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REVIEW

The emerging role of lncRNAs in cancer

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It is increasingly evident that many of the genomic mutations in cancer reside inside regions that do not encode proteins. However, these regions are often transcribed into long noncoding RNAs (lncRNAs). The recent application of next-generation sequencing to a growing number of cancer transcriptomes has indeed revealed thousands of lncRNAs whose aberrant expression is associated with different cancer types. Among the few that have been functionally characterized, several have been linked to malignant transformation. Notably, these lncRNAs have key roles in gene regulation and thus affect various aspects of cellular homeostasis, including proliferation, survival, migration or genomic stability. This review aims to summarize current knowledge of lncRNAs from the cancer perspective. It discusses the strategies that led to the identification of cancer-related lncRNAs and the methodologies and challenges involving the study of these molecules, as well as the imminent applications of these findings to the clinic.

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Cancer is primarily caused by genetic alterations that result in the deregulation of the gene networks that are responsible for the maintenance of cellular homeostasis. Most cancers arise as a result of the interaction of somatic and germline mutations with various environmental factors. Many of these mutations lie within regions of the genome that lack protein-coding capacity[1], yet contain other type of genes that exert their functions as RNA molecules: the noncoding RNAs.

Thanks to the general implementation of tiling microarrays and high-throughput sequencing technologies to whole genomes and transcriptomes, it is now evident that whereas less than 2% of the genome encodes proteins, at least 75% is actively transcribed into noncoding RNAs[2]. Although some of the noncoding transcripts are small, most of them surpass 200 nucleotides in length, and they are therefore catalogued as long (or large) noncoding RNAs (lncRNAs). Most (but not all) lncRNAs are transcribed by RNA polymerase II, and they are thus capped and polyadenylated at their 5′ and 3′ ends respectively. The most recent estimate by the Encyclopedia of DNA Elements (ENCODE) Project Consortium (GENCODE release 23) is that the human genome contains close to 16,000 genes that encode more than 28,000 distinct lncRNA transcripts.

With regard to their role in cancer, lncRNAs have tissue-specific expression and are expressed in a regulated manner, in correlation with distinct gene sets that influence cell cycle regulation, survival, immune response or pluripotency, among other functions, which determine the transformed phenotype of cancer cells[3]. Furthermore, several lncRNAs are transcriptionally regulated by key tumor suppressors or oncogenes[4,5]. Beyond these suggestive observations, more definitive evidence for a role for lncRNAs in oncogenesis has come from functional studies, which have revealed the molecules' active roles in gene regulation governing virtually every physiological process.

LncRNAs are a highly heterogeneous group of transcripts that regulate gene expression by means of diverse mechanisms. Consequently, they are found to be differentially expressed in tumors, and they are directly linked to the transformation of healthy cells into tumor cells. Some of the lncRNAs with known links to cancer are presented in **Table 1** (also see **Supplementary Table 1**). For example, the overexpression of the lncRNA *HOTAIR* promotes the metastasis of breast cancer cells by epigenetically silencing the developmentally important genes in the *HOXD* cluster, among others[6]; overexpression of *ANRIL* (officially known as *CDKN2B* antisense RNA 1 (*CDKN2B-AS1*)), which silences the tumor suppressor locus that comprises *INK4b, ARF and INK4a* (officially known as *CDKN2B, CDKN2AIP* and *CDKN2A*), is linked to poor prognosis in prostate and gastric cancer[7–11]. LncRNAs are thus functional transcripts that contribute to the hallmarks of cancer (**Fig. 1**), and they are therefore becoming attractive potential therapeutic targets[12–14].

Here we present an overview of current knowledge regarding the roles of lncRNAs in cancer, discussing the challenges of studying them, as well as the potential clinical applications that they offer.

Identifying lncRNAs in cancer

Despite the overwhelming presence of lncRNAs in mammalian cells, conceptual and technical limitations have restricted our knowledge of their contribution to cancer. Initially, a conceptual shift had to be made away from the dogma of molecular biology, which implies that the only role of RNA is to encode proteins. In addition, the technological inability to interrogate the noncoding regions of the genome—leading to a lack of reliable lncRNA annotations—needed to be solved. These limitations have been overcome as a result of the general implementation of deep-sequencing technologies and the subsequent functional interrogation of lncRNAs (**Fig. 1**).

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Table 1 Examples of lncRNAs with tumor-suppressor or oncogenic function (see Supplementary Table 1)

