



Long noncoding RNAs in cancer metastasis

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Abstract | Metastasis is a major contributor to cancer-associated deaths. It is characterized by a multistep process that occurs through the acquisition of molecular and phenotypic changes enabling cancer cells from a primary tumour to disseminate and colonize at distant organ sites. Over the past decade, the discovery and characterization of long noncoding RNAs (lncRNAs) have revealed the diversity of their regulatory roles, including key contributions throughout the metastatic cascade. Here, we review how lncRNAs promote metastasis by functioning in discrete pro-metastatic steps including the epithelial–mesenchymal transition, invasion and migration and organotrophic colonization, and by influencing the metastatic tumour microenvironment, often by interacting within ribonucleoprotein complexes or directly with other nucleic acid entities. We discuss well-characterized lncRNAs with *in vivo* phenotypes and highlight mechanistic commonalities such as convergence with the TGF β –ZEB1/ZEB2 axis or the nuclear factor- κ B pathway, in addition to lncRNAs with controversial mechanisms and the influence of methodologies on mechanistic interpretation. Furthermore, some lncRNAs can help identify tumours with increased metastatic risk and spur novel therapeutic strategies, with several lncRNAs having shown potential as novel targets for antisense oligonucleotide therapy in animal models. In addition to well-characterized examples of lncRNAs functioning in metastasis, we discuss controversies and ongoing challenges in lncRNA biology. Finally, we present areas for future study for this rapidly evolving field.

Invasion–metastasis cascade

A stepwise succession of events whereby primary tumour cells adopt a phenotype that promotes local invasion, intravasation into the bloodstream, extravasation and migration to distant organ sites, and colonization of those sites, which often involves physiological adaptation.

Metastatic disease accounts for the vast majority of cancer-associated deaths. Despite advances in the diagnosis and treatment of cancer, the prognosis for patients with metastatic cancer remains poor, with median survival time measured in months in certain cancers¹. The dissemination of cancer cells from primary tumour to distant organs involves an orchestrated, multistep process known as the invasion–metastasis cascade^{2–4}. First, cells of the primary tumour must locally invade surrounding normal tissue. Then, these cells intravasate into the systemic circulation and subsequently extravasate at a distant site, where the metastatic cells are required to proliferate and colonize an often-foreign tissue environment.

Long noncoding RNAs (lncRNAs) are operationally defined as RNA transcripts longer than 200 nucleotides, and there is no evidence that they encode peptides^{5,6} (FIG. 1). While initially described only as intergenic transcripts⁷, lncRNAs now encompass natural antisense transcripts, overlapping transcripts and intronic transcripts, among others, depending on the genomic arrangement of the lncRNA with respect to

nearby protein-coding genes⁸. Tens of thousands of lncRNAs have been identified by high-throughput RNA sequencing^{9–12}, but only a small percentage of these have been functionally characterized. Through differential expression analysis and comparative transcriptomic studies of cancer specimens, various lncRNAs have been prioritized for functional studies, revealing a diversity of phenotypes and mechanisms (FIG. 1).

lncRNAs serve important roles in gene regulation, including modulating gene activation and silencing^{13,14}, X chromosome inactivation^{15,16}, alternative splicing¹⁷ and post-translational regulation^{18–20}. lncRNAs perform these functions through a variety of different mechanisms, including acting as molecular scaffolds that ‘guide’ chromatin-modifying enzymes as in the case of *HOTAIR*^{21–23} or *DLX6AS*^{24,25}, acting as competing endogenous RNAs (ceRNAs) that ‘sponge’ microRNAs or proteins²⁶, facilitating or inhibiting long-range chromatin interactions (for example, *LUNARI* or *CCAT1*)^{27–29}, or even functioning through the act of transcription itself^{14,30–33} (FIG. 1). Additional mechanisms are also emerging, such as orchestration of

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Long noncoding RNAs

(lncRNAs). Noncoding RNA transcripts longer than 200 nucleotides, which are frequently polyadenylated, and that show no evidence that they encode proteins.

Competing endogenous RNAs

(ceRNAs). RNA transcripts (which may be lncRNAs, mRNAs or pseudogenes) that are capable of binding and influencing the activity of microRNAs through complementary base pairing.

Organ-specific tropism

The propensity for disseminated cancer cells to colonize and proliferate at specific organ sites due to diverse physiological and molecular factors.

Antisense oligonucleotides

(ASOs). Exogenous oligonucleotides, often chemically modified to resist degradation, that alter the amount, stability or activity of complementary RNA transcripts, usually through RNase-based mechanisms.

Epithelial–mesenchymal transition

(EMT). A set of molecular processes that convert cancer cells of polarized epithelial phenotypes into more invasive mesenchymal phenotypes.

nuclear architecture, formation of circular lncRNAs and destabilization of interacting mRNAs. These and other mechanisms of lncRNA function have been reviewed elsewhere^{5,6,13,34}, and additional mechanisms are expected to be unearthed. Of note, particular mechanisms such as the ceRNA model have attracted scepticism (BOX 1), primarily owing to stoichiometric imbalances between target mRNAs and their putative binding sites on ceRNAs, and some lncRNAs have multiple mechanisms described^{35–37}, contributing to the complexity of this rapidly evolving field.

lncRNAs have emerged as key regulators of cancer pathways and also as biomarkers of disease^{38,39}. Initially a peculiarity of molecular biology, lncRNAs have now been described in the context of most if not all of the classic hallmarks of cancer — sustained proliferation, replicative immortality, evasion of growth suppressors, induction of angiogenesis, resistance to cell death and metastasis⁴⁰ (FIG. 1).

Importantly, much like protein-coding genes, in which genetic alterations are foundational aspects of oncogenesis, lncRNAs are amplified, deleted or mutated in malignancies^{38,40}. A broad set of lncRNAs is located in recurrent copy number-altered regions in the genomes of tumours, including *FALL1*, whose genomic amplification represses the growth suppressor gene *CDKN1A*. *PVT1* is frequently amplified in many cancers as well^{36,41}, with recurrent mutations found in its promoter region in breast and other tumours³⁶. Although these lncRNAs are appealing to study owing to a potential genetic mechanism of activation or deletion, it is important to evaluate whether the lncRNA gene resides within the minimal common deleted or amplified region, suggesting that its genetic aberration is the result of selective pressure. Epigenetic alteration of lncRNAs also occurs in cancer, as is the case with *CCAT1* activation in the CpG island methylator phenotype^{28,42}. Thus, genetic and epigenetic alterations in lncRNAs might account for the apparent rarity of driver mutations in early cancer genome studies that focused on protein-coding genes⁴³.

In contrast to protein-coding genes in which their putative domains provide insight into their function (and can be disrupted), the regulatory mechanisms of

lncRNAs are diverse and still emerging. Of note, *PVT1* has been shown to have important functions as both a DNA regulatory element (that is, *cis* mechanism of action) and a RNA transcript (that is, *trans* mechanism of action)^{36,41}. This concept is not unique to *PVT1*, and underscores the need for appropriate models to dissect how the DNA or RNA may be affected and its ultimate contribution to tumorigenesis and metastasis^{44–48}. As the lncRNA tumour biology field has evolved, independent groups investigating the few lncRNAs that have been studied have often used different methods (Supplementary table 1) leading to potentially contradictory findings. Although these contradictory findings may cast some doubt on their importance, it also highlights the complexity of lncRNAs and ultimately led to the development of improved strategies for deciphering their functions and potentially resolving existing discrepancies. An experimental framework for studying *cis*- and *trans*-acting lncRNAs has been detailed in a previous review^{5,6}.

Our understanding of how certain lncRNAs regulate the invasion–metastasis cascade and related processes such as organ-specific tropism and how they affect the tumour microenvironment (TME) is steadily increasing (FIGS 2,3). Although lncRNAs in cancer metastasis have been reviewed before^{49–51}, several recent studies have been published, challenging previously held tenets about lncRNA function and mechanism^{35,36,52}. Importantly, new tools such as modified antisense oligonucleotides (ASOs) and CRISPR–Cas9 and its derivatives have transformed the way lncRNAs are studied^{53–57}, elucidating novel revelations about their function^{14,36,44}, as well as lending insight into new therapeutic targets⁵⁸. In this Review, we discuss recent work that has demonstrated *in vivo* evidence of lncRNA function in cancer metastases, along with controversies pertaining to some of these lncRNAs.

Local invasion and EMT

Local invasion of surrounding tissue is a necessary initial step for tumour metastasis. The epithelial–mesenchymal transition (EMT) and its reverse counterpart the mesenchymal–epithelial transition, which are processes normally activated in embryonic development and tissue homeostasis to ensure proper morphogenesis of tissues and organs, are crucial for the execution of the invasion–metastasis cascade^{59–61}. Through activation of master regulators such as SNAI1, TWIST1 and ZEB1, carcinomas with epithelial phenotypes are conferred traits characteristic of mesenchymal cells that allow invasion, intravasation and dissemination to distant sites⁶². Although many lncRNAs have been described to modify growth and invasion, we focus our discussion on lncRNAs with roles in invasion and EMT that are supported by *in vivo* experiments in the context of metastases (FIG. 2).

