CellPress

Review

Long Noncoding RNAs in Cancer: From Function to Translation

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While our understanding of the molecular mechanisms underlying cancer has significantly improved, most of our knowledge focuses on protein-coding genes that make up a fraction of the genome. Recent studies have uncovered thousands of long noncoding RNAs (lncRNAs) that populate the cancer genome. A subset of these molecules show striking cancer- and lineage-specific expression patterns, suggesting that they may be potential drivers of cancer biology and have utility as clinical biomarkers. We discuss emerging modalities of lncRNA biology and their interplay with cancer-associated concepts, including epigenetic regulation, DNA damage and cell cycle control, microRNA silencing, signal transduction pathways, and hormone-driven disease. In addition, we highlight the translational impact of lncRNAs, tools for their mechanistic investigation, and directions for future lncRNA research.

Trends

Thousands of lncRNAs populate the cancer genome and show cancer-specific expression patterns.

lncRNAs drive cancer biology and mediate several cancer-associated concepts, including epigenetic regulation, DNA damage and cell cycle control, miRNA silencing, signal transduction pathways, and hormone-driven disease.

New tools are emerging as powerful methods for lncRNA discovery and mechanistic investigation, including RNA-seq, ChIRP, RAP, RNA-FISH, and icSHAPE.

The MiTranscriptome compendium provides a comprehensive annotation of over 8000 previously undiscovered cancer-associated lncRNAs that may be crucial molecules for future study.

Uncovering lncRNA function may reveal new translational opportunities for biomarker development and therapeutic targeting.

The Emergence of lncRNAs in Cancer

Cancer is a complex disease consisting of multiple factors that lead to the development of malignant tumors [1]. While much progress has been made in identifying the major contributors to cancer progression, the clinical picture remains bleak. Current research efforts aim to better understand the interplay between cancer cells, tumor microenvironments, and defense mechanisms involved in cancer development, immune evasion, and therapeutic susceptibility [1]. However, the majority of these studies focus on protein-coding genes as the crucial components in disease progression, overlooking the vast landscape of noncoding genes.

Among these noncoding transcripts are lncRNAs. lncRNAs are RNA species greater than 200 bp in length commonly characterized by polyadenylation, splicing of multiple exons, promoter trimethylation of histone H3 at lysine 4 (H3K4me3), and transcription by RNA polymerase II [2,3]. lncRNA-mediated biology has been implicated in a wide variety of cellular processes, including pluripotency in mouse embryonic stem cells [4] and X chromosome inactivation [5]. While some lncRNAs, such as *XIST* (X inactive specific transcript) appear to operate exclusively in the nucleus as regulators of gene expression [5,6], others function predominantly in the cytoplasm to regulate signal transduction and the stability of mRNAs [7–9]. Several distinct mechanisms of lncRNA activity have been described. Most prominently, lncRNAs have been shown to collaborate with protein partners to form **ribonucleoprotein complexes** (RNP, see Glossary) [10]. For example, *XIST* interacts with the **Polycomb repressive complex 2** (PRC2), resulting in PRC2 recruitment and subsequent trimethylation of histone H3 at lysine 27 (H3K27me3) of the inactive X chromosome [11]. *Air* and *Kcnq1ot1* bind to G9a, a histone H3 lysine 9 methylase, to regulate gene expression [12,13]. *ANRIL* associates with PRC1 to regulate the *INK4a* locus [14]. Long intergenic noncoding RNA (linc)RNA-*p21* and *PANDA* are

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two p53-regulated lncRNAs that interact with hnRNP-K and NF-YA to regulate transcription [15,16]. *lncRNA-LET* is downregulated across several cancers and functions by binding to and degrading nuclear factor 90 (NF90) protein, which enhances hypoxia-induced cancer cell invasion [17]. Given this tendency to engage proteins, lncRNAs are surfacing as decoys, scaffolds, and guides [18].

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Table 1. Examples of lncRNAs in Cancer

Name	Cancer type(s)	Tumor suppressor/ oncogene	Mechanistic theme(s)[a]	Refs
ANRIL (antisense noncoding RNA in the *INK4* Locus)	Gastric	Oncogene	Cell cycle regulation, epigenetic complex, miRNA regulation	[14,34,85]
BANCR (BRAF activated noncoding RNA)	Melanoma, NSCLC	Oncogene, tumor suppressor	Chemokine signaling, EMT	[99,100]
BCAR4 (breast cancer antiestrogen resistance-4)	Breast, multiple	Oncogene	Hedgehog signaling pathway	[90,104, 105]
CARLo-5 (cancer-associated region lncRNA-5)	Colorectal, gastric, NSCLC, prostate	Oncogene	Apoptosis, cell cycle regulation, EMT	[65–67]
CCAT1 (colon cancer associated transcript 1)	Colorectal	Oncogene	MYC	[119]
CCAT2 (colon cancer associated transcript 2)	Breast, colorectal, esophageal squamous cell, NSCLC	Oncogene	Chromosomal instability, MYC, Wnt signaling pathway	[92–95]
CTBP1-AS (C-terminal binding protein 1-antisense)	Prostate	Oncogene	Hormone-regulated	[113]
DRAIC (downregulated-RNA in androgen-independent cells)	Multiple, prostate	Tumor suppressor	Hormone-regulated	[112]
FAL1 (focally amplified lncRNA on chromosome 1)	Multiple epithelial types, ovarian	Oncogene	Cell cycle regulation, epigenetic complex	[31]
gadd7 (growth-arrested DNA damage-inducible lncRNA)	Non-specific	Tumor suppressor	Cell cycle regulation, DNA damage response	[68]
GAPLINC (gastric adenocarcinoma predictive lincRNA)	Gastric	Oncogene	ceRNA	[84]
GAS5 (growth arrest specific 5)	Non-specific, mesothelioma, prostate	Tumor suppressor	Apoptosis	[71,72,108]
H19	Colorectal, gastric, glioma, osteosarcoma, pancreatic	Oncogene	miRNA interaction, signal transduction	[76–80,91]
HOTAIR (HOX transcript antisense intergenic RNA)	Breast, colorectal, hepatocellular, GIST	Oncogene	Epigenetic complex, miRNA regulation	[19–22]
HOTTIP (HOXA transcript at the distal TIP)	Hepatocellular	Oncogene	Epigenetic complex	[53,54]
HULC (hepatocellular upregulated lncRNA)	Hepatocellular	Oncogene	ceRNA	[82,126, 127]
LED (lncRNA activator of enhanced domains)	Leukemia, non-specific	Tumor suppressor	Cell cycle regulation, epigenetic regulation	[63]
lincRNA-p21	Non-specific	Tumor suppressor	Cell cycle regulation, epigenetic regulation	[15,59]

Table 1. (continued)

