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Long Noncoding RNAs in Cancer Pathways

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Genome-wide cancer mutation analyses are revealing an extensive landscape of functional mutations within the noncoding genome, with profound effects on the expression of long noncoding RNAs (lncRNAs). While the exquisite regulation of lncRNA transcription can provide signals of malignant transformation, we now understand that lncRNAs drive many important cancer phenotypes through their interactions with other cellular macromolecules including DNA, protein, and RNA. Recent advancements in surveying lncRNA molecular mechanisms are now providing the tools to functionally annotate these cancer-associated transcripts, making these molecules attractive targets for therapeutic intervention in the fight against cancer.

Introduction

Cancer is fundamentally a genetic disease that alters cellular information flow to modify cellular homeostasis and promote growth. The discovery of a universal genetic code for protein-coding genes produced countless breakthroughs in understanding how such mutations drive cancer, establishing the scientific principles on which the development of targeted therapies for malignancies are based, such as imatinib for BCR-ABL leukemia or vemurafenib for $BRAF^{V600E}$ melanoma. Furthermore, the clinical application of cancer exome sequencing has identified numerous protein-coding mutations amenable to targeted therapy. However, the coding genome accounts for less than 2% of all sequences, and it has become apparent that aberrations within the noncoding genome drive important cancer phenotypes.

One of the most unexpected findings of the genomics era of biology is the extensive transcription of RNA from non-protein-coding regions of the genome (Morris and Mattick, 2014; Rinn and Chang, 2012). Indeed the list of long noncoding RNAs (lncRNAs), which are functionally defined as transcripts >200 nt in length with no protein-coding potential, number in the tens of thousands, many of which are uniquely expressed in differentiated tissues or specific cancer types (Iyer et al., 2015). In fact, the number of lncRNA genes outnumbers protein-coding genes (Derrien et al., 2012) and more than 90% have no appreciable peptide products (Banfai et al., 2012; Guttman et al., 2013). It is now recognized that lncRNAs are exquisitely regulated and are restricted to specific cell types to a greater degree than mRNA (Cabili et al., 2011) and have frequently evolutionarily conserved function, secondary structure, and regions of microhomology, despite minimal overall sequence similarity (Hezroni et al., 2015; Quinn et al., 2016; Ulitsky et al., 2011). However, the function of the vast majority of these transcripts remains to be identified.

While the discovery of the universal genetic code has provided investigators the tools to recognize how genetic mutations result in functional protein defects, lncRNA biology has so far remained resistant to a rules-based, predictive framework for understanding how sequence affects function. However, recurring molecular mechanisms for lncRNA are now being realized, and emerging technologies are expanding investigators' abilities to functionally annotate cancer-associated lncRNAs. In this perspective, we provide an overview of the current state of lncRNA biomarker identification in cancer phenotypes, catalog the molecular roles for lncRNAs in cellular processes, and review known roles for lncRNAs in cancer pathophysiology.

Identifying lncRNA Signals in the Cancer Transcriptome

We now appreciate that mutations within the noncoding genome are major determinants of human diseases (Maurano et al., 2012). Indeed recurrent somatic noncoding mutations (Melton et al., 2015), epigenetic alterations (Roadmap Epigenomics et al., 2015), or somatic copy-number alterations (Beroukhim et al., 2010) are implicated in multiple cancer types. Mutations in regulatory DNA can broadly affect transcription by altering enhancer and promoter activity or chromatin states, leading to differential lncRNA expression in cancer. Remarkably, several loci that are frequently mutated in cancer encode ultra-conserved noncoding sequences that are deregulated in cancer (Calin et al., 2007). Indeed, SNPs, copy-number alterations, or mutations within the noncoding genome can dramatically alter lncRNA transcription (Table 1). The 8q24 locus is particularly richly populated with cancer-associated SNPs, several of which are implicated in the expression of cancer-associated lncRNAs, including CCAT2 in colorectal cancers and PCAT-1 in prostate cancer (Ling et al., 2015).

The catalog of noncoding genes has grown tremendously over the past few years, in large part due to the identification of extensive lncRNA transcription (Djebali et al., 2012; Harrow et al., 2012; Iyer et al., 2015) arising from active enhancers (Kim et al., 2010), promoters (Seila et al., 2008), and intergenic sequences. These discoveries have depended in large part upon new computation methods for transcriptome assembly and lncRNA annotation (Guttman et al., 2010; Mattick and Rinn, 2015).

It is now widely understood that lncRNAs, serving as signals of specific cellular states or readouts of active cellular programs (Wang and Chang, 2011), could identify cellular pathologies such as cancer, provide prognostic value, or even inform therapeutic options for cancer patients (Table 1). Overexpression of the lncRNA HOTAIR in early-stage, surgically resected breast cancer is highly predictive of progression to metastatic disease and overall survival (Gupta et al., 2010). Subsequent studies showed that HOTAIR deregulation is associated with cancer