H19 in the EMT. H19, one of the earliest described lncRNAs⁶³, is overexpressed in several cancers⁶⁴ and involved in tumour cell invasion (FIG. 3). In bladder cancer cells, it was shown that H19 overexpression drives migration of malignant cells *in vitro*, by associating with

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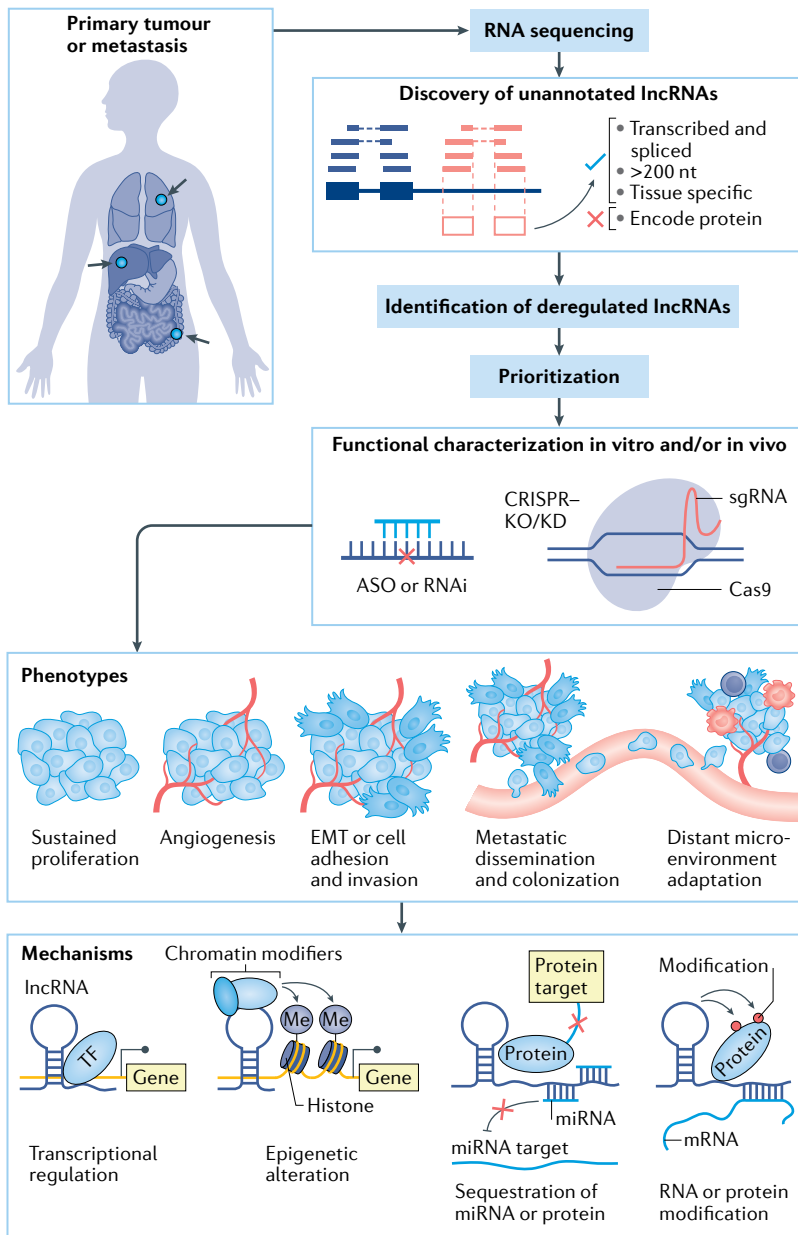


Fig. 1 | Long noncoding RNAs in cancer. Starting from discovery to characterization of their functions and mechanisms. First, tumour specimens and normal tissues sampled from patients with cancer are subject to RNA sequencing. Sequencing reads are then aligned to the reference genome, which allows quantification of RNA abundance and discovery of unannotated long noncoding RNAs (lncRNAs). Comparisons of transcript abundance between tumour and normal and/or between metastasis and primary tumour allow identification of deregulated lncRNAs. Subsequent omics data integration, computational analysis and high-throughput functional screening using techniques such as CRISPRi allow prioritization of lncRNA candidates for further in-depth functional and mechanistic characterization. Perturbation and manipulation of the lncRNAs can be performed via antisense oligonucleotide (ASO), small interfering RNA (siRNA), CRISPR-based methods in vitro and in vivo to generate phenotypic changes that can be observed and evaluated. lncRNAs are known to have both oncogene and tumour suppressor roles and contribute to tumour progression and metastasis. Mechanistically, lncRNAs can regulate the expression of other genes by interacting with other molecules resulting in transcriptional regulation, epigenetic alteration, sequestration of RNA or protein and enhancer regulation. EMT, epithelial–mesenchymal transition; KO/KD, knockout/knock-down; Me, methyl; miRNA, microRNA; RNAi, RNA interference; sgRNA, single guide RNA; TF, transcription factor.

the polycomb repressive complex 2 (PRC2) component EZH2, leading to increased β -catenin activity and decreased E-cadherin, supporting a transition towards a mesenchymal state⁶⁵. A challenge in the interpretation of these results is the tendency of PRC2 to undergo promiscuous RNA interactions⁶⁶, which may obfuscate the primary molecular interactions responsible for H19 function. Nonetheless, H19-mediated transformation towards a mesenchymal identity also occurs in colorectal cancer (CRC) cells, where H19 was shown to function as a ceRNA that sequesters microRNAs miR-138 and miR-200a, leading to the derepression of vimentin, ZEB1 and ZEB2 protein expression⁶⁷. Consistently, H19 expression was higher in mesenchymal subtypes of CRC cells than in epithelial subtypes. The role of H19 in promoting invasion ultimately leading to metastases was further supported in spontaneous mammary tumour mouse models. Here, H19 was specifically overexpressed in clones that are capable of seeding metastases (that is, 4T1, 4T07 and 168FARN lines), and short hairpin RNA (shRNA)-mediated knock-down of H19 in metastasis-capable clones abrogated metastasis from the primary site to distal organs (for example, lung, kidney and liver)⁶⁸. In this study, H19 acted as a ceRNA against miR-200b and miR-200c, leading to derepression of ZEB1 and GIT2 in primary tumour and distal metastases. GIT2 was shown to promote colonization in distal metastases. However, in circulating tumour cells (CTCs), H19 differentially sponged the miRNA let-7b, leading to derepression of CYTH3, which promoted a mesenchymal phenotype conducive to extravasation. This highlights a striking influence of environmental context on lncRNA function, a theme that has been observed as a general property of lncRNA biology⁵⁴. One strength of this study was the use of endogenous Argonaut pulldown, adding biochemical support instead of relying on nucleic acid sequence analysis or indirect measurements of ceRNA activity, and targeted mutagenesis to support the ceRNA role of H19, despite the controversy surrounding ceRNAs in general (BOX 1). Therefore, H19 coordinates a stepwise cascade of metastasis initiation and colonization with potentially different mechanisms of action based on the cellular and environmental context.

These above-mentioned investigations of H19 function, however, do not separate the microRNA sponging capacity of the H19 transcript from its own ability to give rise to miR-675, which is encoded in the first exon of the *H19* gene. In prostate cancer cells, high levels of H19 are unexpectedly associated with reduced metastatic potential⁶⁹, and *H19*-encoded miR-675 expression led to downregulation of transforming growth factor β -induced protein (TGFB1), which is involved in progression of the EMT⁷⁰. Therefore, transcripts derived from the *H19* gene locus may have separable mechanisms for the regulation of the invasion–metastasis cascade. Although it is simpler to ascribe a single regulatory function to a lncRNA, it is plausible that a lncRNA (such as H19) that is altered in multiple cancer types may have a more crucial role and multiple regulatory mechanisms. As such, reconciling these differences requires the use of models that can either separate out each mechanism

(specifically manipulating the PRC2 interaction site or miRNA product) or minimally take into account how each model used could impact each regulatory mechanism (Supplementary table 1). Collectively, this highlights both the importance of H19 and the complexity of studying this and other lncRNAs.

Other competitive endogenous lncRNAs in the EMT. Several additional lncRNAs have been implicated in the EMT. Among these, pro-transition associated RNA (PTAR) has been shown to be associated with the mesenchymal subtype of ovarian cancer and act as a ceRNA against miR-101, resulting in derepression of ZEB1 expression⁷¹. Indeed, shRNA-mediated knock-down of PTAR increased E-cadherin expression and reduced FN1, ZEB1 and vimentin expression in ovarian cancer xenografts. Also, intraperitoneal injection of ovarian cancer cells with stable PTAR knock-down resulted in fewer tumour nodules in infected mice compared

with controls, overall indicative of the role of PTAR in promoting EMT⁷¹.

