Class II HDACs include HDAC4-7, HDAC9, and HDAC10, and these proteins can shuttle between the cytoplasm and the nucleus. Class III HDACs are homologs of Sir2, a yeast transcriptional repressor that requires the cofactor NAD^+ for its deacetylase activity. Most of classes I HDACs are subunits of multiprotein nuclear complexes that are crucial for transcriptional repression [18]. At the E-cadherin promoter, EMT-inducing transcription factor Snail1 binds to the specific sequence called E-box elements, and recruits a repressive complex consisting of HDAC1, HDAC2 and SIN3A. Other EMT-inducing transcription factors Snail2/Slug, ZEB1, and ZEB2 similarly repress E-cadherin transcription by recruiting repressive protein complexes containing different class I HDACs [19].

4.5.3 Histone Methylation

Histone methylation occurs on all basic residues: arginines, lysines and histidines. The best-characterized sites of histone methylation are those that occur on lysine residues. The histone lysine methyltransferases and demethylases are responsible for addition or removal of methyl groups from different lysine residues on histones. These enzymes are highly specific, in that each enzyme regulates mono-, di-, or trimethylation of a single or a few lysine residues on histones. Because lysine methylation does not usually alter the charge of histone proteins, this modification per se has little influence on the interaction between histones and DNA. Instead, the location of the methyl-lysine residue on a histone tail and the degree of methylation (whether mono-, di-, or trimethylation) serve as the docking signal to recruit various reader proteins containing methyl-binding domains. To date, many methyl-binding domains have been identified. The classic methyl-binding domains are the Royal superfamily, including chromodomains, double chromodomains, chromobarrels, Tudor domains, double or tandem Tudor domains and the malignant brain tumor (MBT) repeats. Proteins with other domains, such as PHD finger, WD40 repeats, CW domains, PWWP domains, ankyrin repeats, have also been reported to recognize and bind to methylated lysine residues. Functionally, the combinations of the recruited regulatory proteins ultimately determine whether the transcription of local genes is activated or repressed [20].

The most extensively studied histone methylation sites include histone H3 lysine 4 (H3K4), H3K9, H3K27, H3K36, H3K79 and H4K20. The effects of methylation on gene transcription are diverse and context-dependent. In general, H3K4me3 is associated with active transcription or with genes that are poised for activation, whereas H3K27me3 is associated with repressed chromatin. H3K4me1 is often associated with enhancer function, whereas H3K4me3 is linked to promoter activity [21].

Aberrant histone methylation plays a role in cancer progression. Changes in global levels of certain histone methylation events are correlated with increased cancer recurrence and poor survival [20]. Mutations in or altered expression of histone methyltransferases correlate with various invasive cancers. These enzymes are often critical to the transcriptional regulation of key metastasis-related genes.

The SET-domain containing proteins and DOT1-like proteins have been shown to methylate lysine residues in histone and non-histone substrates. Enhancer of zeste homolog 2 (EZH2) contains a SET domain and is the catalytic component of the PRC2 complex, which is primarily responsible for catalyzing the trimethylation of histone H3 lysine 27 (H3K27me3). EZH2 has been found to be overexpressed in metastatic prostate cancer, and the expression level of EZH2 directly correlates with the aggressiveness of breast cancer. Ectopic expression of EZH2 in immortalized human mammary epithelial cell lines promotes anchorage-independent growth and cell invasion. In the molecular level, EZH2 usually acts as a transcriptional repressor through its H3K27me3 methyltransferase activity. Presumably, EZH2 promotes cancer metastasis by transcriptional inhibition of anti-metastasis genes. EZH2 is recruited to the promoter region of *CDH1* and represses E-cadherin expression in an

invasive prostate cancer cell line DU145 [22]. Interestingly, loss-of-function mutations in EZH2 gene confer a poor prognosis in certain hematopoietic malignancies, suggesting a tumor-suppressive role for EZH2 in these cell lineages [23, 24]. To date, the precise role of gain and loss of EZH2 activity in cancers and the underlying molecular mechanisms are an area of active investigation.

