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The long non-coding RNAs, a new cancer diagnostic and therapeutic gold mine

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The conventional view of gene regulation in biology has centered around protein-coding genes via the central dogma of DNA→mRNA→protein. The discovery of thousands of long non-coding RNAs (lncRNAs) has certainly changed our view of the complexity of mammalian genomes and transcriptomes, as well as many other aspects of biology including transcriptional and posttranscriptional regulation of gene expression. Accumulating reports of misregulated lncRNA expression across numerous cancer types suggest that aberrant lncRNA expression may be a major contributor to tumorigenesis. Here, we summarize recent data about the biological characteristics of lncRNAs in cancer pathways. These include examples with a wide range of molecular mechanisms involved in gene regulation. We also consider the medical implications, and discuss how lncRNAs can be used for cancer diagnosis and prognosis, and serve as potential therapeutic targets. As more examples of regulation by lncRNA are uncovered, one might predict that the large transcripts will eventually rival small RNAs and proteins in their versatility as regulators of genetic information.
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The central dogma of gene expression is that DNA is transcribed into mRNA, which in turn serves as the template for protein synthesis. Intensive investigations over the last few decades have focused on the role of protein-coding genes in the pathogenesis of cancer. However, recent advances in technologies, such as tiling arrays and RNA deep sequencing (RNA-seq), have made it possible to survey the transcriptomes of many organisms to an unprecedented degree, the results of which have led to both great insights and unexpected conundrums. In fact, there are ~20 000 protein-coding genes, only representing <2% of the total genome sequence,[1] whereas at least 90% of the genome are actively transcribed into non-coding RNA (ncRNA), implicating that ncRNAs could have significant regulatory roles in complex organisms (Figure 1).

In general, ncRNAs are grouped into two major classes based on their length. Those transcripts shorter than 200 nucleotides (nt) are usually recognized as small ncRNAs, which include Piwi-interacting RNAs, small-interfering RNAs, microRNAs (miRNAs) and some bacterial regulatory RNAs. The well-documented miRNAs, ~22 nt long, serve as major regulators of gene expression and as intricate components of the cellular gene expression network,[2–5] and have identified critical roles for ncRNAs in cancer.[6,7] Furthermore, miRNA-expression profiling of human tumors has identified signatures associated with diagnosis, staging, progression, prognosis and response to treatment.[7–10] In addition to the relatively well-described miRNAs, the growing knowledge of the mammalian non-coding transcriptome is revealing that the genome is also replete with long ncRNAs (lncRNAs). LncRNAs are mRNA-like transcripts ranging in length from 200 nt to ~100 kb, yet are poorly conserved and do not function as templates for protein synthesis. Some researchers attempted to conduct lncRNA classification based on their genomic proximity to protein-coding genes, including five types: (1) sense, (2) antisense, (3) bidirectional, (4) intronic and (5) intergenic.[11,12] Many identified lncRNAs are transcribed by RNA polymerase II and are polyadenylated, but this is not a fast rule. For example, brain-associated BC200 is transcribed by RNA polymerase III, and not polyadenylated.[13,14] Although initially argued to be spurious transcriptional noise, recent evidence suggests that the proverbial 'dark matter' of the

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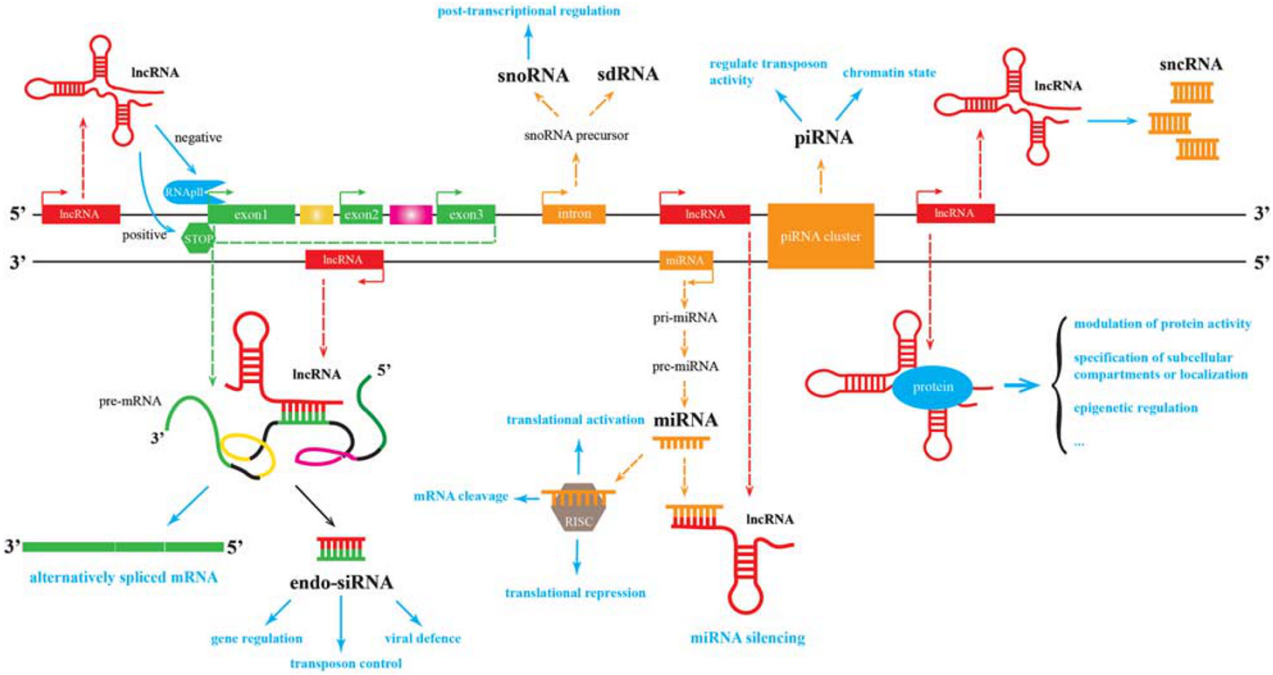