Symbol[a]	Accession no.[b]	Cancer phenotype	Cancer association	Mechanism	Ref.
GAS5	Ensembl ID: ENSG00000234741	Induces cell arrest and sensitizes cells to apoptosis. Alters cell metabolism	Downregulated in breast cancer	Outcompetes DNA binding to the glucocorticoid receptor (GR)	102,103
LINC-PINT (*MKLN1-AS1*)	GEO profile: FLJ43663	Inhibits cell proliferation and promotes apoptosis	Downregulated in colorectal cancer	Interacts with PRC2 to silence gene targets	38,65
MEG3	Ensembl ID: ENSG00000214548	Inhibits cell proliferation	Downregulated in multiple tumor types	Downregulates MDM2 and promotes p53 accumulation. Also controls expression of gene loci through recruitment of PRC2	82,128
NBAT1 (*CASC14*)	GEO profile: LOC729177	Inhibits cell proliferation and invasion, and impairs differentiation of neuronal precursors	High expression predicts a good clinical outcome of neuroblastomas. CpG methylation and a high-risk neuroblastoma-associated SNP contribute to low expression	Promotes the silencing of *REST* by PRC2	32
PR-lncRNA-1	Ensembl ID: ENST00000562178.1	Inhibits cell proliferation, and promotes apoptosis	Downregulated in colorectal cancer	Enhances p53 transcriptional activation	40
PTENP1		Inhibits cell proliferation, migration, invasion and tumor growth	Locus selectively lost in sporadic colon cancer, prostate cancer and melanoma	Decoy for microRNAs that target *PTEN*	111,114
CDKN2B-AS1 (*ANRIL, p15AS*)	Ensembl ID: ENSG00000240498	Promotes cell proliferation	Cancer-associated SNPs and high expression linked to bad prognosis in prostate and gastric cancer	Epigenetic silencing of the locus by interaction with CBX7 and PRC2	7–11
BCAR4	Ensembl ID: ENSG00000262117	Promotes proliferation and migration	Expression correlates with advanced breast cancer, metastasis and anti-estrogen resistance	Activates the hedgehog/GLI2 transcriptional program, by binding to and activating the transcription factors SNIP1 and PNUTS	106,107
HOTAIR	Ensembl ID: ENSG00000228630	Promotes metastasis	Overexpressed in liver, metastatic breast, lung and pancreatic tumors	Acts as scaffold for the chromatin repressors PRC2 and LSD1. Silences *HOXD* and other gene loci	6,76,97
MALAT1 (*NEAT2*)	Ensembl ID: ENSG00000251562	Promotes cell proliferation and metastasis	Overexpressed in lung adenocarcinoma, breast, pancreatic, colon, prostate and hepatocellular carcinomas. SNP linked to hepatocellular carcinoma	Related to alternative splicing and active transcription. Conserved tRNA-like sequence at the 3′ end cleaved off and processed to generate a short tRNA-like ncRNA (mascRNA)	20–25,129
PCAT1	Ensembl ID: ENST00000561978.1	Promotes cell proliferation	Upregulated in prostate cancer. Contains disease-associated SNPs	Interacts with PRC2 and silences genes *in trans*. Also post-transcriptionally activates c-MYC, and inhibits *BRCA2*	89–91
SCHLAP1 (*PCAT11*)	NCBI Reference Sequence: NR_104321.1	Promotes invasion and metastasis	Upregulated in prostate cancer	Interacts with SWIF/SNIF complex and antagonizes its regulatory functions genome-wide	101

[a]Official symbols are given; symbols in parentheses are alternate symbols. [b]Ensembl Genome Browser, Gene Expression Omnibus (GEO), or other accession IDs.

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Alteration of lncRNA expression in cancer cells. The first lncRNAs that were associated with cancer because of their aberrant expression were prostate cancer associated 3 (*PCA3*, also called *DD3*)[15] and prostate-specific transcript 1 (*PCGEM1*)[16], found using differential display analysis of prostate tumors and normal tissue. *PCA3* is currently used as a prostate cancer biomarker[17], and *PCGEM1* is involved in androgen receptor transcriptional activation[18] and *c-MYC* activation, both of which are involved in the development of prostate cancer[19] (**Fig. 2**). The expression of the lncRNA metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) was also identified early on as a prognostic parameter for lung cancer survival[20]. *MALAT1* expression has now been associated with malignancy in multiple types of tumors, including in the liver, breast and colon[21], suggesting that it has a general role in cell proliferation. *MALAT1* is a nuclear lncRNA, highly conserved across mammals and extremely abundant in many cell types. It has been linked to the regulation of alternative mRNA splicing[22] and the modulation of the epigenetic machinery[23], and it is known to be associated with nascent pre-mRNA between the 3′ and 5′ ends of actively transcribed genes[24,25]. Despite being the subject of intensive study, the role of *MALAT1* in cancer is not yet fully understood.

Myriad studies have now used microarray technology to profile different types of cancer samples. However, conventional expression microarray designs contain few probes corresponding to lncRNAs. To compensate for this dearth, and as lncRNA annotation has been refined, arrays have been modified to detect the expression of lncRNAs. This has led to exponential growth in the number of lncRNAs found to have aberrant expression in different cancer types. But possibly the most important progress has been made possible by the application of high-throughput sequencing of RNA (RNA-seq) to an increasing number of tumor samples. The most comprehensive study[26] of lncRNAs so far includes the analysis of poly A^+ RNA-seq data from more than 7,000 samples, including tumors, normal samples and tumor cell lines. The study identifies 58,000 lncRNA genes detected in at least one type of sample, which is twice the number of protein-coding genes identified in this study. The expression of 8,000 out of these 58,000 lncRNAs is associated with a lineage or

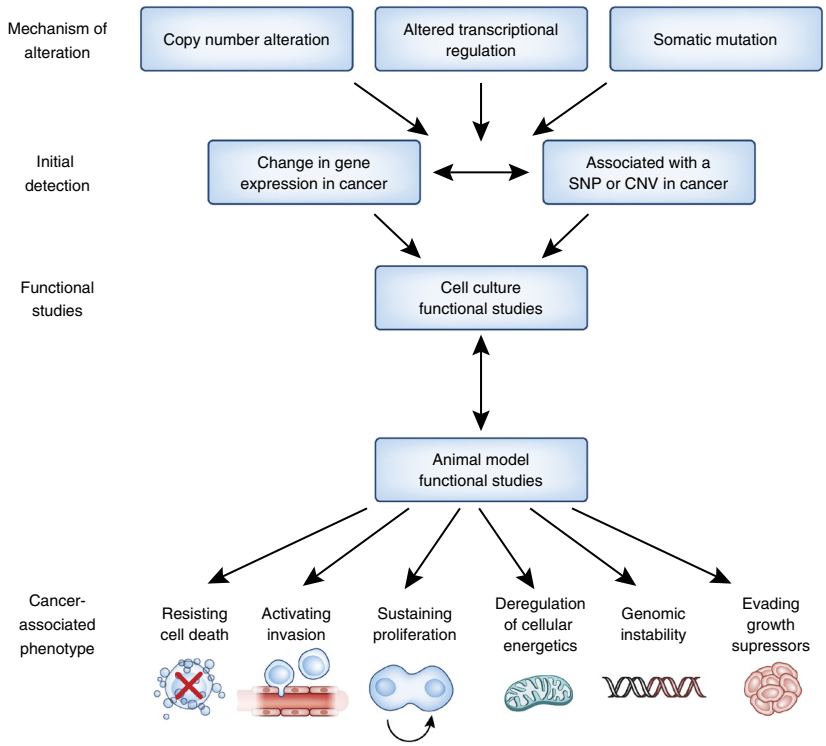

Figure 1 Identification and characterization of lncRNAs with roles in cancer. Alterations in genomic sequence, gene dose and/or expression levels in tumor cells leads to the initial identification of cancer-related lncRNAs. Subsequent studies *in vitro* and *in vivo* have directly linked some of the identified lncRNAs with distinct cancer phenotypes. SNP, single-nucleotide polymorphism; CNV, copy number variation.

