



Long Noncoding RNAs: At the Intersection of Cancer and Chromatin Biology

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Although only 2% of the genome encodes protein, RNA is transcribed from the majority of the genetic sequence, suggesting a massive degree of cellular functionality is programmed in the noncoding genome. The mammalian genome contains tens of thousands of long noncoding RNAs (lncRNAs), many of which occur at disease-associated loci or are specifically expressed in cancer. Although the vast majority of lncRNAs have no known function, recurring molecular mechanisms for lncRNAs are now being observed in chromatin regulation and cancer pathways and emerging technologies are now providing tools to interrogate lncRNA molecular interactions and determine function of these abundant cellular macromolecules.

It has become clear only recently that alterations within the noncoding genome are major contributors to cancer and other 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 (Beroukhi et al. 2010) are implicated in multiple cancer types. One of the most unexpected findings of the genomics era of biology is that the noncoding genome is the source of extensive transcription of noncoding RNA (Rinn and Chang 2012; Morris and Mattick 2014). Remarkably, several cancer-associated loci contain ultraconserved noncoding RNA sequences suggesting that these transcripts play important roles in cancer pathogenesis (Calin et al. 2007).

The catalog of noncoding genes has grown tremendously over the past few years in large part because of the identification of extensive long noncoding RNA (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. Long noncoding RNAs (lncRNAs) are functionally defined as transcripts >200 nucleotides in length with no protein coding potential. These transcripts are biochemically similar to messenger RNA (mRNA) and are typically transcribed by RNA polymerase II, polyadenylated, and are frequently spliced, although they often contain fewer exons than coding genes. It is now recognized that lncRNAs are exquisitely regulated and are restricted to specific cell types to a

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greater degree than mRNA (Cabili et al. 2011). The catalog of identified lncRNAs now includes tens of thousands of genes and many are uniquely expressed in differentiated tissues or specific cancer types (Iyer et al. 2015). Indeed, the number of lncRNA genes outnumbers protein-coding genes (Derrien et al. 2012) with more than 90% having no appreciable peptide products (Banfai et al. 2012; Guttman et al. 2013).

Supporting an important functional role for lncRNAs, some lncRNA genes are evolutionarily conserved, although may display limited sequence similarity. Syntenic genomic sites frequently encode evolutionarily conserved, functional lncRNAs (Chodroff et al. 2010; Ulitsky et al. 2011; Hezroni et al. 2015). In a recent report, identification of regions of conserved microhomology within lncRNAs arising from syntenic sites was used to identify a set of 47 orthologs of the *Drosophila* lncRNA roX in diverse Drosophilid species (Quinn et al. 2016). Despite drastic sequence divergence, roX orthologs maintain regions of conserved secondary structure, retain X-chromosome interactions, and can rescue male lethality in roX-null *Drosophila melanogaster*.

Despite the rapid pace of discovery in the field, the vast majority of annotated lncRNAs have yet to be functionally characterized. However, several recurring lncRNA molecular mechanisms have been observed, particularly in chromatin biology and gene regulation. The emergence of several technologies over the last few years have expanded investigators abilities to functionally annotate cancer-associated lncRNAs and resulted in an exponential increase in our understanding of these enigmatic molecules (Chu et al. 2015). In this article, we provide an overview of the current state of lncRNA function in cellular molecular mechanisms and review the known roles for lncRNAs in cancer pathophysiology. Portions of this review are abbreviated from recent articles by the investigators (Chu et al. 2015; Schmitt and Chang 2016), and readers are referred to these articles for more in depth treatment of lncRNA as cancer biomarkers or lncRNA-centric technologies, respectively.

LONG NONCODING RNA: EMERGENCE OF A DISTINCT CLASS OF REGULATORY RNA

In this section we highlight several of the recurring molecular roles of lncRNAs in cellular processes (Fig. 1). Functionality in part begins with lncRNA cellular localization: nuclear lncRNAs are enriched for functionality involving nuclear structure, chromatin interactions, transcriptional regulation, and RNA processing, whereas cytoplasmic lncRNAs can modulate mRNA stability or translation and influence cellular signaling cascades (Batista and Chang 2013). 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 gene in *trans* on different chromosomes (Huarte et al. 2010; Huarte 2015; Sahu et al. 2015). lncRNAs are widely known to regulate genes in *cis* as enhancer-associated RNAs (Orom et al. 2010), through transcription activation or silencing (Huarte et al. 2010; Zhu et al. 2013; Dimitrova et al. 2014; Trimarchi et al. 2014), 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).