Another intriguing lncRNA, lncRNA-PNUTS, is transcribed from a locus that produces both a lncRNA variant (lncRNA-PNUTS) and a mRNA variant (PNUTS)⁷². While the lncRNA acts as a ceRNA, the mRNA produces a regulator of protein phosphatase 1. Treatment of cells with TGFβ led to PI3K–Akt-dependent phosphorylation and subsequent derepression of lncRNA-PNUTS transcription. lncRNA-PNUTS expression correlated with ZEB1 and/or ZEB2 mesenchymal marker expression and was shown to be a ceRNA that binds to miR-205, leading to derepression of ZEB1/ZEB2 (FIG. 4). Overexpression of lncRNA-PNUTS induced EMT in both lung carcinoma and murine mammary gland cell lines, as evidenced by altered morphology and increased expression of ZEB1/ZEB2 and vimentin, and this effect is abrogated by co-transfection with miR-205, supporting the ceRNA function of lncRNA-PNUTS⁷². ShRNA-mediated knock-down of lncRNA-PNUTS in MDA-231-LM2 breast cancer cells resulted in decreased burden of lung metastases when injected into mammary fat pads of mice.

lncRNA-activated by TGFβ (lncRNA-ATB) is another ceRNA that acts as a regulator of the TGFβ–ZEB1/ZEB2 axis. As a transcriptional target of TGFβ, lncRNA-ATB competitively binds to the miR-200 family, leading to upregulation of ZEB1/ZEB2 (REFS^{73,74}). Consistent with this mechanism, overexpression of miR-200a in hepatoma cells abolished EMT. As a result, lncRNA-ATB induced EMT, invasion, increased levels of CTCs and distant organ colonization in an in vivo orthotopic xenograft model of hepatocellular carcinoma (HCC)⁷⁵. Also in HCC cells, lncRNA ZFAS1 is amplified at the level of genomic DNA (FIG. 4), competitively binds miR-150, derepressing ZEB1, MMP14 and MMP16 gene expression to promote metastasis⁷⁶. Another ceRNA, FTX, acts by competitively sponging miR-374a and inhibiting HCC cell EMT and invasion, in addition to binding DNA replication licensing factor MCM2, thereby impeding DNA replication and inhibiting proliferation in HCC cells⁷⁷.

As discussed above, models of competitive binding between lncRNAs and miRNA are frequently described, and caution should be exercised when interpreting experiments claiming such mechanisms of action. This is because of the potential presence of unbalanced physiological abundances of these transcripts and other concerns (BOX 1). Nonetheless, PTAR, lncRNA-PNUTS and lncRNA-ATB have each been shown to function in multiple cancer types, lending credence to their importance. However, it is possible that as these lncRNAs continue to be mechanistically interrogated additional mechanisms will be revealed that are consistent across cancer types.

EMT regulation by lncRNAs via alternative pathways.

In contrast to lncRNAs interfering with TGFβ signalling and transcriptional EMT pathways (FIG. 3), STAT3-activated lncRNA HOXD-AS1 promoted lymph node metastases in HCC by sequestering miR-130a-3p to derepress SOX4 mRNA expression⁷⁸, ultimately resulting in enhanced migration and invasion via upregulation

Box 1 | The controversy of the competing endogenous RNA hypothesis

The competing endogenous RNA (ceRNA) hypothesis proposes that some RNA molecules with shared microRNA (miRNA) binding sites compete for post-transcriptional control, leading to diminished target gene repression. Despite the growing number of studies focused on long noncoding RNAs (lncRNAs) acting as ceRNAs in metastasis, scepticism remains about whether physiological expression levels of a single lncRNA, which can represent a small fraction of the total miRNA targets, is sufficient to alter miRNA regulation^{156,157}. Others suggest that a ceRNA would need to introduce a similar quantity of miRNA binding sites to the existing pool of targets to alter their repression, or that a ceRNA would need to be at equimolar concentration to all miRNA targets^{158–160}; however, this could be compensated through multiple tandem miRNA binding sites. This highlights the importance of establishing standardized strategies to demonstrate the physiological relevance of ceRNA candidates.

The miRNA prediction methods for establishing a ceRNA interaction can vary significantly between algorithms¹⁶¹. Moreover, sequence content alone does not take into account the physiological expression levels of the ceRNA, miRNA and target genes. Gene expression data across patient cohorts can help to prioritize ceRNAs¹⁶² that have a positive expression correlation with target genes¹⁶³, although it does not discriminate between correlated genes owing to alternative regulatory mechanisms. Another strategy is to prioritize ceRNAs displaying biochemical enrichment with RNA-induced silencing complex (RISC) components¹⁶⁴. Numerous databases leverage various miRNA prediction algorithms, biochemical data and gene expression data to facilitate research on ceRNAs^{165–167}. A caveat of existing databases is that they lack a global perspective on the miRNA target pool size and the fidelity of the miRNA interactions within the target pool that may affect the regulatory ability of the ceRNA. Nonetheless, they provide an opportunity for hypothesis generation.

The most common experimental validation strategies use models of ceRNA overexpression or silencing. Consequently, altered miRNA target gene regulation is assessed by manipulating levels of miRNA or RISC components followed by gene expression analysis. However, it is experimentally challenging to overexpress miRNAs or ceRNAs at physiological levels. Furthermore, direct evidence is necessary to show that the ceRNA–miRNA relationship is dependent on miRNA biogenesis¹⁶⁸. In addition, it is crucial to demonstrate that the miRNA-binding sites within the target genes are dependent on the ceRNA. This can be achieved in an endogenous system via CRISPR genome editing of the miRNA binding site within the target gene and appropriate rescue experiments. Also, although cell lines offer practical advantages, they cannot mimic patient tissues. However, the use of mouse models¹⁶⁹ (if an orthologue exists) or patient-derived xenografts and organoids could serve as more relevant physiological models. Last, future studies will also have to consider the possibility that multiple genes are altered, which collectively increases the abundance of multiple miRNA-binding sites.

Collectively, the ease of identifying ceRNA interactions coupled with a common experimental validation framework may have led to a bias of metastasis-associated lncRNAs being mechanistically characterized as ceRNAs while under-representing alternative regulatory mechanisms.

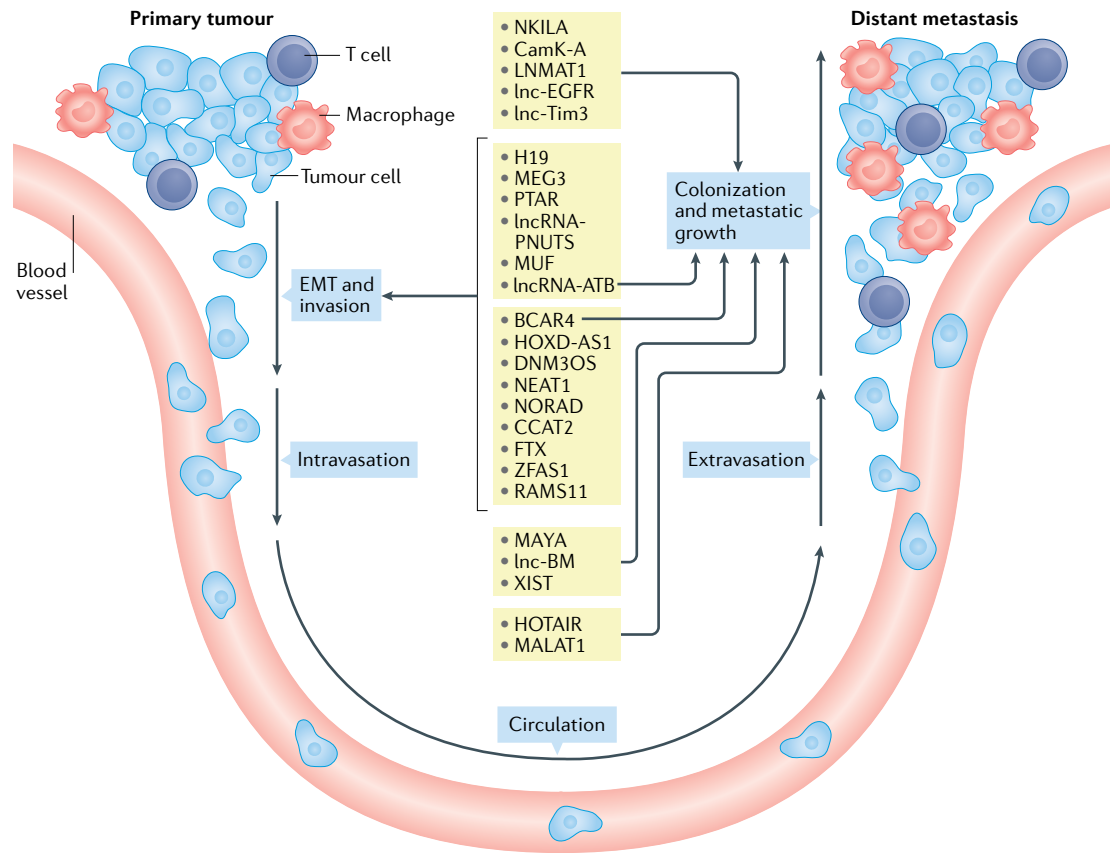


Fig. 2 | Long noncoding RNAs involved in the multiple processes of the invasion–metastasis cascade. Metastasis is a result of a series of orchestrated cellular processes (invasion–metastasis cascade). First, changes in morphology and cellular adhesion are facilitated through epithelial–mesenchymal transition (EMT). Cancer cells then invade the surrounding normal tissue (local invasion) and make way into (intravasation) and out of (extravasation) the systemic circulation to land at a distant site. There, the metastatic cells proliferate and colonize an often-foreign tissue environment. Numerous long noncoding RNAs (lncRNAs) have been reported to regulate one or more of these processes (shown in the centre of the figure with arrows indicating the involvement in the corresponding processes).

of MMP2, EZH2 and other SOX4 signalling targets⁷⁹. Moreover, shRNA-mediated suppression of *HOXD-AS1* in multiple HCC cell lines reduced migration and invasion in vitro (independently of proliferation and apoptosis). In line with this, stable *HOXD-AS1* knock-down in HCC cells led to reduced numbers of lung metastases in a tail vein injection mouse model.