Figure 1 Simplified representation of main ncRNAs biogenesis pathways and their functions. Endo-siRNA, endogenous small-interfering RNA; lncRNA, long non-coding RNA; MiRNA, microRNA; piRNA, PIWI-interacting RNA; sdRNA, sno-derived RNA; sncRNA, small non-coding RNA; snoRNA, small nucleolar RNA.

genome may has a major biological role in cellular development, differentiation and metabolism.[15] The small number of characterized human lncRNAs have been associated with a spectrum of biological processes including epigenetics, alternative splicing, nuclear import, as structural components, as precursors to small RNAs and even as regulators of mRNA decay,[16–21] every level of the gene expression program (Figure 1). Furthermore, accumulating reports of misregulated lncRNA expression across numerous cancer types suggest that aberrant lncRNA expression may be a major contributor to tumorigenesis.[22] In this review, we summarize recent data about the biological characteristics of lncRNAs in cancer pathways. These include examples with a wide range of molecular mechanisms involved in gene regulation. We also consider the medical implications, and discuss how lncRNAs can be used for cancer diagnosis and prognosis and serve as potential therapeutic targets.

Examples of cancer-associated lncRNAs

Cancers are the result of a process where somatic cells mutate and escape the controlled balance exerted by gene expression programs and cellular networks that maintain cellular homeostasis and normally prevent their unwanted expansion. Even the slightest perturbation of these pathways can result in cellular transformation. Genes that affect these processes can be classified into two major groups: tumor-suppressor genes and oncogenes. Tumor-suppressor genes protect cells against deleterious mutations and cellular regulation that could prime transformation. Conversely, genes that initiate the cellular transformation process upon inappropriate activation comprise oncogenes. A role for differential lncRNA expression in cancer had been suspected for many years, however, lacked strong supporting evidence.[23] With advancements in cancer transcriptome profiling and accumulating evidence supporting lncRNA function, lncRNAs are emerging as new factors in the cancer paradigm, demonstrating their potential roles in both oncogenic and tumor-suppressive pathways (Table 1).

Oncogenic lncRNAs

HOTAIR—HOX Antisense Intergenic RNA

Dysregulated lncRNAs may affect epigenetic information and provide a cellular growth advantage, in that their selection may result in the progressive and uncontrolled growth of a tumor. One example of such an oncogenic lncRNA is HOTAIR. HOTAIR, a 2.2-kb gene residing in the mammalian HOXC locus on chromosome 12q13.13, was initially discovered as a gene repressor of *HOXD* genes and underscored the importance of understanding the relationship between epigenetic regulation by lncRNAs and cancer. Elevated expression of HOTAIR was observed in both primary and metastatic breast cancer, demonstrating up to 2000-fold increased transcription over normal breast tissue.[24] In addition, HOTAIR expression level in primary breast tumors was a powerful predictor of patient outcomes such

