



# Epigenetic plasticity in metastatic dormancy: mechanisms and therapeutic implications

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**Abstract:** The overwhelming majority of cancer-associated morbidity and mortality can be ascribed to metastasis. Metastatic disease frequently presents in a delayed fashion following initial diagnosis and treatment, requiring that disseminated cancer cells (DCCs) spread early in tumor progression and persist in a dormant state at metastatic sites. To accomplish this feat, DCCs exhibit substantial phenotypic plasticity that is mediated by the epigenetic regulation of dormancy programs in response to intrinsic (i.e., cellular) and extrinsic (i.e., microenvironmental) cues. The epigenome is a dynamic landscape that encompasses transcriptional regulation via alteration of chromatin architecture, posttranscriptional RNA processing, and the diverse functions carried out by noncoding RNAs. Signals converging on DCCs are transduced through epigenetic effectors. Conversely, epigenetic regulation of gene expression controls the crosstalk between DCCs and cells of the metastatic niche, a phenomenon that is essential for the institution of dormant phenotypes. Importantly, epigenetic effectors can be targeted therapeutically, and the development of novel epigenetic therapies may provide new inroads to combating recurrent metastatic disease. Here we provide an overview of the dynamics of metastatic dormancy and summarize our current understanding of the intersections between dormancy and the epigenome, both mechanistically and therapeutically.

**Keywords:** Dormancy; epigenetics; long non-coding RNAs (lncRNAs); metastasis; targeted therapy

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## Introduction

Cancer is one of the leading causes of premature mortality across the globe. In a given year, over 18 million people will be diagnosed with cancer, with nearly 10 million people succumbing to their disease (1). The vast majority of these deaths can be directly attributed to metastasis, the process by which cancer cells escape from their primary tumor of origin and disseminate to distant sites (2,3). The deadly nature of metastasis encompasses nearly all tumor types, including breast (4), lung (5), and melanoma (5), as well as colorectal and other gastrointestinal (GI) malignancies (6,7). In spite of this clinical imperative, research into the

mechanisms governing metastasis remains under-resourced. Consequently, there is a paucity of therapies capable of specifically targeting metastasis, thereby compelling patients afflicted with metastatic disease to be subjected to cytotoxic chemotherapies (8). Given this tremendous public health burden, there is indeed a pressing need for science and medicine to unravel the molecular mysteries of metastasis.

Further complicating the management and overall outlook for metastatic cancer patients is the emergence of recurrent metastatic disease, which can occur even in the absence of a new primary tumor. Indeed, across the spectrum of human cancers, a substantial proportion of metastatic disease presents years-to-decades following

initial diagnosis and treatment (9,10). This delay in the metastatic outgrowth of disseminated cancer cells (DCCs) that reside in metastatic niches is termed metastatic dormancy, a phenomenon encompassing two distinct stages, namely intrinsic (i.e., cellular) and extrinsic (i.e., microenvironmental) dormancy (11). In intrinsic dormancy, DCCs arrest in  $G_0$  in response to mitogens, growth factors, and microenvironmental cues to which these cells were previously unresponsive and naïve (12-16). The imposition of such conditions activates prosurvival and stress response mechanisms in dormant DCCs (17,18). In contrast, extrinsic dormancy is defined by the formation of micrometastases by DCCs whose rates of proliferation and apoptosis are roughly equivalent (11). Exit from  $G_0$  is accomplished as DCCs adapt to their new microenvironments. However, these cells remain susceptible to immune surveillance (19) and may be reliant upon an insufficient vascular supply (20), thereby limiting their ability to give rise to overt metastatic lesions. Overcoming both intrinsic and extrinsic dormancy requires DCCs to home to and continually remodel specific niches within the metastatic microenvironment (21). DCCs may also co-opt preexisting niches that generate resident tissue stem cells (22). In either case, DCCs must integrate diverse signals arising from parenchymal, stromal, immune, and vascular cells in order to thrive at particular metastatic sites.

DCCs can develop the ability to respond to this milieu of signals through traditional evolutionary processes or via epigenetic control mechanisms. Emerging evidence indicates that DCCs spread early from their primary tumor of origin and undergo limited genetic divergence in metastatic niches (23-25). Thus, the epigenome serves as a critical platform for the acquisition of dormant phenotypes. Herein we summarize major epigenetic regulatory mechanisms and their roles in flexibly maintaining cell identity and dictating metastatic cell behavior, as well as the prospects for targeting these mechanisms therapeutically.