Nuclear Organization

Deranged nuclear architecture has been a defining feature of cancer cells for >100 years since Beale described abnormal nuclear structure in pharyngeal carcinoma cells in the mid-19th century. Nuclear atypia remains an important cytopathologic feature in diagnosing and grading of malignant cells such as in the case of Pap test for cervical neoplasia and in breast carcinoma. Nuclear organization relies heavily on non-coding nuclear RNA (Quinodoz and Guttman 2014). RNase treatment and transcription inhibitors substantially disrupt nuclear organiza-

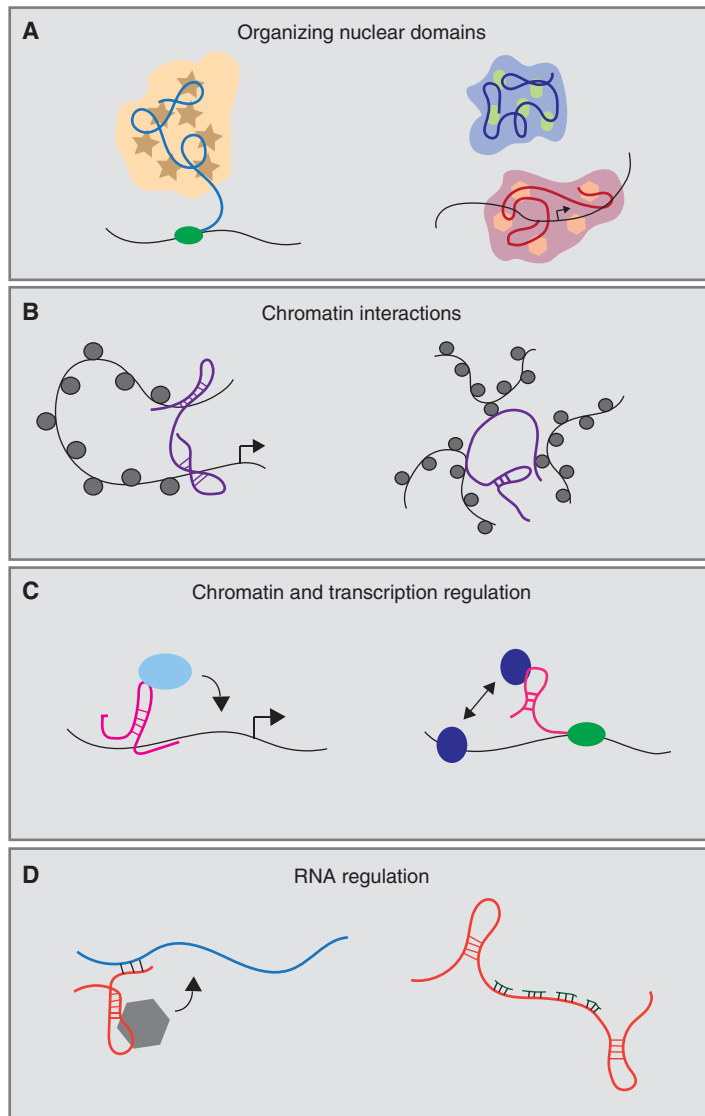


Figure 1. Molecular mechanisms of long noncoding RNAs (lncRNAs). LncRNAs interactions with DNA, RNA, and protein mediate a wide array of cellular functions including (A) nuclear organization, (B) chromatin interactions, (C) chromatin and transcriptional regulation, and (D) RNA regulation.

tion, whereas translation inhibitors did not (Nickerson et al. 1989), pointing to important structural roles for nuclear RNA. It is now clear that nuclear lncRNAs interact with protein, chromatin, and RNA to organize nuclear domains and maintain structure.

