LncRNAs in breast cancer: a link to future approaches

Nikolaos Sideris, Paola Dama, Salih Bayraktar, Thomas Stiff and Leandro Castellano

© Crown 2022

Breast cancer affects millions of women each year. Despite recent advances in targeted treatments breast cancer remains a significant threat to women’s health. In recent years the development of high-throughput sequencing technologies has advanced the field of transcriptomics shedding light on the role of non-coding RNAs (ncRNAs), including long ncRNAs (lncRNAs), in human cellular function and disease. LncRNAs are classified as transcripts longer than 200 nt with no coding potential. These transcripts constitute a diverse group of regulatory molecules essential to the modulation of crucial cellular processes, which dysregulation of leads to disease. LncRNAs exert their regulatory functions through their sequences and by forming complex secondary and tertiary structures that interact with other transcripts, chromatin and/or proteins. Numerous studies have provided evidence of the involvement of LncRNAs in tumor development and disease progression. They possess multiple characteristics that make them novel therapeutic and diagnostic targets. Indeed, the discovery of a novel mechanism by which lncRNAs associated with proteins can induce the formation of phase-separated droplets broadens our understanding of the spatiotemporal control of cellular processes and opens up developing a new treatment. Nevertheless, the role and the molecular mechanisms of many lncRNAs in the regulation of cellular processes and cancer still remain elusive. This is due to the absence of a thorough characterization of the regulatory role of their loci and the functional impact of their aberrations in cancer biology. Here, we present some of the latest advances concerning the role of LncRNAs in breast cancer.

INTRODUCTION

Data from the World Health Organization (WHO) indicates breast cancer as the most frequent malignancy affecting women worldwide [1]. It is a leading cause of mortality in developing countries and the second leading cause of cancer death in American women. In 2020 alone, the WHO recorded over 2.3 million breast cancer cases among women globally and 685,000 deaths, even if there is a significant variation in estimated incidence rates worldwide with a remarkable difference between Australia, North America, Europe and the rest of the world. The difference in mortality rates however are less pronounced [2]. Breast cancer is a very dynamic disease that occurs due to genetic and environmental cues.

Breast cancer exhibits great heterogeneity at both molecular and clinical levels. Each subtype presents varying biological traits with distinct pathological features and different clinical outcomes impacting the treatment planning [3]. Genetic/epigenetic data and protein markers are general criteria to classify breast tumors into subtypes [4].

Breast tumors are historically classified based on specific molecular signatures such as Progesterone Receptor (PR), Estrogen Receptor (ER) and Human Epidermal Growth Factor Receptor 2 (HER2) by classical immunohistochemical assay [5]. Further classification has been accomplished with the use of transcriptional profiling methods of a large set of tumors, revealing five major molecular subtypes i.e. luminal A, luminal B, HER2 overexpression, basal and normal-like tumors. Breast malignancies usually start out from luminal or basal cells of the duct lobular units of the breast. Tumor subtypes represent different biological entities consistent with the cell type of origin where the gene expression profile mirrors the molecular complexity of the tumors [6, 7]. Based on specific molecular subtypes the stratification of the patients results in distinct clinical outcomes and responses to the treatment [6, 7]. Of those, Luminal tumors (A and B) are primarily ER-positive with a slower rate of expansion but a larger incidence of relapse [8]. These are treated with a combination of chemotherapy and endocrine therapy to counteract the hormone receptor overexpression [9]. The HER2 positive subtype is marked by overexpression of the HER2 gene and poor prognosis. Treatment involves chemotherapeutic agents and targeted approaches aimed specifically against HER2 [10]. The basal-like subtype most commonly observed in triple-negative tumors, is the most aggressive subtype with the worst patient outcome [11]. When diagnosed at the earlier stages it can be cured in approximately 80% of patients [12–14].

Unfortunately, a significant percentage of women diagnosed with early-stage breast cancer experience the development of more aggressive phenotype months or even years after the initial treatment. One of the causes of refractory cancer is the cellular heterogeneity of the tumor. For example, cancer stem cells are subpopulation of cells within the tumor that do not respond to the conventional treatment, even the current approaches fail short when it comes to the more aggressive subtypes [15]. Furthermore, distant organ metastasis is the greatest challenge to modern
oncotherapy, with invasive breast cancer being characterized as incurable despite current treatments, making the development of new more efficient therapies essential [16–18].

The development of next generation sequencing technologies enabled the accurate characterization of the human genome. Despite the fact that the majority of nucleotides are transcribed under specific conditions, it has been proven that only a mere fraction of the transcribed genome encodes for proteins. Indeed, the associated transcriptome—which is found pervasively transcribed, led to the discovery of a new class of non-coding transcripts, named long non-coding RNAs (lncRNAs) [19], indicating that the majority of transcripts may function as non-coding molecules [20].

This new subset of RNA molecules has been found to act primarily as gene expression regulators [19]. There is mounting evidence that dysregulation of lncRNA loci can devastate the normal transcriptional landscape resulting in aberrant gene expression and ultimately malignant transformation. A thorough characterization of this new species of RNA could provide insights into new avenues of therapy and disease management. The fundamental characteristics of these molecules such as their highly specific expression patterns and functional tertiary structure make them ideal for use as diagnostic biomarkers, and promising targets for the development of pharmacological inhibitors. In this review we will survey the latest breakthroughs regarding the involvement of lncRNAs in breast cancer and explore their clinical potential.

NON-CODING RNAs (NCRNAS)

lncRNAs have been classified into two groups based on the size of the transcripts. lncRNAs are transcripts longer than 200 nucleotides [21]. Similar to mRNAs, lncRNAs are directly transcribed from genes and can undergo alternative splicing to produce numerous isoforms [22, 23]. Further similarities between lncRNAs and mRNAs on the transcript level include transcription by RNA-polII, a 5' end cap and a 3' poly-A tail, loci exhibiting all the aspects of a bona fide protein coding gene including promoter conservation, indicative chromatin structure and regulation via transcription factors and epigenetic remodelers [24, 25].

However, lncRNAs possess some fundamental differences which make them unique. When compared to protein-coding genes, apart from their lack of protein coding potential, lncRNAs exhibit relatively lower levels of expression and poor evolutionary conservation among species [19, 20, 25]. The most distinctive character they possess however, would be their ability to exert their regulatory functions through elaborate structures [26–28]. Interestingly, while conservation of their primary sequence varies and is generally poor, many lncRNAs present conservation at the tertiary structure level [29, 30]. It is also worth noting that lncRNAs are expressed in most tissues and cell types (including but not limited to stem cells, immune cells, brain cells, tumor cells). Generally, the overall tissue expression levels of lncRNAs are lower compared to those of mRNAs. Nevertheless, lncRNAs can be highly expressed, and are easy to detect in some cell types; demonstrating higher cell/tissue specific patterns of expression than those of protein coding genes, a finding consistent with their role in regulating the cell’s transcriptional landscape [31, 32]. These transcripts are involved in regulating post-transcriptional activity, chromatin remodeling, mRNA integrity and protein interactions amongst others. Therefore, ultimately being responsible for coordinating essential processes like: metabolism, development and differentiation [33–35].

LOCALIZATION OF LCNRNAS

lncRNAs have been shown to localize both in the nucleus and cytoplasm, acting through a wide range of mechanisms with distinct but equally important functions. However, the underlying mechanisms of most lncRNAs remains a mystery.

A significant number of lncRNAs function exclusively in the nucleus. Their expression dysregulation can wreak havoc on cellular homeostasis and lead to malignant transformation.

Notable mechanisms in the nucleus involve interactions with epigenetic remodelers, transcription factors and spliceosomes, where the lncRNAs act as guides, scaffolds or stabilizers to influence chromatin architecture alteration and gene expression [36, 37] (Fig. 1).

More specifically, lncRNAs in the nucleus can guide transcription factors and epigenetic remodelers to their target genes, or even sequester them to promote or inhibit target gene expression, while also controlling protein activities in the nucleus by regulating their ubiquitination and mRNA stability. LncRNAs are also capable of altering chromatin in 3D-space by initiating and maintaining chromosomal looping to bridge distant enhancer elements and gene promoters (Fig. 1).

