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Review

Long noncoding RNAs and their expanding roles in cancer

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ABSTRACT

Protein-coding genes only account for about 2% of the total human genome. Recent technologies (e.g., tiling arrays and RNA-sequencing) have revealed that about 70% of the human genome is transcribed into noncoding RNAs. Long noncoding RNAs (lncRNAs), which comprise of a class of RNA molecules greater than 200 nucleotides in length, have been emerging as critical players in a broad range of cellular processes. More recently, a dozen of human lncRNAs have been implicated in driving cancer development through their oncogenic or tumor suppressive functions. This review aims to broadly define the functions and mechanisms of lncRNAs in the context of cancer, as well as discuss their potential future applications in the clinic.

KEYWORDS: Long noncoding RNAs, lncRNAs, oncogene, tumor suppressor, cancer, metastasis

INTRODUCTION

The central dogma of molecular biology traditionally defines that DNA is transcribed to RNA, and RNA serves as a template to craft a functional protein. However, since the completion of the Human Genome Project, scientists have continually mapped and organized about 3 billion bases to a precise number of protein-coding genes, whose number is often cited to be around 20,500 to 25,000 in total [1, 2]. It is interesting to note however that these protein-coding genes (i.e. exons, introns and untranslated regions (UTRs)) only account for ~2% of the totality of the genomic DNA.

Accumulating evidence indicates that ~70% of the genome can be transcripted to noncoding RNAs, which include small RNAs (e.g., microRNA, snoRNA, piRNA etc.) and long noncoding RNA (lncRNA) [3-6]. The first lncRNA H19 was discovered in 1989 and the X-inactive specific transcript (XIST) was subsequently identified in 1990 [7, 8]. It is now estimated that the lncRNAome consists of more than 30,000 RNA genes, surpassing the estimated number of protein-coding genes [5, 9]. In just the past few years our understanding of lncRNAs has vastly expanded and there are now several examples of well-defined lncRNA genes. It is now known that lncRNAs can be either oncogenic or tumor suppressive, directly promoting or inhibiting tumor formation, growth and metastasis [10-12]. LncRNAs have also been demonstrated to be clinically relevant as diagnostic and prognostic indicators in a variety of cancers [13, 14].

Defining lncRNA

The current and general parameters defining lncRNAs are the following: 1) polyadenylation at 3' UTR in a majority of lncRNAs, 2) epigenetic marks including H3K4me3 at the promoter and H3K36me3 along the gene body, 3) active transcription via RNA polymerase II (RNA pol II), 4) regulation by established transcription factors, 5) processing via canonical exonic splice sites, 6) >200 bp in length without open-reading frame and 7) tissue-specific expression (Figure 1) [15, 16]. There are exceptions however, and examples of non-polyadenylated lncRNAs transcribed via RNA polymerase III have been reported [17, 18].

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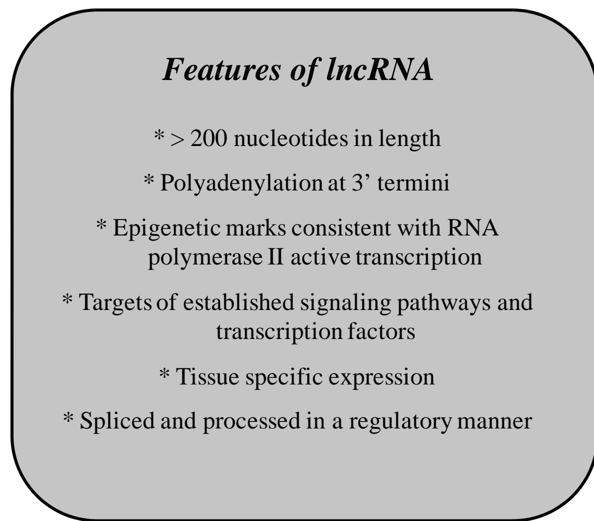

Figure 1. There are several biological features and regulatory processes which distinguish lncRNAs as a class.