Formation of the nuclear paraspeckle, a poorly understood nuclear domain, is dependent on the ncRNA Nuclear enriched abundant

transcript 1 (NEAT1). NEAT1 serves as a nidus for formation of the paraspeckle where, on NEAT1 transcription, soluble protein components of the paraspeckle localize to regions of NEAT1 accumulation thus forming the nuclear domain (Clemson et al. 2009; Mao et al. 2011; Shevtsov and Dundr 2011). This domain has been implicated in nuclear retention of A-to-I hyperedited transcripts (Chen and Carmichael

2009) and the sequestration of a transcription repressor from its chromatin targets (Imamura et al. 2014). Overexpression of NEAT1 in breast cancer specimens is associated with poor patient survival in which a hypoxic tumor micro-environment induces NEAT1 transcription, increasing paraspeckle formation, enhancing cancer cell survival and stimulating proliferation (Choudhry et al. 2015).

lncRNAs associated with functional nuclear domains can provide anchorage points for the localization of genes undergoing dynamic regulation. The serum response, the activation of which in breast cancer is associated with poor survival (Chang et al. 2004), regulates the localization of dynamically regulated genes between transcriptionally inactive and active nuclear domains. Serum stimulation recruits the demethylase KDM4C to select promoter regions that are regulated by E2F where it demethylates human Polycomb 2 homolog (Pc2), also known as Chromobox 4 (Yang et al. 2011). Methylated Pc2 interacts with the lncRNA TUG1 in Polycomb bodies, whereas demethylated Pc2 binds MALAT1/NEAT2 localized in interchromatin granules, essentially creating a posttranslational switch for Pc2 interaction with domain defining lncRNAs.

Remarkably, mouse genetic loss of function models of lncRNAs *Neat1* and *Malat1* have revealed no clear phenotypes in normal animal development (Nakagawa et al. 2011, 2012; Eissmann et al. 2012; Zhang et al. 2012). The contrast of these results with observed in vitro cellular phenotypes suggests that redundancy or compensatory mechanisms may be at work. Yet, *Malat1* knockout or depletion by antisense oligonucleotides in a mouse MMTV-PyMT breast cancer model slows tumor growth and inhibits metastasis. *Malat1* loss of function promotes cystic differentiation of breast cancer cells and enhances adhesion and inhibits migration (Arun et al. 2016).

Chromatin Interactions

The three-dimensional organization of chromatin facilitates contacts between distantly spaced intra- or interchromosomal sites allowing for

transmission of regulatory information between distant loci. Chromatin looping facilitates the interaction of the *cis* interaction of the regulatory lncRNA HOTTIP, which is transcribed from the margin of the HOXA locus, bringing HOTTIP into proximity with other HOXA locus genes and supporting transcription at the locus. HOTTIP, transcribed from the 5' end of the locus, coordinates transcription along the multi-gene locus by interacting with WDR5 and recruiting the WDR5/MLL complex to target genes within the locus, increasing H3K4 trimethylation and gene transcription (Wang et al. 2011). Similarly, a class of lncRNAs referred to as noncoding RNA-activating recruit Mediator to the site of lncRNA transcription and thereby promote gene transcription of neighboring genes in close proximity because of chromatin looping (Lai et al. 2013).

Distant intra- and interchromosomal chromatin interactions are also mediated by lncRNAs. Enhancer RNAs are a subset of lncRNA transcribed from active enhancer sites. In response to estrogen exposure, enhancer RNAs transcribed from estrogen receptor α (ER α) bound enhancers facilitate the enhancer-promoter looping induced by estrogen signaling (Li et al. 2013). Similarly, the prostate cancer-specific lncRNA PCGEM1 enables chromatin looping between androgen receptor occupied enhancer regions and the promoters of target genes to facilitate their regulation. PCGEM1 bridges the enhancer and promoter regions by binding separately to the androgen receptor (AR) at enhancer sites and pygopus 2, a H3K4 trimethyl “reader” protein bound to the promoters of AR-regulated genes (Yang et al. 2013b). The X-linked lncRNA Firre interacts with hnRNPU to organize interactions between five chromosomes (Hacisuleyman et al. 2014).

Regulation of Chromatin

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). X-inactive specific transcript (*Xist*), one of the first functionally anno-



tated lncRNAs, regulates dosage compensation in female mammals by localizing to the X chromosome and recruiting multiple factors directly and indirectly, including the polycomb repressive complex 2 (PRC2), 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).