Name	Cancer type(s)	Tumor suppressor/ oncogene	Mechanistic theme(s)[a]	Refs
lncRNA-ATB (lncRNA-activated by TGF-β)	Hepatocellular	Oncogene	ceRNA, EMT, TGF-β signaling	[83]
lncRNA-HEIH (lncRNA-high expression In HCC)	Hepatocellular	Oncogene	Cell cycle regulation, epigenetic complex	[33]
lncRNA-LET (low expression in tumor)	Colorectal, hepatocellular, lung squamous	Tumor suppressor	Hypoxia, metastasis	[17]
lncTCF7	Hepatocellular	Oncogene	Epigenetic complex, Wnt signaling pathway	[42]
MALAT1 (metastasis-associated lung adenocarcinoma transcript-1)	Endometrioid endometrial, lung, renal cell	Oncogene	EMT, metastasis, Wnt signaling pathway	[96–98, 129–132]
MEG3 (maternally expressed 3)	Colorectal, gastric, hepatocellular, meningioma, NSCLC	Tumor suppressor	DNA damage response, miRNA interaction	[60,61]
MIR31HG	Melanoma	Tumor suppressor	Cell cycle regulation, OIS	[64]
NBAT-1 (neuroblastoma associated transcript-1)	Neuroblastoma	Tumor suppressor	Epigenetic complex	[32]
NEAT1 (nuclear enriched abundant transcript-1)	Breast, multiple solid types, prostate	Oncogene	Epigenetic regulation, hormone-regulated, hypoxia	[106,107]
NKILA (NF-κB interacting lncRNA)	Breast	Tumor suppressor	Inflammation in tumor microenvironment, regulation of signal transduction	[122]
PANDA (P21-associated ncRNA DNA damage activated)	Non-specific, leukemia	Tumor suppressor	Cell cycle regulation, DNA damage response	[16,40]
PCAT-1 (prostate cancer associated transcript 1)	Prostate	Oncogene	miRNA-like function, regulation of DNA damage repair, repression of a tumor suppressor	[69,70,86]
PCAT29 (prostate cancer associated transcript 29)	Prostate	Tumor suppressor	Hormone-regulated	[111,112]
PCGEM1 (prostate-specific transcript 1)	Prostate	Oncogene	Hormone-regulated, MYC	[114–116, 118]
PRNCR1 (prostate cancer associated ncRNA 1)	Prostate	Oncogene	Hormone-regulated	[114,115]
PVT1	Colorectal	Oncogene	MYC	[120]
SChLAP1 (second chromosome locus associated with prostate 1)	Prostate	Oncogene	Epigenetic complex	[41,50–52]
TARID (TCF21 antisense RNA inducing demethylation)	Non-specific	Tumor suppressor	DNA demethylation, epigenetic regulation	[23]
TUG1 (taurine upregulated 1)	Esophageal squamous cell, NSCLC	Oncogene	DNA damage response, epigenetic complex	[35]
XIST (X inactive specific transcript)	Breast, hematologic	Tumor suppressor	Epigenetic complex	[11,36]

[a]Abbreviations: ceRNA, competing endogenous-RNA; EMT, epithelial–mesenchymal transition; GIST, gastrointestinal stromal tumor; NSCLC, non-small cell lung cancer; OIS, oncogene induced senescence.

Glossary

Chromatin remodeler: a protein complex that physically changes DNA architecture to allow or restrict access of regulatory proteins and transcription machinery to DNA. The function of chromatin remodelers is often carried out by epigenetic modifications using the energy of ATP hydrolysis.

Enhancer RNA (eRNA): a class of non-coding RNAs that plays a role in enhancer DNA-mediated transcriptional regulation. eRNAs have been shown to recruit RNA polymerase II to these DNA regions to assist in the initiation of gene transcription.

Epigenetics: the heritable variations in gene expression that result due to differences in how DNA is read rather than in the DNA sequence itself. Epigenetic alterations include DNA methylation and histone modifications.

Epithelial–mesenchymal transition (EMT): the cellular mechanism by which epithelial cells gain migratory and invasive properties to form mesenchymal cells. This phenomenon is often seen when cancer cells gain the ability to invade and metastasize to distant organs. EMT can also occur in normal biological processes, such as wound healing.

Hedgehog signaling pathway: a cellular signaling pathway that is required for the regulation of embryonic cell development. Aberrations in Hedgehog signaling may result in developmental or growth defects.

Oncogene-induced senescence (OIS): a sustained induction of the Rb and p53 tumor suppressive pathways in response to an activating mutation in an oncogene or loss of tumor-suppressive activity within a cell. The mechanism by which OIS occurs has not been fully elucidated; however, it is known to protect against the progression to cancer in response to oncogenic stress.

Polycomb repressive complex 2 (PRC2): a multiprotein complex containing the core components of SUZ12, EED, RbAp48, and EZH2. PRC2 primarily functions as a histone methyltransferase, adding a trimethyl group to histone H3 on lysine 27 (H3K27me3) to produce transcriptionally silent chromatin.

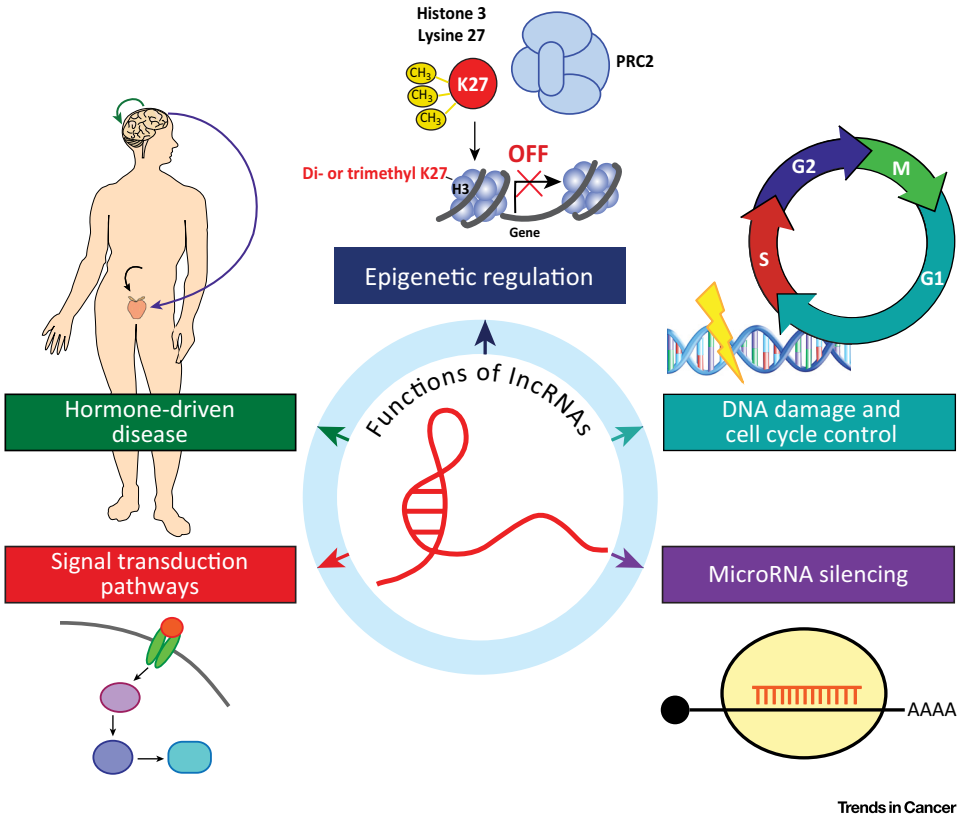

Figure 1. lncRNAs Play a Crucial Role within Major Areas of Cancer Progression and Metastasis. Long noncoding RNA (lncRNAs) mediate several cancer-associated processes, including epigenetic regulation, DNA damage and cell cycle control, microRNA (miRNA) silencing, signal transduction pathways, and hormone-driven disease.