Table 1 Examples of potential oncogenic and tumor-suppressor lncRNAs

Types	*LncRNA*	*Size*	*Gemomic location*	*Cancer type*	*Function*	*References*
Oncogenic lncRNAs	HOTAIR	2.2 kb	Intergenic, Hox C locus	Breast and liver	Gene silencing by interacting with PRC2 and LSD1/CoREST complexes. Metastasis	24,26,86,87
	ANRIL	~3.9 kb	Antisense of INK4b-ARF-INK4a and p15/CDKN2B	Prostate, leukemia	Gene silencing of INK4b-ARF-INK4a and p15/CDKN2B by recruitment of PRC1 and PRC2	34,35
	MALAT1	~7 kb	Intergenic, Chr11q13	Breast, lung, uterus, pancreas, colon, prostate, liver, osteosarcoma	Sequesters SR splicing factors to regulate alternative splicing. Metastasis	37,40,41,42,43,49
	H19	2.3 kb	Imprinted at the Igf2 locus in Chr11	Bladder, lung, liver, breast, esophagus, choricarcinoma, colon	Control of imprinting. Containing miRNA miR-675	50–56,59,60
Tumor-suppressor lncRNAs	LincRNA-p21	~3.1 kb	Intergenic, upstream of p21/Cdkn1a	Induced by p53 upon DNA damage	Global gene repression in the p53 transcriptional response by binding hnRNP-K, inducing cellular apoptosis	61,62
	GAS5	0.6–1.8 kb	Intergenic, Chr1q25.1	Breast	Binding to GR as a decoy and blocking transcriptional induction by GR, induces growth arrest and apoptosis	64
	CCND1	≥200–300 nt	Transcribed from the promoter region of *cyclin D1* gene	Induced by DNA damage	Binding to TLS protein induces TLS allosteric change, allowing interaction with cyclin D1, inhibiting CBP and p300 activity, and silencing *cyclin D1* gene expression	67

as metastasis and survival rate, linking a lncRNA with cancer invasiveness and patient prognosis.[24] Furthermore, if cells expressing HOTAIR were grafted into mouse mammary fat pads, a modest increase in the rate of primary tumor growth was observed, while depletion of HOTAIR from cancer cells led to a reduced invasiveness of cells that express a high level of polycomb proteins.[24] Interestingly, there are reports indicating that numerous lncRNAs are transcribed from the HOX locus, suggesting that HOTAIR may be only one example of a global regulatory phenomena.[25]

HOTAIR has been demonstrated to be intimately associated with the mammalian polycomb-repressive complex 2 (PRC2), which is comprised of the H3K27 methylase EZH2, SUZ12 and EED.[24,26,27] Polycomb group proteins mediate repression of transcription of thousands of genes controlling differentiation pathways during development, and have roles in stem cell pluripotency and human cancer.[24,28–31] HOTAIR guides as well as serves as a scaffold for PRC2 and LSD1/CoREST complexes at their endogenous target genes. The 5′ region of HOTAIR binds the PRC2 complex responsible for H3K27 methylation, whereas the 3′ region of HOTAIR binds LSD1, a histone lysine demethylase that mediates enzymatic demethylation of H3K4Me2,[26] leading to the loss of an activating histone mark (ie, H3K4 dimethylation) and the gain of a repressive histone mark (H3K27 trimethylation) at genes targeted by HOTAIR. Although the precise mechanism of HOTAIR activities remains to be elucidated, it is tempting to speculate that the functional interdependency between HOTAIR and PRC2 could contribute to promote cancer invasiveness by regulating epithelial-to-mesenchymal transition.

ANRIL—Antisense NcRNA in the INK4 Locus

Global transcriptome analysis shows that up to 70% of protein-coding transcripts have antisense partners, and the perturbation of the antisense RNA can alter the expression of the sense gene.[32] Some of these genes encode tumor-suppressor proteins that can become epigenetically silenced by the expression of the antisense ncRNA. One example of a tumor-suppressor gene that is epigenetically silenced by an antisense RNA is ANRIL, which is altered in an estimated 30–40% of human tumors.[33] INK4b-ARF-INK4a locus, encodes three tumor-suppressors, $p15^{INK4b}$, $p14^{ARF}$ and $p16^{INK4a}$, has central roles in cell cycle inhibition, senescence and stress-induced apoptosis. Previous studies have shown that overexpression of ANRIL in prostate cancer results in the silencing of INK4b-ARF-INK4a and p15/CDKN2B by heterochromatin formation.[34,35] Like the lncRNA HOTAIR, which binds both the PRC2 and LSD1 complex, ANRIL binds and recruits two polycomb repressor complexes modifying complexes, PRC1 and PRC2,[35,36] resulting in the targeting of this complex to the chromatin and the establishment of repressive epigenetic marks.[35] These observations suggest that lncRNA-mediated silencing of tumor-suppressor genes may be a major mechanism driving tumorigenesis.