Although 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 and colleagues developed chromatin isolation by RNA purification (ChIRP), in which short complementary biotinylated oligonucleotides hybridize to target RNAs and copurify chromatin cross-linked to the target RNA (Chu et al. 2011). Similar methods including capture hybridization analysis of RNA targets (CHART) and RNA antisense purification (RAP) showed 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 X chromosome inactivation (Simon et al. 2011, 2013; Engreitz et al. 2014).

Transcriptional Regulation

Cancer transcriptional programs are also modulated by lncRNA recruitment to distant promoters and enhancers. ChIRP revealed that Paupar, a central nervous system (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). 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 ER α (Chakravarty et al. 2014).

ChIRP revealed that NEAT1 localizes to promoters of genes involved in prostate cancer growth and increased chromatin marks of active transcription at these sites, driving androgen-independent prostate cancer growth. T helper 17 cells are proinflammatory T cells, and the lncRNA RMRP was shown by ChIRP to coassociate with the key Th17 transcription factor ROR- γ t 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).

Regulation of RNA Processes

lncRNA modulation of RNA metabolism is an emerging theme with described roles in the control of mRNA stability, splicing, and translation. 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 necessary for STAU-1 binding by duplexing with an Alu element of a cytoplasmic lncRNA (Gong and Maquat 2011), thus a lncRNA can confer specificity on the STAU-1-mediated mRNA decay. STAU-1–mRNA interactions are also modulated by terminal differentiation-induced noncoding RNA (TINCR), which drives epidermal differentiation and is down-regulated in poorly differentiated cutaneous squamous-cell carcinomas (Kretz et al. 2013). TINCR recruits STAU-1 to mRNA bearing the 25 nucleotide TINCR Box, somewhat unexpectedly, stabilizing these messages and facilitating the translation of several genes involved in keratinocyte differentiation. DNA damage-induced lncRNA-p21 has been shown to inhibit translation of certain mRNAs such as JUNB and CTNBN1 through a direct RNA–RNA interaction mechanism (Yoon et al. 2012).

Multiple lncRNAs have also been implicated in regulating posttranscriptional mRNA pro-

cessing. The prostate cancer-specific lncRNA PCA3 (Bussemakers et al. 1999), which is the first lncRNA assayed in an FDA-approved clinical test for prostate cancer, is an intronic lncRNA transcribed antisense to the protein coding gene *PRUNE2*. Expression of PCA3 in prostate cancer cells leads to formation of a double-stranded RNA heteroduplex with *PRUNE2* that is a template for ADAR to perform adenosine-to-inosine RNA editing and down-regulation of this tumor suppressor (Salameh et al. 2015). In line with prior observations that MALAT1 can regulate alternative splicing through interactions with serine/arginine splicing factors and pre-mRNA, Malat1 binds to nascent strands of pre-mRNA, localizing Malat1 to localize at the proximal chromatin region of transcriptionally active genes (Tripathi et al. 2010; Engreitz et al. 2014).

lncRNAs and pseudogene RNA have also been postulated to act as competing endogenous RNA (ceRNA) or “RNA sponges,” interacting with microRNAs 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, although 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 (Poliseno et al. 2010; Karreth et al. 2011; Tay et al. 2011). Indeed, lncRNAs serving as RNA sponges appear to regulate a diverse array of prostate cancer driver genes (Du et al. 2016). Similar ceRNA mechanisms have been reported for lncRNAs H19 and HULC (Wang et al. 2010; Keniry et al. 2012; Kallen et al. 2013). However, quantitative analyses of miRNA and target mRNA abundance suggest that the ceRNA mechanism could only operate given appropriate stoichiometry between ceRNA, mRNA, and miRNAs. Denzler and colleagues provided compelling quantitative evidence that very high ceRNA copy number thresholds exist for derepression of mRNAs targeted by a miRNA. Their data supports a model in which the number of endogenous miRNA target sites exceeds the number of miRNA copies per cell, resulting in

a high fraction of miRNAs already bound to target sites. This model implies that derepression of mRNAs by a ceRNA mechanism requires the number of decoy target sites encoded in a ceRNA to equal or exceed the total number of all miRNA target sites in the cell, which in the case of miR-122 is $\sim 10^5$ (Denzler et al. 2014). Thus, quantitative experimental analyses and knock-out studies are needed beyond the presence of matching miRNA seed sequences to validate ceRNA mechanisms.