### The dynamics of metastatic dormancy

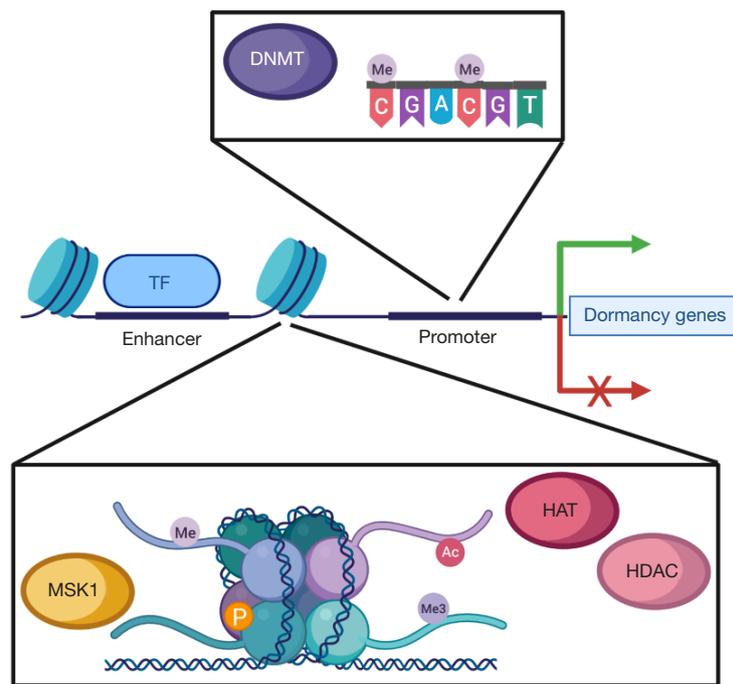
Individual cancer cells do not simultaneously possess all the characteristics required for their dissemination and metastatic outgrowth. Rather, these cells maintain a degree of plasticity that permits them to adopt features that are important at different stages of the metastatic cascade (26-28). Similarly, dormancy requires that DCCs residing within metastatic niches be poised to adapt to the challenges imposed by foreign microenvironments, doing so by dynamically responding to and altering their internal and

external states (29,30). Faced with the selective pressures that accompany the metastatic cascade, DCCs do undergo natural selection, which enriches for cells that are well-suited to survive in specific niches at sites of dissemination (31). However, the evolutionary mechanisms that underlie DCC adaptation are limited, particularly in the context of dormancy. There exists a fundamental conflict between quiescence (i.e., cellular dormancy) and proliferation, which is essential for the propagation of *de novo* mutations. Indeed, quiescence serves as a protective mechanism against genome instability, which ordinarily drives tumor evolution (32,33). Although evolutionary forces may play a more substantial part in extrinsic dormancy (34), micrometastases frequently exhibit significant genetic overlap with their corresponding primary tumors (35,36). Additionally, dormancy requires extensive crosstalk between DCCs and the cells that comprise their microenvironments, including endothelial cells, tissue-resident and circulating immune cells, mesenchymal stem cells, and stromal fibroblasts (37). In general, these cells are viewed as poor substrates for co-evolution with DCCs (38). Hence, Darwinian forces alone are insufficient to give rise to the phenotypic plasticity that is inherent to dormancy.

The tumor-initiating capability of dormant DCCs is intimately linked to their acquisition of cancer stem cell (CSC) traits (39). Dormant DCCs display transcriptional and phenotypic features of CSCs across multiple cancer types (40). Conversely, CSCs are sustained within metastatic niches via signaling pathways that also activate dormancy programs, including Wnt, Notch (39), bone morphogenetic proteins [BMPs; (14,41)], Hedgehog (42), and transforming growth factor- $\beta$  [TGF- $\beta$ ; (43)]. Several of these pathways converge on common intracellular effector molecules, such as the mammalian target of rapamycin [mTOR; (44)] and the mitogen-activated protein kinases (MAPKs) ERK and p38 (12,45). Ligands that modulate flux through these pathways may be the product of cell-cell communication between DCCs (46); they are also derived from stromal cells (14,41,47), immune cells (19,48), or the extracellular matrix (38,49). Given the distributive nature of these inputs and the need to respond to intrinsic and environmental cues in real time, dormant DCCs must mobilize epigenetic effectors in order to persist indefinitely, retain their self-renewal capacity, and preserve their ability to assume new phenotypes, as we presently outline.