TGFβ-responsive lncRNA MEG3 promotes EMT through interacting with EZH2 and indirectly upregulating ZEB1 (REFS^{80,81}). lncRNA-MUF promotes EMT in CRC cells through activation of WNT–β-catenin signalling⁸², while it leads to activation of the TGFβ–SMAD2/SMAD3 signalling pathway through inhibition of SMAD4 protein degradation⁷⁵. Several additional lncRNAs have also been demonstrated to regulate the EMT via alternative pathways^{75,82–86} and are summarized in FIG. 3.

Promotion of invasion and migration. lncRNA NORAD, initially discovered for its role in maintaining genomic stability by sequestering cytoplasmic Pumilio proteins and thus maintaining expression of mitotic and DNA repair transcripts^{20,87}, also functions in invasion and metastases in cancer (FIG. 4). NORAD deficiency is

associated with lymph node metastasis in samples from patients with lung and breast cancer⁸⁸. In breast and lung cancer cells in vitro, it has been shown that ectopic NORAD expression inhibited invasion and migration. In the same study, when intravenously injected into immunodeficient mice, breast cancer cells harbouring *NORAD* knock-down exhibited greater seeding of lung metastases. Mechanistically, NORAD acted as a molecular decoy, binding to and inhibiting the action of S100P, thereby suppressing S100P-mediated pro-metastatic signalling, which includes invasion activated by cathepsin D and cofilin⁸⁸.

In patients with colon cancer, CCAT2 has been shown to be overexpressed in microsatellite-stable CRC and metastatic CRC⁸⁹. It has been shown that CCAT2 can activate *MYC* transcription and WNT signalling through its interaction with TCF7L2 (observed using RNA immunoprecipitation in CRC cells). Consistent with these results, retroviral vector-mediated over-expression of CCAT2 increased migration of HCT116 cells in vitro and increased the frequency of liver metastasis when cells were injected into the spleen. Interestingly, other studies described CCAT2 facilitating metastases through metabolism, by promoting a pro-glycolysis

isoform of the enzyme glutaminase through regulation of alternative splicing⁹⁰.

More recently, using transcriptome sequencing, the lncRNA *RAMS11* was found to be overexpressed in liver metastasis compared with primary tumour from patients

with metastatic CRC⁹¹. Genomic deletion of *RAMS11* using CRISPR–Cas9 decreased cellular invasion and migration of colon cancer cells in vitro. Further, *RAMS11* knockout in both tail vein and hemi-splenectomy orthotopic models reduced liver metastasis and lung

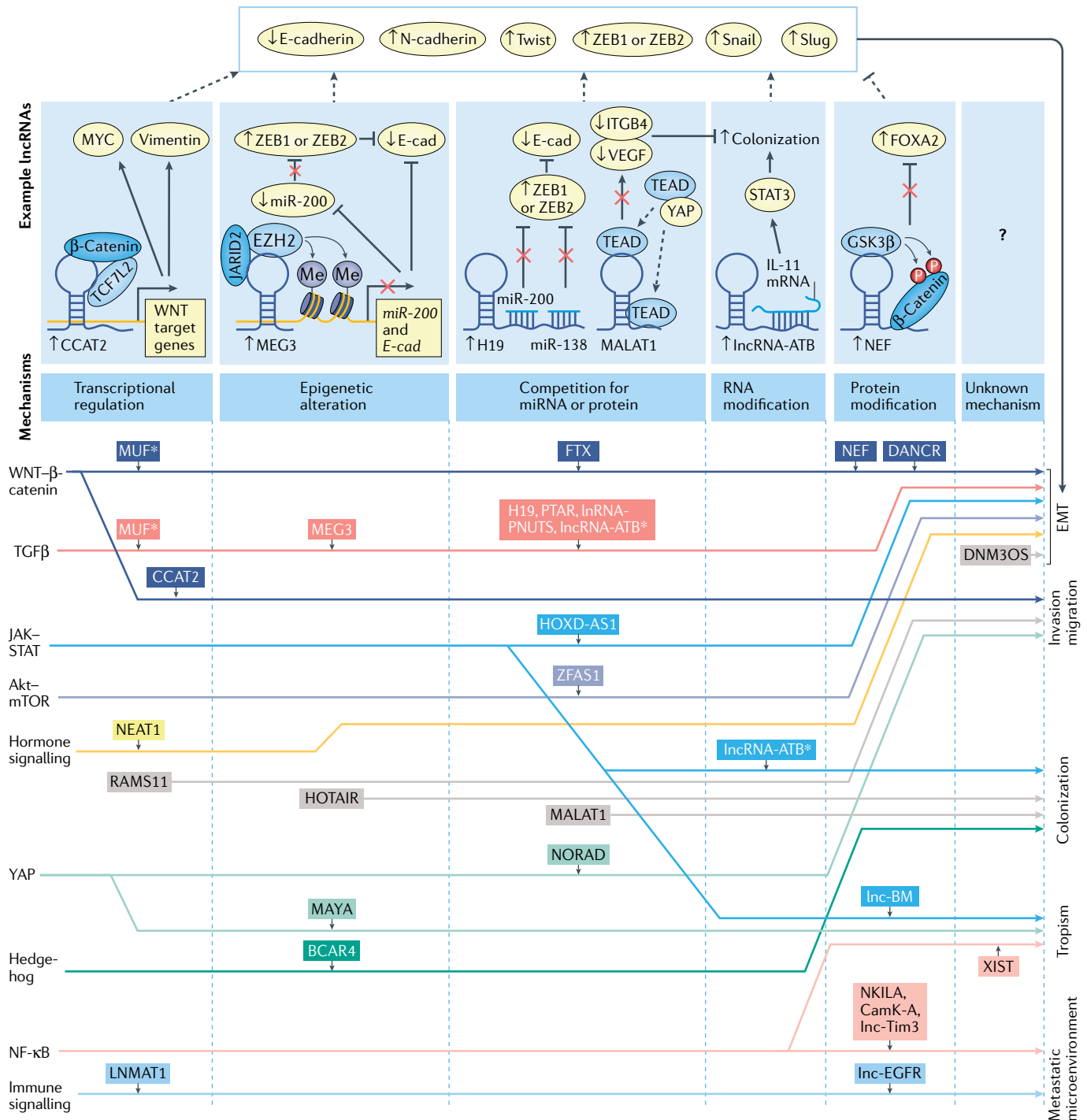


Fig. 3 | Long noncoding RNAs regulate metastasis via various pathways using diverse mechanisms. Shown are long noncoding RNAs (lncRNAs) with their associated pathways (left) and the biological processes in metastasis that they regulate (right) linked by lines to show lncRNAs interacting with specific pathways to regulate certain biological processes. LncRNAs are grouped from left to right by their downstream mechanism shown on the top panel. For each downstream mechanism, an example

detailed mechanism of a lncRNA is presented. LncRNAs in grey boxes are those for which consensus pathway involvement has not been characterized. An asterisk (*) indicates lncRNAs that are involved in multiple pathways or that regulate multiple biological processes in metastasis. In the top panel downstream mechanisms are shown by which representative lncRNAs regulate epithelial–mesenchymal transition (EMT). NF-κB, nuclear factor-κB.