MALAT1—Metastasis-Associated Lung Adenocarcinoma Transcript 1

The lncRNA MALAT1 was first associated with high metastatic potential and poor patient prognosis

during a report employed a subtractive hybridization approach to determine differences in gene expression between primary non-small cell lung cancer tumors of five patients that were cured by surgery and tumors of four patients that subsequently metastasized.[37] This lncRNA, localized in nuclear speckles, was widely expressed in normal human tissues[37,38] but was found to be upregulated in six other types of cancer, including hepatocellular carcinoma, breast, pancreas, osteosarcoma, colon and prostate cancers.[39–43] In addition, increased expression of MALAT1 has been recently shown to be an independent prognostic factor for HCC following liver transplantation.[44] Notably, the MALAT1 locus at 11q13.1 has been identified to harbor chromosomal translocation breakpoints associated with cancer.[45–47]

Following the correlation between high levels of MALAT1 expression and cancer, a number of studies have implicated MALAT1 in the regulation of cell mobility. For example, RNA interference-mediated silencing of MALAT1 reduced the *in vitro* migration of lung adenocarcinoma cells by influencing the expression of motility-related genes.[48] Similarly, short hairpin RNA inhibition of MALAT1 in human cervical cancer cells was shown to suppress cell proliferation and invasion.[49] The lncRNA MALAT1 is thought to regulate alternative splicing by modulating serine/arginine (SR) splicing factor phosphorylation.[19] Depletion of MALAT1 alters splicing factor localization and activity, leading to altered pattern of alternative splicing for a set of pre-mRNAs.[19] In sum, these studies reinforce the role of MALAT1 as an oncogenic lncRNA, and suggest that MALAT1 regulates the invasive potential of metastatic tumor cells through their interaction with and modulation of splicing factor proteins.

H19

The *H19* gene encodes a 2.3-kb lncRNA that is exclusively expressed from the maternal allele, and it has an important role in genomic imprinting during growth and development.[50] H19 is reported to be reactivated during adult tissue regeneration and tumorigenesis. Loss of imprinting at the H19 locus resulted in high H19 expression in cancers of the esophagus, choricarcinoma, liver, breast, bladder and with hepatic metastases.[51–55] The product of the MYC oncogene is widely deregulated in cancer and functions as a regulator of gene transcription. c-Myc significantly induces the expression of the H19 lncRNA in diverse cell types, including breast epithelial, glioblastoma and fibroblast cells. The c-Myc oncogene directly induces the H19 lncRNA by allele-specific binding to potentiate tumorigenesis.[56]

It is unclear what proportion of lncRNAs may be precursors for small RNA species. Previous genomic and transcriptomic surveys, including recent RNA-sequencing surveys, have now confirmed that a significant fraction of long unannotated transcripts could be natural precursors for miRNA-like small RNAs.[57,58] Indeed, H19 transcripts also serve as a precursor for miR-675, a miRNA involved in the regulation of developmental genes.[59] MiR-675 is processed from the first exon of H19 and functionally downregulates the tumor suppressor gene retinoblastoma (*RB1*) in human colorectal cancer.[60] H19 RNA harbors protumorigenic properties; thus, the *H19* gene behaves as an oncogene and may serve as a potential new target for antitumor therapy.

In short, these studies point to the possibility of 'oncogenic lncRNAs' that upon misregulation could either silence tumor-suppressor genes or induce the expression of oncogenes priming the cell for transformation.

Tumor-suppressor lncRNAs

Tumor-suppressor lncRNAs could phenotypically affect cells by promoting tumor-suppressor pathways, and when their function is compromised, cells are prone to develop cancer. In support of this notion, a few new studies have elucidated several examples of 'tumor-suppressor lncRNAs'.

LincRNA-p21

In response to DNA damage, recent studies identified numerous lncRNAs that are induced by the p53 tumor-suppressor pathway,[61,62] suggesting the complex p53 transcriptional network includes numerous regulatory lncRNAs. In particular, one of these lncRNAs, named lincRNA-p21, an ~3.1-kb transcript located in the proximity of the cell cycle regulator gene *Cdkn1a*, was directly induced by p53 to have a critical role in the p53 transcriptional response. Interestingly, the mechanism by which lincRNA-p21 repressed of p53 target genes required the association with heterogeneous nuclear ribonucleoprotein K (hnRNP-K). Loss of lincRNA-p21 led to hnRNP-K mislocalization and resulted in a similar tumor-suppressor phenotype to p53.[62] Although lincRNA-p21 has not been directly associated with disease, this study underlines the importance of well-tuned regulation of lncRNAs to orchestrate transcriptional programs that maintain cellular homeostasis, it is tempting to speculate that loss of function of some lncRNAs could be an important factor contributing to cancer initiation because it may trigger cell death through induction of apoptosis. It remains to be determined how the repressive complex associated with lincRNA-p21 recognizes targeted gene loci and how this complex silences transcription.