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cancer type, highlighting the specific linkage of some lncRNAs to cancers of different tissue origin[26]. It remains to be determined how many of these lncRNAs have a functional role in oncogenesis. This will require systematic experimental analyses to assess the effect of their activity (or lack of it) in normal and transformed cells.

Association of lncRNAs with genetic alterations and single nucleotide polymorphisms. Many lncRNAs are located inside frequent somatic copy number alterations (SNCAs). These are amplified or deleted genomic regions that result from the inherent genomic instability of cancer cells[27]. At least in some cases, there is evidence that a change in the gene dosage of amplified or deleted lncRNAs is connected to malignancy. The search for lncRNAs present in SNCAs led, for instance, to the identification of the focally amplified lncRNA on chromosome 1 gene (*FAL1*, officially known as *FALEC*), a lncRNA frequently amplified in epithelial tumors. *FALEC* acts as an oncogene by inhibiting the expression of the important cell-cycle regulator cyclin-dependent kinase inhibitor 1a (*CDKN1A*) by stabilizing the polycomb complex protein BMI1 that binds to the *CDKN1A* promoter to repress its expression[28]. Studies analyzing somatic point mutations that affect the expression of lncRNAs are sparse, however, in part because of a lack of sequence data. The sequences from whole cancer genomes are now becoming available[29,30] as large consortia such as the International Cancer Genome Consortium actively work to generate comprehensive data sets that describe the mutational landscape of cancer in the noncoding regions of the genome. For instance, a recent study[30] that analyzed the sequence of hundreds of chronic lymphocytic leukemia cases has found recurrent mutations in noncoding regions. These data will predictably enable the identification of the sequence elements that are critical to the function of lncRNAs.

In addition, more than 85% of single nucleotide polymorphisms (SNPs) that are associated with disease occur in noncoding regions[31]. For example, a high-risk neuroblastoma-associated SNP lies within the lncRNA neuroblastoma-associated transcript 1 (*NBAT1*), and it is associated with *NBAT1* differential expression. *NBAT1* inhibits cellular proliferation and invasion by silencing the neuronal-specific transcription factor REST (also known as NRSF, encoded by *REST*)[32].

Other well-known lncRNAs with cancer-associated SNPs in their genomic loci are the antisense RNA genes *CDKN2B-AS1* (ref. 10) and *H19* (ref. 33). *H19* is an imprinted gene encoding a fetal lncRNA that is downregulated postnatally. Although *H19* has been found to suppress metastasis, it is reactivated in liver[34], bladder[33], breast, esophageal and colorectal tumors among others[35]. SNPs in the *H19* gene are associated with a decreased risk of developing non-muscle-invasive bladder cancer[33] and, in the Han Chinese population, increased susceptibility to gastric cancer[36].

Functional analysis of lncRNAs in cancer

***In silico* predictions of lncRNA functions in cancer.** The elucidation of the role of lncRNAs in cancer represents a major challenge; aberrant expression of lncRNAs in tumor samples is not sufficient evidence to ascribe to them an oncogenic or tumor suppressor role. To predict the putative function of lncRNAs, bioinformatic methods such as 'guilt by association'[3] or GiTools[37] have been applied to associate specific lncRNAs with biological processes, by carrying out analyses by correlating a common expression pattern of lncRNAs with protein-coding genes across multiple cell types and tissues. These approaches have identified lncRNAs whose expression correlates with key cancer pathways, such as those controlled by the tumor suppressor p53, the pro-proliferation and survival factor NFκB (nuclear factor κ-light-chain-enhancer of activated B cells) or mTOR (mammalian target of rapamycin)[3,38]. Although the analyses are mere correlations, they suggest that several lncRNAs may be implicated in these cellular responses, an idea that has been corroborated by studies that have linked the expression of lncRNAs to the activation of factors such as p53 (refs. 39–41), E2F (ref. 42), c-MYC (refs. 43,44), androgen receptor[45–47] or estrogen receptor[48]. The results confirm that lncRNAs, similarly to protein-coding genes, are transcriptionally regulated by factors that control key tumor suppressor and oncogenic pathways.

Loss-of-function and gain-of-function lncRNA studies. The determination of the role of lncRNA transcripts in cancer necessitates experimental analyses that will delineate the contribution of an lncRNA transcript to the phenotype of cancer cells or to the transformation of non-tumoral cells (i.e., proliferation, cell cycle, cell death, migration, invasiveness, tumorigenesis, etc.). To this end, RNA interference (RNAi) has been used to knock down lncRNAs with success in

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many loss-of-function studies[49]. However, RNAi frequently does not efficiently reduce lncRNA levels. It has been argued that the predominantly nuclear localization of some lncRNAs impedes their targeting by the RNAi machinery, which is mainly localized to the cytoplasm. Thus, other oligo-mediated RNA knockdown strategies are used as an alternative, such as modified antisense oligonucleotides (ASOs) or 'gapmers', which bind to the target RNA to form a DNA-RNA hybrid, promoting RNA cleavage by ribonuclease H (RNase H)[50,51]. Still, these oligo-based techniques share some limitations with RNAi, in which knockdown is incomplete, has unpredictable off-target effects and only provides temporary inhibition, which limits loss-of-function analysis to a transient system.