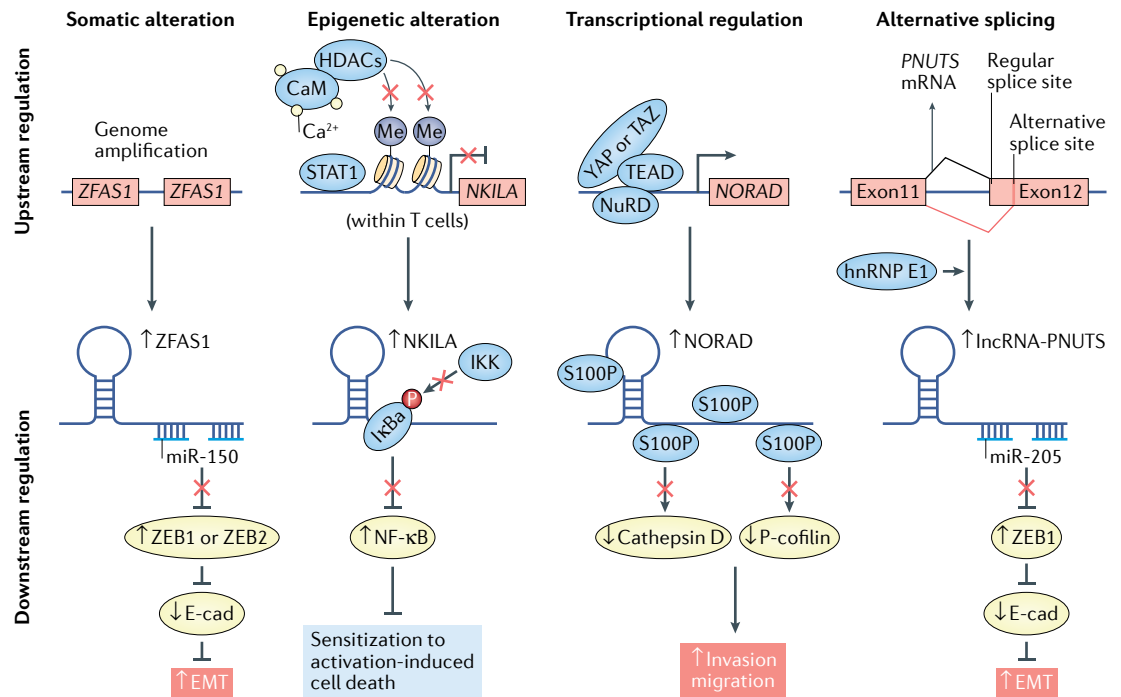


Fig. 4 | **Metastasis-associated long noncoding RNAs are regulated by various upstream mechanisms.** Shown are examples of metastatic long noncoding RNAs (lncRNAs) activated by representative mechanisms (from left to right). For each mechanism, the mechanistic model of an example lncRNA is presented (top: upstream regulation of the lncRNA; bottom: regulation of the downstream target and metastatic phenotype). EMT, epithelial–mesenchymal transition; hnRNP, heterogeneous nuclear ribonucleoprotein; NF-κB, nuclear factor-κB; miR, microRNA.

metastasis, respectively. These metastatic phenotypes were associated with RAMS11-dependent chromo-box homologue 4 transcriptional activation of DNA topoisomerase II-α (*TOP2A*).

Unanswered questions about lncRNA regulation of invasion, migration and the EMT. An emerging theme is that lncRNAs interface with known master regulators of the EMT, such as TGFβ, ZEB1, SNAI1 and the WNT-β-catenin pathway to enhance or repress metastatic potential. These interactions may be direct through ribonucleoprotein complexes, or indirect through epigenetic regulators or miRNA sequestration (FIGS 1, 3). LncRNAs such as NORAD, CCAT2 and RAMS11 can induce invasion and migration phenotypes independently of these pathways. Although these and several other lncRNAs have been reported to contribute to these processes, they do not function in isolation but instead via well-established protein-coding regulators, supporting the notion that lncRNAs tend to function as ‘fine tuners’ rather than master regulators of metastases. Future work will be aimed at corroborating the mechanism of these lncRNAs in contributing to EMT, invasion and migration, and elucidating the mechanisms of action of lncRNAs that use various other pathways. Furthermore, whether these above-described lncRNAs function during other steps of the invasion–metastasis cascade remains to be explored.

Regulation of metastatic colonization

Malignant cells that escape the primary tumour through invasive and mesenchymal phenotypic transformations

must intravasate into the bloodstream, overcome anoikis during haematogenous spread, extravasate at a distal site and colonize distant organ sites³. Although little is known about the role of lncRNAs in the first three of these cellular processes in vivo, several lncRNAs have been implicated in promoting the overall ability of metastatic cells to colonize and flourish at distant organ sites.

MALAT1 in metastatic colonization. MALAT1, one of the first lncRNAs to be described in the process of cancer metastasis, is associated with lung cancer metastases and is also prognostic for survival in patients with early-stage non-small cell lung cancer⁹². Promoter deletion or ASO-mediated knock-down of MALAT1 in a mouse mammary tumour virus (MMTV)–polyomavirus middle T antigen (PyMT) model of human luminal B breast cancer results in decreased formation of lung metastases, greater tumour differentiation (as evidenced by cystic and encapsulated tumour appearance) and elevated E-cadherin (supporting an epithelial phenotype)⁹³. MALAT1 can regulate these functions by localizing to nuclear speckles and altering co-transcriptional alternative splicing based on these initial descriptions.

Furthermore, when MALAT1 expression was inhibited in A549 lung carcinoma cells (via insertion of a premature polyadenylation signal into the *MALAT1* locus), this led to decreased expression of various metastasis-associated genes (for example, *ROBO1*, *MIA2*, *GPC6*, *LPHN2* and *ABCA1*) and reduced migration in vitro compared with control cells⁹⁴. Also, *MALAT1* knock-down led to fewer lung metastases upon tail vein injections in mice, and cells were more

Anoikis

A subtype of programmed cell death triggered by inappropriate cellular or extracellular matrix interactions, often in the setting of tumour cells escaping their primary environment.

Micrometastases

Collections of metastasized tumour cells that are clinically detectable but typically under 2 mm in diameter in human patients.

Comprehensive

identification of RNA-binding proteins by mass spectrometry (ChIRP-MS). A method of identifying endogenous RNA–protein interactions with high specificity, using *in vivo* crosslinking, RNA pulldown and mass spectrometry.

likely to remain within blood vessels compared with control cells, suggesting their inability to intravasate into tissue. Cells that did invade distal lung sites formed micrometastases, implying ineffective colonization following *MALAT1* knockout. Suggesting therapeutic potential for *MALAT1* targeting in metastatic lung cancer, subcutaneous administration of *MALAT1* ASO decreased the burden of lung nodules in pulmonary mouse models of metastatic non-small cell lung cancer⁹⁴. Therefore, perturbation of both the genetic locus and the RNA transcript resulted in concordant phenotypes in this individual study⁹⁴, an agreement not universally observed in lncRNA studies, some reasons for which are dependent on the methodologies used to study lncRNAs (Supplementary table 1).

Although *MALAT1* is generally implicated as a pro-tumour metastasis lncRNA, some evidence has suggested that *MALAT1* can contribute to suppressing metastasis. Transcriptional inactivation of *MALAT1* in MMTV-PyMT mouse models using insertion of premature polyadenylation sequences promoted lung metastasis³⁵, in stark contrast to earlier experiments that also used premature termination (in addition to ASO targeting) as methods of lncRNA inactivation⁹⁴. Furthermore, previous experiments that used promoter deletion or transcript knock-down of *MALAT1* implicate the lncRNA in regulation of mRNA splicing and neighbouring gene expression^{93,95}, adding to the range of differing mechanisms proposed for this gene. The pro-metastasis effects of *MALAT1* depletion observed by Kim and colleagues³⁵ were rescued by transgenic overexpression of *MALAT1* (REF.³⁵). In this study, it was identified that *MALAT1* can bind to and inactivate transcriptional activity of the pro-metastatic TEAD transcription factor family, when analysed using modern techniques such as comprehensive identification of RNA-binding proteins by mass spectrometry (ChIRP-MS). This can result in the abolition of *ITGB4* and *VEGFA* expression, which may be required for migration and invasion³⁵. These disparate results suggest that the phenotype of lncRNA function may depend not only on the cellular context⁵⁴, as is the case when comparing *MALAT1* function in breast cancer³⁵ versus lung cancer⁹⁴, but also on the dominant mechanism of action (for example, regulation of gene expression and splicing^{93,95}, or inactivation of transcription factors³⁵). *MALAT1* also illustrates a major challenge in lncRNA research. As it is not a priori established whether the genomic locus or the RNA gene product (or both) is responsible for lncRNA function, the lncRNA mechanism must be interpreted within the context of the methods used to disrupt varying degrees of the genomic locus or RNA transcript (Supplementary table 1), as different methods may lead to conflicting conclusions if taken at face value.

Pro-colonization pathways connecting lncRNA and cytokines. Metastatic colonization also exploits non-canonical hedgehog signalling, whereby GLI transcriptional networks are activated independently of the PTCH1-SMO transmembrane receptors⁹⁶. In breast cancer, GLI2 activation of pro-metastatic

transcriptional programmes is dependent on BCAR4 (REF.⁹⁷), a lncRNA initially discovered for its role in anti-oestrogen resistance⁹⁸. In response to CCL21 chemokine signalling, BCAR4 interacts with SNIP1 and PPP1R10 to epigenetically derepress GLI2 transcriptional targets. *In vivo* knock-down of *Bcar4* using either shRNA or ASOs suppresses lung metastases derived from orthotopically implanted MDA-MB-231 breast cancer cells in mice⁹⁷. Clinically, BCAR4 expression is correlated with metastatic burden of breast cancer and is prognostic of patient survival, highlighting its biological role in metastatic dissemination and its potential as a disease marker.

lncRNA-ATB, which in addition to its role in promoting the EMT through upregulation of ZEB1 and/or ZEB2 (REF.⁷⁴), induces distant organ colonization of subcutaneously transplanted HCC cells in mice⁷³. This pro-colonization function is attributed to lncRNA-ATB binding and stabilizing *IL11* mRNA, leading to STAT3 signalling, which inhibits apoptosis⁹⁹. Therefore, this lncRNA may represent an important mediator of multiple steps in the invasion–metastasis cascade, despite the challenges of resolving ceRNA function (BOX 1).