GAS5—Growth Arrest-Specific 5

The ability of a lncRNA to modulate the effects of a transcription factor can lead, in some cases, to significant changes in gene expression and subsequently profound effects on the cells ability to respond to external stimuli. GAS5 is highly expressed in cells that have arrested growth and can sensitize a cell to apoptosis by regulating the activity of glucocorticoids in response to nutrient starvation.[63,64] GAS5 functions by interacting directly with the DNA-binding sites of the glucocorticoid receptor (GR), a specific class of nuclear receptors, preventing GR interaction with cognate glucocorticoid response elements, thus reducing cell metabolism,[65] thereby in effect acting as a molecular decoy. This may turn out to be an integral component of the regulatory machinery for modulating steroid hormone activity in target tissue. Interestingly, GAS5 has also been observed to be downregulated in breast cancer, perhaps to keep cancer cells active even under low-nutrient conditions.[63,64] Further studies are needed to determine the underlying mechanisms of how a lncRNA, once activated, modulates the activity of transcription factor(s) to allow the cells to respond to their environment.

CCND1

Another tumor-suppressor lncRNA is involved in the regulation of *cyclin D1*/*CCND1* gene expression. Cyclin D1 is a cell cycle regulator frequently mutated, amplified and overexpressed in a variety of tumors.[66] A set of single-stranded, low-abundance lncRNAs, produced from the cyclin D1 (CCND1) promoter region, have previously been shown to allosterically modulate the activity of an RNA-binding protein known as translocated in liposarcoma (TLS), which is a key transcriptional regulatory sensor of DNA damage signals.[67] Upon binding these lncRNAs, the TLS protein changes from an inactive to an active conformation, so that it binds and inhibits the enzymatic activities of the histone acetyltransferases such as CREB-binding protein and p300,[67] thus silencing cyclin D1 gene expression.

Collectively, these studies show that tumor-suppressor lncRNAs can be rapidly induced by cellular stress to regulate gene expression. Possibly, RNA molecules, due to their quick turn over rate, are ideal effectors when a rapid response is required to protect cells from external insults.

LncRNAs as diagnostic and therapeutic tools

Cancer is a multi-factorial, multi-step and complicated disease. It is known that some desirable molecular markers of malignancy are important diagnostic and prognostic tools, which can help patient management in the oncology clinics. Numerous proteinaceous molecular markers for various cancer have been discovered and validated over the last few decades. However, a clear advantage in the diagnostic use of ncRNA detection *versus* that of protein-coding RNAs is that in the former the RNA itself is the effector molecule, thus its expression levels may be a better indicator of the intrinsic characteristics of the tumor. Identification of lncRNAs correlated to cancer has benefited from the development of a number of effective high-throughput expression analyses technologies as well as from the increasing realization that lncRNAs are emerging as new factors, demonstrating their potential roles in both oncogenic and tumor suppressive pathways. Dozens of lncRNAs have been found to be dysregulated in prostate cancer, breast cancer, colorectal cancer, hepatocellular carcinoma, bladder cancer, lung cancer and others types of cancer (Table 2),[68–84] raising the possibility that lncRNAs may become a promising biomarker in cancer diagnosis and prognosis (Figure 2a). For example, increased expression of lncRNA HOTAIR was shown to be associated with metastasis in breast cancer patients, having a unique association with patient prognosis.[24] In addition, HOTAIR expression levels was found to correlate with metastasis in colorectal cancer,[85] and to predict tumor recurrence in HCC patients who have undergone liver transplant therapy.[86]

Ideally, biomarkers should be easily accessible such that they can be sampled non-invasively. Therefore, biomarkers that can be sampled from body fluids, such as serum or urine, are particularly desirable. Circulating nucleic acids (CNAs), both RNA and DNA species, are extracellular nucleic acids found in cell-free serum, plasma and other body fluids from healthy subjects, as well as from patients.[87] In addition, there is evidence of a good correlation between tumor-associated changes in genomic, epigenetic or transcriptional patterns, and alterations in CNA levels,[88] strongly pointing to the utility of this blood biomarker class as promising clinical tools. Our recent studies and others have suggested that cancer-specific miRNAs are stable and detectable in the blood, sputum and urine of cancer patients.[89–92] At present, few lncRNAs have been characterized as potential biomarkers in human body fluids. For example, lncRNA PCA3 in patient urine samples has been demonstrated as a more specific and sensitive marker of prostate cancer than the widely used serum prostate-specific antigen (PSA),[93,94] highlighting its advantages over PSA and enable noninvasive diagnose.[95] Similarly, the highly expressed hepatocarcinoma-associated lncRNA HULC can be detected in the blood of hepatocarcinoma patients by conventional PCR methods.[96] We speculate that the human body fluids such as serum, plasma, sputum and urine might contain a considerable