Some of the best-documented examples include HOXAIR and MALAT1. HOXAIR regulates the expression of the HOX gene cluster by guiding PRC2 and GASS. These interact directly with activated glucocorticoid receptors, thus preventing the binding of the target genes to them [37], blocking the receptors from binding their target genes and MALAT1, which regulates alternative splicing via controlling serine/arginine splicing factor phosphorylation [38–40].

lncRNAs in the cytoplasm regulate mRNA stability by directly controlling de-adenylation, as well as protecting mRNA transcripts from miRNA mediated degradation by acting as molecular decoys, a process known as miRNA sponging [41, 42]. A novel example of this mechanism is lncRNA SNHG7 which promotes tumourigenesis by acting as a competing endogenous RNA, sequestering a number of tumor suppressor miRNAs in a variety of cancers [43, 44].

LNCRNA AND EPIGENETICS CROSSTALK

The earliest and perhaps best described example of nuclear lncRNA would be Xist, which facilitates X-chromosome inactivation by interacting with and guiding methyltransferases to the X chromosome in females [45]. Interestingly a new study has also shown that Xist can regulate cell proliferation and migration in breast and ovarian cancer by mediating macrophage polarization through competition with miR-101 for the regulation of C/EBPα and KLF6 [46]. HOTAIR, as mentioned earlier, is another well-characterized nuclear lncRNA which binds to the PRC2 complex of the epigenetic apparatus to modulate histone modifications of target genes in the HoxD cluster in trans, thus conflerring transcriptional silencing [47]. It has been shown that HOTAIR is capable of altering the state of chromatin in tumor metastasis, and has been found to be upregulated in metastatic breast carcinomas resulting in an altered pattern of PRC2 occupancy from breast epithelial cells to that of embryonic fibroblasts [48, 49].

Regulation of the transcriptional landscape facilitated by interactions between lncRNAs and chromatin remodelers such as members of the polycomb complex are a common function of nuclear lncRNAs in cancer [50]. A recent study has shown that overexpression of lncRNA PANDAR in breast cancer cells promotes cell proliferation by regulating G1 to S phase transition. Knockdown of the lncRNA induced cell cycle arrest at the G1 phase. Further chromatin and RNA immunoprecipitation (ChIP and RIP) experiments showed PANDAR to interact with the Bmi1 component of the PRC1 complex to downregulate the expression of p16KIA, a known cell cycle regulator, by facilitating Bmi1 binding to the p16 promoter [51, 52]. Similarly, researchers have discovered an overexpressed oncogenic lncRNA in ER-negative breast cancer: linc00511. linc00511 worsens patient prognosis by inhibiting apoptosis and accelerating the G1/S transition. This
effect is achieved by repressing CDKN1B expression; the gene which encodes for the p27 tumor suppressor protein [53]. Overexpression of linc00511 is shown to be triggered directly by the deficiency of ER and activated by the TFAP-2 transcription factor. EZH2, the catalytic subunit of PRC2, is recruited by linc00511 to the promoter of CDKN1B. Silencing of the lncRNA suppressed tumor growth in mice while in vitro ChIP assays confirmed that the knockdown inhibited EZH2 association with the CDKN1B promoter and limited the deposition of H3K27me3 without affecting EZH2 expression [54].

The flexibility of IncRNA mediated regulation and their functional variability in tumourigenesis has been observed through studying the upregulation of IncRNA TINCR by STAT3 [55]. Through bioinformatic analyses utilizing the GEPIA tool, researchers observed TINCR to be significantly upregulated in various cancer types. TINCR correlated to poor prognosis in breast cancer patients with EGFR involvement. A series of in vitro and in vivo knockdown experiments showed that TINCR promoted tumourigenesis via upregulation of EGFR. TINCR was found to be present and active in both the cytoplasm and the nucleus. In the cytoplasm TINCR acted as a ceRNA (competing endogenous RNA) to sponge miR-503-5p, which downregulates both EGFR mRNA and TINCR. In the nucleus however, bisulfite sequencing revealed that TINCR epigenetically silenced the miRNA by recruiting DNMT1 to its promoter and thus creating a positive feedback loop for the expression of EGFR and the lncRNA itself [55].

Thorough investigation into the impact of the IncRNA Stem Cell Inhibitory Transcript (SCIRT) on tumor-initiating cell (TIC) transcriptional programs in 3D breast cancer cultures yielded some rather intriguing data, demonstrating the complexity of IncRNA regulatory circuits [56–58]. TICs are slow proliferating and therefore widely resistant to chemotherapy. TICs are also highly metastatic cells with stemness properties capable of promoting tumor heterogeneity [59–61]. RNA-seq of cells cultured in adherent and sphere conditions at specific time-points showed a reduction of proliferative gene expression, accompanied by an increase in self renewal expression signatures during the transition to spheres. The NGS data suggested SCIRT as a possible regulator of this process. Knockdown and overexpression assays revealed SCIRT’s role as a tumor suppressor; it can restrain stemness in vitro, and tumor formation in mice. Combined with analysis of ChiP data, a series of Capture Hybridization Analysis of RNA Targets (CHART)-seq, RIP, epigenetic mark screening by ChiP-seq experiments, revealed that SCIRT binds globally to promoter/enhancer regions to increase cell-cycle gene expression and decrease self-renewal gene expression by interacting with EZH2 (catalytic component of PRC2), SOX2 (TF essential for self-renewal) and FOXM1 (TF critical for proliferation associated transcription). On the molecular level, SCIRT utilizes a G4-quadruplex in its 5’ region to bind EZH2 and colocalizes with EZH2 and SOX2 at Cpg islands of target gene promoters. Mechanistically SCIRT directly antagonizes EZH2 and SOX2 activity at the promoters of stemness related genes tipping the scale towards self-renewal suppression. On the other hand, SCIRT increases the affinity of EZH2 for FOXM1 at the promoters of cell-cycle promoting genes, thus recruiting FOXM1 in a protein-protein interaction dependent manner to overcome EZH2-mediated expression repression. SCIRT appears to be overexpressed in aggressive breast cancer samples according to TCGA and GTEx cohort datasets, conferring a more favorable outcome [56]. Taken together, these findings provide a novel regulatory network that could be further utilized with great implications in a clinical setting, and lead to new prognostic and therapeutic targets (Fig. 2).

**LncRNA Interactions with TF and Transcription Mediators**

Apart from their significant interaction with chromatin remodelers, lncRNAs are also capable of associating with transcription factors...
and mediators to exert their oncogenic functions with great implications to disease progression. A novel mechanism of breast cancer brain metastasis promotion was discovered, where Inc-BM seems to play a critical role in JAK/STAT signaling in a mutation independent manner. Jak2 is a non-receptor tyrosine kinase involved in cell growth and proliferation control, frequently mutated in cancers [62]. In many cases Jak2 hyperactivation leads to the promotion of oncogenic inflammation pathways through the phosphorylation of STAT3 [63]. Researchers have found that Inc-BM plays an important part in breast cancer brain metastasis, via coordinating cell to cell communication between breast cancer cells and the brain microenvironment. Inc-BM was observed to directly bind to JAK2, conferring a more active structural conformation to the kinase, this coincides with enhanced JAK2/STAT3 signaling in BCBM. This hyperactivated signaling leads to upregulation of ICAM1 promoting cancer cell adherence in the brain, and the secretion of CCL2 which attracts macrophages to the lesion, prompting them to secrete signal enhancing mediators [64]. Remarkably while Inc-BM could promote metastasis in murine models, nanoparticle encapsulated siRNAs were successful in treating the disease via lnc-BM downregulation, thus providing murine models, nanoparticle encapsulated siRNAs were successful in treating the disease via lnc-BM downregulation, thus providing a potential new therapeutic approach. Another notable example of IncRNA dependent phosphorylation was observed in in vitro and in vivo TNBC experiments where DANCR was shown to bind RXRA and increase its association with glycosyn synthase kinase-3b (GSK-3b), thus increasing serine phosphorylation of RXRA and promoting tumourigenesis via enhanced PI3K/AKT signaling [65].