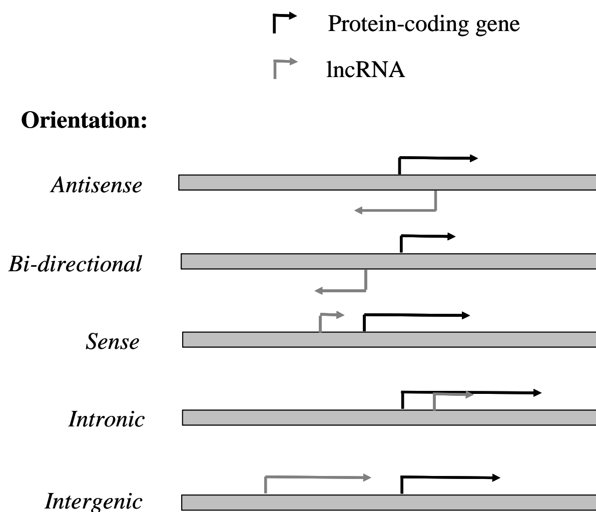

Figure 2. LncRNAs (grey) can be transcribed in various orientations and this is defined in relationship to the nearest protein-coding gene (black).

The majority of these features emulate characteristics of messenger RNAs (mRNAs), emphasizing the notion that lncRNAs are under precise transcriptional control and processed in the same manner as functional protein-coding transcripts. The directionality of lncRNA is usually defined with reference to the nearest protein-coding gene, and can exhibit antisense, bidirectional, sense, intronic and intergenic orientations (Figure 2).

Conservation of lncRNA

The conservation of lncRNAs has been the topic of extensive debate and study. The significance of linking conservation to lncRNAs would provide strong evidence for functionality since these transcripts were retained through selective pressures. By and large, however, lncRNAs exhibit poor sequence conservation across species in comparison to protein-coding genes and microRNAs [19, 20]. One study reported that starting with a pool of tissue-specific human lncRNAs, 80% contained an orthologue transcript in chimpanzee, and this decreased to just 39% in mice [21]. Thus, given the fact that the protein-coding genomes are almost entirely conserved across higher organisms, the noncoding genome likely is a key contributor to species identity and complexity. From an evolutionary perspective, it has been suggested that lncRNAs are rapidly evolving regulatory elements of high plasticity, responsible for continually mobilizing mammalian evolution [20]. This concept is very striking given the short evolutionary distance between humans and the chimpanzee, with lncRNAs accounting for about half the genetic differences between the two genomes [22].

It is also important to remember that selecting for a protein-coding gene and lncRNA needs to be considered as independent and mutually exclusive processes. Conservation of a functional protein-coding gene requires high sequence preservation to ensure proper amino acid coding and secondary protein folding. Any disruption in the sequence can drastically alter the biological function of a protein, and this likely contributes to the high conservation of proteins across several species. On the contrary, lncRNAs do not require significant sequence preservation along the entire body of the transcript to retain the appropriate architecture and secondary structure necessary for function. LncRNA function can occur through a protein binding site, a miRNA seed site, mRNA recognition sequence, or DNA binding element, all of which require relatively short stretches of sequence for utility. A prime example is XIST, a 17-kb transcript in humans in which conservation is only retained in exon 1, and this conserved region is critical for proper function [23]. Also, despite lack of lncRNA sequence conservation in and of itself, the promoters

of lncRNAs demonstrate very high sequence conservation and are capable of binding established transcription factors [24].

Mechanisms of lncRNAs

Epigenetic and chromatin modification

LncRNAs can regulate gene expression within a few megabases along the same chromosome or from a distance at a different chromosome. This regulation is referred to as acting in "cis" or in "trans", respectively. The X-inactive specific transcript (XIST) is a very well studied lncRNA that functions in "cis" (Figure 3). In developing mammalian females, X-chromosome inactivation is a critical compensatory mechanism to ensure gene dosage equivalency between males and females along the X-chromosome. XIST mediates this process through its exclusive expression on the inactive X-allele via the X-chromosome inactivation center (Xic) [23, 25]. The first exon of XIST contains a double hairpin motif capable of binding polycomb recessive complex 2 (PRC2), and recruitment of this histone methyltransferase complex results in trimethylation of lysine-27 at H3 (H3K27me3) and transcriptional silencing along the inactive X-chromosome [26]. Interestingly, the active X-chromosome represses XIST expression through interaction with an antisense lncRNA known as TSIX; however an exact mechanism is yet to be determined for this modulation [27].