G9a (also known as KMT1C or EHMT2) catalyzes dimethylation of histone lysine 9 (H3K9me2). In TGF-β induced EMT cell line models, it has been demonstrated that transcription factor Snail recruits G9a and DNA methyltransferases to the *CDH1* promoter, leading to the inhibition of E-cadherin expression and the induction of EMT. Knockdown of G9a restores E-cadherin expression, inhibits cell migration and invasion, and reduces lung colonization of breast cancer metastasis [25].

SET8 (also known as PR-Set7/9, SETD8, KMT5A), a member of the SET domain-containing methyltransferase family, catalyzes monomethylation of H4K20. Recent studies indicate that SET8 and the transcription factor Twist are functionally interdependent to promote EMT and enhance the invasiveness of breast cancer cells in vitro and in vivo. SET8 is recruited to the *CDH1* promoter by Twist and repress E-cadherin expression; interestingly, SET8 and Twist are also present at the N-cadherin promoter, where they enhance the transcription of the gene. Together, SET8 and Twist coordinately promote EMT and cell invasiveness, and the dual function of SET8 at different promoters reinforce the notion the effect of histone methylation on gene transcription is context-dependent, relying on the combinations of histone modifications nearby and the distinct sets of regulatory proteins recruited [26].

4.5.4 Histone Demethylation

Two families of demethylases, the amine oxidases and jumonji C (JmjC)-domain-containing, iron-dependent dioxygenases, have been identified thus far to demethylate methyl-lysines [27]. Lysine-specific demethylase 1 (LSD1) is an amine oxidase that catalyzes the removal of mono- and di-methylation from histone H3 lysine 4 (H3K4). LSD1 regulates several intracellular signaling pathways including that of TGFβ1, which plays a critical role in cancer metastasis as we mentioned earlier in this chapter. In breast cancer tissue samples, the level of LSD1 is negatively correlated with that of TGFβ1. LSD1 downregulates TGFβ1 and inhibits the invasiveness and metastasis of breast cancer cells in vitro and in vivo [28]. Responsible for the removal of H3K4 trimethylation, JARID1B/PLU-1 belongs to the JmjC family of demethylases. Through its enzymatic activity, JARID1B removes the active H3K4me3 marks at the promoter regions of various genes, including CCL14, an epithelial derived chemokine. JARID1B inhibits the expression of CCL14 and suppresses the angiogenic and metastatic potential of breast cancer cells [29].

In many cases, multiple histone and DNA modification enzymes collaborate to regulate target gene expression. These factors may form a stable protein complex; alternatively they can assemble and dissemble dynamically at different chromatin

locations. H3K27me3 methyltransferase EZH2 physically interacts with DNA methyltransferases at the promoters of certain target genes, leading to the silence of these genes. The transcriptional repressive protein complex NuRD contains multiple components with different chromatin-related activities, and these components include chromatin remodeling factors (metastasis tumor antigen, MTA), histone deacetylases (HDAC1 and HDAC2), histone binding proteins (RbAp46 and RbAp48), methyl CpG-binding proteins (MBD2 and MBD3), DNA helicase/ATPase (Mi-2α/β), and in certain circumstances histone demethylases (LSD1 and JARID1B). Except for the core components, multiple NuRD subunits could be dynamically incorporated into the big protein complex at different chromatin locations, allowing more specific and fine-tuned regulation of different target genes [30, 31].