Table 2 LncRNAs differentially expressed in human cancer

LncRNA	*Size*	*Cytoband*	*Cancer types*	*References*
HOTAIR	2.2 kb	12q13.13	Breast, liver	24,26,86,87
ANRIL	~3.9 kb	9p21.3	Prostate, leukemia	34,35
MALAT1	~7 kb	11q13.1	Breast, lung, uterus, pancreas, colon, prostate, liver, osteosarcoma	37,40,41,42,43,49
H19	2.3 kb	11p15.5	Bladder, lung, liver, breast, esophagus, choricarcinoma, colon	50–56,59,60
GAS5	0.6–1.8 kb	1q25.1	Breast	64
HULC	500 nt	6p24.3	Liver, hepatic colorectal metastasis	96,105
BC200	200 nt	2p21	Breast, cervix, esophagus, lung, ovary, parotid, tongue	13,14
PRNCR1	~13 kb	8q24.2	Prostate	102
PCGEM1	1.6 kb	2q32.2	Prostate	106,107
UCA1	1.4, 2.2, 2.7 kb	19p13.12	Bladder, colon, cervix, lung, breast, stomach	68,69
DD3	0.6, 2, 4 kb	9q21.22	Prostate	108,109
MEG3	~1.6 kb	14q32.2	Myeloid leukemia, multiple myeloma and pituitary tumors	70–72
PTENP1	~3.9 kb	9p13.3	Prostate	73,74
SRA-1	875 nt	5q31.3	Breast, uterus, ovary	75–79
BIC	1.6 kb	21q11.2	B-cell lymphoma	80
LOC285194	2105 nt	3q13.31	Osteosarcoma	81
ncRAN	2186, 2087 nt	17q25.1	Bladder, neuroblastoma	82,83
LSINCT5	2.6 kb	5p15.33	Breast, ovary	84
PTCSC3	1154 nt	14q13.3	Thyroid	110

