

Title: Long non-coding RNAs within the tumour microenvironment: Role in tumour-stroma cross-talk and the phenotype of cancer associated stromal cells.

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Abstract

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Abbreviations:

CAF: cancer-associated fibroblast

lincRNA: long intergenic non-coding RNA

RNPs: ribonucleoprotein complexes

MRE: microRNA response elements

ceRNA: competing endogenous RNA

UBC: urinary bladder cancer

NOF: normal fibroblast

PFKFB2: 6-phosphofructo-2kinase/fructose-2,6-biphosphatase 2

HIF-1A-AS2: hypoxia-inducible factor 1-alpha antisense RNA 2

GEC: glioma associated endothelial cells

VASH-2: vasohibin-2

ATG7: autophagy-related protein 7

FOXM1: forkhead box-1 protein

NPC: nasopharyngeal carcinoma cells

GRP78: glucose-regulated protein 78

ANG-2: angiopoietin-2

HLA-G: human leukocyte antigen-G

PCAT29: prostate cancer associated transcript 29

CRNDE: colorectal neoplasia differentially expressed

GAS5: growth arrest specific transcript 5

MV: microvesicle

HBMEC: human brain microvascular endothelial cells

IncARSR: lncRNA activated in RCC with sunitinib resistance

TGFBI: TGF β induced protein

Introduction:

Long non-coding RNAs (lncRNAs) belong to a family of non-coding RNA (ncRNA) molecules which lack protein-coding capacity. They are distinguished from shorter ncRNAs and microRNAs (miRNAs) by their length (which exceeds 200 nucleotides) and their biological functions and mechanism of action. According to recent data from the GENCODE database, the total number of lncRNA genes currently identified in the human genome surpasses 15,000, coded in more than 27,000 lncRNA transcript loci (www.genecodegenes.org). A large proportion of these ncRNAs are yet to be properly annotated, which reflects the volume of information potentially contained within the non-coding region of the genome.

lncRNAs originate in the nucleus where they are transcribed by RNA Polymerase II. They are capped and polyadenylated at their 3' and 5' ends, respectively; however, post-transcriptional processing can also produce alternative features such as 3' end cleavage or 5' end capping and circular RNA structures.¹⁻³ Emerging data suggests that, like other ncRNA species, lncRNAs participate in key biological processes in both physiology and disease, and that deregulated lncRNA expression may have important consequences during malignant transformation.⁴⁻⁸

Of parallel importance in recent times is recognition of the role of the tumour microenvironment during tumour progression. In the malignant state, the stroma surrounding a cancer stimulates a physiologically distinct 'active' tumour microenvironment, maintained by various tumour associated host cells including inflammatory cells, immunocytes, macrophages and cancer associated fibroblasts (CAFs)(Figure 1).⁹⁻¹² The dynamic and reciprocal interaction between these cells and the malignant epithelium promotes tumour growth/invasion, extracellular matrix (ECM) remodelling and angiogenesis.^{13,14}

The role of miRNAs within the tumour microenvironment is increasingly well understood, however the role of other ncRNA species and lncRNAs in particular has been relatively neglected. Here we briefly describe the structure and function of lncRNAs before looking in depth at their potential roles in carcinogenesis and metastatic progression within the tumour microenvironment. In so doing we will provide the most contemporaneous account of lncRNA activity within the stroma and in tumour-stroma signalling pathways.

lncRNAs: Structure classes and mechanisms of action

Currently, there are two main methods of classifying lncRNAs based on either their position in the genome or mechanism of action. According to their genomic localization, lncRNAs can be grouped in intronic, intergenic, enhancer, bidirectional, sense-overlapping and antisense genes.

Intronic lncRNAs are generated from intronic sequences within protein-coding genes whilst intergenic lncRNAs (lincRNAs) originate from a region between protein coding genes.¹⁵ Enhancer lncRNAs are localized within enhancer sequences of gene promoters and may act by altering the 3-dimensional configuration of DNA;¹⁶ bidirectional lncRNAs are situated in proximity to coding transcript sequences but on the opposite DNA strand. Sense-overlapping lncRNAs are complementary to and overlap with introns and exons of protein-coding genes in the sense strand of the DNA, whereas antisense lncRNAs are transcribed in the antisense direction.¹⁷⁻¹⁹

In terms of molecular actions, lncRNAs can be categorized into a number of groups including signalling, scaffold, guide and decoy molecules (Figure 2).²⁰ Signalling lncRNAs are molecules whose expression is associated with specific signalling pathways or events. Scaffold lncRNAs act as platforms on to which multiple proteins can be assembled in functional units, such as ribonucleoprotein complexes (RNP) capable of inducing or suppressing transcriptional activity.²¹