In cancer, lncRNAs are emerging as a prominent layer of previously underappreciated transcriptional regulation that function as both oncogenes and tumor suppressors [2] (Table 1). For example, overexpression of the *HOTAIR* lncRNA correlates with aggressive breast [19], colorectal [20], hepatocellular [21], and gastrointestinal stromal tumors [22], while lncRNA *TARID* prevents cancer formation through promoter demethylation at tumor suppressors [23]. In this review we discuss emerging themes of lncRNA-mediated function within major areas of cancer progression and metastasis, focusing on advances made over the past several years (Figure 1).

Epigenetic Regulation

Cancer results from an accumulation of modified genes, either by mutation or **epigenetic** alterations such as methylation, acetylation, and phosphorylation [24]. Growing evidence suggests that key cellular genes involved in proliferation, apoptosis, and stem cell differentiation are epigenetically modified in cancer [25]. However, the mechanisms underlying precise epigenetic control are poorly understood.

An evolving model of lncRNA activity centers on their ability to bind to and regulate epigenetic complexes [26]. Specifically, several lncRNAs have been shown to function by interacting with Polycomb group complexes [18]. This is especially relevant in cancer because PRC1 and 2 are known oncogenic drivers in several types of malignancies [27–30]. For example, *FAL1* (focally amplified lncRNA on chromosome 1), a novel oncogenic lncRNA present across several epithelial tumors, associates with BMI1, a core subunit of the PRC1 complex [31]. In ovarian cancer, *FAL1* was shown to mediate cancer progression and was associated with decreased patient survival. *FAL1* interaction with BMI1 stabilizes the PRC1 complex by preventing BMI1

Ribonucleoprotein complex (RNP): a cellular complex containing RNAs and proteins.
Transforming growth factor-β (TGF-β) signaling pathway: a cellular signaling pathway involved in numerous physiological processes, including cell differentiation, cell growth, and apoptosis. Ligand binding initiates a cascade of signaling through serine/threonine receptor kinase activity.
Wnt signaling pathway: a cellular signaling pathway initiated by binding of Wnt ligand to a Frizzled family receptor, resulting in transmission of this signal to Dishevelled within the cell. This leads to downstream regulation of genes involved in embryonic development and in cell differentiation, migration, and proliferation.

degradation, allowing PRC1 to occupy and repress the promoters of target genes such as *p21*, resulting in loss of cell cycle regulation and increased tumorigenesis.

Similarly, *NBAT-1*, *lncRNA-HEIH*, *HOTAIR*, *ANRIL*, *TUG1*, and *XIST* have all been shown to interact with the enzymatic subunit of the PRC2 complex, EZH2, to modulate the repressive H3K27me3 histone mark on downstream target genes. This subsequently leads to either oncogenesis or tumor suppression in a multitude of cancer types, including neuroblastoma [32], hepatocellular [33], breast [19], gastric [34], non-small cell lung carcinoma (NSCLC) [35], and hematologic malignancies [36], respectively. In fact, up to 20% of all lncRNAs have been implicated in PRC2 binding [37], suggesting that PRC2 promiscuously binds to lncRNAs [38]. Recent studies have shown both specific and non-specific binding of PRC2 to lncRNAs, and emerging evidence suggests that these activities are not mutually exclusive [39]. However, the *in vivo* binding specificity of PRC2 remains to be elucidated.

One of the most-studied lncRNAs, *HOTAIR* (HOX transcript antisense RNA), recruits the PRC2 complex to a set of genes involved in suppressing breast cancer metastasis [19]. This genome-wide retargeting of PRC2 results in repression of genes that prevent cancer progression. In addition, *HOTAIR*-mediated genetic reprogramming results in gene expression signatures that resemble embryonic fibroblast gene signatures, and this promotes cell migration, invasion, and metastasis. lncRNAs can also interact with Polycomb group complexes indirectly. For example, *PANDA* (P21 associated ncRNA DNA damage activated) physically interacts with scaffold-attachment-factor-A (SAFA) to indirectly recruit both the PRC1 and PRC2 complexes to the promoters of genes involved in cellular senescence [40]. This suggests that lncRNAs can facilitate epigenetic changes through interaction with protein intermediates.

In addition to Polycomb group complexes, several lncRNAs have been linked to the SWI/SNF nucleosome-remodeling complex in cancer and other diseases [41–44]. SWI/SNF is a multi-subunit complex that uses the energy of ATP hydrolysis to redistribute and rearrange nucleosomes to influence gene expression [45,46]. In cancer, SWI/SNF is widely considered to be a tumor suppressor because deleterious mutations are present in approximately 20% of all cancers [45,47–49]. Indeed, *SChLAP1* (second chromosome locus associated with prostate-1), a prostate cancer-specific lncRNA that is highly expressed in 15–30% of localized and metastatic tumors [41], is significantly associated with poor clinical outcomes and lethal disease. Moreover, *SChLAP1* expression enhances tumor invasion and metastasis, in part, by interacting with and abrogating genome-wide binding of the SWI/SNF complex. Subsequent studies have defined *SChLAP1* as one of the best prognostic genes in prostate cancer and have also shown the clinical utility of *SChLAP1* as both a tissue- and urine-based biomarker [50–52]. Comparably, *lncTCF7* is highly expressed in hepatocellular carcinoma (HCC) and is required for the maintenance of self-renewal capacity in liver cancer stem cells (CSC) [42]. Functionally, *lncTCF7* triggers the Wnt signaling pathway by binding to and recruiting the SWI/SNF complex to the *TCF7* promoter to activate gene expression. This preserves the self-renewal capabilities of liver CSCs and promotes tumor initiation in HCC. lncRNA-mediated SWI/SNF regulation has also been described in other cellular and disease processes. For example, polymerase V-transcribed lncRNAs indirectly interact with the SWI/SNF complex to mediate transcriptional silencing [43]. In addition, the cardio-protective lncRNA *Mhrt* directly interacts with BRG1, the catalytic subunit of SWI/SNF, to prevent cardiac hypertrophy [44]. Taken together, these studies suggest that lncRNAs play an important role in SWI/SNF regulation, and systematic efforts to characterize similar lncRNA mediators of SWI/SNF in other cancers are warranted.

In addition, *HOTTIP* (HOXA transcript at the distal tip) is another lncRNA upregulated in HCC [53]. *HOTTIP* expression is associated with clinical progression of HCC and is also an independent predictor of overall survival. Mechanistically, *HOTTIP* regulates the HOXA locus by

interacting with the WDR5/MLL epigenetic complex to drive H3K4me3 [54]. Previous studies have identified an RNA binding pocket on WDR5 [55], suggesting that direct binding of lncRNAs to WDR5/MLL may similarly promote other cancers.