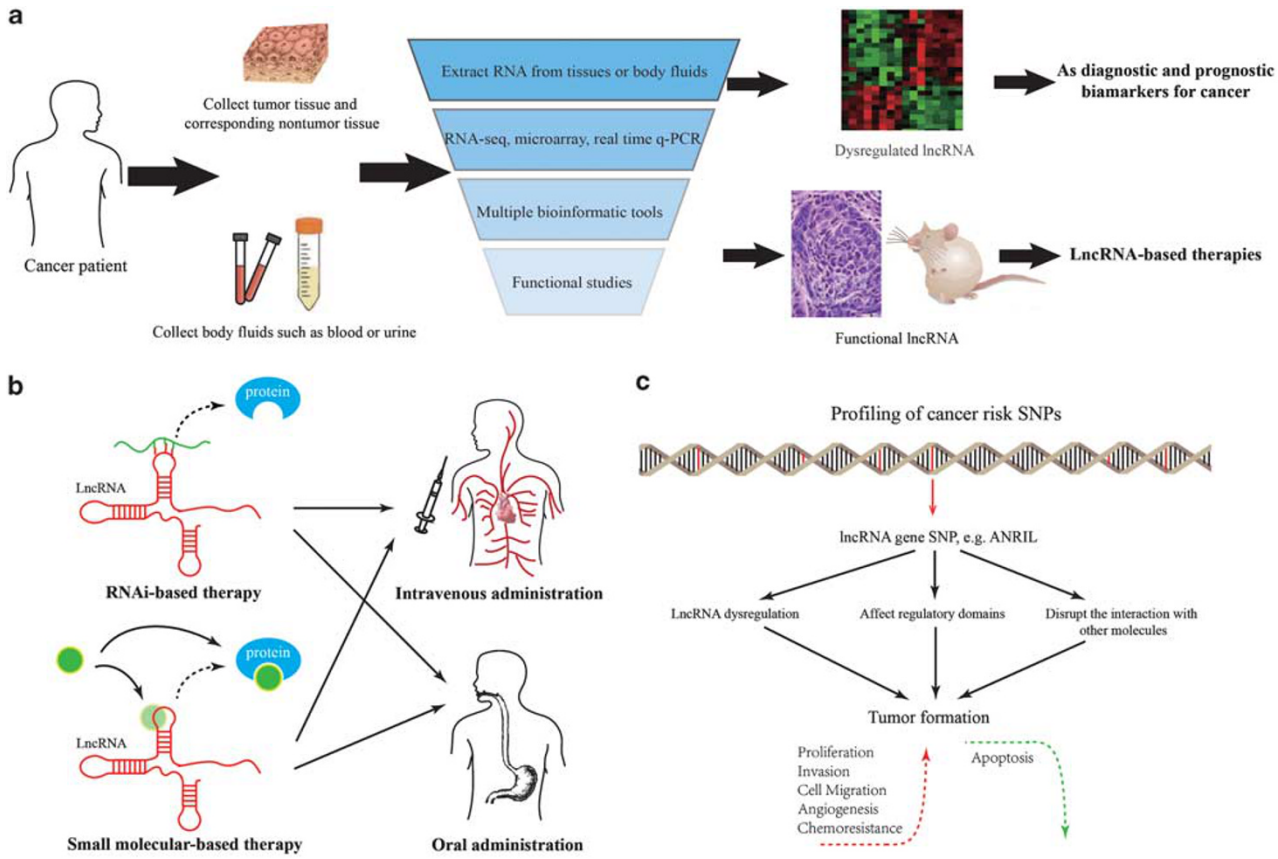

Figure 2 An overview of lncRNA research for isolation, detection and clinical applications. (**a**) Identification of lncRNAs correlated to cancer has benefited from the development of a number of effective high-throughput expression analyses technologies. Some desirable molecular markers could potentially be used for cancer diagnosis and prognosis and serve as therapeutic targets. (**b**) LncRNA-based therapies may target the lncRNA by utilizing RNA interference (RNAi) therapeutic molecule and/or use small molecular inhibitors of their protein partners. These therapeutic avenues may be appropriate for systemic therapy by either intravenous or oral administration. (**c**) Structural variations in *lncRNA* genes, such as single-nucleotide polymorphisms (SNPs), can affect the lncRNA structure and function. Future studies are required to elucidate the mechanism by which mutations in lncRNA functional motifs can affect their regulatory domains and compromise its ability to interact with other molecules, thereby leading to tumor formation. This may help to predict an individual patient's clinical risk for cancer development, aggressiveness or response to therapy in the oncology clinics.

amount of lncRNAs that will eventually be detected by the use of simple quantitative reverse transcriptase PCR or unbiased high-throughput technologies such as microarrays or RNA-seq deep-sequencing of samples. Comparative studies of lncRNAs in human body fluids from cancer patient large cohorts and from normal subjects will possibly reveal novel circulating lncRNAs as potential biomarkers in many types of cancer (Figure 2a). However, such approaches should be subjected to the same controls regarding pre-analytical variables,[97] including the reduction of contaminant hematopoietic cells in the isolation and quantization of circulating lncRNAs.

The release of nucleic acids into the blood is thought to be related to the apoptosis and necrosis of cancer cells in the tumor microenvironment and is also the result of secretion. Circulating miRNAs are detectable in the serum and plasma of cancer patients, being surprisingly stable in spite of the fact that high amounts of RNAses circulate in the blood of cancer patients. This implies that miRNA may be protected from degradation by its packaging into microparticles, which include exosomes, microvesicles, apoptotic bodies and apoptotic microparticles.[98] Although circulating lncRNAs may be promising biomarkers for cancer diagnosis and prognosis, however, this was only the tip of the iceberg, it is timely to ask important questions such as: (i) How stable are circulating lncRNAs and is their stability altered in various disease states? (ii) The reported RNA content of microvesicles and exosomes thus far includes primarily small miRNAs and long protein-coding mRNAs.[99] LncRNAs, ranging in length from 200 nt to ~100 kb, are also packaged into microparticles in a manner similar to miRNAs, only ~22 nt long? (iii) A considerable amount of circulating lncRNAs may be dysregulated in various human diseases and disorders, these lncRNAs are causing the disease or they become altered as a consequence of the disease itself? (iv) How do circulating lncRNAs exert their effects? This could represent an unexpected and yet unexplored gold mine of potential diagnostic and prognostic biomarkers.