Guide lncRNAs regulate gene expression by physically directing RNPs to specific genomic regions. Conversely, decoy lncRNAs sequester transcription factors through direct linkage, reducing their bioavailability. MicroRNAs too can be subjected to this regulatory mechanism by interacting with miRNA response elements (MRE) within the lncRNAs' structure. Acting as "molecular sponges", in this way lncRNAs actively compete with specific protein-coding mRNAs to interact with the common intracellular pool of miRNAs. For this reason lncRNAs and mRNAs are often described as competing endogenous RNAs (ceRNAs) for miRNAs.²² This represents a further novel level of post-transcriptional gene regulation, as lncRNAs act to titrate miRNA, mRNA and ultimately protein availability.

Messenger RNA (mRNA) stability is another key process regulated by lncRNAs. Recent studies have demonstrated that certain lncRNAs localise in subnuclear compartments called nuclear paraspeckles where mRNA can be temporarily stored before ribosomal translation.²³ In these bodies lncRNAs are able to regulate mRNA processing, and in particular pre-mRNA splicing.²⁴ Alternatively, lncRNAs have been shown to form RNA-RNA duplexes with mRNAs mediating their subsequent degradation.²⁵

Finally, lncRNAs which contain open reading frames (ORFs) which may be transcribed have recently been identified.^{26,27} These findings offer a completely new perspective of lncRNAs capabilities, and suggests that some "non-coding" RNAs are actually more like mRNAs for a novel class of small polypeptide, the biological significance of which is only just beginning to emerge.

The mechanistic and topographical variation of lncRNAs within the cell, implies a high degree of biological importance of this class of molecule. To date, the majority of research in this field has focused on the role of lncRNAs within tumour cells, however a growing body of work suggests deregulated lncRNA expression within the tumour microenvironment may play a parallel role during malignant transformation and disease progression.

LncRNAs and stromal cells

Emerging data suggest that lncRNAs regulate the phenotype of cancer associated stromal cells within the tumour microenvironment (Figure 1).

Cancer Associated Fibroblasts

The key cellular constituent of the transformed tumour microenvironment are cancer associated fibroblasts. CAFs are a heterogeneous population of cells which include myofibroblasts. The two terms are often used interchangeably because in effect, they represent phenotypically similar cell populations.⁹ CAFs originate from a variety of stroma-resident progenitor cells which may include fibroblasts, smooth muscle cells, stellate cells, adipocytes and epithelial cancer cells and from migratory bone marrow derived MSCs.²⁸⁻³⁰ TGF β 1 produced by the malignant epithelium is the dominant and best characterised fibroblast-to-myofibroblast transdifferentiation signal. Mature CAFs reciprocate by modulating the makeup of the tumour microenvironment to enhance invasion and metastatic progression.³¹

Zuang et al., demonstrated in vitro that a novel lncRNA called ZEB2NAT is involved in regulating transforming growth factor-1 (TGF- β 1) secretion in CAFs. TGF- β 1 is a potent inducer of EMT-related genes in cancer and promotes increased invasion of urinary bladder cancer (UBC) cells in co-culture experiments with CAFs.³² In a second study, a panel of lncRNAs were found to be differentially expressed between normal fibroblasts (NOFs) and paired CAFs of ovarian origin. Computational analysis based on known lncRNA-transcription factor and lncRNA-gene interactions highlighted a network of signalling pathways through which stromal lncRNA may influence metastatic progression, although it is important to emphasise a purely in-silico approach was used in this instance.³³ The lncRNA LINC00092 was also found to be upregulated in ovarian cancer, a trend which correlates with poor prognosis. The authors of this study suggested LINC00092 induction was triggered by release of the chemokine CXCL14 by adjacent CAFs, leading to altered glycolysis within cancer cells by LINC00092 binding to the fructose-2,6-bisphosphatase PFKFB2. In turn, this enhanced the metastatic potential of ovarian cancer cells through a positive feedback loop which sustained and supported CAF function within the local tumour microenvironment.³⁴