Epigenetic control by lncRNAs is not only exercised via interactions with **chromatin remodelers**. For example, *TARID* (TCF21 antisense RNA inducing demethylation) directs promoter demethylation of the tumor-suppressive transcription factor TCF21 [23]. *TARID* is normally expressed in benign lung, oral, and ovarian epithelium but suppressed in cancer owing to hypermethylation of its promoter. *TARID* acts as a scaffold to recruit GADD45A, a DNA demethylator, to the *TCF21* promoter, resulting in demethylation of the *TCF21* promoter through the base-excision repair pathway. The physical interaction between the *TCF21* promoter, *TARID*, and GADD45A is crucial for TCF21 expression and tumor suppression.

Insight into the biology and mechanism of lncRNAs provides a basis for the understanding of the global epigenetic modifications that occur in cancer.

DNA Damage and Cell Cycle Regulation

Proper responses to DNA damage and appropriate regulation of cell cycle checkpoints are essential for maintenance of cell integrity [56]. With alterations in more than 50% of all cancers, the p53 tumor suppressor mediates responses to DNA damage to prevent tumor-associated changes in cell metabolism, cell cycle checkpoint regulation, and cell motility during cancer development [57]. While our current knowledge of these pathways is guiding targeted drug development in cancer [58], a thorough understanding of the mechanisms governing p53-related function in early tumorigenesis remains elusive.

lncRNAs have surfaced as important regulators of p53 action and cell cycle regulation in cancer. For example, *lincRNA-p21* is regulated by p53 and serves as a repressor in p53-dependent transcriptional responses by physically associating with and guiding hnRNP-K to precise genomic targets [15]. Functionally, *lincRNA-p21* is crucial for p53-mediated apoptosis in response to DNA damage. *lincRNA-p21* recruits hnRNP-K in *cis* to promote p53-dependent transcription of p21, which is a well-known checkpoint regulator in the p53 pathway [59]. The absence of *lincRNA-p21* compromises the G1/S checkpoint and results in increased proliferation.

Several lncRNAs are related to p53 regulation in response to cell stress, including *MEG3*, *TUG1*, *PANDA*, and *LED*. The imprinted lncRNA *MEG3* (maternally expressed gene 3) regulates cell proliferation and apoptosis by activating p53 in meningioma [60] and NSCLC [61]. *TUG1* [35] and *PANDA* [16] are directly regulated by p53 binding to their promoters following DNA damage, and *TUG1* and *PANDA* expression are reduced in primary lung and breast tumors, respectively, compared to normal tissue. Mechanistically, *TUG1* recruits PRC2 to the promoter of *HOXB7*, reducing HOX-mediated cell proliferation; *PANDA* binds to and abrogates chromatin binding of NF-YA, leading to repression of apoptotic gene expression programs.

Upon cellular stress, p53 also directly regulates **enhancer RNAs** (eRNAs), which function by altering the expression of neighboring genes [62]. While many p53-induced eRNAs have p53-binding sites, some do not, suggesting that another mediator is involved in regulating this subset of p53-responsive eRNAs. Recently, *LED* (lncRNA activator of enhancer domains) was identified as a p53-induced lncRNA that associates with and activates several of these remaining enhancers [63]. *LED* prominently associates with the p21 enhancer, and *LED* knockdown significantly influences G1 checkpoint arrest and increases cell proliferation. Mechanistically, *LED* impacts eRNA production by epigenetically increasing the deposition of the active enhancer histone mark, H3K9ac, at specific loci. Interestingly, *LED* expression is downregulated by

hypermethylation in 44% of cancer cell lines, suggesting that *LED* is a p53-responsive lncRNA that regulates the p53 transcriptional response and has tumor-suppressive function.

Other lncRNAs play a vital role in mediating senescence and cell cycle arrest. The lncRNA *MIR31HG* is upregulated during **oncogene-induced senescence** (OIS) and antagonizes the tumor-suppressive function of $P16^{INK4A}$, resulting in decreased cell progression to S phase of the cell cycle [64]. *MIR31HG* functions by mediating Polycomb group protein-mediated repression of the *INK4A* locus. *CARLo-5* (cancer-associated region long non-coding RNA), a lncRNA implicated in colorectal cancer [65], prostate cancer [65], gastric cancer [66], and NSCLC [67], functions by blocking cell cycle arrest at the G1 phase, resulting in uninhibited cell proliferation. lncRNA *gadd7* (growth-arrested DNA damage-inducible gene 7) inhibits the G1/S cell cycle transition, and its expression is induced in response to DNA-damaging agents, including UV irradiation, cisplatin, and growth arrest [68]. Prostate cancer-specific lncRNA *PCAT-1* (prostate cancer-associated transcript 1) is involved in the transcriptional repression of many genes related to mitosis and the cell cycle [69]. *PCAT-1* expression is inversely correlated with BRCA2 levels, and cells overexpressing *PCAT-1* accumulate double-strand breaks (DSB) after treatment with DNA-damaging agents, suggesting its involvement in homologous recombination and DSB repair [70]. Downregulation of the tumor-suppressive lncRNA *GAS5* (growth arrest-specific 5) promotes cell proliferation, in part, by regulating cell cycle factors such as CDK6, E2F1, and p21 [71,72].

Taken together, these mechanisms suggest that a subclass of lncRNAs are crucial gatekeepers of DNA damage repair, cell cycle progression, and apoptosis, and that lncRNA dysregulation in this context contributes, in part, to cancer cell immortality.

miRNA Silencing

miRNAs are small transcripts that have emerged as a prominent class of regulatory genes in numerous diseases, including cancer [73]. miRNAs bind to complementary sequences on target RNAs, leading to repressed gene expression and blocked protein synthesis. Several lncRNAs mediate cancer progression by altering miRNA function. In the competing endogenous RNA (ceRNA) model, lncRNAs that harbor miRNA response elements can bind to and sequester miRNAs, preventing target transcript degradation [9,74]. While some experimental evidence has questioned the validity of the ceRNA hypothesis [75], many lncRNAs function via miRNA pathways, both directly and indirectly.

The *H19* lncRNA has been studied for decades as an important genetic factor in development and cancer [76]. Two miRNA-based mechanisms have been described regarding its function. First, *H19* encodes for and produces miR-675 to promote gastric cancer [77], colorectal cancer [78], and glioma [79]. Next, *H19* modulates the let-7 family of miRNAs [80], which have vital roles in development, cancer, and metabolism [81]. Specifically, *H19* was found to harbor both canonical and non-canonical binding sites for let-7 and acts as a miRNA sponge to sequester and regulate the let-7 family of miRNAs.