To avoid some of these limitations, gene knockout generated by directed targeting nucleases[52–54] provides a powerful tool for elucidating the function of lncRNAs both *in vitro* and *in vivo*. In particular, genome editing by clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated endonuclease 9 (Cas9)[54] is now carried out in a rapid and efficient manner to generate total or partial deletion of lncRNAs[55,56], or to block lncRNA expression by the targeted interruption between the promoter and the lncRNA sequence through insertion of polyadenylation signals[57]. Furthermore, CRISPR-Cas9 can be applied to achieve lncRNA overexpression from its endogenous locus by inserting a strong promoter upstream of the gene[58], or by targeting transcriptional activator complexes to the promoter[59]. However, the most widely applied strategy for gain-of-function studies is the induction of ectopic overexpression using transient or stable transfection or viral transduction. This may be used to enhance the effect of lncRNAs when they exert their functions *in trans*, i.e., at a different location from the locus of transcription. Different technological approaches can thus complement each other when investigating the function of lncRNAs.

Animal models for the study of lncRNAs. Mounting evidence from *in vitro* cell-based assays has linked numerous lncRNAs to cellular processes relevant to cell transformation[39,60,61]. Moreover, human cancer cells with manipulated expression of specific lncRNAs have altered tumor-forming capacity when injected into mice, lending support to the idea that they contribute to the cancerous phenotype[6,18,62,63]. For instance, the overexpression of *lncRNA-LET* (officially known as NPTN intronic transcript 1, *NPTN-IT1*), reduces the invasion and generation of abdominal metastases of human hepatocellular carcinoma cells when injected into nude mice, consistent with the decreased expression levels of *NPTN-IT1* in human liver tumors[63].

However, the field is still in its early stages and only a handful of animal models have been developed to assess the roles of lncRNAs *in vivo*[64]. Several of these knockout mice, such as those lacking Foxf1 adjacent noncoding developmental regulatory RNA (*Fendrr*); long intergenic non-protein coding RNA, Trp53-induced transcript (*Linc-Pint*); or *Linc-Brn1b* (officially known as *Pantr2*, POU domain, class 3, transcription factor 3 adjacent noncoding transcript 2), show important developmental phenotypes[65,66], but their cancer-related phenotypes remain poorly investigated. A remarkable exception is the lncRNA inactive X-specific transcript (*Xist*). One recent study[67] showed a strong connection between *Xist* and cancer after conditionally deleting *Xist* RNA in the blood lineages of mice. This resulted in overexpression of the X-chromosome and a fulminant hematologic cancer known as myeloproliferative neoplasm, myelodysplastic syndrome (mixed MPN/MDS)[67].

A limitation of animal models for the identification of lncRNA function is that many lncRNAs are not highly conserved in evolutionarily distant species, which often hinders the identification of mouse orthologs[68]. Nevertheless, in some cases, partial conservation of human lncRNAs in other mammals exists[69], as it does in some lower vertebrates[70–73]. Conservation is usually higher at the promoter regions of lncRNAs, similar to those of protein-coding genes[3,69]. These observations suggest the possibility of a conserved function of lncRNAs, making animal models useful not only for investigating of the role of lncRNAs *in vivo*, but also for identifying conserved lncRNA sequences or structural elements that account for it.

In contrast, the *in vivo* mouse models could deliver unexpected results. For instance, mice lacking a region containing both *Hoxc* and *Hotair* show neither changes in *Hoxd* expression, nor in histone H3 lysine 27 trimethylation (H3K27me3) in embryos[74], which was the phenotype expected on the basis of studies[75] of human *HOTAIR* in cell culture. However, mice in which only *Hotair* is deleted present phenotypes with skeletal defects, and when mouse cells lacking *Hotair* are placed in culture, substantial differences in their *Hoxd* gene expression and H3K27me3 profiles are detected[76], suggesting the presence of compensatory mechanisms in mice lacking a larger portion of the *Hoxc* gene cluster. A few other examples of knockout mice of cancer-related lncRNAs, including *Malat1* (refs. 57,77) or *Neat1* (refs. 78,79) knockouts, either do not show phenotypes or display subtle ones, which may be the result of compensatory mechanisms. It also remains possible that the gene-regulatory functions of some lncRNAs result in mild and specific phenotypic effects that can only be identified after thorough analyses.

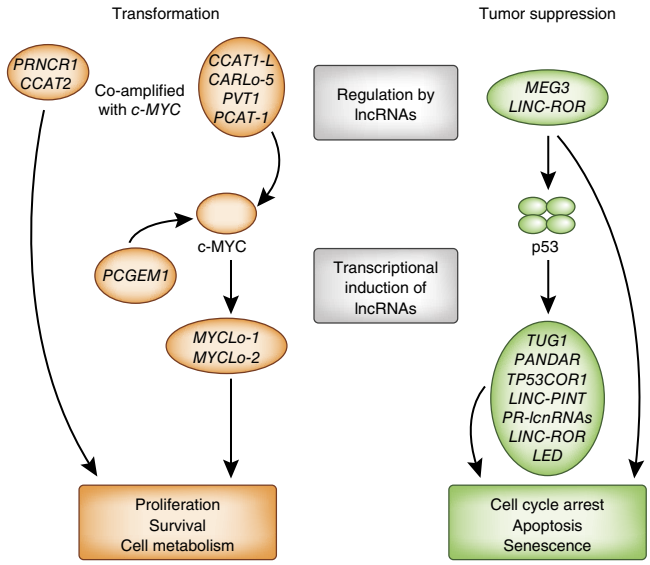

Figure 2 LncRNAs are part of the c-MYC oncogenic and p53 tumor suppressor networks. A number of lncRNAs regulate c-MYC or p53 (MEG3, LINC-ROR) by affecting their expression, protein levels or activity. Several of the c-MYC–regulator lncRNAs (*CCAT1-L, CARLo-5, PCAT1, PRNCR1, CCAT2, PVT1*) are localized to the same genomic region and co-amplified with *c-MYC*. *PCGEM1* regulates the expression of c-MYC but is not coamplified. Additionally, some lncRNAs are direct transcriptional targets of c-MYC (*MYCLo-1, MYCLo-2*) or p53 (*TUG1, PANDAR, TP53COR1, LINC-PINT, PR-lcnRNAs, LINC-ROR, LED*), also contributing to the functional output of their oncogenic and tumor suppressor responses. PR-lncRNAs = p53-regulated lncRNAs, ref. 40; PR-lncRNAs, p53-regulated RNAs.