4.6 Epigenetic Therapy for Cancer Metastasis

Unlike genetic defects, epigenetic aberrations that lead to cancer metastasis are theoretically reversible, making them the ideal drug targets for cancer therapeutics. By inhibition of the particular modification enzymes that are responsible for the addition or removal of the chemical groups from DNA or histones, we may adjust the aberrant epigenome and the abnormal gene expression in cancer cells (Fig. 4.6). Although effective inhibitors of many DNA or histone modification enzymes have been developed, these epigenetic drugs have certain embedded problems that may limit their applications until we get better understanding of the molecular mechanisms of cancer epigenetics. To what degree an inhibitor is specific to a particular enzyme? To what degree inhibition of this enzyme is specific to regulate growth- and metastasis-associated genes in cancer cells? Does inhibition of epigenetic modification enzymes affect the biological function of non-cancer cells? These problems directly affect the efficacy and toxicity of the potential epigenetic anti-cancer drugs. Despite the above concerns and the lack of detailed molecular mechanisms, there are several epigenetic drugs that have been shown to effectively reverse metastatic phenotype in different cancer cell lines, and many clinical studies have evaluated specific enzyme inhibitors in the treatment of cancer metastasis [6, 32, 33].

As we have repeatedly stated earlier, the E-cadherin encoding gene *CDH1* is a critical metastasis suppression gene. Transcription of *CDH1* gene is silenced or reduced in many cancers with high metastasis potential. Reactivation of the *CDH1* gene expression is an important anti-metastasis therapeutic strategy. It has been demonstrated that inhibition of the activity of either DNMTs or histone modification enzymes that remove certain repressive histone modifications can effectively upregulate E-cadherin expression. Treatment of several invasive cancer cell lines with the DNMT inhibitor 5-aza-2′-deoxycytidine leads to restoration of E-cadherin expression and reversion of these cells to the epithelial phenotype. DNMT inhibitors 5-azacytidine and 2′-deoxy-5-azacytidine have been approved for the treatment of myelodysplastic syndrome, a pre-leukemic bone marrow disorder. It is important

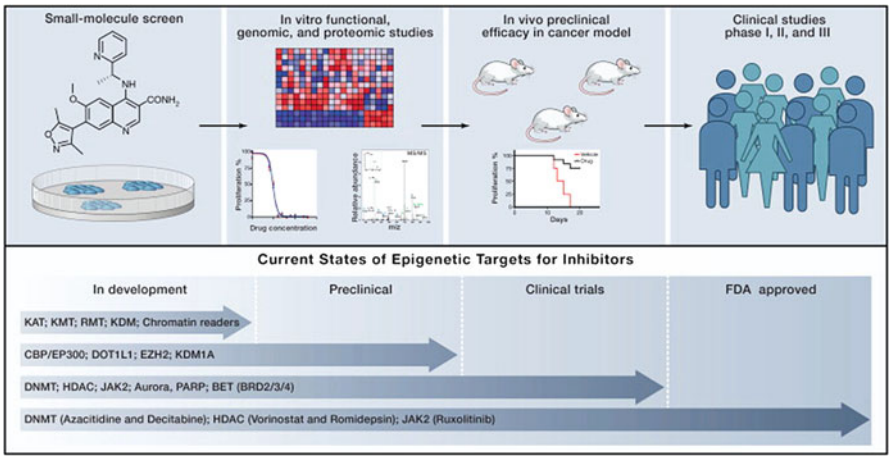

Fig. 4.6 The process to develop epigenetic drugs and the current status of various epigenetic therapies in cancer. Candidate small-molecule inhibitors are first tested in vitro in malignant cell lines for specificity and phenotypic response. These may, in the first instance, assess the inhibition of proliferation, induction of apoptosis, or cell-cycle arrest. These phenotypic assays are often coupled to genomic and proteomic methods to identify potential molecular mechanisms for the observed response. Potentially effective inhibitors are then tested in vivo in animal models of cancer to ascertain whether they may provide therapeutic benefit in terms of survival. Animal studies also provide valuable information regarding the toxicity and pharmacokinetic properties of the drug. Based on these preclinical studies, candidate molecules may be taken forward into the clinical setting. *KAT* histone lysine acetyltransferase, *KMT* histone lysine methyltransferase, *RMT* histone arginine methyltransferase, *PARP* poly ADP ribose polymerase (From M.A. Dawson and T. Kouzarides. Cancer epigenetics: From mechanism to therapy. *Cell* 150: 12–27, 2012. Reprinted with kind permission from Elsevier Limited)