In HCC, *HULC* (highly upregulated in liver cancer) and *lncRNA-ATB* (activated by TGF-β) have been shown to function by miRNA-facilitated modalities. *HULC*, one of the most highly expressed lncRNAs in HCC, is a CREB (cAMP response element binding protein)-regulated transcript that acts as a miRNA sponge to downregulate several miRNAs, including miR-372, leading to decreased translational repression of *PRKACB* and induced activation of CREB [82]. This results in an auto-regulatory loop in which *HULC* promotes its own expression. *lncRNA-ATB* enhances **epithelial–mesenchymal transition** (EMT), leading to cancer progression and tumor metastasis [83]. High *lncRNA-ATB* expression is correlated with decreased recurrence-free survival and overall survival in HCC patients. *lncRNA-ATB*

interacts with several miR-200s which have been previously shown to play a role in EMT suppression. Increased *lncRNA-ATB* expression results in decreased miR-200 levels, suggesting that *lncRNA-ATB* functions as a microRNA sponge. Remarkably, *in vivo* xenograft studies showed that mutating miR-200 target sites on *lncRNA-ATB* decreased the abundance of circulating tumor cells in mice [83].

In gastric cancer, *GAPLINC* (gastric adenocarcinoma predictive lincRNA) was identified as the most upregulated lncRNA in cancer compared to normal tissue and correlates with poor patient outcomes [84]. Mechanistically, *GAPLINC* regulates cell migration pathways by acting as a decoy for miR211-3p, a miRNA implicated in CD44 oncogene degradation.

Moreover, lncRNAs can alter miRNA biology indirectly. The lncRNA *ANRIL* (antisense noncoding RNA in the *INK4* locus), which is known to function in tumor development and progression [85], is highly overexpressed in gastric cancer and correlates with worse disease prognosis [34]. *ANRIL* binds to PRC2 and is required for PRC2-mediated silencing of miR-99a and miR-449a. Downregulation of these miRNAs releases inhibition of E2F1 and CDK6, allowing cell cycle progression and cell proliferation. Subsequently, E2F1 reactivates ANRIL, forming a positive auto-regulatory loop.

In addition, *PCAT-1*, one of the most differentially expressed lncRNAs in prostate cancer compared to benign tissues [69], promotes cell proliferation, in part, by interfering with the regulation of *MYC* by miR-34a [86]. Studies showed that *PCAT-1* binds to the *MYC* 3′ untranslated region (3′-UTR), preventing miR-34a from engaging its target sequence. When *PCAT-1* was knocked down or a *PCAT-1*-specific miRNA was introduced into cells, MYC stabilization was compromised, suggesting that *PCAT-1* plays a crucial post-transcriptional role in MYC regulation.

These studies suggest that lncRNAs significantly influence miRNA biology by acting as a precursor for miRNAs, directly binding to and sequestering miRNAs, or indirectly interfering with miRNA expression and regulation. While the ceRNA hypothesis remains controversial, it is clear that miRNAs are one of several avenues by which lncRNAs mediate cancer progression and metastasis.

Signaling Pathways

The aberrant activation and propagation of cellular signals is a well-documented phenomenon in cancer. lncRNAs that function in these signaling pathways are becoming a major component of cancer mechanisms. As a key target of drug development, further investigation in this area will potentially reveal therapeutic vulnerabilities that can be targeted with novel compounds.

Cellular Signaling

Several studies have highlighted the role of **transforming growth factor**-β (TGF-β) [87], **Hedgehog** [88], and **Wnt** [89] **signaling pathways** in tumor development. For example, TGF-β signaling promotes cancer cell metastasis in HCC via *lncRNA-ATB*. In addition to regulating miRNAs, *lncRNA-ATB* is induced by TGF-β and stabilizes IL-11 mRNA [83]. This allows increased IL-11 secretion and downstream IL-11/STAT3 signaling in an autocrine fashion, leading to enhanced cell colonization at distant metastatic sites.

In breast cancer, *BCAR4* (breast cancer antiestrogen resistance 4) was recently identified as the most upregulated lncRNA expressed in stage III breast cancer versus normal tissue, and increased expression was seen in later stage and metastatic samples, correlating with shorter survival time in breast cancer patients [90]. *In vitro* and *in vivo* experiments showed that *BCAR4* increases breast cancer cell migration and invasion through interactions with two transcription

factors, leading to the activation of a non-canonical Hedgehog signaling pathway. In addition, overexpression of lncRNA *H19* as a result of aberrant Hedgehog signaling promotes osteosarcoma development in mice [91].

The lncRNAs *CCAT2* (colon cancer-associated transcript 2) and *MALAT1* (metastasis-associated lung adenocarcinoma transcript 1) drive tumor progression and metastasis in breast [92], NSCLC [93], esophageal [94], colorectal [95], renal cell [96], endometrial [97], and lung cancers [98] through general activation of the Wnt signaling pathway. Moreover, *lncTCF7* (described above) recruits the SWI/SNF complex to promote Wnt signaling in HCC [42].

In addition, some lncRNAs are involved in chemokine signaling. For example, *BANCR* (BRAF-regulated lncRNA 1) is upregulated in cancer tissues with the active $BRAF^{V600E}$ mutant and increases cell migration in melanoma through increased CXCL11 chemokine signaling [99]. *BANCR* also increases cell migration and invasion in NSCLC by regulating E-cadherin, N-cadherin, and Vimentin, which play key roles in EMT [100]. These studies suggest that lncRNAs may promote tumorigenesis through varying mechanisms of signal transduction.

Hormonal Regulation

Several cancers are driven by hormone regulation [101]. In particular, estrogen and androgen steroid hormones stimulate breast and prostate cancers [102,103]. Given the pivotal role of these hormone receptor pathways in propelling cancer progression, it comes as no surprise that lncRNAs are also involved in their function.

Before being described as a mediator of non-canonical Hedgehog signaling (described above), *BCAR4* was identified in a functional screen for genes involved in tamoxifen resistance [104]. Subsequent studies found that *BCAR4* expression is associated with shorter metastasis-free survival and overall survival in breast cancer patients, and *BCAR4* mediates estrogen-independent tumor growth [105]. In prostate cancer, *NEAT1* (nuclear enriched abundant transcript 1), a lncRNA necessary for nuclear paraspeckle formation [106], was identified as an estrogen receptor α (ER-α)-regulated lncRNA, with increased expression in prostate cancers compared to normal tissues [107]. *NEAT1* coordinates prostate cancer oncogenesis by interacting at promoters of prostate-cancer associated genes. Importantly, prostate cancers expressing high levels of *NEAT1* are unresponsive to androgen antagonists, suggesting that *NEAT1* may play a role in metastatic castrate-resistant prostate cancers (mCRPC). In addition, lncRNA *GAS5* mediates apoptosis in hormone-driven prostate and breast cancers through binding to steroid receptors [108].

The androgen receptor (AR) plays a central role in establishing an oncogenic cascade that drives prostate cancer progression [109]. In fact, the mainstay of treatment for prostate cancer involves androgen deprivation therapy (ADT) [110]. *PCAT29* (prostate cancer-associated transcript 29) [111] and *DRAIC* (downregulated RNA in cancer) [112] are two androgen-suppressed lncRNAs located within 20 kb of each other on chromosome 15q23. Upon androgen stimulation, AR binds to the promoters of both lncRNAs to repress their transcription. Lower *PCAT29* and *DRAIC* expression correlates with poor prognostic outcomes in prostate cancer patients. Tumors treated with ADT showed higher levels of *PCAT29*, and tumors that progressed after ADT had lower expression of *DRAIC*, suggesting that these lncRNAs may play a role in mediating mCRPC.