Table 1. Cancer-Associated lncRNAs

lncRNAs Associated with Common Cancer Genomic Alterations			
lncRNA	Cancer Type	Genomic Alteration	References
PVT1	colorectal	8q24 amplification	Tseng et al., 2014
PCAT-1	prostate	8q24 SNPs	Eeles et al., 2008; Prensner et al., 2011
CCAT2	colorectal	8q24 SNPs	Ling et al., 2013b; Tomlinson et al., 2007
PTCSC3	thyroid	rs944289	Jendrzejewski et al., 2012
HULC	hepatocellular	rs7763881	Liu et al., 2012
ANRIL	various	9p21.3 SNPs	Pasmant et al., 2011
TERC	oral cavity	3q26 amplification	Dorji et al., 2015
GAS5	hepatocellular	rs145204276	Tao et al., 2015
lncRNAs in Cancer Diagnosis and Monitoring			
lncRNA	Cancer Type	Bioavailability of lncRNA	References
H19	Gastric	blood	Zhou et al., 2015
HULC	hepatocellular	blood	Xie et al., 2013
AA174084	Gastric	gastric secretions	Shao et al., 2014
PCA3	prostate	urine	Bussemakers et al., 1999
SeCATs	Sézary	tumor	Lee et al., 2012
SPRY4-IT1	melanoma	tumor	Khaitan et al., 2011
Prognostic lncRNAs			
lncRNA	Cancer Type	Prognostic Information	References
FAL1	ovarian	poor prognosis	Hu et al., 2014a
HOTAIR	breast	increased risk of metastasis	Gupta et al., 2010
HOTTIP	hepatocellular	increased risk of progression	Quagliata et al., 2014
MEG3	meningioma	associated with tumor grade and risk of progression	Zhang et al., 2010
NBAT-1	neuroblastoma	good prognosis	Pandey et al., 2014
NKILA	breast	decreased risk of metastasis	Liu et al., 2015
SCHLAP1	prostate	increased risk of metastasis	Prensner et al., 2014b
lncRNAs Predicting Therapeutic Responsiveness			
lncRNA	Cancer Type	Therapeutic Agent	References
CCAT1	colorectal	BET inhibitors	McCleland et al., 2016
HOTAIR	ovarian	platinum chemotherapies	Teschendorff et al., 2015

progression in 26 human tumor types (Bhan and Mandal, 2015) and can predict differential sensitivity of ovarian cancer patients to two platinum chemotherapies, potentially guiding clinical decision making (Teschendorff et al., 2015). Differential display analysis of human prostate cancers identified Differential display 3, also known as PCA3, as a specific biomarker of prostate cancer (Bussemakers et al., 1999). Subsequently, PCA3 was approved by the Food and Drug Administration (FDA) for prostate cancer diagnosis, the first instance of an FDA-approved test based on an lncRNA. As a biomarker of prostate cancer in the urine, it has become a useful, noninvasive test for prostate cancer with improved specificity, positive predictive value, and negative predictive value compared with serum prostate-specific antigen testing (Wei et al., 2014). Similarly, analyses of gastric secretions from patients with gastric cancer identified lncRNA-AA174084 as a biomarker capable of differentiating between gastric cancer and benign disorders of the gastric epithelium (Shao et al., 2014). Recent genome-wide approaches have identified thousands of lncRNAs that are differentially transcribed between normal tissues and tumors arising from the same organ (Brunner et al., 2012; Iyer et al., 2015; Yan et al., 2015), suggesting enormous potential for further development of lncRNA biomarkers for specific cancer histologies.

lncRNA expression profiles can also identify clinically relevant cancer subtypes that predict tumor behavior and disease prognosis (Du et al., 2013). Overexpression of the lncRNA SChLAP1 in men treated with radical prostatectomy was associated with 2.45-fold increased odds of developing metastatic progression, similar to the risk associated with high-grade prostate cancer (Gleason 8–10 on histology) versus low- to intermediate-grade disease (Gleason $\leq$7 on histology) (Prensner et al., 2014b). Additional studies have identified lncRNAs with specificity for prostate cancer primary tumor stage or associated with lymph node metastases (Bottcher et al., 2015). Furthermore, recent reports are indicating that lncRNAs may be predictive of responsiveness to specific types of cancer therapy. Expression of HOTAIR in ovarian cancer predicts poor survival following treatment with a carboplatin, yet this group had improved responses to cisplatin relative to HOTAIR-negative ovarian cancer patients (Teschendorff et al., 2015). Validation of these observations may provide an lncRNA biomarker to guide the selection of platinum-containing regimens in patients with ovarian cancer.

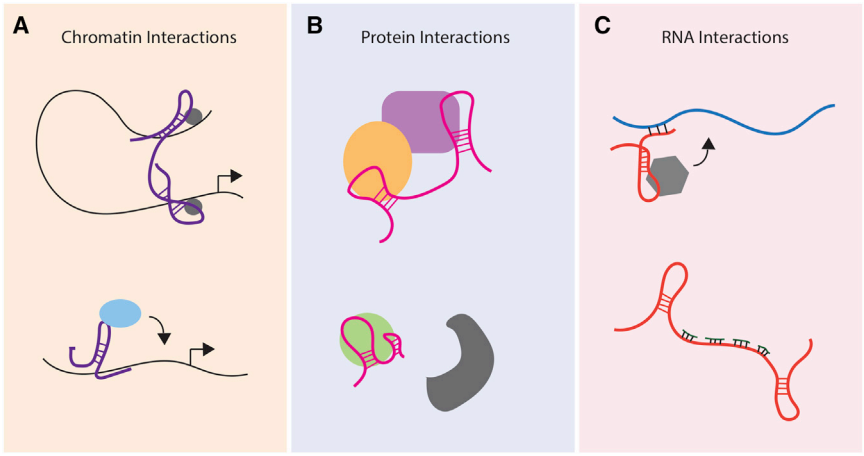

Figure 1. lncRNA Mechanisms Rely on Interactions with Cellular Macromolecules
(A) Chromatin-bound lncRNAs can regulate gene expression by controlling local chromatin architecture (above) or directing the recruitment of regulatory molecules to specific loci (below).
(B) lncRNA interactions with multiple proteins can promote the assembly of protein complexes (above) or impair protein-protein interactions (below).
(C) mRNA interactions with lncRNA can recruit protein machinery involved in multiple aspects of mRNA metabolism to affect splicing, mRNA stability, or translation (above) or sequester miRNA away from target mRNA (below).