lncRNA DRIVERS OF CANCER PHENOTYPES

Cancer cells modulate intracellular signaling networks to proliferate, overcome cytostatic and tumor-suppressor pathways, enhance viability, and promote invasion and metastasis (Hanahan and Weinberg 2000; Hanahan and Weinberg 2011). lncRNAs are key regulators of pathways involving each of the hallmarks of cancer, as described by Hanahan and Weinberg (2000) (Fig. 2). In this section, we review the myriad of cellular activities of lncRNAs in driving classic cancer phenotypes.

Cell Growth and Tumor Suppression

Normal tissue homeostasis requires a careful balance of the imperative of cellular proliferation and repopulation on the one hand with the need to limit the expansion of deranged cell populations on the other. 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 up-regulate insulin like growth factor 1 receptor expression and signaling (Trimarchi et al. 2014). Estrogen responsive genes depend on enhancer lncRNAs to facilitate local chromatin interactions that promote transcriptional regulation by ER α (Li et al. 2013). Furthermore, androgen signaling in prostate cancer also relies on a number of lncRNAs that are implicated in prostate cancer proliferation that act through direct interactions with the androgen receptor (Yang et al. 2013b; Zhang

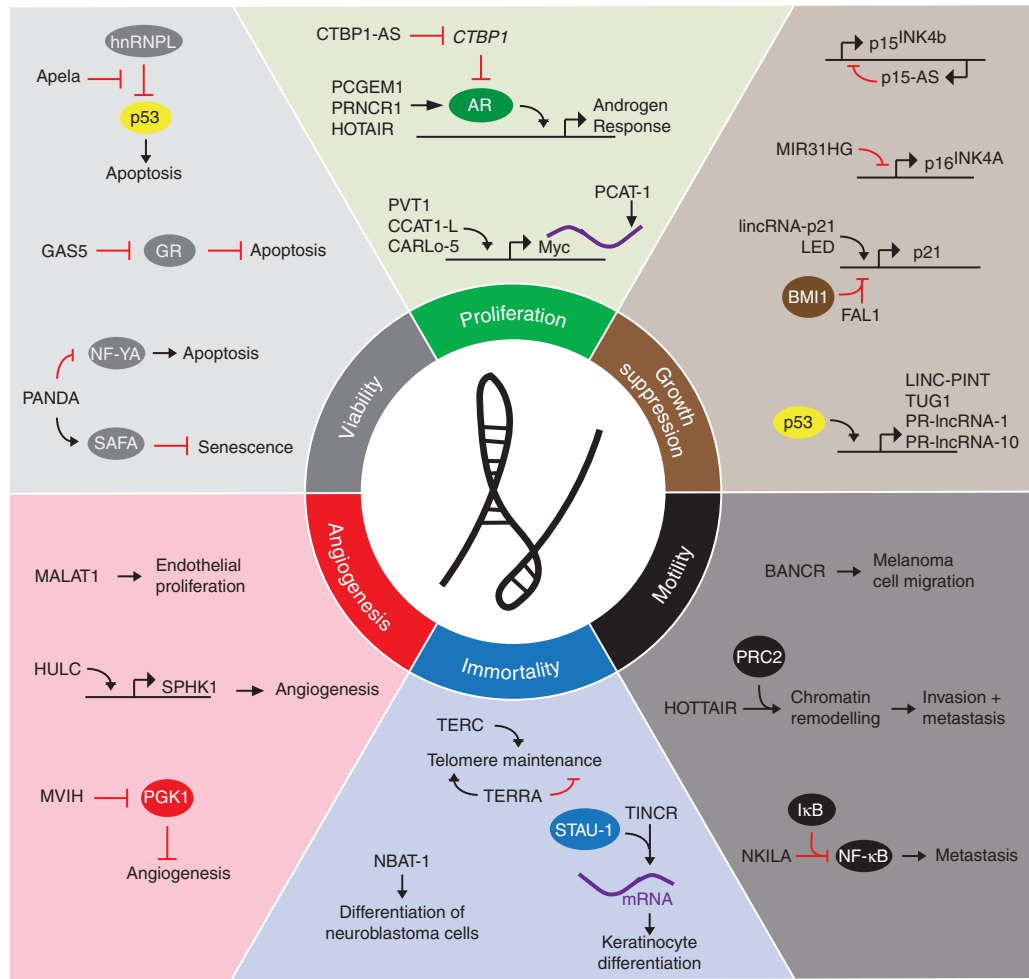


Figure 2. Long noncoding RNAs (lncRNAs) in cancer phenotypes. LncRNAs contribute to each of the six hallmarks of cancer defined by Hanahan and Weinberg. Selected examples of lncRNAs and their molecular partners or genomic targets are shown for proliferation, growth suppression, motility, immortality, angiogenesis, and viability cancer phenotypes. (From Schmitt and Chang 2016; reprinted, with permission, from the authors.)

et al. 2015) or by inhibiting repressors of the androgen receptor (Takayama et al. 2013).

The *myc* proto-oncogene is the subject of extensive regulation by lncRNAs. *Myc* transcription is activated in *cis* by the colon cancer-associated lncRNA *CCAT-1*, also known as *CARLo-5*, which promotes long-range interactions between *myc* and an enhancer element (Kim et al. 2014; Xiang et al. 2014). The prostate specific lncRNA *PCGEM1* binds to *myc* enhancing *myc*'s transcriptional activation of sev-

eral genes that regulate metabolic processes in 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).

Amplification of the 8q24 locus is a well-characterized oncogenic event in human malignancies that amplifies *MYC* copy number. *PVT1* is a lncRNA gene that is at the breakpoint of the t(2:8) translocation in Burkitt 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 multigene segment encompassing *Myc* and the lncRNA *Pvt1* promoted efficient tumor development (Tseng et al. 2014). Coamplification of *Pvt1* and *Myc* increased *myc* protein levels, whereas depletion of *PVT1* in *myc*-driven human colon cancer cells impaired proliferation.