CTBP1-AS is another androgen-regulated lncRNA that mediates AR activity by directly inhibiting the expression of the AR co-repressor CTBP1 [113]. *CTBP1-AS* functions by recruiting histone deacetylases via the RNA-binding PTB-associated splicing factor (PSF) to target gene promoters. *CTBP1-AS* knockdown suppresses androgen-dependent cell proliferation *in vitro* and

reduces xenograft tumor growth *in vivo*. Furthermore, upregulation of *CTBP1-AS* and downregulation of *CTBP1* is detected in primary and metastatic prostate cancer samples, but not benign tissues, suggesting that this lncRNA directly contributes to prostate cancer progression.

Other lncRNAs have also been shown to directly mediate AR activity in prostate cancer. *PRNCR1* and *PCGEM1* are two lncRNAs that bind successively to AR to strongly enhance both ligand-dependent and ligand-independent AR-mediated gene activation and proliferation in prostate cancer cells [114]. However, the crucial role of these lncRNAs in mCRPC remains questionable because subsequent studies found extremely low levels of *PRNCR1* expression in metastatic prostate tumors and lack of lncRNA binding to AR in prostate cells [115]. Nevertheless, *PCGEM1* may play a role in mediating disease progression during the early stages of prostate cancer [116], and further experimentation is necessary to delineate the precise role of these lncRNAs in mediating AR function.

Downstream Mediators

The *MYC* proto-oncogene is a downstream effector of many signal transduction pathways and alterations in *MYC* are known to be oncogenic [117]. The human chromosomal 8q24 region includes a gene desert that contains enhancer elements that regulate MYC activity through long range chromatin looping. lncRNAs are also implicated in these processes. In prostate cancer, *PCAT-1* mediates *MYC* regulation [86] and *PCGEM1* (prostate cancer gene expression marker 1) co-activates AR and *MYC* to regulate tumor metabolism [118]. The colorectal cancer-specific lncRNA *CCAT1-L* mediates chromatin looping to allow the *MYC* promoter to interact with its enhancer elements [119]. lncRNA *PVT1* is transcribed from the gene desert associated with *MYC*, and *PVT1* expression is required for the oncogenic potential of *MYC*-driven human cancers [120]. Specifically, in 98% of *MYC*-amplified human tumors, *PVT1* expression is also upregulated, and *PVT1* knockdown abolishes the tumorigenicity of cancers with *MYC* amplification. However, *PVT1* itself is not sufficient to cause tumor development without concurrent

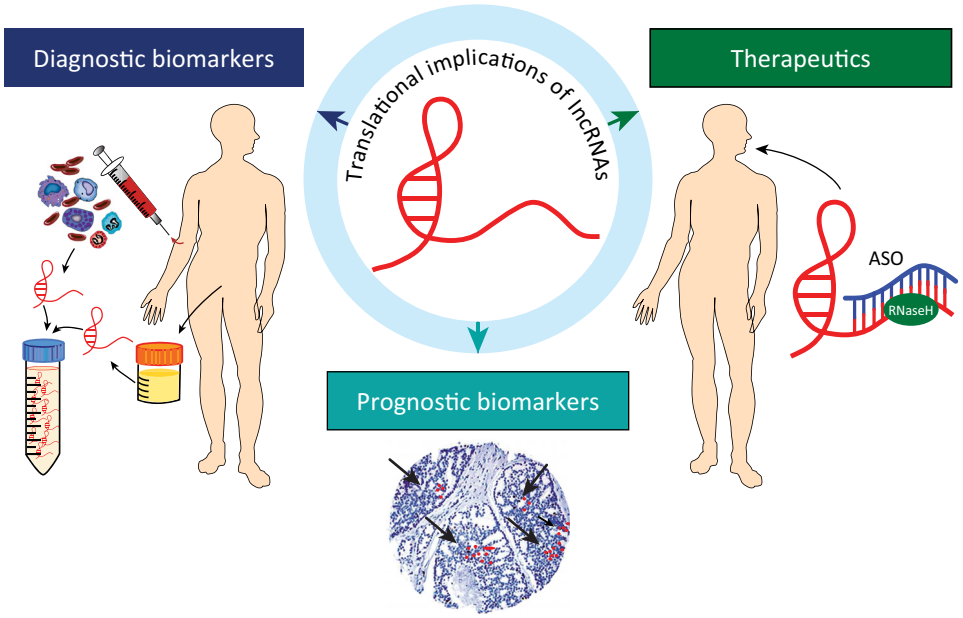

Figure 2. Translational Implications of lncRNAs. Long noncoding RNAs (lncRNAs) are emerging as both diagnostic and prognostic biomarkers that can be detected in tissue, serum, and urine. Antisense oligonucleotides (ASOs) can be used to directly target lncRNAs and are a promising therapeutic strategy in cancer.

MYC upregulation, suggesting that a synergistic effect exists between *PVT1* and *MYC* in cancer development.

The transcription factor NF-κB is also highly upregulated in a variety of cancers and plays a role in tumor microenvironment inflammation, resulting in cancer development, metastasis, and invasion [121]. *NKILA* (NF-κB Interacting lncRNA) is upregulated by NF-κB and binds to NF-κB/IκB to form a stable complex, preventing degradation of IκB and subsequent NF-κB activation [122]. Low *NKILA* expression is correlated with cancer metastasis, advanced stage, higher grade, increased tumor size, and decreased patient survival, suggesting a clinically important function of *NKILA* in mediating inflammation-stimulated breast cancer.

Translational Implications of lncRNAs

lncRNAs are beginning to show translational utility as both biomarkers and therapeutic targets (Figure 2 and Table 2). Dozens of lncRNAs show promise as diagnostic and prognostic markers across several types of cancers [123]. In general, lncRNAs show higher tissue- and disease-specific expression compared to protein-coding genes [124]. In cancer, lncRNAs show striking cancer- and lineage-specificity, suggesting that these molecules may be powerful biomarkers in the clinical setting [125]. In addition, lncRNAs can be measured in blood, urine, and tissue,