One of the better-characterized lncRNAs that functions in epigenetic and chromatin modification in "trans" is the HOX antisense intergenic ncRNA (HOTAIR). HOTAIR is located on chromosome 12 and is oriented antisense to the HoxC cluster, and transcriptionally represses the HoxD locus on chromosome 2 [28]. There are two functional domains of HOTAIR, a PRC2-binding domain at the 5' end which interacts with subunit Suz12, and an LSD1/CoREST1-binding domain at the 3' end. It is suspected that these two complexes use HOTAIR as a modular scaffold for collaboration and recruitment to target gene locations [29]. The precise mechanisms of how "trans" functioning lncRNAs target specific genes on distant chromosomes are still not well understood. There have been several additional mechanisms reported for lncRNA function (Table 1).

Sponge for miRNAs

It is becoming more apparent that some lncRNAs contain multiple miRNA response elements (MREs), which layers ncRNA regulation through competition. This theory is known as the competing endogenous RNA (ceRNA) hypothesis [30]. LncRNAs can

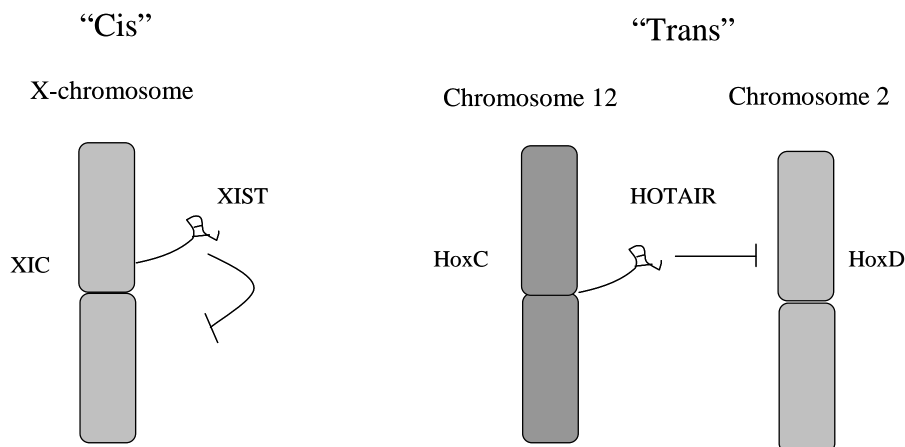

Figure 3. LncRNAs, like XIST, can function in "cis" and regulate target gene expression locally and within a few megabases along the same chromosome from which it is transcribed. In females, XIST is transcribed at the X-chromosome inactivation center (XIC) and recruits PRC2 complex to shut off local gene expression. LncRNAs can also function in "trans" and regulate target gene expression from a distant chromosome. HOTAIR is transcribed in the antisense direction in the HoxC locus in chromosome 12, yet functions to negatively regulate HoxD gene cluster at chromosome 2.

Table 1. Biological mechanisms of lncRNAs.

Mechanism	Specific lncRNA mechanism	Reference
Epigenetic and chromatin modification	**XIST**: Exclusively expressed on the inactive X chromosome, recruits PRC2 to X-chromosome inactivation center (XIC) for gene silencing	26, 47-50
Sponge for miRNAs	**H19**: Contains 4 Let-7 binding sites throughout transcript body to competitively bind and down-regulate Let-7 in Ago2 dependent manner.	31
Messenger RNA processing and protein translation	**ZEB2-AS**: Transcribed antisense to ZEB2 mRNA, binds ZEB2 transcript and prevents splicing of 5' intron preventing protein translation.	37
Enhancers and transcriptional activation	**CCAT2**: Located ~330 kb away from C-MYC promoter binds TCF7L2 to transcriptionally activate C-MYC	41
Decoy for protein binding	**PANDA**: Induced in DNA damage response, binds and sequesters transcription factor NF-YA to prevent induction of pro-apoptotic gene targets	43