One of the main characteristics of IncRNAs is a general lack of conservation at the primary sequence level among species, although there are some exceptions [30–66]. Researchers have identified the nuclear enriched Linc01271 as the human ortholog of murine MATAR25, and linked it to metastatic invasion in breast cancer with poor patient prognosis through regulation of TNS1 expression. TNS1 is strongly suppressed in cancer, especially cancer with poor patient prognosis through regulation of TNS1 expression [73]. Expression of TNS1 is regulated through poly-purine sequence-specific interactions, and positively modulates cell migration and invasion [67, 68]. MATAR25 knockouts in aggressive breast cancer cell lines generated via CRISPR-Cas9 lead to a reduction in cell proliferation, migration, and invasion capabilities. These findings were corroborated by decreased tumor progression and metastasis in tumor bearing KO-mice. Conversely, restoring the expression of MATAR25 restored the proliferative and invasive phenotype in mice. A combination of MATAR25-KO cell RNA-seq and CHIRP-seq data found TNS1 to be the most promising downstream target, further confirmed by its expression levels during these conditions [69]. On a molecular level, MATAR25 exerted its function by binding to PURB, a transcriptional co-activator, by acting as a scaffold for the IncRNA/PURB/TNS1 interaction, as confirmed by antisense oligonucleotide pulldown (RAP) and PURB-KO experiments. Genome synteny studies identified Linc01271 as a potential ortholog in humans, which was then confirmed when ectopic expression of Linc01271 in mouse MATAR25-KO cells rescued the proliferative and invasive phenotype. The high level of expression of Linc01271 in metastatic breast cancer according to TCGA, in combination with successful tumor size reduction in MATAR25-ASO mediated silencing in mice, highlights Linc01271 as a prominent therapeutic target requiring further investigation [69].

In another study nuclear IncRNA EGOT1 was found to be downregulated in breast cancer tissues and its involvement in microtubule-associated function hinted to a potential impact on paclitaxel treatment, a microtubule disruptor utilized as a chemotherapeutic agent [70]. Overexpression of EGOT1 in mouse xenografts was shown to sensitize the cells to paclitaxel, resulting in reduced tumor volume and weight, while its downregulation seemed to protect the cells. EGOT1 is transcribed from the intronic regions of the ITPR1 gene in an antisense direction, its expression was positively correlated with ITPR1 mRNA levels. Further investigation showed overexpression of EGOT1 induced autophagy through increased ITPR mediated autophagic signals and vesicles, while EGOT1 knockdown had the opposite effect. Ectopic expression of EGOT1 upregulated endogenous pre-ITPR1 mRNA, and increased stability of the pre-mRNA. RNA-FISH and treatment with RNase-H subsequently revealed that EGOT1 could directly hybridize with the ITPR1 pre-mRNA, forming a dsRNA to regulate ITPR1 expression. Interestingly, RIP results showed that the dsRNA could physically associate with the RNA binding protein hnRNPl through a specific region of EGOT1. Knockdown of hnRNPl reduced EGOT1 and ITPR1 transcript levels, potentially implicating it in regulation of alternative splicing. This data demonstrated the ability of EGOT1 to function both in cis and in trans for the regulation of ITPR1 expression thereby sensitizing breast cancer cells to paclitaxel [70] (Fig. 3).

**LncRNA AS CHROMATIN ARCHITECTS**

Many previous studies have focused on elucidating the functional impact of IncRNA-protein interactions both in the nucleus and the cytoplasm. Yet, their ability to associate with double stranded DNA has remained relatively unexplored [68]. IncRNAs have been observed to interact with and bind to specific double stranded DNA regions. Through poly-purine sequence-specific recognition and hydrogen bond interactions, they form RNA-DNA triplex structures thanks to specific triplex-forming oligonucleotide sequences (TFOs) [71–73]. These triplex formation interactions have been reported to play a role in the regulation of gene expression in cancer, with the potential of providing new targets for therapeutic approaches [73, 74].

In 2019 a comprehensive investigation into the ability of IncRNA MIR100HG to promote TNBC cell proliferation revealed its capability of forming an RNA-DNA triplex at the promoter of the p27 gene to regulate its expression [75]. Overexpression and knockdown of the IncRNA in vitro showed increased cell proliferation and cell cycle arrest at the G1 phase, with similar results occurring in mouse xenografts. RNA-seq revealed p27 to be among the genes affected by MIR100HG silencing, resulting in reduction at both the transcript and protein level. Predictive bioinformatic analysis provided three TFOs (namely TFO1-3) present in the IncRNA sequence, and a triplex targeting site in the 5’UTR of the p27 gene. On a molecular level it was revealed that p27 regulation by MIR100HG was TFO1 dependent. TFO1

---

**Fig. 2** Representative example of IncRNA and epigenetics crosstalk. PANDAR interacts with BMI1 to recruit PRC1 to the p16 promoter leading to downregulation of gene expression.
Fig. 3  Visual representation of IncRNA-protein interactions. Lnc-BM directly binds to JAK2 conferring a more active state and enhancing signaling activity.

binding and triplex forming ability was observed in vitro and in vivo via chromatin isolation by RNA purification (CHIRP) of biotin labeled TFO1 in TNBC cell lysates. This showed significant enrichment of p27 and TFO1, providing a novel mechanism by which MIR100HG could potentially recruit chromatin remodelers or TFs to the promoter of p27 [75]. An earlier example of this mechanism was observed during a thorough examination of the function of MEG3 in breast cancer. It was revealed that MEG3 regulated the TGF-β pathway, where it exerts its function through the formation of an RNA-DNA triplex at the TGFR1 promoter via a GA-rich motif, facilitating the formation of an R-loop between the promoter and distal regulatory elements [76].

Apart from the functional role of lncRNA transcripts in gene expression regulation, it appears that lncRNA regulatory elements have an independent role of their own. Investigation of the role of the lncRNA PVT1 gene has yielded some rather intriguing results. PVT1 has been implicated in the promotion of tumourigenesis and metastasis in various cancers. In gallbladder cancer PVT1, recruiting DNMT1 and EZH2, promoted the methylation of miR-18b-5p leading to epigenetic silencing [77]. In Clear Cell Renal Cell Carcinoma (ccRCC) a PVT1/HIF2a positive feedback loop has been demonstrated, whereby PVT1 stabilizes HIF2a bound to the enhancer to transactivate its expression [78].

In triple negative breast cancer, PVT1 interacts with KLF5 and enhances its binding to the BAP1 de-ubiquitinase, increasing its stability, and promoting TNBC cell growth through beta catenin signaling upregulation [79]. However, what is truly remarkable about this gene is the recent discovery of its promoter's capability to function as an autonomous regulator of c-MYC expression, independently from the lncRNA itself.

In sharp contrast to the oncogenic activity the PVT1 transcript demonstrates, a combination of 4C-seq, ATAC-seq and Hi-ChIP experiments has proven that promoter of PVT1 can act independently in breast cancer as a tumor suppressor by limiting c-MYC activity as it competes with the c-MYC promoter for a group of enhancers [80, 81]. Moreover, the extremely high frequency of PVT1 promoter mutations in numerous cancer types indicates its significance to the regulatory process [82]. In conclusion, this novel example shows that lncRNA mediated regulation is not limited to the transcript level. Regulatory elements of lncRNAs can act independently, have their own significant role to play in transcriptional regulation, and have provided a new insight into transcriptional regulatory processes (Fig. 4).

**LNCRNAS AS MEDIATORS OF PHASE SEPARATION**

Extensive studies have provided a new framework for how biological matter can be organized within the cell, through formation of biomolecular condensates enriched in RNA and proteins, which then form a number of membraneless organelles (cajal bodies, paraspeckles, RNP granules among others) to mediate a series of biological functions [83, 84]. Known as liquid-liquid phase separation (LLPS) or simply as phase separation, this phenomenon results from the interaction of proteins containing peptide segments with insufficient hydrophobic amino acids to mediate co-operative folding. Aforementioned proteins can react with each other or repetitive nucleic acid sequences, forming aggregates with defined boundaries [84, 85]. These droplet-like compartments allow for spatiotemporal control of various biochemical reactions and cellular functions, including signal transduction, RNA splicing and chromatin organization [86-88]. For example, it has been observed that the TAZ transcription factor and other TFs can facilitate gene expression by compartmentalizing the transcription machinery through LLPS activity of specific domains [89, 90]. Despite the early stages of LLPS research, accumulating evidence suggests its importance in cellular homeostasis and the impact of aberrant condensates in human pathologies. These include: neurodegeneration, cancer, and infectious diseases, where IncRNAs are emerging as potent modulators [91-93].

A well-established example of lncRNA mediated phase separation occurs via NEAT1, a transcript pivotal for the assembly and maintenance of paraspeckles in the nucleus, whose aberrations have been described in various cancers including breast [94-96]. A recent study revealed that NEAT1 possesses redundant sequences in its middle domain, these are used to recruit NONO protein dimers to initiate the oligomerization of DBHS proteins for the...
formation of an RNP complex, which during phase separation assembles the paraspeckle. Functional analysis of these subdomains showed they were essential to the assembly process, as deletion mutants were incapable of recruiting NONO, while assessment of aggregate formation exhibited their ability to induce a higher order assembly of paraspeckle proteins [97]. IncRNA SNHG9, a transcript which can interact and bind to phosphatidic acid (PA), has been clinically correlated with disease progression and poor prognosis in breast cancer with enhanced YAP activity, by decreasing phosphorylation through LATS1, a member of the Hippo pathway. SNHG9 was found to control the kinase activity by interacting with PA and binding to LATS1 through the C-terminal to facilitate protein aggregation, thus promoting phase separation via the formation of liquid droplets in a dose dependent manner [98].