Differential regulation of lncRNAs relative to coding genes may underlie the high degree of tissue-specific lncRNA expression. Intriguingly, genome-wide characterization of lncRNA and protein-coding promoters revealed that these classes of genes are under distinct regulatory regimes (Quinn and Chang, 2015). lncRNA promoters are enriched for A/T nucleotides and diminished in CpG patterns, contain distinct transcription factor binding sequences, and have a unique pattern of chromatin marks relative to protein-coding genes (Alam et al., 2014). Furthermore, lncRNAs contain a higher density of methylation at transcriptional start sites (TSS) than at the TSS of protein-coding genes, regardless of expression levels, highlighting the context-dependent manner of gene silencing by methylation (Sati et al., 2012). Finally, the Dicer-microRNA (miRNA) pathway and the Myc oncogene differentially regulate the expression of mRNA and lncRNAs (Zheng et al., 2014). Dicer production of miR-295 drives Myc expression, upon which Myc is recruited to lncRNA genes with a high density of Myc binding sites, thereby activating lncRNA transcription initiation and elongation. Remarkably, lncRNA genes are substantially more sensitive to this Dicer-miRNA-Myc circuit than protein-coding genes with Myc binding sites.

Molecular Mechanisms of lncRNAs

In this section we highlight some of the recurring molecular mechanisms that govern how lncRNAs regulate cellular processes (Guttman and Rinn, 2012). Functionality in part begins with lncRNA cellular localization: nuclear lncRNAs are enriched for functionality involving chromatin interactions, transcriptional regulation, and RNA processing, while cytoplasmic lncRNAs can modulate mRNA stability or translation and influence cellular signaling cascades (Batista and Chang, 2013). We also highlight recent innovations in molecular tools to survey lncRNA interactions with other cellular macromolecules and describe how these tools have dramatically accelerated the discovery of lncRNA functions (Chu et al., 2015a). These methods are now elucidating how lncRNAs elicit functional outcomes through interactions with DNA, chromatin, signaling and regulatory proteins, and a variety of cellular RNA species (Figure 1).

lncRNAs Localizing to Chromatin

lncRNA transcriptional regulation at the level of chromatin is a widely observed mechanism that can involve activities affecting neighboring intrachromosomal genes in *cis* or targeting of genes in *trans* on different chromosomes (Huarte, 2015; Huarte et al., 2010; Sahu et al., 2015). lncRNAs are widely known to regulate genes in *cis* as enhancer-associated RNAs (Orom et al., 2010), through transcriptional regulation (Dimitrova et al., 2014; Huarte et al., 2010; Trimarchi et al., 2014; Zhu et al., 2013), transcription factor trapping (Sigova et al., 2015), chromatin looping (Wang et al., 2011), and gene methylation (Schmitz et al., 2010) to name just a few mechanisms. lncRNAs also regulate distant genes through modulation of transcription factor recruitment (Hung et al., 2011; Yang et al., 2013b), chromatin modification (Wang and Chang, 2011), and serving as a scaffold for assembly of multiple regulatory molecules at single locus (Tsai et al., 2010).

One particularly well-characterized mechanism by which lncRNAs regulate gene expression both in *cis* and in *trans* involves interaction with chromatin to facilitate histone modification (Khalil et al., 2009). Xist, one of the first functionally annotated lncRNAs, regulates dosage compensation in female mammals by localizing to the X chromosome and recruiting multiple factors directly and indirectly to execute X chromosome inactivation (XCI) (Gendrel and Heard, 2011; Lee and Bartolomei, 2013). That human malignancies frequently have X aneuploidy suggested an important role for dosage compensation of X-linked genes in preventing malignant transformation (Pageau et al., 2007). Confirming this, female mice with *Xist* deletion in the hematopoietic compartment develop an aggressive myeloproliferative disorder with full penetrance (Yildirim et al., 2013).

While Xist coating of the inactive chromosome is required for X silencing, annotation of Xist interaction domains is a necessary prerequisite for clarifying the role of Xist. To define the genomic interactions of lncRNAs, Chu et al. (2011) developed chromatin isolation by RNA purification (ChIRP), whereby short biotinylated oligonucleotides complementary to the lncRNA transcript purify chromatin bound by the target RNA. Similar methods including capture hybridization analysis of RNA targets (CHART) and RNA antisense purification (RAP) demonstrated that Xist initially binds gene rich islands on the X chromosome that are in close three-dimensional proximity, then spreads to gene-poor regions during de novo XCI (Engreitz et al., 2014; Simon et al., 2011, 2013).