interact with miRNAs through these repeat MREs along the length of the transcript to influence global gene expression. For example, within the body of lncRNA H19 resides four canonical and non-canonical MREs for Let-7 [31]. It was demonstrated that enforced expression of H19 results in more Let-7 binding at these MREs in an Ago2-dependent manner. Through competitive binding and sequestering of Let-7, downstream targets of Let-7 such as DICER and HMGA2 increased their expression [31].

Linc-ROR, a regulator of reprogramming, can also act as a sponge and shares MREs with several miRNAs that target the core transcription factors in maintaining embryonic stem cells [32, 33]. Expression patterns of linc-ROR are consistent and are associated with the undifferentiated stem-like state. Linc-ROR contains MREs for miR-145, miR-181, and miR-99-3p, which target Oct4, Nanog, and Sox2. Enforced expression of linc-ROR competitively binds these miRNAs relieving target repression, enhancing protein expression of these core transcription factors. Loss of linc-ROR leads to decreased expression of Oct4, Nanog, and Sox2 and movement towards the differentiated state [33].

Messenger RNA processing and protein translation

LncRNAs can physically interact with mRNAs to influence stability of the transcript or translation in dynamic ways [34, 35]. This relationship is especially apparent in the case of protein-coding genes which contain overlapping natural antisense transcripts (NATs). Uchl1 protein gene overlaps with antisense lncRNA Uchl1-AS at the 5' region and also contains an inverted SINE2B repeat element downstream [36]. In stress response, Uchl1-AS will rapidly shuttle from the nucleus to the cytosol where it binds to and stabilizes the Uchl1 mRNA transcript along the 5' UTR region leading to increased translation. Detailed underlying mechanism is not very well understood, but the SINE2B element is critical for enhanced translation during this process indicating a possible novel function for transposable elements within the genome [36].

In another example, Snail1-induced epithelial to mesenchymal transition (EMT) does not activate Zeb2 mRNA transcription, but rather promotes Zeb2 translation through splicing of a large intron at the 5' UTR region. This intron contains an internal ribosome entry site (IRES) necessary for the expression of Zeb2. Maintenance of 5' UTR Zeb2 intron is dependent on the expression of Zeb2-AS1 that overlaps the 5' splice site in the intron. In the non-EMT state this intron is spliced out, which does not affect mRNA level yet does prevent protein translation. In the case of Snail1-induced EMT, Zeb2-AS1 is transcribed and its complementary base pairs with the 5' UTR of Zeb2 prevents splicing of the 5' IRES sequence allowing for Zeb2 translation [37].

Enhancers and transcriptional activation

Enhancers are regulatory DNA elements that activate gene transcription in "cis" through chromosomal looping and recruitment of cofactors [38, 39]. There is now evidence that these regulatory regions frequently contain lncRNAs which have target gene specificity and promote cofactor recruitment. In one study, enhancer-associated ncRNA-a7 was characterized as being able to specifically regulate SNAI1 transcription, yet had no relationship to RNF114, UBE2VI, TMEM189, or CEBPB which reside within the same 100 kb radius [40]. It was also shown that SNAI1 migration is driven through ncRNA-a7, providing strong evidence that lncRNAs play a key regulatory role in gene expression and downstream biological processes [40].

Another lncRNA, CCAT2, which locates ~334 kb away from C-MYC transcriptional start site was shown to be over-expressed in colorectal cancers and promote chromosomal instability, invasion, and metastasis [41]. CCAT2 binds to transcription factor TCF7L2 for recruitment to the enhancer region and results in activation of C-MYC transcription through chromosomal looping and interaction at the promoter. Interestingly, CCAT2 has a single nucleotide polymorphism (SNP) that associates with increased risk of developing colon cancer. This study suggests that this SNP is functional through the RNA product, and enhances TCF7L2 recruitment to the C-MYC promoter, prompting a paradigm shift that SNPs are typically functional through DNA elements [41].