Interrogation of the role of the interaction between IncRNA DIGIT and BRD3 has yielded some interesting findings with regards to transcriptional regulation in endoderm differentiation. ChIP-seq screening showed BRD3 recognizes and binds to H3K18ac throughout the genome and facilitates gene expression by occupying enhancer elements, while its capacity to form phase-separated droplets was demonstrated with titration in vitro. Researchers discovered that DIGIT interacts with BRD3 through its bromodomains, guiding it to specific genes involved in driving endoderm differentiation, where it promotes the formation of BRD3 aggregates to modulate transcriptional activity. Loss of BRD3 blocked differentiation, and similarly depletion of DIGIT blocked differentiation by impairing BRD3 to key target genes [99]. In a different study damage-induced IncRNAs (dlincRNAs) transcribed at sites of double stranded breaks, were found to recruit DNA-damage response (DDR) proteins like 53BP1 and assemble LLPS condensates in the form of DDR foci for the regulation of DSB signaling. ASO-mediated knockdown of the dlincRNAs attenuated the formation of the DDR foci and blocked DSB repair, highlighting their crucial role in modulating this process (Table 1) [100].

**CLINICAL RELEVANCE IN BREAST CANCER**

**IncRNAs as a driving force of chemoresistance and metastasis**

While breast cancer is treatable if detected early, the occurrence of metastatic and chemoresistant phenotypes as a result of tumor heterogeneity are a major hindrance to therapeutic intervention [101–103]. The clinical importance of IncRNAs has been demonstrated on multiple occasions through their capacity to act as promoters of tumourigenesis as well as tumor suppressors with a marked impact on disease progression and outcome through a plethora of mechanisms [104, 105]. For instance, the oncogenic properties of HOTAIR and its correlation to poor prognosis in various cancers has been well documented [49, 106, 107]. Recently, researchers demonstrated that activation of HOTAIR, triggered by the uptake of signaling mediators, could promote breast cancer EMT and lung metastasis in mice via activating CDK5 signaling therefore highlighting the importance of paracrine signaling in disease progression and providing new potential therapeutic targets. Specifically, it was discovered that secretion of TGF-β1 by cancer associated fibroblasts (CAFs) led to direct binding of SMAD2-4 to the promoter of HOTAIR in breast cancer cells thus causing its expression. HOTAIR subsequently activated CDK5 signaling by recruiting the components of PRC2 complex to the promoter of CDK5RAP1 and facilitating methylation of the promoter region. HOTAIR activation was attenuated after treatment with TGF-β1 inhibitors while RNAi mediated knockdown of HOTAIR in mice abrogated the metastatic phenomenon [108].

Involved in EMT transition and metastasis, Linc-ROR has also been shown to promote estrogen-independent growth of breast cancer cells by regulating the ERK-specific DUSP7 phosphatase, thus enhancing MAPK/ERK signaling with potential implications for tamoxifen resistance [109, 110]. IncRNA TROJAN, associated with poor survival, confers CDK4/6 inhibitor resistance, and promotes proliferation in ER+ breast cancer via upregulating CDK2 expression [111]. Other examples include: LINK-A which enhances AKT/HIF1α signaling and downregulates antigen presentation gene expression, facilitating immune escape and drug resistance; AGAP2-ASI which regulates fatty acid oxidation through the formation of IncRNA/HuR/CPT1 complex to promote stemness and trastuzumab tolerance [112, 113]. Conversely, SNORD3A functions as a ceRNA to protect UMPS expression via sponging miR-185-5p, thereby sensitizing breast cancer cells to 5-FU [114]. Expression of IncRNA ANCR has been shown to reduce breast cancer cell invasion and migration capabilities by directly regulating EZH2 stability, binding to and marking it for proteasome degradation [115]. NORAD, which is under the transcriptional control of the YAP protein, is capable of suppressing metastasis by binding to S100P and sequestering it in the cytoplasm [116].

**IncRNA implementation in precision diagnostics and therapeutics**

Functional characterization and understanding of the underlying mechanisms governing IncRNA mediated regulation in human diseases could provide us with novel opportunities to revolutionize our existing arsenal of diagnostic and therapeutic tools. These transcripts possess several qualities which make them ideal for combatting cancer.

Their tissue and cell type specific expression patterns highlight their potential for use as highly accurate biomarkers [117]. IncRNAs can be utilized on their own or in complement with
| lncRNA   | Mechanism                  | Localization | Expression Rate | Effectors                                      | Function/Pathways                                      | Biological Processes                                | Reference |
|---------|----------------------------|--------------|-----------------|------------------------------------------------|------------------------------------------------------|-------------------------------------------------------|-----------|
| HOTAIR  | Epigenetic Crosstalk       | Nucleus      | Increased       | HOX Gene cluster: PRC2 and GASS             | Chromatin remodeling                                   | EMT transition and Metastasis                        | [38, 40] |
| MALAT1  |                            | Nucleus      | Increased       | SRSF1, TDP43, and PRC2 components, including EZH2 | Alternative splicing; serine/arginine phosphorylation | Metastasis and tumor progression                     | [39, 132]|
| Xist    |                            | Nucleus      | Decreased       | C/EBPα and KLF6                               | Macrophage polarization                               | Cell proliferation and migration                     | [45, 46] |
| PANDAR  |                            | Nucleus      | Increased       | BMI1/P16Nk4/C/EBPα/KLF6                       | Regulation of G1 to S phase transition                | Cell proliferation and Cell cyle                     | [51, 52] |
| linc00511 |                       | Nucleus      | Increased       | CDKN1B                                        | G1/S transition                                       | Apoptosis inhibition                                 | [53]      |
| TINCR   |                            | Cytoplasm/Nucleus | Increased     | EGFR/miR-503-5p                               | ceRNA/DNMT1 recruitment                              | Tumorigenesis                                        | [55]      |
| SCRT    |                            | Nucleus      | Increased       | EZH2; SOX2; FOXM1                              | Self renewal expression signatures                    | Cell Cycle                                           | [56–58]  |
| Inc-BM  | Transcription Factors interaction | Cytoplasm | Increased       | JAK2/ICAM1/CCL2                              | JAK/STAT signaling                                   | Brain Metastasis                                     | [62–64]  |
| DANCR   |                            | Cytoplasm/Nucleus | Increased     | RXRA                                          | PI3K/AKT signaling                                   | Proliferation; Suppressor of cell differentiation    | [65]      |
| linc01271 |                        | Nucleus      | Increased       | TNS1                                          | Fibrillar adhesion; cell migration and evasion       | Metastasis                                           | [67, 68] |
| EGOT1   |                            | Cytoplasm    | Decreased       | IPTR1                                         | pre-mRNA stability                                   | Autophagy                                            | [70]      |
| MEG3    | Chromatin Architect       | Nucleus      | Decreased       | PRC2/TFGR1                                    | TGF-β signaling pathway                              | Cell proliferation and progression; Autophagy        |          |
| PVT1    |                            | Cytoplasm/Nucleus | Decreased     | miR-128-5p; KLFS; c-Myc                       | Epigenetic Silencing; Beta Catenin signaling         | Cell proliferation and progression                   | [77, 78] |
| NEAT1   | Phase Separation          | Nucleus      | Increased       | pS4(NONO)                                     | G1/S transition; Paraspeckles formation              | Apoptosis and Cell cycle; Cancer stenness            | [95–97]  |
| SNHG9   |                            | Nucleus      |                | PA/LATS1                                      | Kinase activity                                       | Protein aggregation                                  | [98]      |
| DIGIT   |                            | Nucleus      |                | BRD3                                          | Endoderm differentiation                              | Trascriptional activity                              | [99]      |
Other biomarkers to assess a patient’s status or possible response to specific therapies and are detectable in tissue samples, such as formalin embedded samples (FFPE), as well as bodily fluids [118, 119]. Isolation and studies can be achieved through classical RNA extraction protocols involving sample preparation and TRI-agents paired with RNA sequencing and qPCR [120]. While FFPE samples can be routinely obtained during biopsies, the procedure is invasive and generates heterogeneous samples prone to nucleic acid degradation which can jeopardize the reliability of results [121]. A number of approaches are being developed to better detect and analyze IncRNAs in these samples including the use of target enrichment methods as well as laser micro dissection to limit the sample heterogeneity in breast cancer [121, 122]. The majority of researchers however are focusing on detecting IncRNAs or transcript fragments in bodily fluids such as serum or urine in order to discover and develop less invasive approaches, however the mechanisms which control IncRNA secretion are still poorly understood and their biological functions in cancer still under investigation [118, 120].