Cancer transcriptional programs are also modulated by lncRNA recruitment to distant promoters and enhancers. ChIRP

revealed that Paupar, a CNS-restricted lncRNA located adjacent to *PAX6*, interacts with numerous promoters to regulate the cell cycle and maintain the dedifferentiated state of neuroblastoma (Vance et al., 2014). Nuclear enriched abundant transcript 1 (NEAT1), a component of nuclear paraspeckles (Clemson et al., 2009), is associated with an increased risk of biochemical progression and metastasis of prostate cancer and is a downstream transcriptional target of estrogen receptor α (Chakravarty et al., 2014). ChIRP revealed that NEAT1 localizes to promoters of genes involved in prostate cancer growth and increases chromatin marks of active transcription at these sites, driving androgen-independent prostate cancer growth. T-helper 17 cells (Th17) are pro-inflammatory T cells, and the lncRNA RMRP was shown by ChIRP to co-associate with key Th17 transcription factor ROR-γt for inflammatory effector function (Huang et al., 2015). Nascent RNA transcripts in the vicinity of enhancers and promoters can also bind the transcription factor YY1, promoting local accumulation of YY1 in the vicinity of the regulatory sites, supporting the engagement of YY1 with these elements, and driving a positive feedback loop that maintains active transcription (Sigova et al., 2015).

lncRNA Interactions with Protein

lncRNAs interact with proteins to modulate protein function, regulate protein-protein interactions, or direct localization within cellular compartments. These interactions are central to determine lncRNA functional effects, yet characterizing protein-lncRNA has presented significant challenges. RNA chromatography has been successfully used to identify protein interactions for lncRNAs of interest as an initial handle for mechanistic characterization. In this approach, proteins bound to genetically or biochemically modified bait RNA are retrieved by affinity purification and proteins identified by mass spectrometry or western blot. Using this strategy, Huarte et al. (2010) identified that the p53-inducible lincRNA-p21 bound to hnRNP-K to mediate gene repression in response to DNA damage. Using similar methods, Li et al. (2015) identified that Apela RNA positively regulates DNA-damage-induced apoptosis in mouse embryonic stem cells by binding to hnRNPL, inhibiting its ability to sequester p53 away from the mitochondria. While RNA chromatography has yielded important insights into lncRNA molecular mechanisms, artifacts resulting from nonphysiologic interactions that take place with misfolded bait RNA or only in nonphysiologic in vitro condition can complicate analyses. Recently, multiple groups developed methods to overcome these limitations. By coupling ChIRP and RAP with mass spectrometry, two different groups of investigators discovered dozens of novel protein components of the Xist complex that are involved in XCI (Chu et al., 2015b; McHugh et al., 2015). CHART-mass spectrometry has also been used to characterize the protein interaction landscape of NEAT1 and MALAT1 (West et al., 2014).

Complementary, protein-centric methods of characterizing ribonucleoprotein complexes have also identified unique lncRNA activities in cancer cells. Using RNA immunoprecipitation (RIP), two prostate-specific lncRNAs, PCGEM1 and PRNCR1, were found to associate with androgen receptor in prostate cancer cells (Yang et al., 2013b) and promote transcriptional regulation by androgen receptor both in androgen-sensitive and castration-resistant prostate cancer cell lines. However, another study of PCGEM1 and PRNCR1 suggests that the role of these lncRNAs in prostate cancer may be more limited (Prensner et al., 2014a). HOTAIR, initially found to define chromatin boundaries at the *HOXD* locus (Rinn et al., 2007), was reported to bind androgen receptor, which prevents binding to and subsequent MDM2 ubiquitinylation and protein degradation, thereby promoting androgen receptor transcriptional regulation and driving castration-resistant prostate cancer (Zhang et al., 2015).

RNA Targets of lncRNA Actions

lncRNA modulation of RNA metabolism is an emerging theme with described roles in the control of mRNA stability, splicing, and translation. lncRNA Staufen-1 (STAU-1) mediates mRNA decay through interaction with a double-stranded RNA in the 3′ UTR of select mRNAs. However, a subset of STAU-1 regulated mRNAs can only form the requisite double-stranded RNA stem by duplexing with an Alu element of a cytoplasmic lncRNA (Gong and Maquat, 2011), thus a lncRNA can confer specificity on the STAU-1 pathway. STAU-1-mRNA interactions are also modulated by terminal differentiation-induced noncoding RNA (TINCR), which drives epidermal differentiation and is downregulated in poorly differentiated cutaneous squamous cell carcinomas (Kretz et al., 2013). Using ChIRP retrieval of TINCR and associated RNAs, TINCR was found to bind several mRNAs containing a 25-nt TINCR box motif. TINCR recruits STAU-1 to mRNA bearing the TINCR box, and, somewhat unexpectedly, stabilizes these messages and facilitates the translation of several genes involved in keratinocyte differentiation. A similar technique, RNA antisense purification of RNA (RAP-RNA), identified a MALAT1 interaction with pre-mRNA that directs MALAT1 to localize at the proximal chromatin region of transcriptionally active genes (Engreitz et al., 2014), in line with prior observations that MALAT1 can regulate alternative splicing through interactions with serine/arginine splicing factors and pre-mRNA (Tripathi et al., 2010). lncRNA-p21 also interacts with JUNB and CTNNB1 mRNA to selectively impair translation of these messages (Yoon et al., 2012).