Mesenchymal Stem cells

MSCs typically reside in bone marrow, but are recruited to sites of injury as part of the inflammatory response by pro-inflammatory mediators such as chemokines/cytokines and growth factors.^{35,36} They

have the potential to differentiate into a variety of stromal cells³⁷ and they play an important role during wound healing.³⁸ MSC “homing” is also triggered by cancer; ‘the wound that never heals.’ MSCs within the tumour microenvironment have diverse roles and may transdifferentiate into cancer associated stem cells or even cancer associated fibroblasts (CAFs) in response to paracrine signals such as TGF β from the transformed epithelium. By exerting a potent immunosuppressive influence and reciprocally triggering epithelial-to-mesenchymal transition in cancer cells, MSCs have been shown to enhance tumour growth and invasion and promote metastatic progression.³⁹

Two recent studies have suggested lncRNAs influence MSC phenotype and differentiation status within the tumour microenvironment. In one study, MSCs were found to stimulate an increasingly aggressive mesenchymal phenotype in HCC cells in co-culture experiments. The presence of MSCs increased the expression of a novel lncRNA called MUF in HCC cells which in turn induced EMT by inhibiting miR-34a expression.⁴⁰ In the second study, Mineo and colleagues identified a Glioblastoma Multiforme (GBM) MSC specific pattern of lncRNA expression which included the lncRNA candidate hypoxia-inducible factor 1-alpha antisense RNA 2 (HIF1A-AS2).⁴¹ This lncRNA appears regulate GSC tumour growth and survival and molecular programming in a hypoxia-dependent manner, leading to the suggestion that individual lncRNAs in MSCs may orchestrate an adaptive response to hypoxic stress within the tumour microenvironment.

Endothelial cells and angiogenesis

Angiogenesis is the term used to describe the generation of new blood vessels.⁴² In cancer this process is essential to sustain tumour progression, and endothelial cells residing in the stroma are important regulators of invasive tumour growth.

Recent data has implicated lncRNAs in several angiogenesis pathways. Glioma-associated endothelial cells (GEC) cultured in glioma-conditioned medium showed significant upregulation of the lncRNA H19. This in turn was associated with enhanced GEC proliferation and migration and the enhanced ability to form proto-vascular tubular structures in vitro. After H19 knockdown, these effects were reversed, as H19 suppression was associated with miR-29a induction and the subsequent downregulation of and vasohibin-2 (VASH-2), a potent proangiogenetic factor. Thus, it can be postulated that lncRNA H19 is able to regulate glioma angiogenesis by inhibiting expression of miR-29a which, in turn triggers endothelial activity through enhanced expression of VASH-2 protein.⁴³ Similarly, a study by Ma and colleagues showed that lncRNA PVT1 is upregulated in glioma vascular endothelial cells and is associated with low expression of miR-186.⁴⁴ PVT1 negatively regulates miR-

186 expression which reduces miR-186 suppression of ATG7 and Beclin1, which are autophagy associated genes which contribute to enhanced vascular endothelial cells survival in hypoxic conditions.⁴⁵

MALAT-1 is another lncRNA considered to have proangiogenic effects and which in human umbilical vein endothelial cells (HUVEC) cells causes reduced transcription of the protein FOXM1 and reduced vascular endothelial cell proliferation. MALAT-1 is a ce-RNA which sequesters miR-320a a translational suppressor of FOXM1,⁴⁶ but it may modulate neovascularisation via other mechanisms also. In neuroblastoma for example, new vessel growth in hypoxic conditions *in vitro* is associated with MALAT1 dependent fibroblast growth factor-2 (FGF-2) expression.⁴⁷

HOTAIR has been implicated in the regulation of angiogenesis in nasopharyngeal carcinoma cells (NPC). Researchers identified upregulated expression of HOTAIR in NPC cells and clinical tumour samples and demonstrated attenuated angiogenesis by knocking down HOTAIR *in vitro* and *in vivo*. At a molecular level, HOTAIR was able to alter the expression of the master angiogenesis-regulator vascular endothelial growth factor A (VEGFA) directly through its promoter sequence or alternatively by modifying the levels of glucose regulated protein-78 (GRP78) and Angiopoietin-2 (ANG-2) ⁴⁸.

Tumour neo-angiogenesis is an imperfect process which gives rise to incomplete, leaky and dysfunctional blood vessels.⁴⁹ Imperfect neovascularisation combined with rapid tumour growth lead to low oxygen tension, an increasingly acidic tumour microenvironment and as a consequence, further neovascularisation.⁵⁰ Oral squamous cancer cells (OSCC) exhibit higher levels of lncRNA HAS2-AS1 when cultured in hypoxic conditions compared to normoxic ones, suggesting that HAS2-AS1 may act as a marker of tumour hypoxia and therefore potentially a marker of a more aggressive tumour phenotype. Furthermore, a paper by Zhu et al. suggested increased expression of HAS-AS1 in OSCC in hypoxic conditions leads to incremented production of hyaluronan synthase. This sensitizes OSCC cells to signals which promote epithelial-to-mesenchymal transition (EMT), increasing their migratory and metastatic potential.⁵¹