Roles of lncRNAs in cancer

Tumor suppressor and oncogenic lncRNAs. Multiple lncRNAs are subjected to transcriptional regulation by factors that control

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fundamental aspects of cellular homeostasis. Much of the evidence for this comes from genome-wide studies that reveal that transcription factors, such as p53 (refs. 4,40), MYC[43,44], or the estrogen receptor[48], or signaling cascades such as Notch[80], specifically regulate the expression of a substantial number of lncRNAs. For example, after DNA damage or oncogenic stress, the transcription factor p53, a preserver of cellular homeostasis, initiates a tumor suppressor program that involves the induction of many genes, including dozens of lncRNAs. Some of these are direct transcriptional targets of p53 (**Fig. 2**). Among them, the mouse tumor protein p53 pathway corepressor 1 *lincRNA-p21* (officially known as *Trp53cor1*) promotes apoptosis by contributing to p53-dependent transcriptional repression through its interaction with the protein heterogeneous nuclear ribonucleoprotein K (Hnrnpk)[4]. In addition, the human lncRNAs (*PANDAR*)[41] and *LINC-PINT*[38] act as regulators of p53-dependent apoptosis and cell cycle arrest, depending on the cellular context, by mediating transcriptional and epigenetic repression of gene expression, respectively. In contrast, the lncRNA induced by p53 lncRNA activator of enhancer domains (*LED*), contributes to p53 transcriptional regulation by interacting with p53 transcriptional enhancers[81]. Consistent with their role in the p53 response, several human p53-regulated lncRNAs are downregulated in colorectal cancer[38,40], or, similarly to *LED,* epigenetically silenced in acute lymphocytic leukemia, among other tumor types[81], suggesting a role for them as tumor suppressors. In addition, other lncRNAs are involved in the p53 network without necessarily being transcriptional targets of p53, such as the maternally expressed gene 3 (*MEG3*) lncRNA, which is downregulated in multiple cancers, is involved in p53 regulation and has a concomitant effect on cell survival and proliferation[82].

In contrast to lncRNAs that are involved in the p53 tumor-suppressor pathway, the expression of numerous lncRNAs is regulated by the proto-oncogene *MYC*[43,44,83] (**Fig. 2**). Some of them, such as *MYCLo-1* and *MYCLo-2* (officially known as ELFN1 Antisense RNA 1, *ELFN1-AS1*), upregulated in colorectal cancer, are involved in *MYC*-dependent gene repression of some cell-cycle regulator genes[44]. *MYC* resides in the 8q24 genomic region, which is the most frequently amplified region in human cancers[27]. This 2-Mb region also contains several cancer-associated SNPs within enhancers that form tissue-specific long-range chromatin loops with the *MYC* gene[84,85]. Several lncRNAs are expressed from this transcriptionally active region[58,86–89]. Among these, prostate cancer–associated transcript 1 (*PCAT1*)[89] and prostate cancer–associated noncoding RNA 1, *PRNCR1* (ref. 18), contain SNPs that confer predispositions to prostate and both breast and prostate cancers, respectively, and the rs6983267 SNP, which resides in the colon cancer–associated transcript 2 *CCAT2* gene and predisposes a host to prostate and colorectal cancer, shows allele-specific effects on the expression levels of another lncRNA contained in the region, colon cancer associated transcript 1, *CCAT1*, also known as *CARLo-5* (ref. 87). Several of the lncRNAs in the amplified region regulate the transcription of *MYC*[58,86,87]. For instance, *CCAT1* (refs. 58,87) has a role in *MYC* transcriptional regulation by promoting long-range chromatin looping. In contrast, the oncogenic lncRNA *PCAT1,* which promotes the proliferation of prostate cancer cells and is also co-amplified with *MYC*, induces *MYC* expression by a post-transcriptional mechanism and impairs double-stranded DNA break repair by inhibiting expression of the breast cancer 2 gene, *BRCA2* (refs. 90,91).

Also contained inside the 8q24 genomic region is *PRNCR1*. This has been linked to prostate cancer, and together with *PCGEM1,* it induces cell proliferation. They both interact with the androgen receptor protein, promoting androgen-receptor–mediated gene activation programs[18]. These results, nonetheless, have been questioned in another study[92]. Finally, a recent study performed in mice using chromosome engineering demonstrated that amplification of the lncRNA plasmacytoma variant translocation 1 *Pvt1,* which is contained in the same genomic region and adjacent to *Myc*, correlates with c-*Myc* gene copy number gain, and that such a gain in *Pvt1* increases Myc protein levels, indicating a probable *cis*-regulatory mechanism of *Myc* regulation by *Pvt1* (ref. 88). Although these few studies represent only a small sample, they illustrate the large diversity of strategies by which lncRNAs may regulate tumor suppressors or oncogenes to influence cancer phenotypes.