lncRNAs and pseudogene RNA have also been postulated to act as competing endogenous RNA (ceRNA) or ''RNA sponges,'' interacting with miRNAs in a manner that can sequester these molecules and reduce their regulatory effect on target mRNA (Tay et al., 2014). Transcription of the *PTEN* pseudogene, *PTENP1* and other transcripts, while unable to serve as a template for PTEN translation, nonetheless can increase PTEN protein levels through an RNA-dependent mechanism that involves binding and sequestering PTEN-targeting miRNA (Karreth et al., 2011; Poliseno et al., 2010; Tay et al., 2011). Similar ceRNA mechanisms have been reported for lncRNAs H19 and HULC (Kallen et al., 2013; Keniry et al., 2012; Wang et al., 2010). However, quantitative analyses of miRNA and target mRNA abundance suggest that the ceRNA mechanism can only operate given appropriate stoichiometry between ceRNA and miRNAs (Denzler et al., 2014). Experimental analyses and knockout studies are thus needed beyond the presence of matching miRNA seed sequences to validate ceRNA mechanisms.

lncRNA Drivers of Cancer Phenotypes

Cancer consists of a constellation of phenotypes resulting from (1) dysfunctional intrinsic cellular regulatory networks and (2) intercellular communication to generate the tumor microenvironment (Hanahan and Weinberg, 2000, 2011). Intracellular

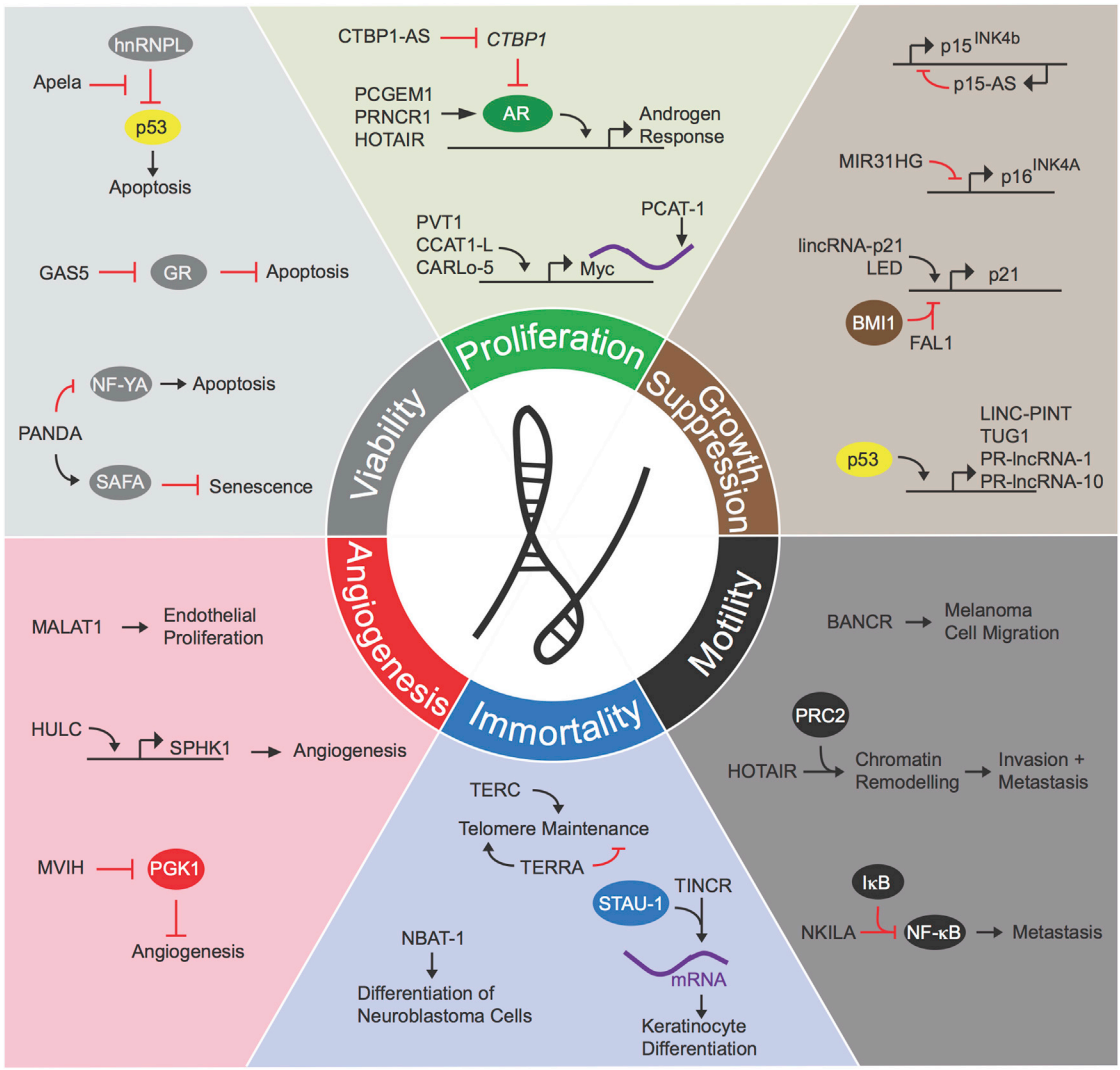

Figure 2. lncRNAs in Cancer Phenotypes
lncRNAs contribute to each of the six hallmarks of cancer (diagram adapted from Hanahan and Weinberg, 2000). Selected examples of lncRNAs and their molecular partners or genomic targets are shown for proliferation, growth suppression, motility, immortality, angiogenesis, and viability cancer phenotypes.

signaling networks are modulated in cancer to sustain proliferation, impair cytostatic and differentiation signals, enhance viability, and promote motility. As lncRNAs have primarily been studied in the context of intracellular networks, our review focuses on the role of lncRNAs in these specific cancer phenotypes. Indeed, each of the hallmarks of cancer, as described by Hanahan and Weinberg in 2000, is modulated by the activity of multiple lncRNAs (Figure 2).