Other evidence suggests that lncRNA expression is linked to hypoxia-inducible factor 1 α (HIF-1 α), a master regulator of the hypoxic cellular adaptive response. Under hypoxic conditions, HIF-1 α stimulates expression of lncRNA UCA1 by direct binding to its promoter, which in bladder cancer cell lines promotes tumour invasion *in-vitro*.⁵² Similarly, in a second study, HIF-1 α was shown to bind directly to two responsive sites on the promoter of the lncRNA HOTAIR gene in non-small cells lung cancer (NSCLC).⁵³ HOTAIR overexpression in hypoxic conditions led to reduced apoptosis and enhanced invasion in NSCLC cells suggesting that this lncRNA is also involved in driving a more

malignant phenotypes in a hypoxic tumour microenvironment. Equally, HIF-1 α activity may be stimulated by other hypoxia-related lncRNAs. Oral cancer cell lines characterised by high levels of the lncRNA HIFCAR were analysed in a paper by Shih et al. HIFCAR forms a complex with HIF-1 α and as a co-activator facilitates recruitment of HIF-1 α and other co-factors to target promoter sequences. These data suggest that HIF-1 α not only regulates hypoxia-related lncRNAs but is also regulated by them.⁵⁴

Immune cells

The concept of immunosurveillance is based on the theory that immune cells can identify and eradicate tumour cells due to antigenic differences of neoplastic cells.⁵⁵ However, cancer cells may evade the immune response through cancer immunoediting, a process which disrupts the innate and adaptive immune response and the equilibrium between immunogenicity and immune tolerance, and which ultimately may lead to tumour progression.⁵⁶

Immune dysfunction in cancer has been linked to epigenetic changes within malignant cells and the role of lncRNAs in the differentiation and function of various immune cells is increasingly well understood.⁵⁷ In contrast, the role during tumour progression of deregulated lncRNAs within infiltrating immune cells is only just beginning to emerge.

In gastric cancer, studies have identified lncRNAs involved in systemic immune dysfunction. Xiong et al. demonstrated that increased distribution of circulating regulatory T cells (Treg) in gastric cancer is associated with increased expression of lnc-POU3F3. *In vitro* experiments supported the role of lnc-POU3F3 in increasing Treg distribution by up-regulating TGF β signalling.⁵⁸ HOTAIR has also been linked to immune cell evasion in gastric cancer through increased HLA-G expression. HLA-G is a non-classical MHC family, which plays important role in cancer-induced immune dysfunction by inhibiting cytotoxic activity of CD8⁺ T and NK cells, and CD4⁺ T cell proliferation.⁵⁹ Song and colleagues demonstrated that increased expression of HOTAIR correlates directly with and in-fact regulates HLA-G expression and secretion in-vivo.⁶⁰

In HCC, Jiang et al. identified lnc-EGFR as a key factor in influencing tumour infiltrating lymphocytes. This study demonstrated that in HCC patients, there are high basal levels of lnc-EGFR which stimulates the generation and persistent activation of Tregs within the tumour microenvironment. lnc-EGFR specifically binds to EGFR which it stabilizes through blocking c-CBL. Downstream, this leads to Treg differentiation and consequently to cytotoxic T cell inhibition.⁶¹

In ovarian cancer, lncRNAs RP11-284N8.3.1 and AC104699.1.1 have been implicated in T cell activation and differentiation. Additionally, the increased expression of these lncRNAs correlated with improved survival in ovarian cancer, due to an improved anti-tumour immune response.⁶²

In colorectal cancer, lnc-sox5 influences cytotoxic T lymphocyte infiltration of tumour tissue. Wu et al. found that lnc-sox5 knock-down promoted infiltration and cytotoxicity of CD8+ T cells based on cell lines and *in vivo* in animal studies.⁶³

lncRNAs and Tumour-Stroma signalling

Intracellular communication is essential for cells to carry out their normal biological functions in health however, this process may become corrupted in cancer. Tumour cells which have the capacity to influence the phenotype other cells within the tumour microenvironment, may through aberrant signalling hijack the function of these cells to enhance their own survival or promote disease progression.

Paracrine signalling pathways

Cytokines, chemokines and growth factors constitute an important mechanism of communication between tumour and stromal cells and may contribute to a pro-inflammatory tumour microenvironment which promotes disease progression in solid organ tumours. lncRNAs have been identified both as down-stream effectors and up-stream regulators of these important intracellular signalling pathways.