LncRNAs and *trans*- and *cis*-gene regulation in cancer. The epigenetic alterations that tumor cells undergo are a determining factor in cancer development[93]. Many lncRNAs affect this aspect of cell transformation, as they interface with the epigenetic machinery influencing gene expression (reviewed in refs. 94,95). Several can associate with the polycomb repressive complex 2 (PRC2), a chromatin repressor complex that catalyzes H3K27 trimethylation and which is tightly linked to the aberrant proliferation of cancer cells. Several lncRNAs have been shown to modulate PRC2 targeting of distantly located genes to silence them[94–96]. An example of this tripartite interplay between chromatin, lncRNA and PRC2 is *HOTAIR*, which is deregulated in different cancers[97], and the expression of which is correlated with poor outcome in primary breast tumors[6]. *HOTAIR* forms a macromolecular complex with PRC2 and the lysine-specific demethylase 1 (LSD1) that silences specific gene loci, leading to metastasis[98] (**Fig. 3**).

Besides *HOTAIR*, a number of cancer-altered lncRNAs, such as *NBAT1* (ref. 32) or the p53-regulated—and downregulated in colorectal cancer—lncRNA *LINC-PINT*[38], and the taurine upregulated 1 lncRNA, *TUG1,* upregulated in lung cancer[99], affect cell proliferation by regulating gene expression through their interaction with PRC2. It has been debated whether the binding of PRC2 to lncRNAs only reflects the unspecific affinity of this protein complex for RNA[99]. In contrast, several studies[32,38,96,99,100] show the functional relationship between lncRNAs and PRC2. Nonetheless, deeper functional and structural studies are needed to understand the principles of this RNA-protein interplay, and hence the role of PRC2-interacting lncRNAs in cancer.

PRC2, however, is not the only chromatin complex that has been linked with the function of lncRNAs. Besides the studies associating *TUG1* with PRC2 (refs. 96,99), this lncRNA has been shown to localize to repressive chromatin by interacting with the methylated form of the polycomb 2 (PC2) protein[23]. Furthermore, instead of promoting the activity of chromatin complexes, some nuclear lncRNAs can antagonize their function. For example, the oncogenic SWI/SNF complex antagonist associated with prostate cancer 1 lncRNA (*SCHLAP1*), whose expression levels predict poor outcomes in prostate cancer, antagonizes the genome-wide localization and regulatory functions of the SWI/SNF chromatin remodeling complex[101]. Similarly, acting as a molecular decoy, the tumor suppressor lncRNA growth arrest–specific 5 (*GAS5*) outcompetes DNA binding to the glucocorticoid receptor (GR), which impedes GR-dependent gene activation and reduces cell metabolism[102,103].

While the lncRNAs cited above affect expression of genes located on different chromosomes, other lncRNAs act *in cis*, i.e., on gene loci proximal to the locus where the lncRNA is transcribed, and they can sometimes spread their effects over long distances along the same chromosome. Some of the *cis*-acting lncRNAs activate the transcription of closely located genes by promoting chromatin looping from

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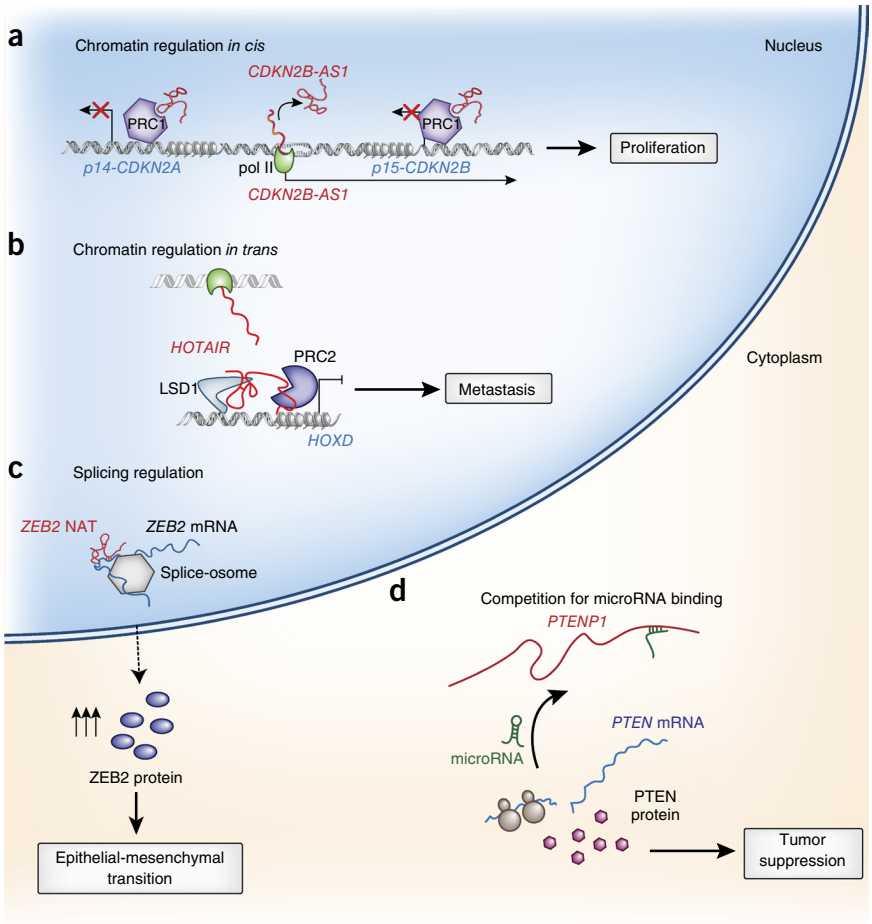