HOTAIR promotes colonization. The *HOX* loci lncRNA HOTAIR is a relatively well-established regulator of metastasis³⁸. Initially discovered as a *trans* repressor of certain *HOXD* cluster genes in embryonic development²², HOTAIR is overexpressed in metastatic breast cancer and is also prognostic of patient survival²¹. In patients with CRC, high HOTAIR expression correlates with the presence of liver metastases¹⁰⁰. Ectopic expression of HOTAIR in non-metastatic SK-BR3 breast cancer cells and metastasis-competent MDA-MB-231 cells results in lung colonization following tail vein injection into mice, with MDA-MB-231 cells exhibiting persistent metastatic colonization and expansion²¹. By acting as a molecular scaffold for the PRC2 and histone H3K4 demethylase LSD1 (REF.²³), HOTAIR reprogrammes the epigenome of breast cancer cells to resemble those of embryonic fibroblasts²¹. Interestingly, the metastatic potentiation of HOTAIR in breast cancer cells can be enhanced in a paracrine mechanism¹⁰¹. TGFβ1 secreted by carcinoma-associated fibroblasts (CAFs) *in vitro* is capable of upregulating HOTAIR expression levels through transcriptional activation of *HOTAIR* by SMAD2, SMAD3 or SMAD4, thereby inducing EMT in breast cancer cells (MDA-MB-231 and MCF-7). Consistent with this observation and prior studies, *HOTAIR* knock-down decreases lung metastases¹⁰¹. Although HOTAIR was one of the first lncRNAs studied in metastasis, and therefore more groups have been able to continue dissecting its role, it has nonetheless been broadly found to promote metastasis across cancer types.

As tumour cells disseminate towards distant organs, the lncRNAs described in this can promote colony expansion in what are often foreign microenvironments. Further investigation is warranted to determine whether this effect is a generalized consequence of enhanced growth and proliferation, or whether these and other lncRNAs strengthen the relative fitness of metastatic cells within specific niches.

Regulation of site-specific tropism

Metastatic cells exhibit site-specific tropism, as various organ environments require specific adaptations for the survival of disseminated tumour cells^{102–105}. In other words, specific organ environments are more hospitable for the survival of disseminated tumour cells. This has been supported by expression of specific protein-coding genes that facilitate organ-specific metastatic tropism (such as connective tissue growth factor (CTGF) in bone metastases¹⁰⁶), which allow disseminated tumour cells to overcome the demands of the tissue microenvironment at distant sites. An outstanding question in such organotropic metastasis is how breast cancer, the most common malignancy in women, gives rise to metastases that are distributed to distant organs (for example, bones, lungs, central nervous system (CNS), lymph nodes and gastrointestinal tract) in non-random ways. For instance, up to 70% of breast cancer metastases are disseminated to the bone, while 10–30% of metastases localize to the brain¹⁰⁷. As such a pervasive disease, breast cancer has motivated the development of several well-characterized *in vivo* mouse genetic models^{108–110}, enabling dissection of this crucial decision-making process. Certain lncRNAs have been identified to direct these organ preferences, the principles of which may be applicable to lncRNAs in other primary malignancies (FIG. 5).

Bone metastases. Within bone metastases derived from primary breast cancer, paracrine release of CTGF from malignant cells activates the YAP pathway in osteoclasts to induce differentiation and osteolysis, thereby promoting bone metastasis colonization¹⁰⁶. Using a

high-throughput RNA interference (RNAi) screen, it was shown that the lncRNA MAYA was required for YAP1 activation in MCF-7 breast cancer cells¹¹¹ (FIG. 5). Knock-down of MAYA in cells derived from human breast cancer bone metastases (BoM-1833) led to abrogation of CTGF secretion, which impaired cancer cell-induced osteoclast differentiation and bone resorption. Mechanistically, it was shown that MAYA functions in a molecular complex with LLG2 and the methyltransferase NSUN6, which methylates the Hippo pathway component MST1. Methylation of MST1 then leads to activation of YAP1-regulated genes¹¹¹. Tumours in mice derived from BoM-1833 cells with MAYA knock-down showed a reduced burden of bone metastases compared with controls. Furthermore, intravenous injection of locked nucleic acids (LNAs) against MAYA reduced the bone metastasis burden in mice that had previously been inoculated with breast cancer cells or A549 lung cancer cells, suggesting therapeutic targeting of MAYA as a precise way of treating bone metastases¹¹¹.

Brain metastases. By contrast with bone metastases, breast cancer cells that metastasize to the CNS exploit different pathways to achieve site-specific colonization. Brain metastases derived from primary breast cancer express L1 cell adhesion molecule (LICAM) to co-opt vascular endothelia, endowing metastatic outgrowth throughout the brain¹¹². Lnc-BM is a lncRNA enriched in brain metastases compared with lung or bone metastases derived from orthotopically introduced breast cancer cells¹¹³ (FIG. 5). Lnc-BM expression was inversely associated with survival in patients, and positively

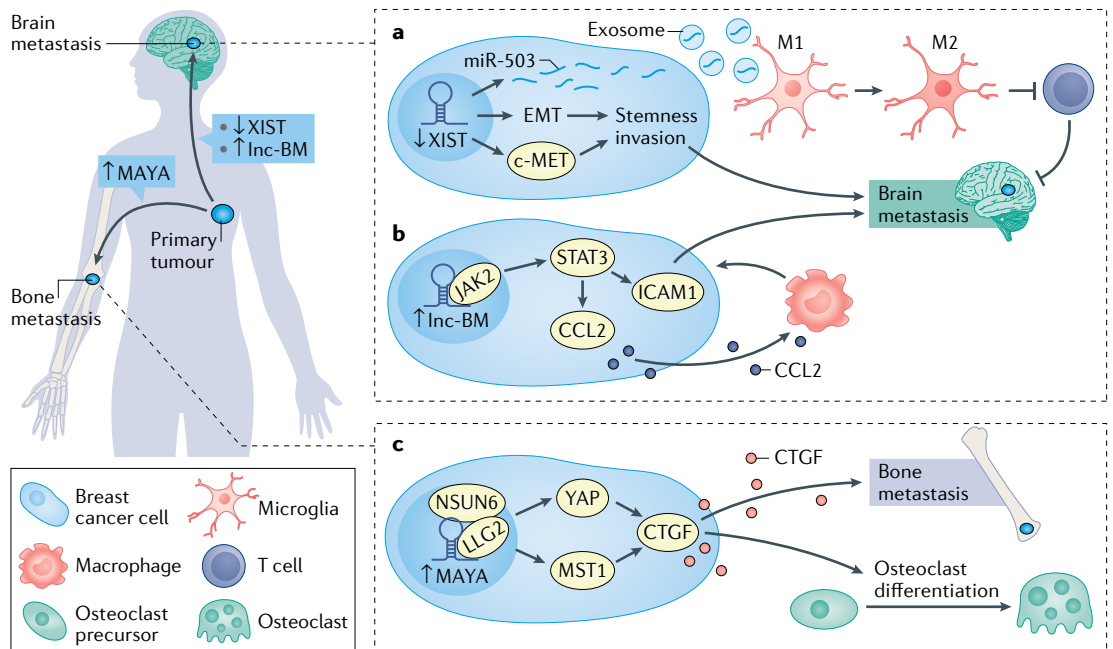


Fig. 5 | Long noncoding RNAs in metastasis site-specific tropism. a | Downregulation of XIST expression levels activates epithelial–mesenchymal transition (EMT) and MET, leading to stemness, invasion, increased production and release of miR-503, which subsequently induces M2 microglia polarization and T cell responses in breast cancer brain metastases. **b** | lnc-BM interacts with JAK2 to activate STAT1/STAT3 leading to activation of ICAM1, thereby permitting malignant cell co-option of brain endothelial cells. This also activates CCL2 and induces CCL2-dependent macrophage recruitment to brain metastases. **c** | MAYA promotes bone metastases via activation of YAP1 and methylation of the Hippo pathway gene MST1, leading to increased connective tissue growth factor (CTGF) and osteoblast differentiation.