### Epigenetic regulation of metastatic dormancy

Several mechanisms for epigenetic regulation of gene



**Figure 1** Regulation of DCC plasticity and metastatic dormancy by DNA and histone modification. Signals that control metastatic outgrowth by DCCs do so by modulating the expression of dormancy-related genes through covalent modification of both genomic DNA (upper inset) and histones (lower inset). Promoter DNA is methylated by DNA methyltransferases (DNMTs), such as DNMT1, at CpG dinucleotides. Histones undergo a variety of modifications, including methylation (mono- and trimethylation are depicted), acetylation, and phosphorylation. Histone acetylation is maintained by the coordinated actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Histone phosphorylation is accomplished by numerous kinases, including the stress-responsive kinase MSK1, whose function is associated with the induction of dormancy. Collectively, these modifications alter chromatin structure and determine the propensity of dormancy-related genes to be bound by transcription factors (TFs) at important regulatory elements (e.g., enhancers). TF binding, in turn, stimulates (green arrow) or inhibits (red arrow) expression of target genes to generate phenotypic plasticity in DCCs. DCCs, disseminated cancer cells.

expression exist in DCCs. These processes exert their influence at the transcriptional, posttranscriptional, translational, and posttranslational stages of gene expression. In turn, each of these mechanisms possesses unique characteristics that ensure high-fidelity control of cell identity and phenotypic plasticity across the events of intrinsic and extrinsic dormancy.

### *Covalent modification of DNA and histones*

Nucleosomes are composed of genomic DNA that packed around histones, thereby representing the basic units for regulating chromatin accessibility and gene transcription. Covalent modification of the DNA or histone components of nucleosomes at specific genomic loci can dramatically alter gene expression and cellular phenotypes (Figure 1). In

dormant DCCs, a transcriptional network responsible for choreographing the G1/S transition is readily suppressed by the DNA methyltransferase DNMT1, instituting cell cycle arrest and quiescence (50). Furthermore, methylation of histone H3 at key residues (H3K4, H3K9, H3K27) results in diminished expression of growth-promoting and CSC-related genes, such as SOX9. In turn, H3 methylation is orchestrated by the orphan nuclear receptor NR2F1, which is commonly epigenetically silenced in multiple cancers via promoter hypermethylation but becomes highly expressed in models of dormancy (51). Similarly, methylation of histone H4 by the histone methyltransferase SMYD5 is required for dormancy in breast DCCs that have disseminated to the lungs (52). These data reveal the versatile nature of nucleosome methylation in coordinating transcriptional programs that are central to establishing

dormant and CSC-like identities in DCCs.

Histone modification takes a number of forms in addition to methylation, and many of these play critical roles in regulating dormancy (*Figure 1*). For instance, NR2F1 and retinoic acid receptor  $\beta$  (RAR $\beta$ ) act in concert to direct the removal of acetyl groups from histone H3 by histone deacetylases (HDACs), and this axis is associated with the presence of dormant DCCs in patients (51,53,54). Histone acetylation also appears to drive the emergence of a growth-arrested cell population during the development of therapeutic resistance and disease recurrence in glioblastoma (55). Apart from this, the mitogen- and stress-activated protein kinase 1 (MSK1), acting downstream of p38, controls the expression of markers of stemness and differentiation by phosphorylating histone H3 at serine 10 or serine 28 (56). In addition to posttranslational modification, proteolytic processing of a variant histone H3 (H3.3) provokes quiescence concomitant with increased production of TGF- $\beta$ 2 and BMP4 (57). Thus, DCC plasticity is profoundly intertwined with chromatin structure and nucleosome modification, and specific chromatin states program these cells to engage other DCCs and their microenvironments to regulate metastatic outgrowth.