As mentioned before, clinical studies have revealed a link between HOTAIR and metastasis. Overexpression of this IncRNA in breast cancer samples especially of ER-positive patients has been associated with poor prognosis, indicating its potential use as a novel biomarker to predict metastasis. Additionally, serum levels of circulating HOTAIR were capable of differentiating between breast cancer patients and healthy individuals [123, 124]. Furthermore, through encapsulation and exosomal dissemination, in breast cancer IncRNAs such as ACO73352.1, HISLA, SNHG14, and SNHG16 are involved in promoting tumorigenesis, chemoresistance, as well as modulation of tumor microenvironment, piquing the interest of researchers worldwide [125–128]. Identification and detection of tumor-derived circulating exosomal IncRNAs could significantly expand our diagnostic toolkit, and provide new avenues for precision medicine. Aside from their expression patterns their sequence polymorphisms can also provide valuable insights such as those of MEG3 in breast cancer which have been investigated both as a means by which to silence IncRNAs driving malignant transformation and disease progression in breast cancer have made them extremely valuable targets in combatting this complex disease. Their overall lower levels of expression compared to protein coding genes combined with their distinguished expression patterns, make them ideal for nucleic acid-based strategies [130]. Such strategies include the use of siRNAs for targeted knockdown of transcripts via RISC as well as antisense oligonucleotides (ASOs), which hybridize with the target RNA blocking secondary structure formation and mediate degradation via RNase-H [131]. For instance, classic RNAi has been successfully implemented for the in vivo targeting of Malat1 and HOTAIR [106]. In a similar fashion the use of MALAT1 targeting ASOs was capable of blocking breast cancer progression via MALAT1 knockdown [132–135]. Despite the efficiency of siRNAs in targeting cytoplasmic transcripts they are a bit unpredictable when it comes to nuclear IncRNAs due to problems with nuclear localization. On the other hand well designed ASOs can efficiently target IncRNAs regardless of their localization and may be better suited for dealing with aberrant nuclear IncRNAs [136]. While nucleic acid-based approaches have great potential there are still significant limitations to overcome before clinical application such as the inert instability of nucleic acids which necessitates extra molecular modifications for stability and efficiency, off target effects due to sequence pairing, immunogenicity due to immune recognition by Toll-like receptors (TLRs) [137, 138]. The main challenge however is the engineering of efficient delivery systems such as advanced nanoparticles or exosomes to ensure correct tissue and intercellular localization and avoid uptake in the wrong organs or endosome retention [138–140].

Gene editing tools such as the CRISPR-CAS9 system present many opportunities in IncRNA based therapeutics. CRISPR is being investigated both as a means by which to silence IncRNAs driving malignant transformation through CRISPRi, as well as to restore expression of transcriptionally dormant IncRNAs with tumor suppressor properties like ANCR [141, 142]. Other applications of this technique could include the fusion of CAS-9 to transcriptional repressors to target IncRNA promoters via guide RNAs for target specific transcription repression. It would be interesting to see the

| IncRNA      | Mechanism/Pathway signaling                                      | Clinical relevance                          | Reference |
|-------------|------------------------------------------------------------------|--------------------------------------------|-----------|
| HOTAIR      | EMT Transistion                                                  | Poor prognosis and metastasis              | [49, 106, 107, 123, 124] |
| EGOT1       | Induced autophagy through increased IPTR mediated autophagic signals and vesicles | Paclitaxel treatment                       | [70]      |
| linc-ROR    | Promote estrogen-independent growth MAPK/ERK signalin             | Tamoxifen resistance                       | [109, 110]|
| Linc-TROJAN | Proliferation in in ER+ breast cancer CDK2 upregulation          | CDK4/6 inhibitor resistance                | [111]     |
| LINK-A      | Enhances AKT/HIF1-a signaling and downregulates antigen presentation gene expression | Immune escape and drug resistance          | [112]     |
| AGAP2-ASI   | Regulates fatty acid oxidation IncRNA/HuR/ CPT1 complex          | Promote stenness Trastuzumab tolerance     | [113]     |
| SNORD3A     | ceRNA to protect UMP S expression via sponging miR-185-5p         | Sensitizing breast cancer cells to 5-FU     | [114]     |
| ANCR        | Regulating EZH2 stability, binding to and marking it for proteasome degradation | Reduce breast cancer cell invasion and migration capabilities | [115]     |
| NORAD       | YAP pathway                                                     | Metastasis suppressor by binding to S100P and sequestering it in the cytoplasm | [116]     |
| ACO73352.1, HISLA, SNHG14, and SNHG16 | Modulation of tumor microenvironment | Promoting tumorigenesis, chemoresistance | [125–128] |

Table 2. List of clinical relevant IncRNAs in breast cancer.
effects of such a system in regulating independently functional IncRNA regulatory elements like the PVT1 promoter.

However, the most promising platform for modulating oncogenic IncRNA mediated tumourigenesis would be to target their tertiary structure through which they associate with proteins, RNA, DNA and exert their regulatory effects [143]. In that context small molecular inhibitors or aptamer-based approaches could be utilized to antagonize the direct interactions between IncRNAs and their interactors [144, 145]. Indeed, a small molecular inhibitor dubbed AC1Q3QWB has been developed and tested in breast cancer derived xenografts, resulting in efficient disruption of PRC2 recruitment by HOTAIR without off target effects [146]. Another example of IncRNA structure targeting would be the TMPyP4 small molecule which disrupts the association of the NEAT1 transcript with the NONO protein through targeting of the secondary G4 structures NEAT1 uses for its interaction with NONO [28, 147]. Similar approaches are being tested for the disruption of G4 structure mediated MALAT1 interactions in associated cancers with the small molecule pyridostatin as well as peptides and aptamers [148]. The development of the RNA targeting CRISPR-CAS13 system could also be used in a similar fashion to attenuate IncRNA oncogenic functions (Table 2) [149].

CONCLUDING REMARKS
The advancements in the fields of transcriptomics and genomics in the last decade have elevated IncRNAs from "transcriptional noise" to functional multidimensional entities responsible for the regulation of cell fate and homeostasis. Their discovery has revealed a new complex framework of regulatory processes governing the initiation and progression of human diseases including breast cancer, with the potential to revolutionize the way we diagnose and treat it. Discovery of deregulated IncRNAs is currently ongoing, but despite the availability of cancer related genomic/transcriptomic data, very few IncRNAs have been functionally characterized due to a lack of throughput analyses of their loci and their aberrations. These transcripts have been found to function through interactions with other transcripts, DNA, and proteins facilitated by their complex tertiary structure, often assembling RNP complexes capable of forming condensates promoting LLPS [27, 150]. Mounting evidence demonstrates the importance of nuclear enriched IncRNAs in regulating chromatin organization, transcription, and DNA damage repair, with major implications for malignant transformation and metastasis in breast cancer [151].

Limitations in our understanding of IncRNA biology stem in part from the difficulty of studying their interactions in cancer. Their lack of conservation makes it difficult to establish representative mouse models, requiring the use of xenografts to bridge the gaps in our knowledge [152]. This is further complicated by the ability of some IncRNAs to perform multiple different functions within the cell, as well as having opposing roles in different cell types or disease stages [153]. IncRNA databases are being compiled to realize their true potential.