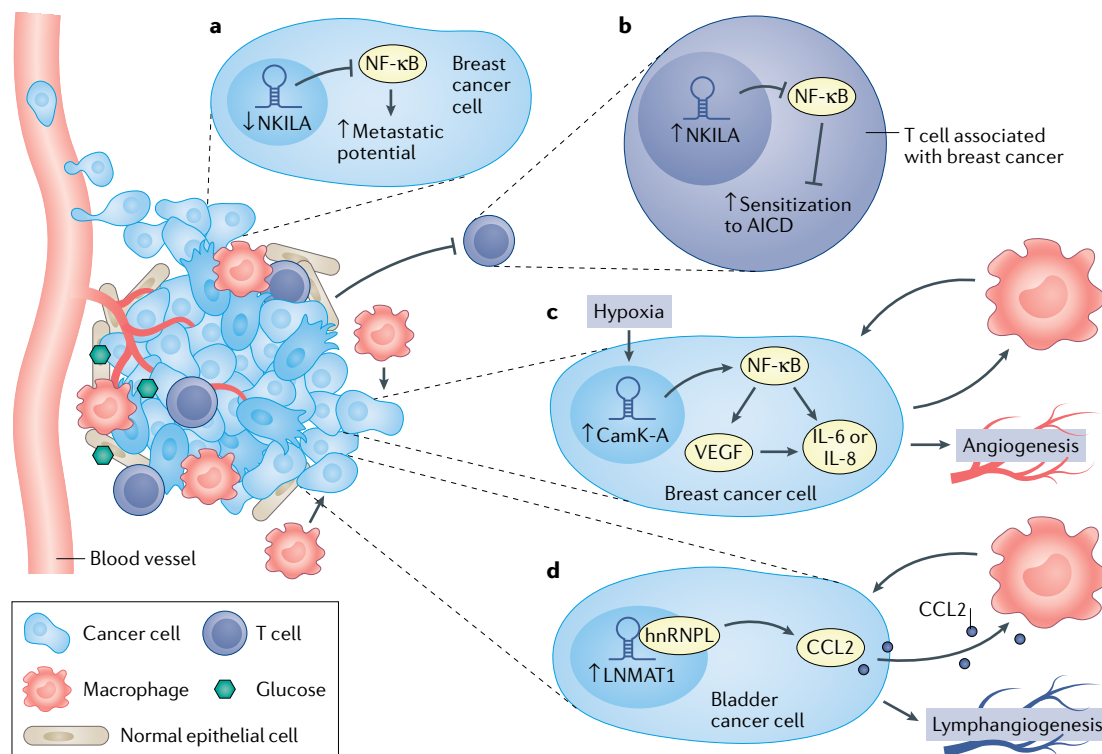


Fig. 6 | Long noncoding RNAs and tumour microenvironment. **a** | NKILA inactivates the nuclear factor- κ B (NF- κ B) pathway in breast cancer cells, and NKILA downregulation leads to decreased metastatic potential. In tumour-infiltrating T lymphocytes, NKILA leads to sensitization to activation-induced cell death. **b** | CamK-A activates NF- κ B in response to microenvironmental hypoxia, leading to activation of IL-6/IL-8 and VEGF, which increase macrophage recruitment and angiogenesis. **c** | LNMAT1 activates CCL2 and CCL2-dependent macrophage recruitment in bladder cancer metastasis. hnRNPL, heterogeneous nuclear ribonucleoprotein L.

correlated with CNS recurrence. Intracardiac injection of mice with breast cancer cells that were depleted of lnc-BM (via RNAi or CRISPR–Cas9) reduced the burden of brain metastases, along with reducing co-option of blood vessels within the brain by cancer cells. Mechanistically, lnc-BM activates expression of ICAM1 through phosphorylation of STAT1/3, thereby permitting malignant cell co-option of brain endothelial cells in an analogous manner to LICAM^{112,113}. Furthermore, once cells colonized within the brain, lnc-BM mediated JAK2 activation to recruit macrophages via CCL2, thereby regulating multiple pathways conducive to brain metastases.

In a departure from its role in X chromosome inactivation^{16,114}, lncRNA XIST was shown to be repressed in breast cancer brain metastases in mice, when compared with metastases in bone, liver or lung¹¹⁵. In line with this, silencing of XIST in MCF-7 and SK-BR3 breast cancer cells that were intracardially injected into mice resulted in increased burden of brain metastases. Further, genomic knockout of Xist enhanced brain metastases in MMTV-PyMT mouse models of spontaneous breast cancer metastasis, through induction of EMT and activation of MET, promoting a stem cell phenotype¹¹⁵. XIST knock-down can also lead to release of exosomal miR-503, which induces polarization of M2 microglia, thereby altering the metastasis microenvironment¹¹⁵.

As the expression and function of lncRNAs are generally cell type specific (with notable exceptions such

as MALAT1 and XIST)^{9,54}, it is perhaps unsurprising that lncRNAs play important roles in the organ-specific tropism of cancer metastases. However, it is unclear whether site-specific lncRNA regulatory programmes are triggered before metastatic colonization, thereby acting as directors of cell fate. Alternatively, lncRNAs may exert their influence on the maintenance of metastatic clones through the microenvironment.

lncRNAs in the metastatic microenvironment

On arrival at distant organ sites, metastatic cells form and interact with the TME, which includes diverse processes such as angiogenesis¹¹⁶, suppression and/or co-option of the innate and adaptive immune system^{117–119}, and the reprogramming of stromal populations to promote metastatic outgrowth^{120,121}. The establishment and maintenance of a supportive microenvironment, including resident stromal cells and innate and adaptive immune cells, is required for the outgrowth of metastatic colonies^{2,122}. Through suppression of the immune system, recruitment of angiogenesis, paracrine signalling and deposition of pro-metastatic extracellular matrices, the TME contributes to cancer metastases in diverse ways. The roles of lncRNAs in the tumour and metastatic microenvironment are emerging (FIG. 6).

NF- κ B-associated lncRNAs in the microenvironment.

Initially discovered as a cytoplasmic lncRNA that directly inhibits the NF- κ B complex within breast

cancer cells, leading to increased metastatic potential when downregulated¹²³ (FIG. 6a), the NF- κ B-interacting lncRNA (NKILA) also regulates intrinsic antitumour activity in T lymphocytes in a STAT1–histone acetylation-dependent mechanism¹²⁴ (FIGS 4, 6b). In breast tumour-infiltrating T cells, NKILA suppresses NF- κ B signalling, leading to activation-induced cell death of those T cells (FIG. 6b). Knock-down of *NKILA* using shRNA in CD8⁺ T cells increased their penetration into lungs when adoptively transferred into breast cancer xenograft mouse models, and those T cells upregulated perforin and CD107a expression and exhibited increased antitumour activity¹²⁴. CD8⁺ T cell exhaustion can also be induced through lnc-Tim3, which was shown to promote activation of p53 and RelA transcriptional targets¹²⁵.

Whereas NKILA negatively regulates the NF- κ B pathway in both tumour cells and immune cells, lncRNA CamK-A was shown to activate the NF- κ B pathway in breast cancer cells through degradation of I κ B in response to hypoxia-induced calcium influx¹²⁶ (FIG. 6b). This led to activation of gene transcription of *IL6*, *IL8* and *VEGF* (among other genes), which ultimately promoted macrophage recruitment and angiogenesis in patient-derived breast cancer flank xenograft models. Consistently, conditioned medium from CamK-A-deficient breast cancer cells failed to induce angiogenesis in HUVEC endothelial cell culture, whereas angiogenesis was induced in conditioned medium from CamK-A-proficient cells¹²⁶.

Immune cell regulation by lncRNAs. Regulatory T cells, which contribute to avoidance of immune surveillance in tumours, are also influenced by lncRNA activity. In CD4⁺ T cells, lnc-EGFR was shown to bind to cytoplasmic EGFR, thereby stabilizing it for downstream signal transduction¹²⁷. This led to induction of FOXP3, signifying differentiation towards the regulatory T cell lineage. Consistent with the expansion of this suppressive immune population, lnc-EGFR overexpression in T cells led to increased tumour growth compared with controls when these cells were implanted in xenograft models of HCC¹²⁷.

In bladder cancer, *LNMAT1* is overexpressed in lymph node-positive bladder cancer and is prognostic of overall survival in patients with bladder cancer¹²⁸. ShRNA-mediated knock-down of *LNMAT1* reduced lymph node metastases of bladder cancer cells injected into footpads of nude mice. However, the function of *LNMAT1* was not intrinsic to the tumour cell, as conditioned medium from *LNMAT1*-transduced bladder cancer cells was capable of stimulating macrophage activation, and this effect could be abrogated by CCL2-neutralizing antibodies (FIG. 6c). Interestingly, *LNMAT1* activated the expression of *CCL2* by forming a DNA–RNA triplex with the *CCL2* promoter and facilitated the deposition of the transcriptionally active histone modification, H3K4 trimethylation. Furthermore, *LNMAT1* overexpression increased lymphangiogenesis in vitro through macrophage-dependent upregulation of VEGF-C¹²⁸. Therefore, lncRNAs can have potent non-cell-autonomous effects on the microenvironment.

lncRNAs are crucial regulators of differentiation and function in immune cells belonging to the progenitor, innate and adaptive lineages¹²⁹. Furthermore, lncRNAs are necessary for the development and physiology of neural, pulmonary, cardiac and embryonic tissues^{130,131}. As all these cell types can contribute to the metastatic microenvironment or premetastatic niche, it is conceivable that additional lncRNAs will be implicated in these processes as well.