### RNA processing

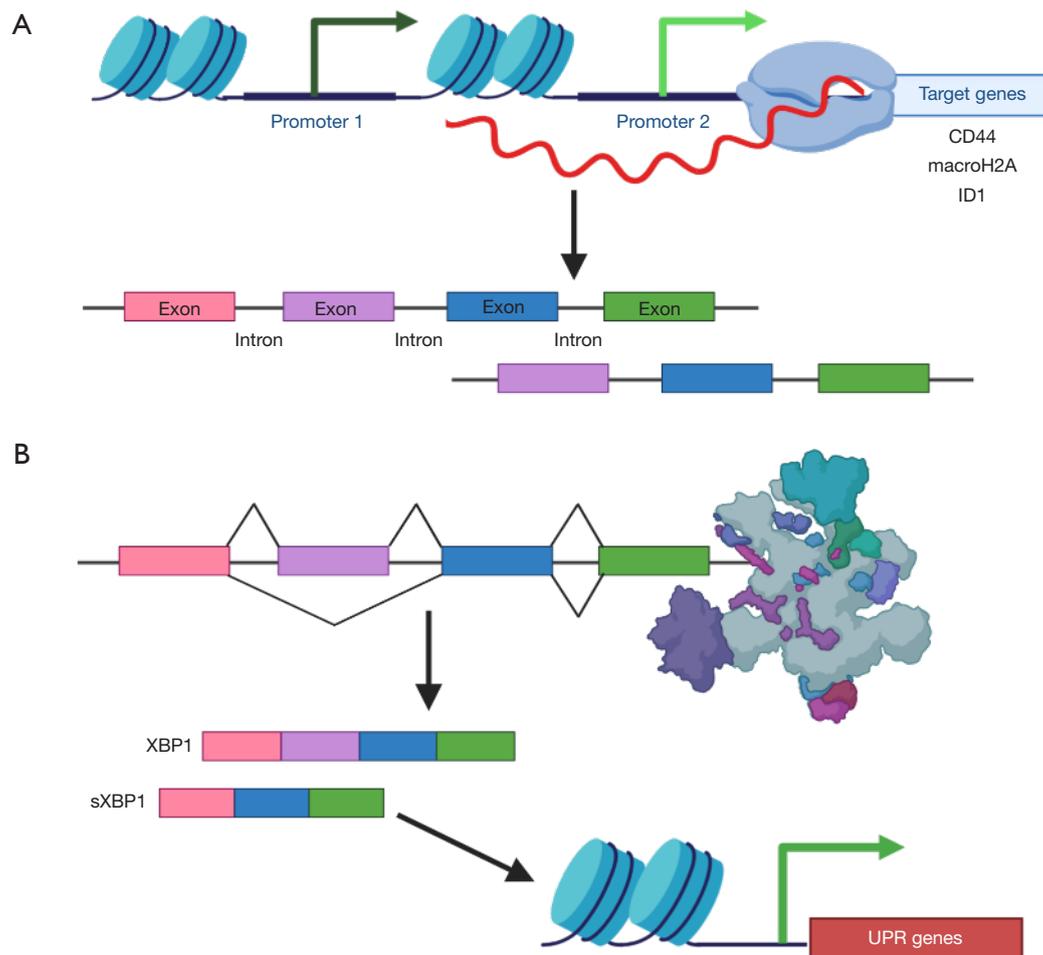
In dormancy, epigenetic regulation via chromatin remodeling not only influences gene expression globally, but also impacts the expression of alternative RNA isoforms in particular (*Figure 2A*). For example, chromatin structure dictates isoform expression of the CSC surface marker CD44 (58). In addition, upregulation of specific isoforms of the histone macroH2A (macroH2A1.1 and macroH2A2) is indicative of a dormant state and can act as a biomarker for lung cancer recurrence (59). Similarly, the inhibitor of differentiation 1 (ID1) isoform ID1b promotes dormancy by inducing cell cycle arrest at G<sub>1</sub>/G<sub>0</sub> both *in vitro* and *in vivo* (60). ID1b represses ERK activation and simultaneously increases the expression of the tumor suppressor p27 and CSC markers NOTCH1 and ALDH1A1. In contrast, the ID1a isoform promotes proliferation, thereby antagonizing the function of ID1b. Interestingly, while the majority of transcript variants associated with dormancy appear to be the result of chromatin remodeling, some posttranscriptional splicing events generate RNAs that directly regulate chromatin architecture (*Figure 2B*). For instance, the X-box binding protein 1 (XBP1) isoform sXBP-1 activates the unfolded