Decoy for protein binding

LncRNAs can also function as decoys through binding protein targets and thereby preventing normal function and localization of the protein. For example, in response to DNA damage a subset of lncRNAs will be induced in a p53-dependent manner including 2 bidirectional transcripts upstream of the p21 promoter, lincRNA-p21 and PANDA [42, 43]. During this response PANDA will bind to the transcription factor NF-YA and prevent its localization at promoters of pro-apoptotic target genes. Thus, PANDA allows for cell cycle arrest and DNA repair rather than programmed cell death in the p53 response [43]. Another example can be seen in extra-coding CEBPA lncRNA (termed ecCEBPA), an ~4.5kb transcript encompassing the entire ~2.6 kb portion of the CEBPA gene in the sense direction [44]. When ecCEBPA is expressed it will competitively bind and exclude DNMT1 for the occupying chromatin at the CEBPA promoter, thus preventing DNA methylation and activating transcription of the CEBPA [44].

LncRNAs in human malignancy

LncRNAs regulate genes that participate in a wide range of biological processes, and for this reason they can act as oncogenes and tumor suppressors. Aberrant expression of lncRNA can be the deciding point in cancer etiology, promote tumor onset and disease progression, as well as drive metastasis. LncRNAs are also a novel and untapped resource for pathological and clinical utility for use as novel biomarkers. Although a number of lncRNAs have been associated with cancer in literature, there is a clear need for further identification and characterization of more candidates. The following section will discuss the function and clinical significance of specific lncRNAs reported in human cancer to date (Table 2).

HOTAIR

HOTAIR, as discussed above, locates on chromosome 12 and is responsible for "trans" silencing the HoxD genes of chromosome 2 through recruitment of PRC2 complex [28]. HOTAIR expression has been shown to be up-regulated in both primary and metastatic breast cancers, in some cases as high as 2000-fold over-expression. Furthermore, the elevated expression correlates with increased risk of metastasis, as well as poorer metastasis-free and overall survival [14]. *In vitro* studies demonstrate that knockdown of HOTAIR can inhibit invasive capacity. Enforced expression of HOTAIR *in vivo* results in a greater capacity for breast cancer cells to colonize the lung following tail vein injection [14].

In non-small cell lung cancer (NSCLC), HOTAIR has been shown to have elevated expression levels when comparing 42 lung carcinomas to matched normals [45]. Elevated HOTAIR in NSCLC patients also associates with late stage tumor, positive lymph node metastasis, and poor overall survival. This study also demonstrates that HOTAIR can

Table 2. Cancer-associated lncRNAs.

LncRNA	Function	Mechanism	Interactors	Cancer Type	References
HOTAIR	Oncogene	Chromatin modification	SUZ12, LSD1	Breast, lung, renal	14, 28, 45, 46
XIST	Tumor Suppressor	Chromatin modification	EZH2	Myeloproliferative, breast, ovarian	26, 47-50
MEG3	Tumor Suppressor	Transcriptional activator	p53	Pituitary, neuroglioma, hepatocellular	51-54
MALAT1	Oncogene	Transcriptional repressor	Paraspeckle proteins (PSPs)	Lung	55-57
LincRNA-p21	Tumor Suppressor	Transcriptional repressor	hnRNPK	Lung	42, 58
PCAT1	Oncogene	Transcriptional repressor	PRC2	Prostate, colorectal	59-61
ANRIL	Oncogene	Transcriptional repressor	CBX7	Prostate, gastric	62-67
CCAT2	Oncogene	Enhancer	TCF7L2	Colorectal	41
Carlo-5	Oncogene	Enhancer	unknown	Lung	72
PVT1	Oncogene	Protein stability	C-MYC	Colorectal	73
FAL1	Oncogene	Transcriptional repressor	BMI1	Ovarian	74
lncRNA-ATB	Oncogene	miRNA sponge	miR-200a/b/c, miR-141, miR-429	Hepatocellular	75

control cell migration, invasion, and metastasis *in vitro* and *in vivo*, and shows that knockdown of HOTAIR leads to apoptosis. Depletion of HOTAIR resulted in significant loss of expression of known metastasis promoting genes MMP2 and MMP9, and increased expression of HOXA5, which has been shown to be important in inhibiting lung cancer metastasis [45].