Figure 3 Diverse mechanisms of cancer-related lncRNAs. (**a**) Acting in the nucleus of the cell, some lncRNAs affect the expression of proximally located genes, such as *ANRIL (CDKN2B-AS1)*, which mediates the epigenetic silencing of two genes on the same locus, *CDKN2A* and *CDKN2B*, inducing cell proliferation (refs. 7,9). (**b**) *HOTAIR* promotes metastasis in breast cancer by targeting distant genes, such as those in the *HOXD* cluster, for epigenetic silencing by the PRC2 complex (refs. 6 and 75). (**c**) Other nuclear lncRNAs act post-transcriptionally, such as the NAT of *ZEB2* mRNA, *ZEB2*. The *ZEB2* NAT blocks splicing of *ZEB2* mRNA, promoting the use of an internal ribosome entry site (IRES) for translation initiation and delivering high ZEB2 protein levels, which induces epithelial-to-mesenchymal transition (EMT) (ref. 108). (**d**) In contrast, a number of cytoplasmic lncRNAs may act as microRNA sponges. For instance *PTENP1* binds to microRNAs that otherwise bind to the 3′ untranslated region (UTR) of *PTEN* mRNA, reducing its expression and tumor suppressor activity (ref. 111). Red text, lncRNAs; blue text, protein-coding genes.

transcriptional enhancers, such as the previously discussed *CCAT1* (refs. 86,87), which enhances *MYC* transcription. In contrast, others promote gene repression. *XIST,* for instance, controls the epigenetic silencing of one female X chromosome in the process of dosage compensation, which involves binding to PRC2, among other chromatin factors. Aberrant expression of *XIST* and X-chromosome overexpression are linked to multiple types of human cancers[104]. Another remarkable example of *cis*-acting lncRNA is *CDKN2B-AS1*, which binds to chromobox 7 (CBX7) within the polycomb repressive complex 1 and to SUZ12, a core subunit of PRC2. Through these interactions, *CDKN2B-AS1* is involved in the repression of the tumor suppressor genes in the locus[7–9] (**Fig. 3**). Interestingly, SNPs that alter the expression of *CDKN2B-AS1* are associated with many diseases, including several types of cancers[10].

Post-transcriptional regulation by lncRNAs. Although the majority of characterized lncRNAs are directly involved in transcriptional regulation, some affect gene expression through post-transcriptional events. Indeed, different lncRNAs have been implicated in the splicing, turnover, export or translation of mRNAs, as well as in the stability and post-translational modification of proteins[105]. An example of the latter is the lncRNA breast cancer anti-estrogen resistance 4 (*BCAR4*), the expression of which correlates with metastatic advanced breast cancer and anti-estrogen resistance[106,107]. *BCAR4* interacts with Smad nuclear interacting protein 1 (SNP1) and serine/threonine-protein phosphatase 1 regulatory subunit 10 (PNUTS), promoting the acetylation of the proteins and transcriptional activity on genes of the hedgehog/GLI2 transcriptional program that promotes cell migration[107].

In addition, alternative splicing is a process in which lncRNAs can be active in influencing cancer progression. Many mRNA-splicing isoforms are developmentally regulated, and they are preferentially re-expressed in tumors. The natural antisense transcript (NAT) zinc finger E-box binding homeobox 2, *ZEB2*, for instance, regulates alternative splicing according to its ability to interact with *ZEB2* mRNA. It overlaps and binds to an intronic 5′ splice site of the *ZEB2* protein-coding gene, preventing its splicing. The retained intron contains an internal ribosome entry site (IRES) that is necessary for the increased translation of ZEB2 protein, and which can subsequently function as a transcriptional repressor of E-cadherin, a cell adhesion protein that inhibits proliferation, invasion and metastasis (**Fig. 3**). A strong association has been demonstrated between the expression of the NAT *ZEB2* and human tumors with low E-cadherin expression[108]. Whereas the NAT *ZEB2* can enhance protein translation, the human *TP53COR1* has been involved in translation inhibition. In HeLa cells, *TP53COR1* associates with the proto-oncogene mRNAs *JUNB* and *CTNNB1*, selectively reducing their translation into proteins[105].

Some lncRNAs such as the noncoding transcripts that function as competing endogenous RNAs (ceRNAs), or RNA sponges[109], base their activity on sequence-specific interactions with other noncoding RNAs. This type of noncoding transcript not only includes lncRNAs[110], but also pseudogenes[111] and circular RNAs[112,113] that contain binding sequences for microRNAs, and that reduce their action on mRNA targets by titrating the amount of free microRNA. An example of this interplay between RNA molecules is the regulation of the phosphatase and tensin homolog (*PTEN*) tumor suppressor by its pseudogene lncRNA, *PTENP1*, a locus deleted in colorectal cancer, prostate cancer and melanoma[114]. Transcribed *PTENP1* positively regulates the levels of PTEN by competing for microRNA binding[111] (**Fig. 3**). This type of mechanism seems to be shared by a number of cancer-related genes that possess pseudogenes, such as the Kirsten rat sarcoma viral oncogene homolog *KRAS*[111], establishing a gene expression

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balance that is perturbed in cancer cells. This crosstalk between different types of regulatory RNAs and 3′ UTR of mRNAs highlights the complex fine-tuning of RNA networks. However, their dynamics and stoichiometry are still far from understood.

lncRNA-based diagnostics and therapies

The existence of a massive number of lncRNAs that may have roles in the transformation and maintenance of cancer phenotypes has important clinical implications. It opens up a whole new range of possibilities for the diagnostics and treatment of cancer. One of the distinctive features of lncRNAs is their highly tissue- and cell type–specific expression patterns[26,115], which could accurately classify different subclasses of tumors or even predict responses to treatments. Beyond detection and quantification of lncRNA expression on tumor biopsies, the application of lncRNAs as biomarkers in daily practice involves challenges, some inherent to their nature as long RNA molecules. The desirable markers should be stable and easily detectable in plasma or other body fluids, to allow noninvasive diagnosis. The lncRNA *PCA3* meets these requirements because it is a more specific and sensitive marker of prostate cancer in patient urine samples than is the widely used serum prostate-specific antigen (PSA)[15,17]. Similarly, the hepatocellular carcinoma (HCC) upregulated lncRNA, *HULC*, is highly expressed in HCC patients and can be detected in the blood by conventional PCR methods[116].