protein response (UPR) in response to cellular stressors typically faced by dormant DCCs, such as hypoxia. sXBP1 is upregulated in CSCs and dormant DCCs, likely because the UPR transcriptional program allows these cells to withstand the stresses imposed by new micrometastatic niches (61,62). RNA processing therefore plays a central role in acclimation and survival of dormant DCCs. Moreover, RNA isoform expression can be interrogated in order to illuminate the mechanisms underlying metastatic recurrence.

### Noncoding RNAs (ncRNAs)

ncRNAs are a class of transcribed RNAs that are not translated into proteins. In the past decade, a wealth of important discoveries has established a myriad of functions for both short and long ncRNAs (63-65). For instance, an important class of short ncRNAs are microRNAs (miRNAs), which are 20–24 nucleotides long and base-pair with target mRNAs to either inhibit their translation or induce their cleavage by the RNA-induced silencing complex [RISC; (65)]. miRNAs can function within DCCs or be carried by cancer cell-derived exosomes to repress gene expression by cells of the tumor microenvironment (66,67) (*Figure 3*). In fact, the abundance of circulating miRNAs, such as miR-222/223, miR-23b, miR-21, miR-34a, miR-127, and miR-197, is correlated with disease progression and dormancy (68-71). The targets of these miRNAs include the chemokine CXCL12, which possesses immunomodulatory functions and controls cancer cell proliferation (71). Similarly, miR-190 is upregulated in dormancy models of glioblastoma, osteosarcoma, liposarcoma, and breast cancer and acts primarily by limiting neoangiogenesis (72,73). Conversely, miR-101 expression is restricted to GI CSCs that give rise to aggressive metastatic disease, rather than those that have adopted a dormant phenotype. A key miR-101 target in mediating its pro-metastatic effects is the histone methyltransferase EZH2, presenting an intriguing intersection between miRNA regulation and chromatin modification in the context of DCC dormancy (74). Taken together, these findings highlight the importance of miRNAs in both intrinsic and extrinsic dormancy at each phase of gene expression.

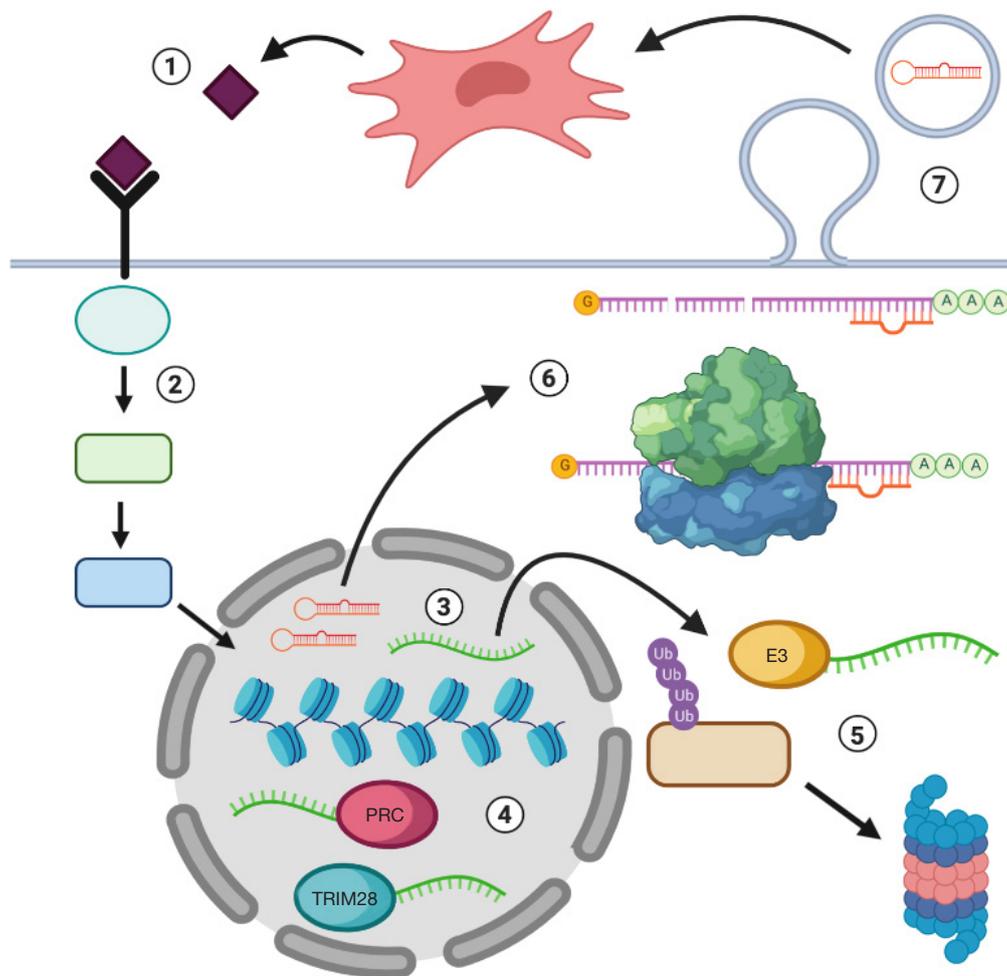
Long non-coding RNAs (lncRNAs) are defined as ncRNAs that are longer than 200 base-pairs and lack an open reading frame; they also have diverse functions in enhancing or suppressing gene expression. In metastatic disease, lncRNAs often carry clinical and functional significance in driving disease progression and recurrence