A recent study shows that in renal carcinoma cells HOTAIR is inversely correlated in expression with miR-141 [46]. This study also shows that HOTAIR plays a critical role in promoting migration, invasion, and survival in renal carcinoma. Interestingly, enforced expression of miR-141 results in significant loss of HOTAIR expression, reduced invasive phenotypes, and increased apoptosis. Further investigation revealed that HOTAIR is a bona fide miR-141 target in an AGO2-dependent manner. This study illustrates the dynamic relationship between ncRNA species regulating each other, and their potential role in kidney cancer [46].

XIST

In one study the contribution of XIST to hematological cancers using a conditional knockout mouse model was evaluated. The majority of the female mice in which XIST is conditionally removed from cells in the blood compartment results in aggressive and rapid onset of multi-lineage hyper-proliferations, myelodysplasia, and myeloproliferative neoplasms [47]. These results suggest a potent tumor suppressor role for XIST in hematopoietic stem cells and potentially other tissues. Deletion of XIST resulted in X chromosome reactivation and aberrant gene expression in several

major tumor suppressive pathways contributing to the rapid onset of disease [47].

A second study used microarray technology to compare global changes in RNA expression in primary ovarian tumor to recurrent ovarian tumors after chemotherapy [48]. Interestingly, in recurrent ovarian cancer XIST was the most significantly downregulated gene in comparison to primary tumor [48]. From a therapeutic standpoint, this suggests that XIST can potentially play a role in sensitizing patients to taxol and cisplatin based chemotherapies. XIST can also serve as a marker for chemo-resistant ovarian cancer.

XIST has also been linked to the BRCA1 tumor suppressor gene in patients with breast or/and ovarian cancers. Initial reports indicated that breast cancers with deficient BRCA1 expression or function have defects in X chromosome inactivation, and enforced expression of BRCA1 gene can initiate XIST expression [49]. This view was later challenged using a BRCA1 conditional knockout mouse model which demonstrated that depleting BRCA1 had no affect on X inactivation and gene dosing, as well as showed no evidence of BRCA1 and XIST colocalization [50]. The relationship between BRCA1 and XIST in breast cancer will require future investigation to be better established.

MEG3

Maternally expressed gene 3 (MEG3), a paternally imprinted gene comprised of 10 exons, is expressed in many normal tissues [51]. There are 12 different MEG3 gene transcripts, generated by alternative splicing. They contain the common exons 1-3 and exons 8-10, but each uses one or more of exons 4-7 in a different combination in the middle. All MEG3 RNA isoforms contain three distinct secondary folding motifs M1, M2, and M3 that are critical for activation of p53. MEG3 isoform expression patterns are tissue and cell type specific. However, each isoform stimulates p53-mediated trans-activation and suppresses tumor cell growth. MEG3 retains the ability to activate p53 in spite of replacing regions unrelated to M1, M2, or M3, thus providing evidence that a proper folding structure of the MEG3 RNA molecule is critical for its biological functions and that only short stretches of sequence conservation are vital to maintain function [52]. In addition to transcriptionally activating p53, MEG3 can also enhance binding of p53 at target gene promoters including GDF15 [53]. MEG3 expression is dramatically lost in several human cancers [54-57]. Reintroduction of MEG3 into NSCLC cells was able to slow colony formation capacity and cell proliferation [56].