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 tumor-suppressor genes are regulated in *cis* by their antisense transcripts. For example, the 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).

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 *p15AS* induces silencing of $p15^{INK4b}$ through heterochromatin formation (Yu et al. 2008) and elevated *p15AS* 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).

LncRNAs in the p53 Pathway

Regulation of the p53 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 pointing to 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; Zhang et al. 2014; Younger et al. 2015). 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 *CKDNIA* enhancer to support cell-cycle arrest by p53. *LED* is silenced in a subset of p53-wildtype leukemia cells indicating a possible tumor-suppressor role for this lncRNA (Leveille et al. 2015).

LncRNA-*p21*, which is induced by DNA damage in a p53-dependent manner, interacts with hnRNA-K to regulate *CDKN1A* in *cis* and arrest the cell cycle in a p21-dependent manner (Huarte et al. 2010; Dimitrova et al. 2014). *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 (Li et al. 2015). The lncRNA *FAL-1*, 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 proapoptotic genes (Hung et al. 2011). *PANDA* has also been shown to regulate senescence through an interaction with *SAFA* and *PRC1* (Puvvula et al. 2014).

Enhancing Cellular Survival

The selective advantage of tumor cells is driven in some cancers by preservation of an undifferentiated tumor cell population. LncRNAs are extensively involved in differentiation circuits and modulation of this functional class of lncRNAs may contribute to the maintenance of cancer stem-cell populations (Flynn and Chang 2014). *LncTCF7* recruits the *SWI/SNF* complex to the *TCF7* promoter to activate *Wnt*

signaling 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).

The replicative potential of differentiated somatic mammalian cells is typically limited by progressive telomere shortening following replication. However, tumor cells adapt mechanisms to maintain telomeres and effectively obtain immortality. The telomere RNA component (TERC) is a critical subunit of the ribonucleoprotein telomerase complex and encodes the template for the hexanucleotide repeats that compose the telomere sequence (Feng et al. 1995). Single-nucleotide polymorphisms at the TERC locus are associated with telomere lengthening and an increased risk of developing high-grade glioma (Walsh et al. 2014). 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 promotes 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).