The association between lncRNAs and TGF β within the tumour microenvironment has been reviewed extensively,⁶⁴ but more recently, the role of lncRNAs within other pro-inflammatory signalling pathways has also been explored.

IL-6, secreted by numerous cancer associated stromal cell types is associated with increased invasive capability of tumour cells.^{65,66} In HCC specifically, the lncRNA lncTCF7 is transcriptionally activated by exposure to IL-6, and promotes epithelial to mesenchymal transition and consequently a more aggressive tumour phenotype. At a molecular level, the main target of IL-6 signalling is STAT3 which binds the lncTCF7 promoter sequence directly⁶⁷. In prostate cancer, high levels of IL-6 is associated with decreased expression of tumour suppressor lncRNA prostate cancer associated transcript 29 (PCAT29) in cultured tumour cell lines. Al Aameri and colleagues found that IL-6 achieved tonic

suppression of PCAT29 by stimulating STAT3 dependent miR-21 expression.⁶⁸

Other studies have highlighted pathways through which lncRNAs appear to regulate cytokine secretion within the tumour microenvironment. Using a tumour astrocyte model, Li and colleagues demonstrated that lncRNA CRNDE induces transcription factor activity within the toll-like receptor signalling pathway, leading to increased expression of a number of cytokines including IL-6, IL-10 and tumour necrosis factor (TNF).⁶⁹ They postulated that CRNDE may trigger inflammation through intense stimulation of the NF- κ B pathway with subsequent release a multiple pro-inflammatory cytokines. In contrast, growth arrest specific transcript 5 (GAS5) which is downregulated in CRC was shown to inhibit the release of IL-10 and VEGF by blocking NF- κ B and ERK1-2 pathways in-vitro and in a colitis associated CRC murine model.⁷⁰

Extracellular vesicles

As well as releasing soluble paracrine signals, tumour cells secrete extracellular vesicles (EV), containing ncRNA species, mRNA, DNA and proteins which are internalised by other cells within the tumour microenvironment. EVs are multi-layered membrane encircled vesicles derived from endosomes within the cytoplasm or from the plasma membrane. According to their size and origin they can be defined as exosomes, microvesicles (MVs) or apoptotic bodies.

A growing body of evidence suggests that EV derived lncRNAs are functional within the tumour microenvironment and may have important biological effects in recipient cells.⁷¹⁻⁷³ LncRNAs within EVs have also generated interest as diagnostic and/or prognostic biomarkers in cancer as specific lncRNAs appear to be enriched in EVs produced by cancer cells⁷⁴⁻⁷⁶ and can be retrieved and reliably profiled from EV extracted from human bio-fluids.^{77,78}

Exosomes which are 30-100nm endosome derived vesicles appear to be the richest reservoir of lncRNA whilst the larger (100-100nm) plasma membrane derived MVs appear to contain the lowest amount.⁷⁶ Tumour derived exosomal lncRNAs may promote angiogenesis within the tumour microenvironment and hence the capacity for malignant cells to metastasise. In HCC, CD90+ cancer stem cells may increase the migration rate of endothelial cells and subsequent formation of vessel-like tubular structures by upregulating the expression of proangiogenic genes VEGF and VEGFR1 in associated endothelial cells in-vitro. Although a mechanistic dissection of this effect was not attempted, the authors of this study found expression of the lncRNA H19 was enriched specifically in cancer exosomes and that overexpression in endothelial cells stimulated angiogenesis.⁷³ Similarly, linc-POU3F3 which is upregulated in glioma tissue is transferred via exosomes from cultured glioma cell lines to human brain microvascular endothelial cells (HBMEC), causing upregulated expression of

pro-angiogenic genes including bFGF, bFGFR and promotes HBMEC migration, proliferation and tubule formation in-vitro.⁷⁹

Within the tumour microenvironment, exosomal lncRNAs may play other roles in angiogenesis. Tumour-associated macrophages (TAM) produce exosomes which negatively regulate endothelial cell migration by upregulating miR-146b-5p in recipient endothelial cells and suppressing TNF receptor associated factor 6 (TRAF6) expression in the NF- κ B signalling pathway. Wu et al., discovered however, that ovarian cancer cell derived exosomes overcome this inhibition and identified two lncRNAs (ENST00000444164 and ENST00000437683) which may contribute to this effect by regulating NF- κ B phosphorylation.⁷¹

In renal cancer, exposure of HUVEC cells to exosomes which contain high levels of lncARSR (lncRNA Activated in RCC with Sunitinib Resistance) exhibited a poor response to sunitinib in tubule retraction assays implying that drug resistance may be propagated by exosomal lncRNAs within the tumour microenvironment.⁸⁰ A recent study has also shown that lung cancer-derived exosomes inhibit the potential for mesenchymal stem cells (MSC) to terminally differentiate and are associated with widespread deregulation of lncRNA expression.⁸¹ This suggests cell types other than vascular endothelial cells within the tumour microenvironment may be reprogrammed by exosomal transfer of lncRNAs.