The discovery of deregulated lncRNAs represents a new layer of complexity in the molecular architecture of human disease. In addition to the imminent use of our knowledge of cancer-associated lncRNAs for diagnosis and prognosis, the use of lncRNAs as therapeutic agents is only beginning to be explored.[100] The tumor expression of certain lncRNAs provides tumor-specific regulatory regions, the targeting of which would reduce the risk of affecting normal tissues during transgene therapy. The progress in the use of RNAi-mediated gene silencing for the treatment of different diseases is encouraging and provides a straightforward approach to selectively silence oncogenic lncRNAs (Figure 2b). For example, targeting human H19 for the treatment of bladder cancer with a plasmid-based system was recently advanced by successes.[101] A novel lncRNA termed 'PRNCR1' (prostate cancer non-coding RNA 1) is upregulated in some of the prostate cancer cells as well as precursor lesion prostatic intraepithelial neoplasia, knockdown of PRNCR1 can attenuate the viability of PC cells and the transactivation activity of the androgen receptor.[102] Although our understanding of the molecular mechanisms of lncRNA function is limited, some features of lncRNAs, including structural scaffolds for protein complexes and complex RNA structural motifs, make them ideal candidates for therapeutic intervention.[103] Preventing the interactions of HOTAIR with the PRC2 or LSD1 complexes, by targeting endogenous HOTAIR and/or using small molecular inhibitors of PRC2, for example, may limit the metastatic potential of breast cancer.[104] Collectively, the recent flurry of studies regarding the roles of lncRNAs in various biological processes clearly suggest the potential roles of lncRNAs-mediated diagnostics and therapies.

Conclusions and Perspectives

In this review, we highlight characterized oncogenic and tumor-suppressor lncRNAs described to have a functional role in cancer-associated processes. Aberrant lncRNA expression participates in carcinogenesis by disrupting major biological processes and, when we have a deeper understanding of their roles in cancer, lncRNAs represent a significant untapped gold mine in cancer as diagnostic and prognostic markers, as well as the potential of developing lncRNA-mediated therapy.

Numerous lncRNAs are misregulated in a variety of human cancers; however, few have thus far been associated with a single cancer type. HULC, for example, is highly expressed in HCC and in colorectal carcinomas that metastasized to the liver,[96,105] but not in the primary colon tumors or in non-liver metastases, whereas other three lncRNAs, PCGEM1, DD3 and PRNCR1, have been associated solely with prostate cancer.[102,106–109] Another tissue-specific lncRNA is papillary thyroid carcinoma susceptibility candidate 3 (PTCSC3), which is thyroid specific and downregulated in tumor tissue compared with unaffected thyroid tissue.[110] A key goal for future progress is to identify lncRNAs that could potentially serve as biomarkers for specific disease states.

The misexpression of lncRNAs in cancer naturally raises the question as to how structural variations in *lncRNA* genes (for example, amplifications, deletions and sequence mutations), either germline or somatic, may contribute to cancer predisposition. Currently, we are only observing the tip of the iceberg. Several lines of evidence have shown that even small-scale mutations, such as single-nucleotide polymorphisms, can affect the lncRNA structure

and function. For example, mutations in ANRIL are associated with cancer and cardiovascular disease and also lead to aberrant ANRIL transcripts.[111,112] Thus, future studies are required to elucidate the mechanism by which mutations in lncRNA functional motifs can affect their regulatory domains and compromise its ability to interact with other molecules, thereby leading to the pathogenesis of disease (Figure 2c). However, compared with mutations in protein-coding genes, where certain single nucleotide mutations (such as a premature stop codon or frame shift) can completely abrogate protein function, structural variations in lncRNAs may have more subtle effects, which makes it more difficult to verify them experimentally.

In contrast to the extensive evidence that links dysregulation of protein-coding genes to disease etiology, to date only a few lncRNAs have been implicated in human disease, therapeutic applications may be possible in a more distant future. Such therapies would be useful in cases where drugs designed to target proteins have failed, or even in conjunction with available drugs to enhance their effects.[82] However, RNA therapeutics face considerable hurdles, including development of reliable delivery systems, dosage regimes and techniques to ameliorate off-target effects.[113,114] In addition, targeting transcripts the size of lncRNAs may seem like a daunting task; one must screen more small-interfering RNAs compared with mRNAs, possibly because of the extensive secondary structures in lncRNAs. Furthermore, few examples of transgenic models of lncRNA have been published to date. There is a clear need to develop genetic model systems to understand lncRNAs' function *in vivo*. When these limitations are overcome, lncRNAs may be attractive therapeutic targets owing to their high turnover rate and direct and specific regulatory functions that, working as riboswitches, control the expression of other 'conventional' genes.

In conclusion, although a lot of key questions remain unanswered, lncRNAs are shedding new light on our understanding of these cancer pathways, the potential roles of lncRNAs in biology and medicine could be tremendous and may be useful as novel diagnostic and prognostic markers for various cancers. In addition, they may have therapeutic applications and will require many years of intensive research before they can be fully deciphered and applied. As more examples of regulation by lncRNA are uncovered, one might easily predict that the large transcripts will eventually rival small RNAs and proteins in their versatility as regulators of genetic information.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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