Table 2. Biomarker Potential of lncRNAs in Cancer

Name	Cancer type(s)[a]	Diagnostic/prognostic	Blood/tissue/urine	Refs
ANRIL	Gastric	Prognostic	Tissue	[34]
BANCR	NSCLC	Prognostic	Tissue	[100]
BCAR4	Breast	Prognostic	Tissue	[90]
CARLo-5	NSCLC	Prognostic	Tissue	[67]
CCAT2	Breast	Prognostic	Tissue	[92]
DRAIC	Multiple, prostate	Prognostic	Tissue	[112]
FAL1	Ovarian	Prognostic	Tissue	[31]
GAPLINC	Gastric	Diagnostic, prognostic	Tissue	[84]
GAS5	Gastric	Prognostic	Tissue	[72]
HOTAIR	Breast, colorectal, GIST, hepatocellular	Prognostic	Tissue	[19–22]
HOTTIP	Hepatocellular	Prognostic	Tissue	[53]
HULC	Hepatocellular, pancreatic	Diagnostic, prognostic	Blood, tissue	[126,127]
lncRNA-ATB	Hepatocellular	Prognostic	Tissue	[83]
lncRNA-HEIH	Hepatocellular	Prognostic	Tissue	[33]
MALAT1	Colorectal, glioma, prostate, renal cell	Diagnostic, prognostic	Blood, tissue	[96,129–132]
MEG3	NSCLC	Prognostic	Tissue	[61]
NBAT-1	Neuroblastoma	Prognostic	Tissue	[32]
NEAT1	Prostate	Prognostic	Tissue	[107]
NKILA	Breast	Prognostic	Tissue	[122]
PCA3	Prostate	Diagnostic	Urine	[128]
PCAT29	Prostate	Prognostic	Tissue	[111]
SChLAP1	Prostate	Prognostic	Tissue, urine	[41,50–52]
TUG1	NSCLC	Prognostic	Tissue	[35]

[a]GIST, gastrointestinal stromal tumor; NSCLC, non-small cell lung cancer.

justifying the development of non-invasive tests [2]. For example, *HULC* is not only associated with poor prognosis in pancreatic cancer [126] but it is also highly detectable in the plasma of patients with HCC compared to healthy controls [127]. In prostate cancer, *PCA3* has proven to be a powerful diagnostic urine marker [128]. Similarly, initial data show *SChLAP1* can be detected in both tissue and urine of patients with more-aggressive prostate cancer [50–52]. In addition to its prognostic value in colorectal carcinoma [129], renal cell carcinoma [130], and glioma [131], *MALAT1* can be detected in patient serum and may serve as a diagnostic marker in prostate cancer [132]. Furthermore, *AA174084* [133] and a set of oral lncRNAs [134] are found in gastric juices and saliva, and may serve as potential non-invasive biomarkers in gastric and oral squamous cell cancers, respectively.

Direct targeting of lncRNAs may be a viable therapeutic strategy in cancer. Antisense technology has gained considerable traction over the past few years as several antisense oligonucleotides (ASOs) have been introduced into clinical trials and some have been FDA-approved for clinical use [135–138]. ASOs function by basepairing to target RNAs, resulting in transcript-specific RNase H-mediated catalytic degradation [139]. ASOs are a particularly attractive therapeutic modality for several reasons, including predictable human pharmacokinetics, prolonged tissue elimination half-lives, enhanced specificity compared to small-molecule inhibitors, and lack of cytochrome P450 enzyme metabolism [91,139–141]. These characteristics are thought to make ASOs safer for patients and also more suitable for combination therapies with other drugs. Given the important role of lncRNAs across several cancer pathways, ASO-mediated therapies are likely to surface as a promising class of new cancer drugs over the next few years.

Box 1. Tools for lncRNA Investigation

Several techniques and tools have been developed to discover and study lncRNAs (see Figure 3 in main text). RNA-seq has emerged as the most powerful method of lncRNA discovery. Recently, a large-scale lncRNA annotation effort identified nearly 60 000 lncRNAs across the cancer genome, suggesting that a large portion of the human transcriptome remains unexplored [125]. The MiTranscriptome portal (www.mitranscriptome.org) was developed using RNA-seq data from over 6500 samples comprising benign and malignant tissues as well as cell lines, making it the most comprehensive lncRNA annotation to date. Furthermore, a sample set enrichment analysis (SSEA) revealed approximately 8000 previously unknown lncRNAs showing tissue- and/or cancer-specificity, providing the scientific community with a vast database of potentially crucial molecules for future study. While this new resource will provide a foundation for lncRNA genomics and cancer disease mechanisms, it is limited to polyadenylated transcripts. Future sequencing and annotation efforts will need to focus on also identifying non-polyadenylated lncRNAs.

Novel methods to isolate RNA *in vivo* have led to the discovery of chromatin, RNA, and protein interacting partners of lncRNA transcripts. RNA pulldown methods such as chromatin isolation by RNA purification (ChIRP) [154] and RNA antisense purification (RAP) [155] have aided in the discovery of lncRNA function. These methods utilize antisense complementary oligonucleotides to isolate a target RNA and its associated molecules. Downstream sequencing and mass-spectrometry analysis can then be used to identify novel interactors in an unbiased manner.

Another useful tool to delineate lncRNA function is direct visualization. Single-molecule RNA-FISH (fluorescence *in situ* hybridization) is a powerful method to localize and visualize lncRNA expression patterns in cells and tissues [156,157]. Multiplexing RNA-FISH with protein immunofluorescence can also be used to identify and confirm RNA–protein interactions.

One of the greatest biological challenges has been the structural analysis of RNA molecules *in vivo*. A new approach, termed icSHAPE (*in vivo* click selective 2′-hydroxyl acylation and profiling experiment) enables RNA structure analysis *in vivo* at nucleotide resolution for all four bases and can identify RNA strandedness [158]. Perhaps the most relevant aspect of this technique to lncRNAs is the ability to differentiate structural changes in RNA at protein-binding sites.

Traditionally, computational methods are used to determine whether RNA is coding or noncoding. A variety of tools analyze sequence features such as open reading frame (ORF) length and the presence of a protein domain within a transcript. A subset of lncRNAs have been classified as TUCPs (transcript of unknown coding potential) [125,143]. This is especially relevant because several examples of novel small peptides produced from putative lncRNAs have been described [159]. Improved bioinformatics tools and experimental methods, such as ribosomal profiling [160], should be employed to thoroughly assess the protein-coding capacity of a transcript.

Concluding Remarks

Although we have attempted to classify lncRNAs by their predominant mechanistic modality, most transcripts could fit into multiple categories, suggesting that lncRNAs may form important regulatory networks that can coordinate numerous aspects of cancer progression simultaneously. Although our understanding of lncRNA-mediated cancer biology has increased significantly in the last several years, we believe this is only the tip of the iceberg (see Outstanding Questions). A continued understanding of the role of lncRNAs in cancer will be enhanced by new tools that uncover novel lncRNAs, better annotate known lncRNAs, as well as assess lncRNA localization, structure, and function (Box 1 and Figure 3).