Extracellular Matrix interactions

In order to metastasise, cancer cells must traverse the extracellular matrix (ECM) and penetrate lymphatic and circulatory vessels before dispersing to distant organs. Overcoming this physical barrier and interacting with ECM components is therefore an essential requirement for cancer cells during disease progression.

ECM is made up of an interstitial matrix of proteoglycans, hyaluronans and growth factors and other structural proteins secreted by epithelial, endothelial and stromal cells. It is a dynamic rather than a static construct, constantly being remodelled by enzymes such as metalloproteinases.

Recently, studies have shown that lncRNAs may be involved both in mechanisms of communication between metastatic cancer cells and ECM and in ECM turnover. Zhu et al, described indirect regulation by lncRNA H19 of TGF β induced protein (TGFB1), an ECM protein which can facilitate cancer cell migration through enhanced adhesion with ECM molecules. They identified that downregulated expression of this non-coding RNA in metastatic prostate cancer cells vs. non-metastatic cells was associated with downregulated expression miR-675 in a lncRNA H19 dependent manner. Further

mechanistic and functional analysis suggested that the lncRNA H19/miR-675 axis acts as a suppressor of tumour cell migration and prostate cancer metastasis in-vitro.⁸²

The lncRNA HOTAIR has been shown to play an important role in invasion and metastasis in breast cancer, however paradoxically, in a study by Li and colleagues, low expression was identified in breast cancer cell lines in 2-dimensional cell culture. Crucially however, in 3-dimensional culture within an extracellular matrix rich in laminin, HOTAIR was significantly upregulated and furthermore, HOTAIR suppression with siRNA reduced tumour cell invasion. The authors of this study suggested that ECM signalling, mediated by integrin $\alpha 2$ and SRC, may determine the transcription of an alternative HOTAIR isoform that stimulates invasion pathways in tumour cells in direct contact with ECM.⁷¹

This use of organotypic 3D culture models has also proved to be effective tool to explore lncRNA activity in lung cancer cells and in particular investigating the role of interstitial ECM components such as collagen 1 (Col-1). Col-1 enriched in the tumour microenvironment and has tumour-promoting effects. In Col-1 supplemented 3D culture, Zhuang and colleagues observed induction of HOTAIR in cultured cancer cells with subsequent disruption of tumour morphology. Specifically, HOTAIR induction by Col-1 disrupted the tissue acinar architecture, a characteristic of well-differentiated lung adenocarcinomas, and contributed to a more invasive cellular phenotype.⁸³

As well as ECM proteins, cancer cells interact directly with cells within the tumour microenvironment which produce ECM and basement membrane components. After demonstrating that metastatic prostate cancer cells cause upregulated expression in osteoblasts of a panel of genes including the *Wnt* inhibitor *Sost* involved in metastatic progression, a reciprocal experiment demonstrated that *Sost* knockout in osteoblasts leads to upregulated expression of the lncRNA MALAT-1 in prostate cancer cells with which they were co-cultured. Although functional analysis was not performed in this study, *MALAT1*, has been implicated in several human cancers including prostate cancer and is linked to enhanced cell proliferation, migration, and invasion.⁸⁴ This suggests that reduced *Sost* expression may stimulate osteoblasts to promote bone metastasis in prostate cancer cells through MALAT-1 up-regulation.⁸⁵

Conclusions

The tumour microenvironment supported by various cancer associated stromal cells plays an important role during tumour growth and metastatic progression. A growing body of evidence describes a high degree of interaction and signalling complexity between malignant epithelial cells

and the surrounding stroma. Nevertheless, many studies continue to focus on the metastatic behaviour of tumour cells in isolation, neglecting their context and ignoring potentially important biological information.

A growing appreciation of the tumour microenvironment in recent years has highlighted the role of non-coding RNAs involved in shaping the phenotype of cancer associated stromal cells and in tumour-stroma cross-talk. Although compared to microRNAs, the knowledge-base around lncRNAs is significantly lacking recent studies have shown that like miRNAs, lncRNAs have prognostic and diagnostic utility and may represent promising molecules to exploit pharmacologically.