In a panel of hepatocellular carcinoma (HCC) cells MEG3 is frequently downregulated, and enforced expression of MEG3 was able to slow cell proliferation, inhibit anchorage-independent growth, and promote apoptosis [58]. This same study also demonstrated that maternal allele of MEG3 is hyper-methylated in a DNMT1- and DNMT3b-dependent manner. Interestingly, miR-29a expression is also commonly lost in HCC, and DMNT1 and DNMT3b are direct targets of miR-29a. Loss of miR-29a results in aberrantly increased DMNT1/3b expression in HCC and provides a novel mechanism for MEG3 downregulation due to hyper-methylation at the promoter [58].

MALAT1

Metastasis associated in lung adenocarcinoma transcript 1 (MALAT1) was initially identified in non-small cell lung cancer (NSCLC) as being preferentially over-expressed in patients with early stage tumors that presented metastasis at diagnosis [59]. High levels of MALAT1 are also correlated with poor overall survival in patients with early stage lung cancer [59]. A later study revealed that MALAT1 is differentially expressed throughout different stages of the cell cycle and is required for the G1/S transition. Depleting MALAT1 in normal immortalized lung cell line causes induction of p53, loss in B-MYB, and cell cycle arrest [60].

In a later study, gene expression analysis showed that MALAT1 functions to inhibit tumor suppressor and metastasis-associated genes MIA2, HNF4G, and ROBO1 [61]. This study also demonstrates that loss of MALAT1 can prevent lung metastasis using tail vein injection mouse model. Interestingly, mice with established subcutaneous tumors and subsequently treated with antisense oligonucleotides (ASOs) against MALAT1 showed decreased lung metastasis compared to the control [61].

These data indicate that lncRNAs can be therapeutic targets in human cancer.

LincRNA-p21

LincRNA-p21 locates ~15 kb upstream of CDKN1A gene (p21) and is induced by p53 [42]. Induction of lincRNA-p21 in p53-dependent manner was observed in several cancer types including lung, sarcoma and lymphoma. The p53 response is dynamic in that the end result can lead to either cell cycle arrest or apoptosis. This study has demonstrated that lincRNA-p21 can bind to hnRNP-K, a component of transcriptional repression, and locates preferentially at the promoters of pro-apoptotic genes for repression in "trans". Thus, in the p53 response, lincRNA-21 shifts the balance to cell cycle arrest and DNA repair, rather than programmed cell death [42].

In a recent report, a conditional knockout mouse model demonstrated that loss of lincRNA-p21 results in defective G1/S checkpoint, increased proliferation rates, and enhanced reprogramming efficiency [62]. This report also showed that by conditionally removing lincRNA-p21 in mouse embryonic fibroblasts (MEFs), a subset of PRC2 target genes are activated in the p53 response. This result suggests that the lincRNA-p21, p53, and the PRC2 downstream target genes are interconnected, and the exact response and interplay is likely dependent on many factors. Interestingly, this study also showed that lincRNA-p21 predominantly functions in "cis" to activate expression of its neighboring gene p21 through recruitment of hnRNP-K as a coactivator for p53-dependent p21 transcription [62].

PCAT1

Prostate cancer associated transcript 1 (PCAT1) was first identified as 1 of 121 dysregulated lncRNAs in a high throughput experiment examining 102 prostate cancer tissues and cell lines using RNA-sequencing [63]. PCAT1 resides upstream of C-MYC oncogene on chromosome 8q24, a region that is associated with risk and amplified in several cancer types [63]. PCAT1 is inversely correlated with EZH2 and is a direct binding partner and target of PRC2 repression. Elevated levels of PCAT1 associate with more aggressive tumor, increased cell proliferation, and cell cycle progression through its regulation of target genes BRCA2, CENPE, and CENPF [63].

It was later established that PCAT1 can interrupt homologous recombination (HR) in the DNA damage response (DDR) through suppression of the BRCA2 tumor suppressor gene [64]. Faulty DNA damage repair is a hallmark of cancer and cancers with faulty HR pathways are sensitive to drugs which target the DDR such as poly-ADP ribose (PARP) inhibitors. This study demonstrates that prostate cancers with elevated PCAT1 are selectively sensitive to PARP inhibitors [64].