REFERENCES
1. Rebecca LS, Kimberly DM, Hannah EF, Ahmedin J. Cancer statistics, 2022. Cancer J Clin. 2022;72:7–33. https://doi.org/10.3332/caac.21708.
2. American Cancer Society. The Cancer Atlas, 2nd Ed. Atlanta, GA: American Cancer Society; 2019.
3. Polyak K. Breast cancer: origins and evolution. J Clin Invest. 2007;117:3155–63. https://doi.org/10.1172/JCI33295.
4. Skibinski A, Kuperwasser C. The origin of breast tumour heterogeneity. Onco- gene. 2015. https://doi.org/10.1038/onc.2014.475.
5. Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, et al. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res. 2015;5:2929–43.
6. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumour subtypes in independent gene expression data sets. Proc Natl Acad Sci USA. 2003;100:8418–23.
7. Perou CM, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, et al. Molecular portraits of human breast tumours. Nature. 2000. https://doi.org/10.1038/35021093.
8. Russnes HG, Lingeaarde OC, Borresen-DAL, Caldas C. Breast cancer molecular stratification: from intrinsic subtypes to integrative clusters. Am J Pathol. 2017. https://doi.org/10.1016/j.ajpath.2017.04.022.
9. Ignatiadis M, Sotiriou C. Luminal breast cancer: from biology to treatment. Nat Rev Clin Oncol. 2013. https://doi.org/10.1038/nrclinonc.2013.124.
10. Goutsoulakis K, Veeraraghavan J, Sethunath V, De Angelis C, Osborne CK, Rimawi MF, et al. Towards personalized treatment for advanced breast cancer: a stage HER2-positive breast cancer. Nat Rev Clin Oncol. 2019. https://doi.org/10.1038/s41571-019-0199-9.
11. Badve S, Dabbs DJ, Schnitt SJ, Baehner FL, Decker T, Eusebi V, et al. Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. Mod Pathol. 2011;24:157–67.
12. Chaffer CL, Weinberg RA. Cancer cell of origin: spotlight on luminal progenitors. Cell Stem Cell. 2010. https://doi.org/10.1016/j.stem.2010.08.008.
13. Gusterson B, Eaves CJ. Basal-like breast cancers: from pathology to biology and back again. Stem Cell Rep. 2018. https://doi.org/10.1016/j.stemcr.2018.04.023.
14. Harbeck N, Penault-Llorca F, Cortes J, GNant M, Houssami N, Poortmans P, et al. Breast cancer. Nat Rev Dis Primers. 2019. https://doi.org/10.1038/s41572-019-0111-2.
15. Marusyk A, Janiszewska M, Polakow K. Intratumour heterogeneity: the Rosetta stone of therapy resistance. Cancer Cell. 2020. https://doi.org/10.1016/j.ccell.2020.03.007.
16. Jin L, Han B, Siegel E, Cui Y, Giuliano A, Cui X. Breast cancer lung metastasis: molecular biology and therapeutic implications. Cancer Biol Ther. 2018. https://doi.org/10.1080/15384047.2018.1456599.
17. Abolghasemi M, Tehrani SS, Yousefzadeh-Darani MA, Fox AH, Fortini E, Moscato P, et al. Critical roles of long noncoding RNAs in breast cancer. J Cell Physiol. 2020. https://doi.org/10.1002/jcp.29442.
18. Scimena M, Trivigno D, Bonfiglio R, Ciuffa S, Urbano N, Schilacci O, et al. Breast cancer metastasis to bone: from epithelial to mesenchymal transition to breast osteoblast-like cells. Semin Cancer Biol. 2020. https://doi.org/10.1016/j.semcancer.2020.01.004.
19. Morris KV, Mattick JS. The rise of regulatory RNA. Nat Rev Genet. 2014;15:423–37.
20. Djabali S, Davis CA, Merkel A, Dobin A, Lasmann T, Mortazavi A, et al. Landscape of transcription in human cells. Nature. 2012. https://doi.org/10.1038/ nature11233.
21. Mongelli A, Martelli F, Farinetti A, Gaetano C. The dark that matters: Long non- coding RNAs as master regulators of cellular metabolism in noncommunicable diseases. Front Physiol. 2019. https://doi.org/10.3389/fphys.2019.00369.
22. Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet. 2016. https://doi.org/10.1038/nrg.2015.10.
23. Chen J, Liu Y, Min J, Wang H, Li F, Xu C, et al. Alternative splicing of IncRNAs in human diseases. Am J Cancer Res. 2021;11:624–39.
24. Clark MB, Johnston RL, Inostroza-Ponta N, Fox AH, Fortini E, Moscato P, et al. Genome-wide analysis of long noncoding RNA stability. Genome Res. 2012. https://doi.org/10.1101/gr.131037.111.
25. Uchida S, Dimmeler S. Long noncoding RNAs in cardiovascular diseases. Circ Res. 2022. https://doi.org/10.1161/CIRCRESAHA.116.302521.
26. van Bakel H, Nislov C, Blencowe BJ, Hughes TR. Most “dark matter” transcripts are associated with known genes. PLoS Biol. 2010. https://doi.org/10.1371/journal.pbio.1000371.
27. van Bakel H, Nislov C, Blencowe BJ, Hughes TR. Response to “The reality of pervasive transcription”. PLoS Biol. 2011. https://doi.org/10.1371/journal.pbio.1001102.
46. Zhao Y, Yu Z, Ma R, Zhang Y, Zhao L, Yan Y, et al. lncRNA-Xist/miR-101-3p/KLF6/
45. Wutz A, Rasmussen TP and Jaenisch R. Chromosomal silencing and localization
43. Li Y, Zeng C, Hu J, Pan Y, Shan Y, Liu B, et al. Long non-coding RNA-SNHG7 acts
42. Carlevaro-Fita J, Johnson R. Global positioning system: understanding long
40. Lucafo M, De Iudicibus S, Di Silvestre A, Pelin M, Candussio L, Martelossi S, et al.
39. Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, et al. The nuclear-retained
33. Moran VA, Perera RJ and Khalil AM. (2012)
36. Sun Q, Hao Q, Prasanth KV. Nuclear long noncoding RNAs: key regulators of
35. Sun W, Yang Y, Xu C, Guo J. Regulatory mechanisms of long noncoding RNAs on
32. Mattick JS, Taft RJ, Faulkner GJ. A global view of genomic information - moving
31. Sun W, Hao Q, Prasanth KV. Nuclear long noncoding RNAs: key regulators of
gen expression in cancers. Cancer Genet. 2017;216:7-105–10.
30. Sun Q, Hao Q, Prasanth KV. Nuclear long noncoding RNAs: key regulators of
gen expression. Trends Genet. 2018. https://doi.org/10.1016/j.tig.2017.11.005.
29. Statolet L, Guo C, Chen L and Huarte M. Gene regulation by long non-coding
RNAs and its biological functions. Nat Rev Mol Cell Biol. 2021. https://doi.org/
10.1038/s41580-020-00315-9.
28. Tassinari M, Richter SN, Gandellini P. Biological relevance and therapeutic
27. Besch R, Giovannangeli C, Degitz K. Triplex-forming oligonucleotides - sequence-
specific DNA ligands as tools for gene inhibition and for modulation of DNA-associated
functions. Curr Drug Targets. 2005.https://doi.org/10.2174/13894501077345100.
26. Zhao Y, Yu Z, Ma R, Zhang Y, Zhao L, Yan Y, et al. lncRNA-Xist/mir-101-3p/KLF6/
C/EBPa axis promotes TAM polarization to regulate cancer cell proliferation and
migration. Mol Ther. 2021. https://doi.org/10.1016/j.therap.2020.12.005.
25. Benencina D, Stampone E, Autilio A, Tramontano A, Barone C, Negri A, et al. A
cancer-associated CDRKN18 mutation induces p27 phosphorylation on a novel
residue: a new mechanism for tumour suppressor loss-of-function. Mol Oncol.
2021. https://doi.org/10.1016/j.molonc.2021.08.0261.12881.
24. Zhang J, Sui S, Wu H, Zhang J, Zhang X, Xu S, et al. The transcriptional landscape of
lncRNAs reveals the oncogenic function of LINCO0511 in ER-negative breast
Cancer Cell Death Dis. 2019;10. https://doi.org/10.1038/s41419-019-1835-z.
23. Wang Q, Liu J, You Z, Yin Y, Liu L, Kang Y, et al. LncRNA TINCR favors tumourigenesis via STAT3–TINCR–EGFR-feedback loop by recruiting DNMT1 and
acting as a competing endogenous RNA in human breast cancer. Cell Death Dis.
2021;12. https://doi.org/10.1038/s41419-020-03188-0.
22. ZagoraC, di GiorGio A, Dabrovskas A, Kalisz M, Casas-Vila N, Cathcart P, et al.
SCITRlncRNA restrains tumourigenesis by opposing transcriptional programs of
tumour-initiating cells. Cancer Res. 2021;81:580–93.
21. Pardini B, Dragomi P. SClTRlncRNA blocks the shot of breast cancer cells self-
renewal mechanism. Cancer Res. 2021:81:535–6.
20. di GiorGio A, Castellano AL. SCITRlncRNA slows the formation of tumour
initiating cells in breast cancer. Oncoscience. 2021;8:74–5.
19. Zhou BBS, Zhang H, Damelin M, Geles KG, Grindley JC, Dirks PB. Tumour
initiating cells: challenges and opportunities for anticancer drug discovery. Nat
Rev Drug Discov. 2009. https://doi.org/10.1038/nrd2137.
18. Meacham CE, Morrison SJ. Tumour heterogeneity and cancer cell plasticity.
Nature. 2013. https://doi.org/10.1038/nature12624.
17. Qureshi-Baig K, Ullmann P, Haan S, Letellier E. Tumour-initiating cells: a critical
review of isolation approaches and new challenges in targeting strategies. Mol
Cancer Res. 2016;14:12943–12962.
16. Gnanasambandan K, Sayeski PP. A structure-function perspective of Jak2
mutations and implications for alternate drug design strategies: the road not
taken. Curr Med Chem. 2012. https://doi.org/10.2174/092986711797379267.
15. Yu H, Parodi D, Jove R. STAT3 in cancer inflammation and immunity: a leading
role for STAT3. Nat Rev Cancer. 2009. https://doi.org/10.1038/nrc2734.
14. Wang S, Liang K, Hu Q, Li P, Song J, Yang Y, et al. JAK2-binding long noncoding
RNA promotes breast cancer cell metastasis. J Clin Investig. 2017;127:4498–
515. https://doi.org/10.1172/JCI91553.
13. Tang J, Zhong G, Zhang H, Yu B, Wei F, Luo L, et al. LncRNA DANC2 upregulates
PI3K/AKT signaling through activating serine phosphorylation of IRX3A. Cell
Death Dis. 2016. https://doi.org/10.1038/cddis.2016.1220-7.
12. Li D, Yang MQ. Identification and characterization of conserved lncRNAs in
human and rat brain. BMC Bioinformatics. 2017. https://doi.org/10.1186/s12859-
017-1890-7.
11. Hall EH, Daugherty AE, Choi CK, Horwitz AF, Brautigan DL. Tensin1 requires
DNA triplex structures. Nat Commun. 2015;6. https://doi.org/10.1038/ncomms10692.
10. Ke H, Wang S, Xiao L, Zou L, Zou L, Zou L, et al. MEG3 long noncoding RNA
blocks the shot of breast cancer cells self-renewal and restrains tumour-initiating
mechanism. Cancer Res. 2021;81:580–93. https://doi.org/10.1158/0008-5472.
9. Hall EM, Mieczkowska K, Vaid R, Enroth S, Uday S, Reinius B, et al. MEG3 long
noncoding RNA regulates breast cancer cell invasion by recruiting the DNA-binding
protein NAB2/PRCC. J Mol Biol. 2021. https://doi.org/10.1016/j.jmb.2021.
8. Hall EM, Mieczkowska K, Vaid R, Enroth S, Uday S, Reinius B, et al. MEG3 long
noncoding RNA regulates breast cancer cell invasion by recruiting the DNA-binding
protein NAB2/PRCC. J Mol Biol. 2021. https://doi.org/10.1016/j.jmb.2021.
7. Hall EM, Mieczkowska K, Vaid R, Enroth S, Uday S, Reinius B, et al. MEG3 long
noncoding RNA regulates breast cancer cell invasion by recruiting the DNA-binding
protein NAB2/PRCC. J Mol Biol. 2021. https://doi.org/10.1016/j.jmb.2021.
6. Hall EM, Mieczkowska K, Vaid R, Enroth S, Uday S, Reinius B, et al. MEG3 long
noncoding RNA regulates breast cancer cell invasion by recruiting the DNA-binding
protein NAB2/PRCC. J Mol Biol. 2021. https://doi.org/10.1016/j.jmb.2021.
5. Hall EM, Mieczkowska K, Vaid R, Enroth S, Uday S, Reinius B, et al. MEG3 long
noncoding RNA regulates breast cancer cell invasion by recruiting the DNA-binding
protein NAB2/PRCC. J Mol Biol. 2021. https://doi.org/10.1016/j.jmb.2021.
1876