In this review, we have explored the findings of the most recently published research involving of lncRNA within the tumour micro-environment. We have described the function of lncRNAs in key cancer associated stromal cell types and in intracellular signalling pathways, and highlighted their contribution to a molecular machinery which produces an increasingly aggressive tumour phenotype. Further research is required in the novel but expanding theme, as a better understanding of the role of non-coding RNAs within the tumour microenvironment has the potential to translate into clinical tools to improve and extend the lives of patients with cancer.

Declaration of interests

None.

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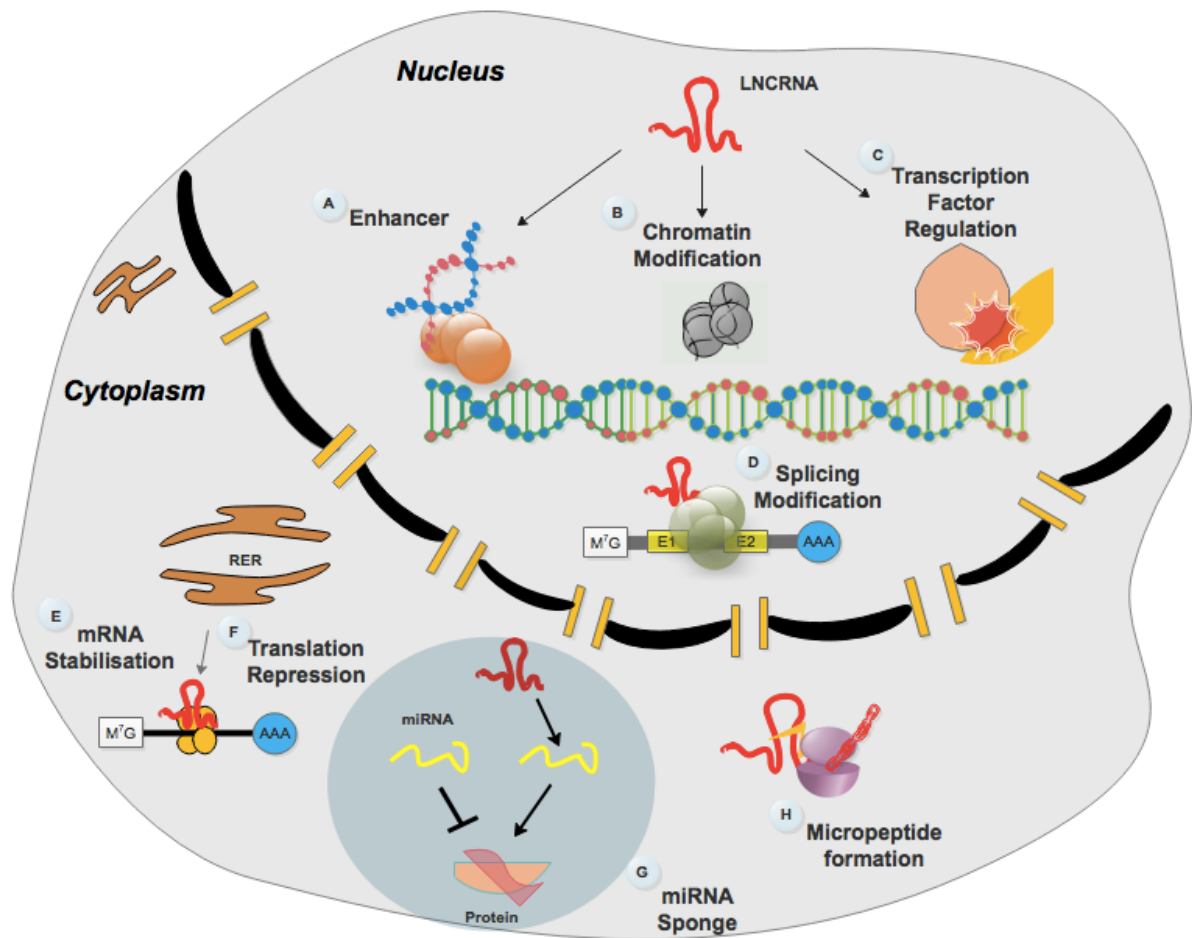


Figure 1. lncRNA mechanisms of action.