Another study evaluated the clinical significance of elevated PCAT-1 expression in 108 colorectal cancer (CRC) tumors compared to 81 matched normal controls, as well as the significance of copy number variation in 17 matched samples [65]. It was concluded that PCAT1 shows elevated expression in > 60% of CRCs, yet few cases demonstrated to be elevated due to copy number variation. This denotes that in addition to 8q24 amplification commonly seen in cancers, alternate mechanisms could be responsible for PCAT1 over-expression. In CRC, high PCAT1 expression is also a prognostic indicator of poor overall survival [65].

ANRIL

Antisense noncoding RNA in the INK4 locus (ANRIL) was initially identified as being deleted in the germ-line deletion of the 9p21 gene cluster [66]. This gene cluster contains several important tumor suppressor genes including, p14ARF, p15INK4B, and p16INK4A that are deleted along with ANRIL across ~403 kb region and results in cancer predispositions [67-69]. ANRIL locates in the antisense direction and overlaps with the p14ARF promoter and 2 exons of the p15INK4B gene body [66]. Although initial reports identified this transcript as associated with deletion, later reports suggest that ANRIL over-expression can also drive cancers [70, 71].

Elevated expression of ANRIL was detected in prostate cancer and inhibiting ANRIL expression resulted in increased INK4A and INK4B gene products, denoting a transcriptional repression in "cis". In this same study it was shown that ANRIL can bind to the CBX7 component of the PRC1 repressive complex and epigenetically

silence the associated tumor suppressive genes on 9p21 gene locus [70]. ANRIL expression is also elevated in human gastric cancers and associates with poor overall survival. This same study demonstrated that ANRIL can silence oncogenic miRNAs, including miR-99a and miR-449a, through binding to the components of PRC2 transcriptional repression complex [71].

Emerging lncRNAs in cancer

The 8q24 chromosomal amplification associates with increased risk for several cancers. Linked within this region resides a gene desert containing no protein-coding genes in addition to the C-MYC onco-protein [72-75]. Several lncRNAs (CCAT2, Carlo-5, and PVT1) have been identified within this region and regulate C-MYC expression through a variety of mechanisms including transcriptional activation, enhancer-associated function, and protein stability [41, 76, 77].

Another interesting lncRNA, FAL1, has recently been identified as locating in a focally amplified chromosome 1q21 region, across several epithelial cancers including breast and ovarian [78]. FAL1 binds to PRC1 core subunit BMI1 to transcriptionally repress tumor suppressor gene p21. Interestingly, FAL1 is a predictor of poor overall survival in ovarian cancer patients, and elevated FAL1 closely associates with chromosome 1q21 DNA copy number gain [78].

TGFβ-induced lncRNA-ATB is also emerging as an important driver in EMT and metastasis in liver cancer. LncRNA-ATB is a potent driver of the EMT by acting as miRNA sponge for the miR200 family [79]. This family is the master regulator of ZEB1/2 expression through 3' UTR mediated repression in an Ago2-dependent manner. Also, lncRNA-ATB promotes cell proliferation and distant colonization through directly binding and stabilizing IL-11 mRNA resulting in activation of phospho-STAT3 [79].

CONCLUSION

LncRNAs are a novel class of RNAs that regulate a broad range of biological processes through a variety of mechanisms. Accumulating evidence indicates that they are directly involved in cancer etiology, tumor progression, and metastasis. While several cancer-associated lncRNAs are better defined and characterized in mechanism and clinical usefulness, there remain a promising group of up and coming lncRNAs which will continue to be evaluated in human malignancy. Due to tissue-specific expression property, lncRNAs have great potential to serve as diagnostic biomarkers for human cancer. In addition, lncRNAs could be critical therapeutic targets for cancer prevention and treatment. Antisense oligonucleotide (ASO) technology has provided the foundation and proof that targeting lncRNA can be effective in treating cancer. It is critical to continue characterizing lncRNA function and the underlying biological mechanisms to translate this new class of RNAs from bench to bedside ultimately improving patient care.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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