Proliferation Circuits

Multiple lncRNAs are downstream targets of chemokine and hormonal pathways (Xing et al., 2014). In T cell acute lymphoblastic leukemia, the Notch1 oncogene drives growth in part by inducing the lncRNA LUNAR1 to upregulate insulin-like growth factor 1 receptor expression and signaling (Trimarchi et al., 2014). Androgen signaling in prostate cancer also relies on a number of lncRNAs implicated in prostate cancer proliferation that act through direct interactions with the androgen receptor (Yang et al., 2013b; Zhang et al., 2015) or by inhibiting repressors of the androgen receptor (Takayama et al., 2013).

Amplification of the 8q24 locus is a well-characterized oncogenic event in many types of human malignancies resulting in *MYC* amplification, but multiple lines of evidence are now implicating lncRNAs in Myc-driven cancers. *PVT1* is a lncRNA gene at the breakpoint of the t(2:8) translocation in Burkitt's lymphoma that brings the human immunoglobulin enhancer to *PVT1-MYC* locus. In a mouse model of Myc oncogenesis, single-copy amplification of *Myc* alone was insufficient to enhance tumor formation, whereas amplification of a multi-gene segment encompassing *Myc* and the lncRNA *Pvt1* promoted efficient tumor development (Tseng et al., 2014). Co-amplification of *PVT1* and *MYC* increased Myc protein levels while depletion of PVT1 in Myc-driven human colon cancer cells impaired proliferation. Furthermore, Myc transcription is activated in *cis* by the colon cancer-associated lncRNA CCAT1, also known as CARLo-5,

by facilitating long-range interaction between Myc and an enhancer element (Kim et al., 2014; Xiang et al., 2014). The prostate-specific lncRNA PCGEM1, also residing at the 8q24 locus, binds to Myc and enhances Myc's transcriptional activation of several genes involved in various metabolic processes required for growth of prostate cancer cells (Hung et al., 2014). Myc also targets numerous lncRNAs for transcriptional regulation (Zheng et al., 2014), which can in turn regulate cell-cycle progression (Kim et al., 2015).

Tumor-Suppressor Circuits

Recently, several lncRNAs have been found to play an extensive role in modulating tumor-suppressor and growth-arrest pathways. The complement of lncRNA transcription is dynamically regulated under differing cell-cycling conditions (Hung et al., 2011) and during senescence (Abdelmohsen et al., 2013). Several lncRNAs regulate the expression of key tumor suppressors from the *CDKN2A/CDKN2B* locus, which encodes $p15^{INK4b}$, $p16^{INK4a}$, and $p14^{ARF}$. The antisense noncoding transcript p15-AS induces silencing of $p15^{INK4b}$ through heterochromatin formation (Yu et al., 2008), and elevated p15-AS expression is associated with low $p15^{INK4b}$ expression in leukemic cells. The lncRNA MIR31HG recruits Polycomb group proteins to the *INK4A* locus to repress its transcription during normal growth but is sequestered away from the *INK4A* locus during senescence (Montes et al., 2015). Expression of the tumor suppressor TCF21 is activated by its antisense RNA TARID, which recruits the GADD45a to the *TCF21* promoter to facilitate demethylation (Arab et al., 2014).

Regulation of the p53 tumor-suppressor pathway by lncRNAs has been a topic of especially intense interest. The maternally imprinted RNA MEG3 binds to p53 and activates p53-dependent transcription of a subset of p53-regulated genes (Zhou et al., 2007). Furthermore, the known complement of p53-regulated lncRNAs is growing rapidly, indicating widespread involvement of lncRNAs downstream of p53 activation (Huarte et al., 2010; Hung et al., 2011; Marin-Bejar et al., 2013; Sanchez et al., 2014; Younger et al., 2015; Zhang et al., 2014). Distant p53-bound enhancer regions generate enhancer RNAs that are required for p53's regulation of multiple genes from a single enhancer site (Melo et al., 2013). Genome-wide profiling of p53-regulated enhancer RNAs identified the p53-induced lncRNA LED, which interacts with and activates strong enhancers including a *CKDN1A* enhancer to supports cell-cycle arrest by p53. LED is silenced in a subset of p53 wild-type leukemia cells, indicating a possible tumor-suppressing role for this lncRNA (Leveille et al., 2015). lncRNA-p21, which is induced by DNA damage in a p53-dependent manner, interacts with hnRNPK to regulate *CDKN1A* in *cis* and arrest the cell cycle in a p21-dependent manner (Dimitrova et al., 2014; Huarte et al., 2010). The lncRNA FAL1, located within a region of chromosome 1 with frequent amplification in cancer, recruits the chromatin repressor protein BMI-1 to multiple genes including *CDKN1A* to promote tumor cell proliferation (Hu et al., 2014a). Additional p53 pathway activities are also regulated by the p53 and DNA-damage-inducible lncRNA PANDA, which inhibits DNA-damage-induced apoptosis by binding to the transcription factor NF-YA and blocking its recruitment to pro-apoptotic genes (Hung et al., 2011). PANDA has also been shown to regulate senescence through, and interaction with, SAFA and PRC1 (Puvvula et al., 2014).