The human genome complex encodes a wide variety of lncRNAs that fulfil a diverse range of regulatory roles. Within the nucleus lncRNAs act (A) by enhancing DNA transcription; (B) by recruiting chromatin-modifying protein to specific sites in the genome; (C) by binding specific transcription factors and altering their function; and (D) regulating alternative splicing. In the cytoplasm lncRNAs (E) bind specific mRNAs to provide stability and control trafficking; (F) bind mRNAs to induce translation repression; (G) suppress the negative regulatory activity of miRNAs; and (H) encode functional micro-peptides.

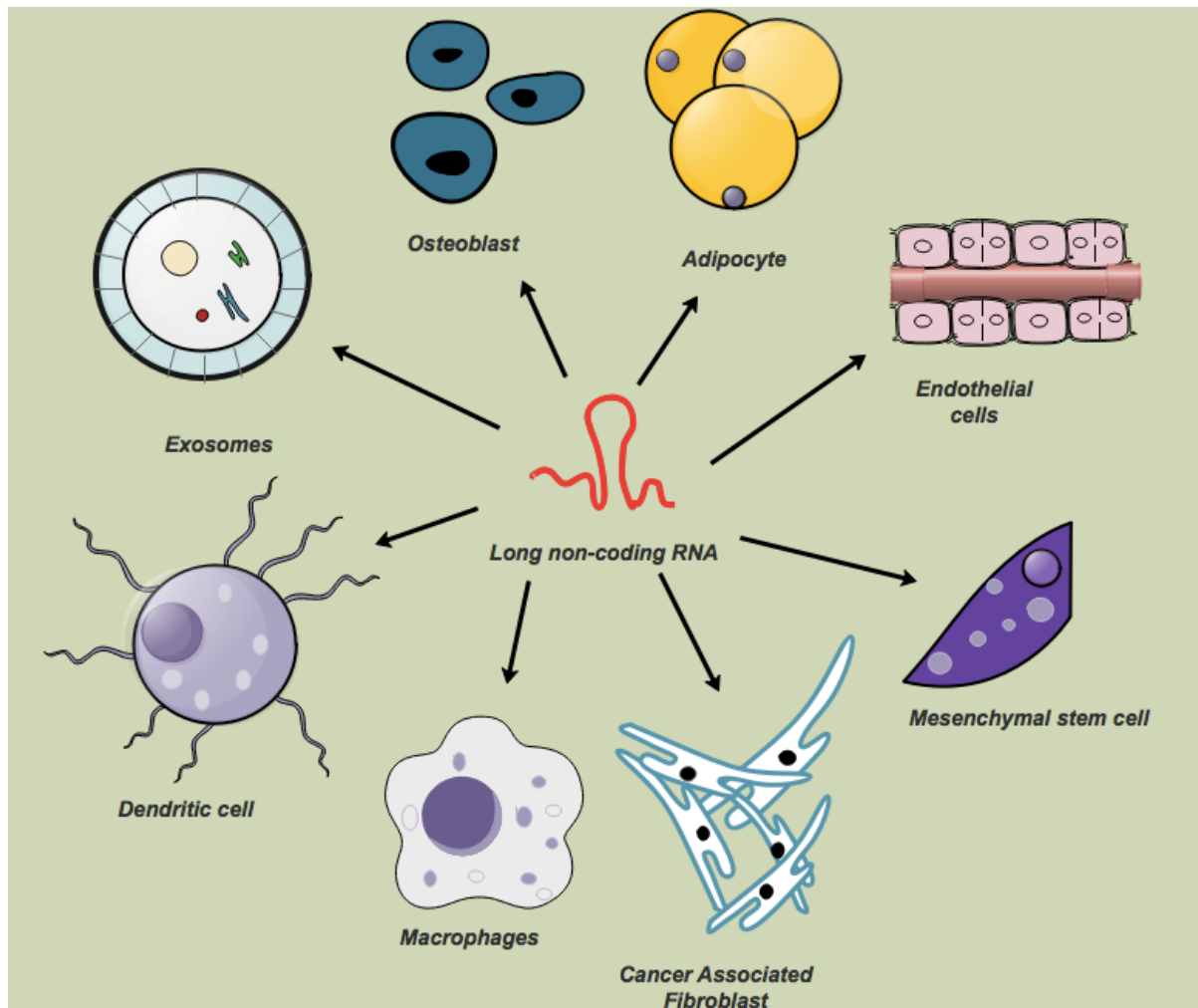


Figure 2. LncRNAs within the tumour microenvironment.

LncRNAs within the tumour microenvironment are functional and play important roles during tumour progression by impacting on tumour stroma signalling and the phenotype of cancer associated stromal cells.