**Figure 2** Regulation of DCC plasticity and metastatic dormancy by RNA processing. (A) Multiple isoforms of dormancy-related genes, such as CD44, macroH2A, and ID1, can be transcribed, and these disparate isoforms harbor divergent functions. Preferential synthesis of specific isoforms (red) can be accomplished, for example, by utilizing noncanonical promoters, thereby generating transcripts with unique open reading frames (represented by the presence or absence of the pink exon). The ability of RNA polymerase II (blue) to engage these promoters is determined in part by chromatin accessibility and modification state. (B) Dormancy-related transcripts are also created *via* alternative splicing. XBP1, for instance, is processed by the spliceosome to yield both long and short mature mRNAs. Subsequently, the short variant (sXBP1) directs aspects of the unfolded protein response (UPR), enabling DCCs to respond to microenvironmental stressors and activate dormancy programs. DCCs, disseminated cancer cells.

(Figure 3). For example, the lncRNA HOTAIR is a strong predictor for breast cancer metastasis and is correlated with recurrence in cancers of the liver, stomach, bladder, and cervix (75-78). At a molecular level, HOTAIR recruits the polycomb repressive complex (PRC) to specific regions of chromatin, thereby coordinating the epigenetic silencing of target genes such as *p21* and Wnt inhibitory factor 1 [*WIF1*; (79,80)]. Along these lines, expression of the lncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is associated with tumor recurrence and poor

prognosis in breast and prostate cancers (81). Like HOTAIR, MALAT1 can assemble the PRC to facilitate histone methylation and control the expression of pro-tumorigenic factors (81,82). The lncRNA BORG (BMP/OP-responsive gene) similarly stimulates metastatic outgrowth in dormant breast DCCs by directing transcriptional repression by the E3 SUMO ligase TRIM28 (83,84). In short, lncRNAs function as primary mediators of the chromatin and transcriptomic alterations that effect metastatic dormancy and reactivation.



**Figure 3** Regulation of DCC plasticity and metastatic dormancy by noncoding RNAs. [1] Cells that compose the metastatic microenvironment produce signaling molecules that are recognized by DCCs. [2] These signals are transduced to alter gene expression, including [3] modulating transcription of specific microRNAs (orange) and lncRNAs (green). [4] lncRNAs can remain in the nucleus bound to chromatin remodelers, such as the polycomb repressive complex (PRC) and the SUMO ligase TRIM28. [5] lncRNAs may also be exported from the nucleus and influence protein turnover by activating E3 ubiquitin ligases (E3), such as DZIP3 and MEX3B, thereby marking target proteins for proteasomal degradation. miRNAs possess both cell-intrinsic and -extrinsic regulatory capacity. [6] Within DCCs, miRNAs control protein production by binding to complementary sequences in target mRNAs, triggering mRNA decay or blocking translation by impeding progression by ribosomes. [7] miRNAs are also packaged into exosomes, which are taken up by cells of the microenvironment. This serves as a critical feedback loop for mediating crosstalk between DCCs and their niches. DCCs, disseminated cancer cells; lncRNAs, long non-coding RNAs.