Viability Circuits

The selective advantage of tumor cells is driven by telomere maintenance, tolerance of nutrient stress, and in some cancers, preservation of an undifferentiated tumor cell population. lncRNA Growth arrest specific 5 (Gas5) is induced in cells arrested by nutrient deprivation or withdrawal of growth factors. Gas5 blocks glucocorticoid responsive gene expression by binding to the DNA binding domain of the glucocorticoid receptor (GR) and acting as a decoy (Hudson et al., 2014; Kino et al., 2010). This blockade of GR decreases expression of the cellular inhibitor of apoptosis 2 (Kino et al., 2010), thereby enhancing apoptosis under stressed conditions in normal cells. However, suppression of Gas5 in human breast cancer cells relative to adjacent normal breast tissue may support the enhanced viability of breast cancer cells in the nutrient-poor tumor microenvironment (Mourtada-Maarabouni et al., 2009).

Tumor cell immortality requires telomere maintenance to avoid replicative senescence. Telomere RNA component (TERC) is a critical part of the ribonucleoprotein telomerase complex and encodes the template for the hexanucleotide repeats that compose the telomere sequence (Feng et al., 1995). While the majority of human tumors maintain telomeres by overexpressing the reverse transcriptase TERT, SNPs at the *TERC* locus are associated with telomere lengthening and an increased risk of developing high-grade glioma (Walsh et al., 2014). Furthermore, TERC copy-number gain strongly predicts the progression of premalignant oral cavity neoplasms to invasive cancer (Dorji et al., 2015). Meanwhile, Marek's disease virus efficiently induces T cell lymphomas in chickens by expressing a viral telomerase RNA to promote telomere lengthening (Trapp et al., 2006). The telomeric repeat containing RNA (TERRA) transcribed from subtelomeric and telomeric DNA sequences exerts both telomerase-dependent and telomerase-independent effects on telomere maintenance (Rippe and Luke, 2015). One role for TERRA involves its dynamic regulation during the cell cycle, which regulates the exchange of single-strand DNA binding protein RPA by POT-1 and, thus, telomere capping (Flynn et al., 2011). Cancer cells lacking the SWI/SNF component ATRX maintain persistent TERRA loci at telomeres as cells transition from S phase to G2. This results in persistent RPA occupancy on the single-stranded telomeric DNA preventing telomerase-dependent telomere lengthening. These cells instead rely on the recombination-dependent alternative pathway of telomere lengthening which requires ATR, rendering ATRX-deficient cancer cells highly sensitive to ATR inhibitors (Flynn et al., 2015).

A recent report has also identified a role for a lncRNAs in the maintenance of genome stability. In the century since Boveri first described aneuploidy, chromosome aberrations have become one of the defining features of cancer. The lncRNA NORAD has been shown to exert a profound effect on chromosomal stability (Lee et al., 2016). NORAD, which is an abundant lncRNA, sequesters PUMILIO proteins away from target mRNA, including those for genes involved in mitosis, DNA repair, and replication. PUMILIO proteins negatively regulate these mRNAs by decreasing mRNA stability and translation. Thus, $NORAD^{-/-}$ cells have hyperactive PUMILIO and subsequently develop genomic instability and aneuploidy.

lncRNAs are extensively involved in stem cell maintenance and differentiation circuits (Flynn and Chang, 2014). That cancer

cells can co-opt these circuits by modulating lncRNAs is now appreciated. lncTCF7 recruits the SWI/SNF complex to the *TCF7* promoter to activate Wnt signaling in order to maintain liver cancer stem cell self-renewal (Wang et al., 2015). Cutaneous squamous cell carcinoma cells repress the lncRNA TINCR, which is required for keratinocyte differentiation by stabilizing differentiation-associated mRNAs through recruitment of STAU-1 (Kretz et al., 2013). The lncRNA NBAT-1 has also been observed to promote neuronal differentiation in neuroblastoma cells through regulation of the neuron-specific transcription factor NRSF/REST, and repression of this lncRNA is associated with high-risk neuroblastoma (Pandey et al., 2014).

Motility Circuits

Overexpression of MALAT1, an evolutionarily conserved, abundant nuclear lncRNA, was found to predict a high risk of metastatic progression in patients with early-stage non-small cell lung cancer (Ji et al., 2003). While MALAT1 loss of function in mouse revealed that it is a nonessential gene in development or for adult normal tissue homeostasis (Nakagawa et al., 2012; Zhang et al., 2012), depletion of MALAT1 in lung carcinoma cells impairs cellular motility in vitro and metastasis in mice (Gutschner et al., 2013), suggesting that MALAT1 overexpression in cancer may drive gain-of-function phenotypes not observed during normal tissue development or homeostasis.