Translational potential of lncRNAs in metastases

Survival of patients with metastatic cancer is poor, and resistance to therapy remains a major barrier to effective treatment. One intriguing approach to improving the treatment of cancer is to leverage tissue- and cancer-specific expression profiles to develop prognostic markers for progression from primary to metastatic disease.

lncRNAs as novel diagnostic and prognostic tools. While lncRNA PCA3 has been identified as a non-invasive upfront diagnostic marker of prostate cancer with reliable test characteristics and is used in clinical practice¹³², overexpression of lncRNA SchLAP1 is independently prognostic of prostate cancer metastasis within 10 years following prostatectomy based on a microarray analysis of 1,008 patients¹³³. Application of SchLAP1 measurement to risk stratification of patients at time of surgery may enable precision medicine strategies for proactive medical treatment of aggressive tumours. Indeed, SchLAP1 expression is a critical component of a genomic classifier used in clinical practice for prostate cancer¹³³. Mechanistically, SchLAP1 binds to and antagonizes the SWI/SNF complex, leading to decreased genome-wide occupancy of SNF5 and decreased expression of its target genes¹³⁴. Consequently, *SchLAP1* knock-down in 22Rv1 prostate cancer cells reduced metastatic seeding when these cells were intracardiacally injected into SCID mouse models. However, this proposed mechanism of SchLAP1 has been challenged, as subsequent investigation demonstrated that overexpression of SchLAP1 did not evict the SWI/SNF complex from chromatin, suggesting a SWI/SNF-independent mechanism of action for SchLAP1 in prostate cancer metastases⁵². It is possible that the mechanism of SchLAP1 exhibits context or cell-type specificity while still maintaining utility as a clinical prognosticator.

Clinical outcomes of patients with metastatic cancer vary dramatically, especially across patients with different histologies of metastatic cancer^{135,136}. In patients with metastatic renal cell carcinoma (RCC), high lncARSR expression was prognostic for shorter overall survival compared with tumours with low lncARSR levels¹³⁷. lncARSR can confer resistance to sunitinib by sequestering miR-34 and miR-449, resulting in derepression of AXL and MET expression. By shuttling across cells via exosomes, lncARSR is capable of promoting drug resistance in a paracrine fashion. In line with lncARSR conferring sunitinib resistance, intravenous administration of LNA ASOs against lncARSR in an orthotopic xenograft model of RCC conferred sensitivity to concurrent sunitinib therapy¹³⁷.

RAMS11 expression was associated with poor prognosis in patients from two independent cohorts of patients with colon cancer⁹¹. Interestingly, a drug screen of FDA-approved agents revealed that increased RAMS11 expression promoted resistance to floxirudine (FUDR), a chemotherapy that is commonly used to treat metastatic CRC, and topoisomerase inhibitors. Indeed, *RAMS11* CRISPR knockout cells showed increased sensitivity to FUDR and topoisomerase inhibitors⁹¹. Further work is necessary to translate the prognostic and predictive utility of RAMS11 to clinical trials.

As a caveat, the biomarker discoveries discussed thus far may still be at an early stage. Whereas SChLAP1 ranked highest for elevated expression in patients with metastatic progression compared with patients without metastatic progression¹³³, many lncRNAs with cancer- or tissue-specific expression profiles may not be highly expressed enough to be used as a reliable biomarker. Moving forwards, the increasing availability of transcriptome sequencing data will greatly facilitate lncRNA biomarker discovery. However, independent validation is crucial and could be more challenging for lncRNAs. Importantly, only a subset of microarray platforms contained probes monitoring RAMS11 and SChLAP1 expression that enabled independent validation across cohorts of patients with long-term clinical outcome^{91,133}. However, many lncRNAs will not be captured by any existing microarray platform. Although some studies may use gene-specific validation in small independent cohorts, more widespread adoption of a biomarker requires a systematic comparison of the diagnostic or prognostic significance of a lncRNA relative to existing protein-coding gene biomarkers, when available. Given the longer follow-up time associated with specific cancer types (for example, prostate cancer) coupled with the need for well-annotated clinical follow-up associated with metastatic end points, this may require leveraging older retrospective cohorts. Further, owing to complex mechanisms of patient progression and response to treatments, a single lncRNA may not be the most clinically useful biomarker, but instead potential lncRNA biomarkers need to be evaluated in conjunction with protein-coding genes. Moving forwards, it will also be important to consider the ability to reliably detect putative lncRNA biomarkers non-invasively to minimize the need for biopsies. This will be particularly important for using lncRNA biomarkers for serial monitoring of patients for whom repeat tumour biopsy is impractical. Overall, SChLAP1, lncARSR and RAMS11 highlight the contributions of lncRNAs in cancer treatment response and their potential uses as prognostic and predictive biomarkers.

Therapeutic targeting of lncRNAs. Through in vivo preclinical models of metastatic disease, lncRNAs have shown promise as therapeutic targets for cancer treatment. For instance, the lncRNAs MAYA, MALAT1 and lncARSR, which we discuss above, have each been targeted for in vivo silencing using ASOs to ameliorate the burden of metastatic disease in mouse models^{93,94,111,137}. However, it will be crucial to ensure minimal off-target effects when targeting lncRNAs using ASO therapeutics^{138,139}, which could pose further

issues given the generally lower abundance of lncRNA transcripts in vivo⁹. Emerging evidence demonstrates that ASOs disrupt target RNAs through premature transcriptional termination^{140,141}, in addition to RNase H-mediated degradation of mature RNA, which should be considered when predicting the activity of ASO therapeutics. Hepatotoxicity has also been observed with certain ASOs¹⁴², and chemical modification of the ASO has been shown to mitigate this and off-target effects as well^{143,144}. A larger practical issue will be the optimization of ASO delivery, which is a general area of development to increase the impact of ASOs targeting lncRNAs or protein-coding genes. Despite these challenges, it is reassuring that protein-coding genes have been targeted using ASOs in both mouse models of cancer^{145,146} and clinical trials, as in the case of *SMN2* in patients with spinal muscular atrophy¹⁴⁷, *STAT3* in treatment-refractory lymphoma¹⁴⁸ and *SMAD7* in Crohn's disease¹⁴⁹. Perhaps the once-lofty vision of targeting lncRNAs for cancer metastasis treatment appears hopeful.

Current limitations

Although many seminal studies demonstrate the contributions of lncRNAs throughout metastasis (FIG. 2), gaps of knowledge remain. In particular, the steps in the invasion–metastasis cascade between cellular transformation and distant colonization, which would include extravasation, haematogenous (or leptomeningeal as may be the case in CNS malignancies) spread, intravasation and formation of micrometastases, have less well-established contributions from lncRNAs. Overcoming these limitations would require additional patient-matched molecular profiling data from granular stages of metastasis (for example, primary tumour, CTCs, micrometastases and well-defined metastases).

In addition, the majority of the lncRNAs discussed in this Review act to enhance metastatic potential. Whether these observations present inherent attributes of lncRNA biology or are a consequence of methodological bias in respective studies remains to be determined (Supplementary table 1). Nonetheless, additional studies of lncRNAs that potentially suppress metastasis are warranted. Generalizing the roles of lncRNAs in metastasis across cancer types has also been elusive, and perhaps this should be expected based on the cell type-specific function of most lncRNAs. Improved methodology to dissect the mechanisms of lncRNAs in experimental models that faithfully recapitulate human disease are also required, as characterization of lncRNAs in cancer still lags behind that of their protein-coding counterparts.

Another conundrum in the field of lncRNA biology is why seemingly disparate lncRNAs tend to converge upon a few firmly established protein-coding pathways, instead of functioning as independent regulators of metastasis. It is possible that decades of research on protein-coding genes have enabled the discovery and convergence of key metastasis regulator genes whereas the comparative infancy of research into many lncRNAs has not yet enabled similar lncRNA discoveries and validation across independent laboratories. However, emerging data suggest that ‘functional conservation’ in lieu of primary sequence or secondary structure

conservation may be a salient feature of lncRNAs^{150,151}. Furthermore, it is possible that multiple RNA species fulfill similar roles interacting with established protein-coding genes, a concept highlighted by the requirement of PRC2 for RNA interaction to properly function¹⁵², despite exhibiting promiscuous protein–RNA interactions⁶⁶. Taken together, it is conceivable that even a menagerie of lncRNAs generated from various locations in the genome with divergent sequences can influence a similar set of potent metastatic pathways.

Conclusions

Metastases are an often-deadly consequence of cancer. lncRNAs are not only important regulators of cancer hallmarks in general^{38,39}, but also key players in the pathogenesis of metastases⁴⁹. As more lncRNAs are rapidly characterized, as has been the case in recent years, it is evident that lncRNAs function at critical junctures in the invasion–metastasis cascade, organotropic colonization of distant organs and TME. By functioning together with pro- or anti-metastatic protein complexes, or by interacting directly with other RNA species or DNA (as in the case of RNA–DNA triple helices), lncRNAs exert their influence on cancer pathways via diverse mechanisms^{5,6,13}. Outstanding questions remain, such as

the role of lncRNAs in CTCs, formation of the premetastatic niche and stromal cell-specific functions^{120,153}. Future studies necessitate investigation into these additional processes and also further delineation of the mechanisms of lncRNAs involved in metastasis. Moving forwards, in addition to selecting appropriate models for inactivating a lncRNA, it will be crucial for labs to perform appropriate genetic rescue experiments. Independent validation of results between different investigators will be necessary as well. While undertaking these endeavours, it is also crucial to understand the impact of various methodologies on interpreting lncRNA function, and to be open minded to different possibilities for how lncRNAs function.

lncRNAs have also emerged as an underappreciated cache of novel therapeutic targets. Their diverse roles in cancer progression provide new opportunities for undermining metastases in the clinical setting. The maturation of technologies that enable in vivo targeting of lncRNAs^{53,154}, such as antisense oligonucleotides that are analogous to those currently used to treat human disease¹⁵⁵, will greatly foster the realization of lncRNA therapeutics against metastases.

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