100. Pessina F, Giavazzi F, Yin Y, Gioia U, Vitelli V, Galbiati A. et al. Functional tran-
94. Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, et al. An
93. Wang B, Zhang L, Dai T, Qin Z, Lu H, Zhang L, et al. Liquid
92. Guh CY, Hsieh YH, Chu HP. Functions and properties of nuclear lncRNAs—from
89. Boija A, Klein IA, Sabari BR, Dall
84. Peng L, Li EM, Xu LY. From start to end: phase separation and transcriptional
83. Li P, Banjade S, Cheng HC, Kim S, Chen B, Guo L, et al. Phase transitions in the
82. Tseng YY, Moriarty BS, Gong W, Akiyama R, Tiwari A, Kawakami H. et al.
79. Tang J, Li Y, Sang Y, Yu B, Lv D, Zhang W. et al.
78. Zhang MX, Zhang LZ, Fu LM, Yao HH, Tan L, Feng ZH, et al. Positive feedback
76. Shin VY, Chen J, Cheuk IWY, Siu MT, Ho CW, Wang X, et al. Long non-coding RNA
75. Daneshvar K, Ardehali MB, Klein IA, Hsieh FK, Siu MT, Ho CW, Wang X, et al.
74. Liao SJ, Horembis P, Malaveida M, He D, et al. CRISPR-based
genome-scale identification of functional long noncoding RNA loci in human cells.
73. Science. 2017. https://doi.org/10.1126/science.aah7111.
72. Jiang M-C, Ni J-J, Cui W-Y, Wang B-Y, Zhou W. Emerging roles of IncRNA in cancer
71. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, et al. Long non-
coding RNA HOTAIR: a chromatin state to promote cancer metastasis. Nature.
70. Yao Y, J I, Wang L. Large intervening non-coding RNA HOTAIR is an indicator of
69. Ren Y, Jia H-H, Xu Y-Q, Zhou X, Zhao X-H, Wang Y-F, et al. Paracrine and
epigenetic control of CAF-induced metastasis: the role of HOTAIR stimulated by TGFR1-activation.
68. Hou P, Zhao Y, Li Z, Yao R, Ma M, Gao Y, et al. LincRNA-ROR induces epithelial-to-
mesenchymal transition and contributes to breast cancer tumourigenesis and metastasis. Cell Death Cell.
67. 2014.11–18. https://doi.org/10.1038/cdxds2014.249.
66. Peng W-X, Huang J-G, Yang L, Gong A-H, Mo Y-Y. Linc-RoR promotes MAPK/ERK
signaling and confers estrogen-independent growth of breast cancer. Mol Cancer. 2015.12:81–88.
65. Jin X, Ge L-P, Li D-Q, Shao Z-M, Di G-H, Xu X-E. et al. LncRNA TROJAN promotes
proliferation and resistance to CDK4/6 inhibitor via CDK2 transcriptional acti-
vation in ER− breast cancer. Mol Cancer. 2020;19:1–18.
64. Hu Q, Ye Y, Chan L-C, Li Y, Liang K, Lin A, et al. Oncogenic lncRNA downregulates
breast cancer cell antigen presentation and intrinsic tumour suppression. Nat Immu-
nol. 2019. https://doi.org/10.1038/s41591-019-0400-7.
63. Han J, Ou H, Han M, Ding Y, Xie M, Hu J, et al. MSC-induced lncRNA AGAP2-A51
promotes stemness and trastuzumab resistance through regulating CPT1 expression
and fatty acid oxidation in breast cancer. Oncogene. 2021;20:833–47.
62. Luo L, Zhang J, Tang H, Zhao D, Huang D, Ling L, et al. LncRNA SNORD3A
specifically sensitizes breast cancer cells to 5-FU by sponging miR-185-5p to enhance
UMPs expression. Cell Death Cell. 2020.11. https://doi.org/ s41419-020-2557-2.
61. Li Z, Hou P, Fan D, Dong M, Ma M, Li H et al. The degradation of EZH2 mediated
by IncRNA ANCR attenuated the invasion and metastasis of breast cancer. Cell Death Differ.
2017;24:59–71.
60. Tan BS, Yang MC, Singh S, Chou YC, Chen HY, Wang MY, et al. LncRNA NORAD
is repressed by the YAP pathway and suppresses lung and breast cancer metas-
tasis by sequestering S100P. Oncogene. 2019;38:5612–26.
59. Qian Y, Shi L, Luoz. Long non-coding RNAs in cancer: implications for diagnosis,
prognosis, and therapy. Front. Med. 2020. https://doi.org/10.3389/fmed.2020.612393.
58. Shi T, Gao G, Cao Y. Long noncoding RNAs as novel biomarkers have a promis-
ing future in cancer diagnostics. Dis Markers. 2016. https://doi.org/10.1155/2016/9085195.
57. Yu Y, Zhang W, Li A, Chen Y, Ou Q, He Z, et al. Association of long noncoding
RNA biomarkers with clinical immune subtype and prediction of immunother-
apy response in patients with cancer. JAMA Netw Open. 2020. https://doi.org/
10.1001/jamanetworkopen.2020.2149.
56. Chandra Gupta S, Nandan Tripathi Y. Potential of long non-coding RNAs in
cancer patients: from biomarkers to therapeutic targets. Int J Cancer. 2017. https://doi.
org/10.1002/ijc.30960.
55. Iraola G, Guzmán S, Brunet-Vega A, Pegueroles C, Saus E, Hovhannisyan H, Casalots
et al. Target enrichment enables the discovery of lncRnas with somatic
mutations or altered expression in paraf
a pilot study. Medicina. 2021. https://doi.org/
10.1007/s10549-013-2776-7.
54. Shin YW, Chen J, Cheuk IWY, Siu MT, Ho CW, Wang X, et al. Long non-coding RNA
NEAT1 confers oncogenic role in triple-negative breast cancer through modu-
lating chemoresistance and cancer stemness. Cell Death Dis. 2009. https://doi.
org/10.1038/s10549-013-2776-7.
53. Yamaizaki T, Souquere S, Chugo T, Kobelke S, Chong YS, Fox AH, et al. Functional
domains of NEAT1 architectural IncRNA induce paraspeckle assembly through
phase separation. Mol Cell. 2018;70:153–67.
52. Li RH, Tian T, Ge OW, He XY, Shi CY, Li JH, et al. A phosphonic acid-binding IncRNA
SNHG9 facilitates LAT51 liquid–liquid phase separation to promote oncogenic YAP
signaling. Cell Res. 2021. https://doi.org/10.1038/s41422-021-00530-9.
51. Daneshvar K, Ardehali MB, Klein IA, Hsieh FK, Kratkiewicz AJ, Mahpour A, et al.
IncRNA DIGIT and BRD3 protein form phase-separated condensates to regulate
endometrial differentiation. Nat Cell Biol. 2020;22:1211–22.
50. Pessina F, Giavazzi F, Yin Y, Gioia U, Vitelli V, Gabbiat A, et al. Functional trans-
scription promoters at DNA double-strand breaks mediate RNA-driven phase
separation of damage-response factors. Nat Cell Biol. 2019;21:1286–99.
49. Ji X, Lu Y, Tian H, Meng X, Wei M, Cho WC. Chemoresistance mechanisms of breast
and their countermeasures. Biomacrophor. 2019. https://doi.
org/10.1007/jbipha.2019.108800.
126. Chen F, Chen J, Yang L, Liu J, Zhang X, Zhang Y, et al. Extracellular vesicle-packaged HIF-1α-stabilizing IncRNA from tumour-associated macrophages regulates aerobic glycolysis of breast cancer cells. Nat Cell Biol. 2019;21:498–510.