Multiple cancer-associated lncRNAs have been implicated in regulating cancer invasion and metastases (Flockhart et al., 2012; Hu et al., 2014b; Huarte, 2015). Transforming growth factor β was found to induce the expression of lncRNA-ATB in hepatocellular carcinoma (HCC) cells, which facilitated epithelial to mesenchymal transition (EMT), cellular invasion, and organ colonization by HCC cells by two distinct RNA-RNA interactions (Yuan et al., 2014). lncRNA-ATB competitively binds miR-200 to activate the expression of ZEB1 and ZEB2 during EMT, while interactions with interleukin-11 mRNA enhances Stat3 signaling to promote metastasis. The breast cancer-associated lncRNA BCAR4 connects extracellular CCL22 to noncanonical Gli2 signaling by binding the transcription factors SNIP1 and PNUTS, stimulating cell migration and metastasis (Xing et al., 2014). lncRNAs also mediate metastasis programs through chromatin deregulation. Overexpression of the HOX-associated lncRNA HOTAIR in breast cancer reprograms the chromatin landscape genome-wide via recruitment of PRC2, enforcing a mesenchymal cellular phenotype which promotes breast cancer metastasis (Gupta et al., 2010), and is associated with poor prognosis in other malignancies as well (Kogo et al., 2011). The prostate cancer lncRNA SChLAP1, associated with poor prognosis and metastatic progression (Prensner et al., 2014b), promotes prostate cancer invasion and metastasis by disrupting the metastasis-suppressing activity of the SWI/SNF complex (Prensner et al., 2013).

Recent identification of metastasis-suppressing lncRNAs has opened a new perspective on a link between the tumor microenvironment and lncRNA modulation of the metastasis phenotype. The lncRNA NKILA, which is induced by nuclear factor κB (NF-κB) in response to inflammatory signaling, mediates a negative feedback loop suppressing NF-κB signaling by binding the cytoplasmic NF-κB/IκB complex and preventing IκB phosphorylation, NF-κB release, and nuclear localization (Liu et al., 2015). NKILA suppression in human breast cancer is linked to metastatic dissemination and poor prognosis. The lncRNA LET connects the hypoxia response to metastasis. Hypoxia-induced histone deacetylase 3 suppresses the LET promoter, impairing its expression and facilitating NF90 accumulation and hypoxia-induced cellular invasion (Yang et al., 2013a).

Therapeutic Promise in Targeting lncRNAs

lncRNAs, as highly tissue-specific drivers of cancer phenotypes, are now set to become prime targets for cancer therapy. The development of RNA-targeting therapeutics provides tremendous opportunities to modulate lncRNAs for anti-cancer purposes. While several strategies have been successfully employed to deplete lncRNAs, a prior knowledge of lncRNA cellular localization is critical for selecting the appropriate strategy to achieve robust lncRNA modulation (Lennox and Behlke, 2016). Small interfering RNAs, upon loading in the RISC complex in the cytoplasm, can efficiently deplete cytoplasmic lncRNAs, although these molecules have had variable success in targeting predominantly nuclear lncRNAs. Antisense oligonucleotides (ASOs), on the other hand, are chimeric RNA/DNA oligonucleotides that direct RNase H to cleave complementary target mRNA or lncRNA (Ideue et al., 2009) and can robustly deplete the transcripts regardless of their cellular localization.

ASOs have already demonstrated success in modulating coding genes involved in a variety of benign disorders, lymphoma, and solid tumors (Buller et al., 2015; Gaudet et al., 2014; Hong et al., 2015; Meng et al., 2015), and are poised for use in lncRNA-targeted therapeutics (Ling et al., 2013a). Preclinical studies are demonstrating the therapeutic efficacy of ASOs targeting cancer-associated lncRNAs. For example, Malat1 may be a viable therapeutic target in some cancers, such as in the mouse MMTV-PyMT breast cancer model where Malat1 drives tumor growth and metastasis. In this model, ASOs targeting Malat1 have demonstrated therapeutic efficacy in vivo by promoting cystic differentiation, increased cell adhesion, and decreased migration (Arun et al., 2016).

Inhibiting lncRNA function by RNA depletion need not be the only mechanism of targeting lncRNAs for cancer therapy. New classes of RNA-based therapeutics (Kole et al., 2012) hold promise for modulating the activity of lncRNAs in diverse ways. Splice-switching oligonucleotides could be utilized to excise lncRNA exons that encode necessary functional domains. Alternatively, steric blocking oligonucleotides may interfere with the interaction of lncRNAs with their binding partners. Future technical innovations in modulating lncRNAs in vivo along with increased insights into lncRNA pathways in cancer biology will offer further opportunities for lncRNA-targeting therapeutics.

Emerging Paradigms and Future Directions

The vast majority of studies in cancer-associated lncRNAs have focused on the cellular effects of altered transcript abundance of lncRNAs. However, structural approaches to evaluating lncRNA functions have revealed that even SNPs can alter local RNA structure at functionally relevant sites involved in miRNA or protein binding (Wan et al., 2014). Application of whole-transcriptome RNA structural analyses in cancer may reveal functional consequences of SNPs or somatic mutations within cancer-associated lncRNAs (Chu et al., 2015a; Spitale et al., 2015). Furthermore, the emerging evidence that some putative

lncRNAs may encode short, translated open reading frames (Anderson et al., 2015; Ingolia et al., 2014), and that coding RNAs can exert translation-independent functional roles (Li et al., 2015), suggests that distinction between mRNA and lncRNA may be less absolute than once thought.

Cataloging the physiological role of cancer-associated lncRNAs will require a transition in investigative approaches from lncRNA annotation and molecular or cellular characterization to animal genetic models of cancer (Sauvageau et al., 2013), as recently suggested (Li and Chang, 2014). Organismal models will be especially critical for elucidating the emerging physiologic roles for noncoding RNA in intercellular signaling, inflammation, angiogenesis, and immune modulation, which are central factors in the cooperation of cancer cells and stromal cells required to support tumor growth and development (Bernard et al., 2012; Lu et al., 2015; Michalik et al., 2014; Satpathy and Chang, 2015; Yuan et al., 2012).

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