A promising area of research focuses on using RNA molecules contained in exosomes, microvesicles, apoptotic bodies and apoptotic microparticles as biomarkers[117]. These membranous particles are released by tumor cells, and they may contain tumor-specific lncRNAs that are protected from the RNases present in body fluids. Current ongoing studies will shed light on the presence of lncRNAs and their functional implications.

As previously discussed, many lncRNAs regulate specific facets of protein activity. Therefore, drugs that target lncRNAs can be more refined and less toxic than conventional protein-targeting drugs. For instance, distinct lncRNAs have been shown to interact with the oncogenic PRC2 complex, promoting the repression of specific subsets of PRC2 gene targets[93]. On the basis of this concept, companies are focusing their efforts on developing synthetic oligonucleotide antagonists that specifically block the binding of PRC2 to an individual lncRNA region to de-repress specific genes[118].

Other therapeutic strategies utilize oligonucleotides for the knockdown of deleterious lncRNAs. As proof of principle, Angelman syndrome, a neuro-genetic disorder characterized by severe intellectual and developmental disabilities, has been cured in mice by inhibiting a lncRNA with antisense oligonucleotides (ASOs)[119]. This syndrome is caused by a lack of expression of the imprinted ubiquitin protein ligase E3a gene (*Ube3a*), which is repressed by its antisense transcript, the lncRNA *Ube3a-ats* and can be targeted in mice by the administration of ASOs[119] that specifically target the antisense lncRNA. Although promising, the greatest challenge ahead for similar applications in cancer is to demonstrate efficient oligonucleotide delivery accompanied by long-lasting effects on tumor cells in human patients, independently of tumor location in the body. Working toward this goal, a number of companies have made substantial progress in developing improved oligonucleotide chemistry for less toxic, more stable, and more efficient targeting *in vivo*, for instance by using locked nucleic acids (LNAs). These improved molecules are aimed not only at mediating the degradation of specific lncRNAs (ASOs, gapmers, small interfering RNAs (siRNAs), etc.), but also at blocking lncRNA activity by binding to the molecules themselves or to their protein partners[118,120].

Beyond these, features of lncRNAs can be exploited for other therapeutic applications that do not necessarily involve targeting them. For instance, some strategies take advantage of the highly specific expression of some lncRNAs in tumor cells to reduce the risk of affecting normal tissues during treatment. The plasmid BC-819 (DTA-H19) has been developed to make use of the tumor-specific expression of the *H19* lncRNA. This plasmid carries the gene for the A subunit of diphtheria toxin under the regulation of the *H19* promoter, and its intratumoral injection induces the expression of high levels of diphtheria toxin in the tumor, resulting in a reduction of tumor size in human trials in a broad range of carcinomas[121]. Also demonstrating the therapeutic application of lncRNA gene regulatory activity, a study[122] has applied transgenic expression of the powerful lncRNA *XIST* to silence one of the triplicated chromosomes 21 in cells derived from a person with Down syndrome. These pioneering studies encourage further exploration of the therapeutic opportunities that lncRNAs offer.

Future directions

The surveillance of human transcriptomes to an unprecedented degree has caused a substantial shift in our understanding of gene regulation. The existence of thousands of lncRNAs taking part in cell regulatory networks has important implications for our conception of cancer, and it will force us to revise our view of the disease, from its causative origins to the design and prescription of treatments. The field is, however, still in its infancy, and we are far from incorporating lncRNAs into the clinic. The functions of lncRNAs—not only in cancer, but also in normal physiological conditions—are still far from fully understood. Despite the rapid increase in the catalog of roles reported for lncRNAs, one of the greatest challenges is identifying the sequence and structural elements that allow long noncoding RNA molecules to carry out their cellular functions. For instance, it is, at present, impossible to predict whether and how a cancer-associated SNP or somatic mutation might affect the function of the lncRNA in which it occurs. The particular characteristics of lncRNAs as long RNA molecules, typically lowly expressed, add technical difficulty to the task. The implementation of reliable computational predictions and experimental modeling of lncRNA structure, together with methodologies that identify genome-wide DNA binding sites of lncRNAs (chromatin isolation by RNA purification, ChiRP; capture hybridization analysis of RNA targets, CHART; and RNA antisense purification, RAP)[123–125], and the combination of these DNA-binding maps with the three-dimensional structure of the genome[125], as well as techniques that detect lncRNA interactions with key RNA-binding proteins (CLIP-seq, PAR-CLIP-seq)[126], when applied to a large number of lncRNAs and proteins will allow us to infer the principles that govern the function of lncRNAs.

A question under debate is the utility of animal models for the study of cancer-related lncRNAs, as lncRNAs are weakly conserved overall. Importantly, the number of lncRNAs encoded by the genome has increased with animal evolution[68], suggesting that the presence of lncRNAs is linked to organismal complexity. Still, many human lncRNAs have syntenic orthologs in lower organisms[127]. Nonetheless, when analyzing lncRNA conservation, it should be taken into account that not just the linear sequence, but also the RNA structure, might determine function. It is therefore possible that despite the limited sequence conservation, a conserved function exists. Thus, the sequence and structural elements involved can be inferred from comparative studies.

LncRNAs hold the promise of tailored therapeutic applications, either by inhibition or restoration of lncRNAs that fine-tune the regulatory networks of cancer cells in a highly cancer type–specific manner.

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How soon this promise will crystallize depends on achieving drug treatments (either oligonucleotide-based or with small molecules) that manipulate lncRNA activities, and delivering them efficiently and with lasting effects on tumor cells. An absolute requirement is a more pronounced understanding of lncRNA functions and mechanisms, both in physiological and pathological conditions. It is now necessary to go beyond the descriptive identification of cancer-related lncRNAs and concentrate on their functional study. Intensive research in high-throughput and large collaborative projects, in parallel with the careful characterization of individual lncRNAs, will lead to the cracking of the lncRNA code.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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