to bear in mind the exact role of the DNMT inhibitors in cancer progression is not fully understood, and in some cases these drugs can elicit opposite response in different cancer cell lines. Recent data showed that treatment of MCF-7 breast cancer cells, which are largely epithelial, with 5-aza-2′-deoxycytidine may increase their ability for invasion and metastasis, concomitant with the upregulation of several pro-invasive genes. These observations raise concerns about the potential use of DNA methyltransferase inhibitors for the treatment of breast cancer [6].

HDAC inhibitors (HDACi) have been shown in preclinical studies to selectively target cancer cells with high specificity. The effects of HDACi include the induction of apoptosis and cell cycle arrest, and suppression of angiogenesis and tumor cell invasion. The anti-metastasis activity of HDACi is at least partially dependent on their potent capacity to upregulate E-cadherin expression. Butyrate, the first HDACi to be identified, induces cell cycle arrest and enhances cell-cell adhesion in two breast cancer cell lines, and these phenotypic changes can be inhibited by the addition of E-cadherin antibodies. Subsequently, butyrate was found to upregulate E-cadherin expression in colon cancer cells and in endometrial carcinoma cells.

As of today, there are at least 20 structurally different HDAC inhibitors in clinical trials, either in monotherapy or in combination therapy trials for hematological and solid tumors. HDAC inhibitors SAHA and romidepsin have been approved for the treatment of cutaneous T cell lymphoma [33, 34].

It is important to notice that the clinical effectiveness of the approved drugs is not necessarily dependent on their function to change the epigenome. Azacytidine is not a specific inhibitor of DNMTs, but a nucleoside analog that affects many cellular pathways. It is presently unclear to what degree the diverse and complex drug effects contribute to clinical responses to azacytidine. On the other hand, although SAHA is a highly specific HDAC inhibitor, the target enzymes are not specific for histones and include a wide range of non-histone proteins that are not involved in epigenetic regulation. Once again, clarifying the molecular mechanisms of epigenetic factors in cancer progression and designing more specific enzyme inhibitors are the key issues in the future application of epigenetic anti-cancer therapy.

4.7 Concluding Remarks and Future Perspective

Metastasis is life-threatening and accounts for 90 % of cancer mortality. Along the invasion-metastasis cascade, the initial commitment for carcinoma cells to move out is the EMT process. Extensive investigations have identified key signaling pathways and transcription factors that mediate EMT. Most of our current knowledge of metastasis epigenetics is essentially about the deregulated epigenome leading to abnormal expression of genes that induce or inhibit EMT. By contrast, while colonization in and adaptation to the distant foreign tissues is the final rate-limiting step for metastasis, our understanding to the molecular mechanisms of colonization is still limited, partly due to the difficulty to establish experimental models of this step. In fact, many patients may already have occult micrometastases at the time of primary cancer diagnosis; therefore targeting the final colonization step is a more appropriate therapeutic strategy to treat metastatic cancers. It is no doubt we should expect more studies to elucidate the molecular mechanisms governing metastatic colonization and the underlying epigenetic factors contribute to this process.

In recent years, we have witnessed the rapid development of many genome-wide based technologies. Global alteration of epigenomics is constantly observed in various types of invasive cancers. The integration of these data with the information coming from genomics and transcriptomics will exponentially expand understanding of cancer metastasis and yield better epigenetic biomarkers for detection, prognosis and therapy prediction. Considering that the effects of most of the epigenetic drugs are still nonspecific and may cause undesirable side effects, it will be necessary to design new agents against specific enzymes of the epigenetic machinery involved in specific types of cancer. At that time, epigenetics will truly enter the center stage of cancer research.

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