While new biological and computational techniques have greatly accelerated our ability to investigate RNAs in cancer research, most lncRNA discovery and annotation efforts in cancer have been severely limited, with poor overlap between different catalogs [142], avoidance of monoexonic transcripts and complex regions of the genome [143], poor bioinformatics tools for *ab initio* assembly of novel transcripts [144], and small cohorts from which to reconstruct the cancer transcriptome [69]. Moreover, several studies continue to rely on microarray-based platforms for the identification of disease-associated lncRNAs [90,145]; however, their use in discovering new lncRNAs is limited because gene expression probes are designed against

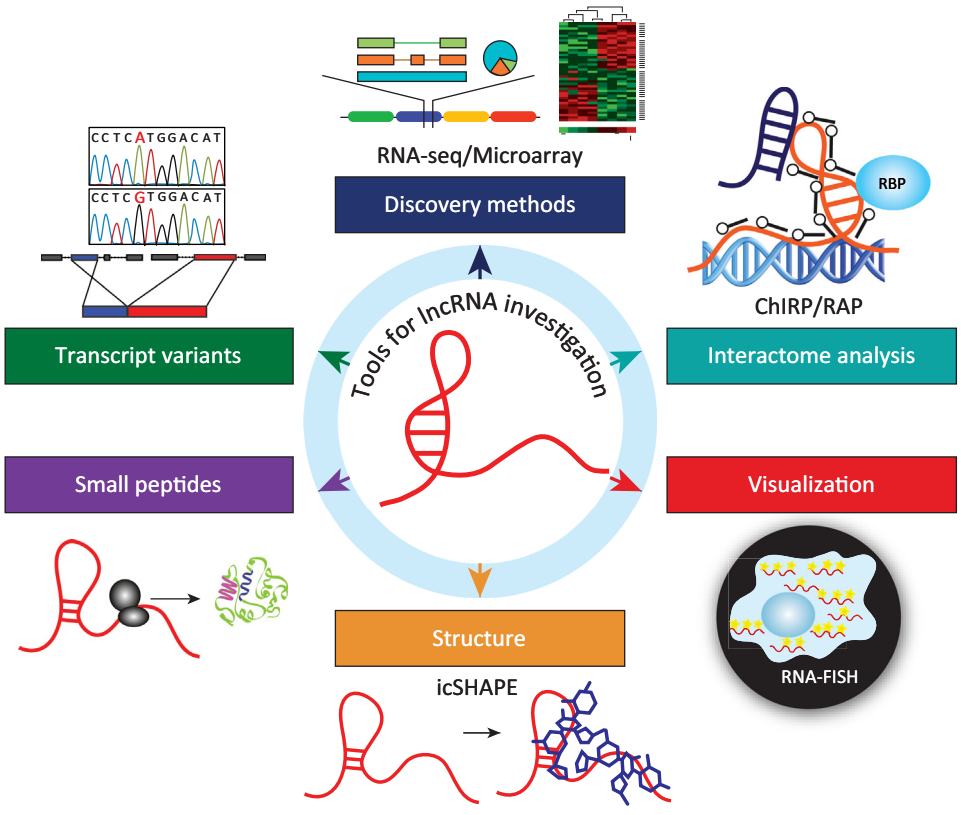

Figure 3. Tools for lncRNA Investigation. Emerging areas for investigation in the long noncoding RNA (lncRNA) field include improved discovery methods, unbiased interactome analysis, transcript visualization and localization, RNA structure determination, discovery of small peptides produced from short open reading frames (sORFs), and the identification and comprehension of lncRNA variants. Abbreviations: ChIRP, chromatin isolation by RNA purification; FISH, fluorescence in situ hybridization; icSHAPE, *in vivo* click selective 2′-hydroxyl acylation and profiling experiment; RAP, RNA antisense purification; RBP, RNA-binding protein.

Outstanding Questions

How do lncRNA variants contribute to cancer progression? Differential expression patterns in cancer have guided lncRNA discovery and investigation thus far. lncRNA transcript variants such as mutations, amplifications, deletions, and fusions remain unexplored.

How do lncRNAs coordinate various molecular networks to drive cancer? The majority of lncRNA studies to date have focused on a single mechanism of action. However, several transcripts have numerous functions, suggesting that lncRNAs may form important regulatory networks that can coordinate many aspects of cancer progression simultaneously.

Do a subset of lncRNAs have protein-coding potential? Some lncRNAs have been identified as TUCPs (transcripts of unknown coding potential). Previously undiscovered small peptides produced from these lncRNAs may have significant roles in cancer biology.

How can lncRNAs be used to guide precision medicine approaches in cancer? An emerging area of cancer therapeutics utilizes genomic signatures to guide treatment choices for patients. Current efforts employ aberrations in protein-coding genes; incorporating lncRNAs into these analyses may improve therapeutic response and patient outcomes.

How can lncRNAs be utilized in the clinical setting? lncRNAs have been identified as powerful diagnostic and prognostic biomarkers. Targeting lncRNAs directly with antisense oligonucleotides may also be a promising therapeutic strategy.

previously annotated transcripts. Therefore, RNA-seq remains the most powerful tool to discover new lncRNAs in an unbiased fashion [146,147].

In addition, almost all lncRNA studies to date have focused on the aberrant expression patterns of novel transcripts in cancer. While this is an essential first step to identify important lncRNAs in cancer, future analyses will need to include transcript variants that populate and drive cancer. Protein alterations such as point mutations, deletions and amplifications, and gene fusions have emerged as key regulators in cancer [148,149]. As our understanding of cancer-associated lncRNAs expands, similar variants will also need to be explored in noncoding transcripts. Furthermore, studying isoform-specific functions may provide new insights into lncRNA gene function [10,125]. Uncovering the precise function of lncRNAs in cell and animal models also cannot be overlooked. While methods to knockdown (ASOs and locked nucleic acids, LNAs [150]) and knockout (CRISPR) [151,152] lncRNA genes are improving, caution should be taken when employing these tools to explore lncRNA function [153].

Less than a decade ago, lncRNAs were mostly ignored, often considered to be 'junk', and were attributed to leaky transcription. Now, functional, mechanistic, and translational insights have revealed the crucial role of lncRNAs in cell biology and disease pathogenesis. Importantly, lncRNAs are emerging as crucial players in cancer progression and metastasis. Given the tissue- and disease-specific nature of these transcripts, their abundance throughout the genome, and the relatively recent discovery of the majority of these transcripts, it is likely that lncRNAs hold answers to questions in cancer that have eluded us for years.

Acknowledgments

We thank Robin Kunkel for assistance with figure preparation and Karen Giles for critically reading the manuscript and for the submission of documents. This work was supported in part by US National Institutes of Health Prostate Specialized Program of Research Excellence grant P50 CA69568, Early Detection Research Network grant UO1 CA111275, US National Institutes of Health grant R01 CA132874 (A.M.C.), and US Department of Defense grant PC100171 (A.M.C.). A.M.C. is supported by the Prostate Cancer Foundation and the Howard Hughes Medical Institute. A.M.C. is an American Cancer Society Research Professor and a Taubman Scholar of the University of Michigan. A.S. is supported by a Prostate Cancer Foundation Young Investigator Award and by a National Institutes of Health Ruth L. Kirschstein National Research Service Award F30 CA180376. A.S. is a Fellow of the University of Michigan Medical Scientist Training Program. U.S. is supported by the Howard Hughes Medical Institute Medical Research Fellows Program. A.M.C. serves on the scientific advisory board of Paradigm. He is a coinventor on patents filed by the University of Michigan covering the diagnostic and therapeutic field of use for T2-ERG in prostate cancer which has been licensed to Hologic, and the diagnostic and therapeutic field of use for SChLAP1 which has been co-licensed to Genome Dx. Paradigm, Hologic, and Genome Dx did not play roles in the design and conduct of this study, in the collection, analysis, or interpretation of the data, or in the preparation, review, or approval of the article.

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