127. Ni C, Fang Q-Q, Chen W-Z, Jiang J-X, Jiang Z, Ye J, et al. Breast cancer-derived exosomes transmit IncRNA SNHG16 to induce CD73/γδ Treg cells. Signal Transduct Target Therapy. 2020.5. https://doi.org/10.1038/s41392-020-0129-7.

128. Kong X, Li J, Li Y, Duan W, Qi Q, Wang T, et al. A novel long non-coding RNA AC073352.1 promotes metastasis and angiogenesis via interacting with YBX1 in breast cancer. Cell Death Dis. 2021. https://doi.org/10.1038/s41419-021-03943-x.

129. Bayarbaa W, Wu Z, Peng J, Wang Y, Xu S, Yan T, et al. Association of LncRNA AC039631.4 with BRCA1 function in breast cancer. Transl Oncol. 2020;5. https://doi.org/10.1016/j.tranon.2020.100219.

130. Slaby O, Laga R, Sedlacek O. Therapeutic targeting of non-coding RNAs in cancer. Biochem J. 2017;474:4219–33.

131. Arun G, Diermeier SD, Spector DL. Therapeutic targeting of long non-coding RNAs in cancer. Trends Mol Med. 2018. https://doi.org/10.1016/j.molmed.2018.01.001.

132. Gutschner T, Hammerle M, Eissmann M, Hsu J, Kim Y, Hung G, et al. The non-coding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. Cancer Res. 2013;73:1180–9.

133. Arun G, Diermeier S, Akerman M, Chang KC, Wilkinson JE, Hearn S, et al. Differentiation of mammary tumours and reduction in metastasis upon Malat1 IncRNA loss. Genes Dev. 2016. https://doi.org/10.1101/gad.270959.115.

134. Hung T, Wang Y, Lin MF, Koegel AK, Kotake Y, Grant GD, et al. Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. Nat Genet Nat Publ Group. 2011;43:621–9.

135. Ren S, Liu Y, Xu W, Sun Y, Liu J, Wang F, et al. Long noncoding RNA MALAT-1 is a new potential therapeutic target for castration resistant prostate cancer. J Urol. 2013;190:2278–87.

136. Youssefi H, Maheronnaghsh M, Molaei F, Mashouri L, Aref AR, Momeny M, et al. Long noncoding RNAs and exosomal IncRNAs: classification, and mechanisms in breast cancer metastasis and drug resistance. Oncogene. 2020;39:953–74.

137. Tiemann K, Rossi JJ. RNA-based therapeutics—current status, challenges and potential solutions. Nat Rev Drug Discov. 2021. https://doi.org/10.1038/s41573-021-00219-z.

138. Paunovska K, Loughey DH, Dahlman JE. Drug delivery systems for RNA therapeutics. Nat Rev Genet. 2022;2021:23465789. https://doi.org/10.1038/s41576-021-00439-4.

139. Winkle M, El-Daly SM, Fabbri M, Calin GA. Non-coding RNA therapeutics — challenges and potential solutions. Nat Rev Drug Discov. 2021. https://doi.org/10.1038/s41573-021-00219-z.

140. Yamada Y. Nucleic acid drugs—current status, issues, and expectations for exosomes. Cancers. 2021. https://doi.org/10.3390/cancers13190502.

141. Goyal A, Myacheva K, Grof M, Klingenberg M, Diederichs S. Challenges of CRISPR/Cas9 applications for long non-coding RNA genes. Nucleic Acids Res. 2017;45:e12.

142. Yang J, Meng X, Pan J, Jiang N, Zhou C, Wu Z, et al. CRISPR/Cas9-mediated noncoding RNA editing in human cancers. RNA Biol. 2018;15:35–43.

143. Bregoli R, Sideris N, Giaidobutts A. LncRNAs as chromatin regulators in cancer: from molecular function to clinical potential. Cancers. 2019;11:1–21.

144. Darfeuille F, Regadas S, Hansen JB, Orum H, di Primo C, Toulme JJ. Aptamers targeted to an RNA hairpin show improved specificity compared to that of complementary oligonucleotides. Biochemistry. 2006;45:12076–82.

145. Fatemi RP, Velmeshev D, Faghihi MA. De-repressing LncRNA-targeted genes to upregulate gene expression: Focus on small molecule therapeutics. Mol Ther Nucleic Acids. 2014;3:e196.

146. Li Y, Ren Y, Wang Y, Tan Y, Wang Q, Cai J, et al. A compound AC1q3qWB selectively disrupts HOTAIR-mediated recruitment of PRC2 and enhances cancer therapy of DoxGEP. Theranostics. 2019. https://doi.org/10.7150/thno.35188.

147. Simko EAI, Liu H, Zhang T, Velasquez A, Tei S, Haeusler AR, et al. G-quadruplexes offer a conserved structural motif for NONO recruitment to NEAT1 architectural IncRNA. Nucleic Acids Res. 2020. https://doi.org/10.1093/nar/gkaa475.

148. Mou X, Liew SW, Kwok CK. Identification and targeting of G-quadruplex structures in MALAT1 long non-coding RNA. Nucleic Acids Res. 2022;50:397–410.

149. Abudayeh OE, Gootenberg JS, Essletzbichler P, Han S, Joung J, Belancio JJ, et al. RNA targeting with CRISPR-Cas13. Nature. 2017. https://doi.org/10.1038/nature24049.

150. Luo J, Wu F, Gao F, Lin J, Liu J, Lin A. LncRNAs: architectural scaffolds or more potential roles in phase separation. Front Genet. 2021;12:1–13.