Cancer cells that overcome intrinsic checks on growth are yet constantly subjected to conditions of nutrient scarcity and hypoxia and must develop compensatory mechanisms to survive under conditions of stress. 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 glucocorticoid receptor's DNA-binding domain and acting as a decoy (Kino et al. 2010; Hudson et al. 2014). This blockade of glucocorticoid receptor decreases expression of the cellular inhibitor of apoptosis 2 (Kino et al. 2010), thereby enhancing apoptosis under stressed conditions in normal cells. Suppression of Gas5 in human breast cancer cells relative to adjacent normal breast tissue has been observed and may support the enhanced viability of breast cancer cells in the nutrient-poor tumor microenvironment (Mourtada-Maarabouni et al. 2009).

Tumor Invasion and Metastasis

Cancer metastasis was one of the first cancer phenotypes associated with dysregulated lncRNAs. Overexpression of MALAT1 in early stage nonsmall cell lung cancer patients was found to predict a high risk of metastatic progression (Ji et al. 2003). Although MALAT1 loss of function in mouse revealed that it is a non-essential 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). Overexpression of the HOX-associated lncRNA HOX transcript antisense RNA (HOTAIR) in breast cancer repro-



grams 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. 2014), promotes prostate cancer invasion and metastasis by disrupting the metastasis suppressing activity of the SWI/SNF complex (Prensner et al. 2013). Transforming growth factor β (TGF- β) was found to induce the expression of lncRNA-ATB in hepatocellular carcinoma (HCC) cells, which facilitated the 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, although interactions with IL-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).

Recent identification of metastasis suppressing lncRNAs has opened a new perspective into a link between the tumor microenvironment regulation of lncRNAs that promote or impair metastasis. The lncRNA LET connects the hypoxia response to the metastasis phenotype. Hypoxia-induced histone deacetylase 3 suppresses the LET promoter, impairing its expression and facilitating nuclear factor 90 accumulation and hypoxia-induced cellular invasion (Yang et al. 2013a). On the other hand, NKILA is a metastasis suppressor lncRNA that is induced by NF- κ B in response to inflammatory signaling. NKILA mediates a negative feedback loop that suppresses NF- κ B signaling by binding the cytoplasmic NF- κ B/I κ B complex and preventing I κ B phosphorylation, NF- κ B release, and nuclear localization (Liu et al. 2015). Downregulation of NKILA expression in human breast cancer is associated to metastatic dissemination of disease and poor prognosis.

FUTURE DIRECTIONS

LncRNAs occupy a unique niche at the interface of chromatin biology and the regulation of cancer phenotypes. That thousands of these transcripts are differentially expressed in cancer suggests that lncRNAs may play a far greater role in cancer pathways that currently appreciated. However, additional layers of lncRNA regulation are clearly at work that may increase the diversity of lncRNA function far beyond what is currently appreciated. The extensive posttranscriptional modifications of RNA, such as N^6 -methyladenosine, are dynamically regulated in mammalian cells and influence cell fate decisions (Batista et al. 2014; Zhou et al. 2015). Furthermore, RNA structural approaches to evaluating lncRNA functions have revealed that even single-nucleotide polymorphisms 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 structural consequences of SNPs or somatic mutations within cancer-associated lncRNAs that may alter lncRNA function (Chu et al. 2015; Spitale et al. 2015). Furthermore, the emerging evidence that some putative lncRNAs may encode short, translated open reading frames (Ingolia et al. 2014; Anderson et al. 2015), 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 physiologic role of cancer-associated lncRNAs will require an integrated approaches utilizing molecular and cellular characterization as well as animal genetic models (Sauvageau et al. 2013), as recently suggested (Li and Chang 2014). Animal models will be especially critical for elucidating the emerging physiologic roles for noncoding RNA in intercellular signaling, inflammation, angiogenesis, and immune modulation (Bernard et al. 2012; Yuan et al. 2012; Michalik et al. 2014; Lu et al. 2015; Satpathy and Chang 2015) and for identification of therapeutic targets in cancer. Multiple RNA-directed technologies, including antisense oligonucleotides, now provide an op-

portunity to rationally design targeted drugs to modulate lncRNAs involved in cancer pathophysiology for therapeutic gain (Gaudet et al. 2014; Buller et al. 2015; Meng et al. 2015).

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