In addition to orchestrating transcriptional events, lncRNAs serve to modulate DCC plasticity at the DNA and protein levels (*Figure 3*). For instance, HOTAIR interacts with the E3 ubiquitin ligases DZIP3 and MEX3B to mark target proteins for proteasomal degradation, particularly in the context of cell cycle arrest (85). On the other hand, BORG promotes the survival of metastasis-initiating cells

in response to chemotherapy, a potent inducer of dormancy in discrete subpopulations of DCCs. Specifically, BORG ensures genome stability by coordinating a treatment-induced DNA damage response through its interaction with replication protein A [RPA; (86)]. Still other lncRNAs, such as nuclear paraspeckle assembly transcript 1 [NEAT1; (87)], are associated with tumor recurrence through mechanisms

that remain to be fully elucidated. Thus, ncRNAs comprise a vast, multifunctional network that regulates phenotypic plasticity in DCCs through an array of modalities, many of which have yet to be elucidated.

### Targeting the epigenome in recurrent and metastatic disease

Because epigenetic mechanisms are paramount in dormancy, they present attractive targets for the development of novel therapeutics against recurrent and metastatic disease. Along these lines, several FDA-approved and commercially available DNMT and HDAC inhibitors have shown promise in preclinical and clinical trials in eradicating CSCs (88), and in improving survival in patients with relapsed and refractory cancers (89). Similarly, DNMT inhibitors in conjunction with poly(ADP-ribose) polymerase (PARP) inhibitors yield clinical benefit in recurrent and resistant breast, ovarian, and urothelial cancers (90,91). Moreover, the DNMT inhibitor azacytidine in combination with the HDAC inhibitor benzamidine demonstrates prolonged progression-free survival in non-small cell lung cancer (NSCLC) patients (92). Trials using DNMT/HDAC inhibitor combinations for metastatic and/or recurrent NSCLC are currently enrolling patients. Ongoing trials are also testing the effects of the HDAC inhibitor belinostat in recurrent B-cell and T-cell lymphomas as well as ovarian cancer (93). Likewise, patients with relapsed multiple myeloma experienced significant survival benefits upon addition of the HDAC inhibitor panobinostat to a treatment regimen containing the proteasome inhibitor bortezomib and dexamethasone (94). Strikingly, next-generation HDAC inhibitors are being designed with high selectivity for specific HDAC isoforms, thereby expanding the promise of these drugs to encompass HDACs that are dysregulated in dormant DCCs (95).

Targeting ncRNAs for the treatment of metastasis presents unique challenges in drug development and delivery. At present, there are no available or experimental therapies that directly target lncRNAs (96). Notably, however, there has been progress made with respect to miRNA therapeutics, particularly those utilizing nanoparticle-conjugated miRNA mimetics to treat multiple solid tumor types (97). This trial represents an exciting advance in the treatment of metastasis, opening the door to synthetic miRNA therapies that preferentially operate in DCCs during dormancy and reactivation. Together with improvements in our molecular knowledge, these novel

clinical approaches herald a new age of understanding the epigenome, shedding light on the mechanisms employed by cancer cells to maintain phenotypic plasticity and exploiting these mechanisms to combat the world's deadliest and most insidious cancers.

### Conclusions

Metastatic dormancy is a primary factor underlying disease recurrence and patient mortality, which remain clinically intractable problems in need of innovative therapeutic approaches. Understanding the dynamic interplay between dormancy and the epigenome will give birth to such innovations by advancing our knowledge of the molecular underpinnings of cell plasticity, and by identifying novel targets for the treatment of metastatic cancers. These advances will ultimately facilitate the development of new therapeutic platforms, such as RNA-based therapeutics, leading to improvements in the survival and quality of life for the innumerable people suffering from the burden of metastasis.

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