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REVIEW

Long noncoding RNA (lincRNA), a new paradigm in gene expression control

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Abstract Long intergenic non-coding RNAs (lincRNAs) are defined as RNA transcripts that are longer than 200 nucleotides. By definition, these RNAs must not have open reading frames that encode proteins. Many of these transcripts are encoded by RNA polymerase II, are spliced, and are polyadenylated. This final fact indicates that there is a trove of information about lincRNAs in databases such as the Gene Expression Omnibus (GEO), which is a repository for RNAseq and microarray data. Recent experiments indicate that there are upwards of 15,000 lincRNAs encoded by the human genome. The term "intergenic" refers to the identification of these transcripts from regions of the genome that do not contain protein-encoding genes. These regions coincide with what was once labeled as the "junk DNA" portions of our genomes, which, upon careful examination by whole genome RNA sequencing experiments, clearly encode RNA transcripts. LincRNAs also contain promoter- or enhancer-associated RNAs that are gene proximal and can be either in the sense or antisense orientation, relative to the protein-coding gene with which they are associated. In this review, we describe the functions of lincRNAs playing roles in biological processes such as gene expression control, scaffold formation, and epigenetic control.

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Introduction

Recent developments in next generation sequencing technologies have led to extensive transcriptomic and bioinformatic analysis of many cell lines and tissues at an unprecedented scale. These analyses show that although more than 75 % of the human genome is selectively transcribed, only a small portion of the transcripts are translated into final protein products (Djebali et al. 2012). The rest of the transcripts that do not possess any protein-coding capacity are annotated as noncoding RNA (ncRNA). These ncRNAs are subdivided into two main categories, depending on their length. Small ncRNAs (sncRNA) are shorter than 200 nucleotides and largely consist of microRNAs (miRNA) and small nucleolar RNAs (snRNA). Long intergenic ncRNAs (lincRNA) are longer than 200 nucleotides (this differentiates them from miRNAs, which tend to be short transcripts encoding small hairpin structures).

As more and more of these noncoding transcripts with different functions are discovered, their nomenclature has evolved. While some sources label these transcripts as lincRNAs, others omit the definition "intergenic" and label them simply as lncRNAs (St. Laurent et al. 2015). Both sncRNAs and lincRNAs have some common properties such as establishing specific RNA-RNA and RNA-DNA interactions (Guttman and Rinn 2012). However, the longer lengths of lincRNAs give them a second layer of functionality, by which they can fold upon themselves, forming complex structures. As a result, they are able to mediate target recognition not only by base-pairing, but also through tertiary structure-defined surface interactions (Rinn and Chang 2012). Also,

even though by definition, lincRNAs must not have open reading frames that encode proteins, recent evidence indicates that short polypeptides may be encoded by these transcripts (Ruiz-Orera et al. 2014).

LincRNA genes are less evolutionarily conserved than protein-coding genes; specifically, their exons are generally more divergent compared to their promoters and some short stretches of their sequences (Derrien et al. 2012; Guttman and Rinn 2012). However, this lack of conservation in the primary sequence does not necessarily indicate an absence of function because the overall secondary and higher order of structures of lincRNAs can mediate specific activities (Fatica and Bozzoni 2014; Pang et al. 2006). Moreover; weaker constraints on lincRNA evolution could allow these sequences to evolve rapidly and gain new levels of complexity, while preserving functional stretches of primary sequence (Ng et al. 2012; Pollard et al. 2006). LincRNAs are generally expressed at low levels compared to protein-coding genes and are more tissue or cell-type specific (Cabili et al. 2011; Derrien et al. 2012). LincRNAs were found to participate in a vast variety of biological processes such as cell proliferation, morphogenesis, pluripotency, development, neuronal processes, and gametogenesis (Guttman et al. 2009). These functions make lincRNAs vital for cellular health; conversely, their dysregulation has been shown to participate in tumorigenesis and in genetically inherited diseases (Batista and Chang 2013; Yan et al. 2015).

The primary characteristics of lincRNAs

Evolution of lincRNAs

Evolutionary conservation of genomic sequences is commonly used as evidence for functionality of the particular locus. The link between evolutionary conservation and function may not necessarily apply for lincRNAs, as structure, rather than sequence, may be more important for their function. LincRNA exons are more conserved than neutrally evolving repeat sequences, on which no known selection pressure applies; however, the extent of lincRNA conservation is less than that for protein-coding genes (Derrien et al. 2012). When different regions of lincRNA genes are analyzed for conservation, promoters are found to be the most conserved parts of the gene, compared to exons and introns. In fact, lincRNA promoters are as conserved as the promoters of protein-coding genes. The lack of conservation in the body of a lincRNA may be because current bioinformatic tools are not trained to detect short conserved sequences that form RNA stem loop structures with large loops. If the functional (e.g., protein binding) part of a lincRNA is the stem structure, the large loops that separate the two strands of the stem may not necessarily be conserved. There is also evidence that lincRNA sequences emerge and decline rapidly within particular evolutionary lineages (Ponting et al. 2009). In this fashion, lincRNAs may evolve differently from protein-coding genes, which generally arise from duplications and subsequent sequence divergence.

LincRNAs can be found in the nucleus or in the cytoplasm

While some studies demonstrate the function of cytoplasmic lincRNAs, they are specifically concentrated in the nucleus, and particularly in the chromatin fraction (Derrien et al. 2012). LincRNAs have been shown to recruit chromatin-modifying enzymes to specific genomic loci to activate or to repress gene expression (Fatica and Bozzoni 2014). Nuclear lincRNAs may also function as decoys in order to sequester transcription factors from their genomic targets, as scaffolds to enable the formation of large multi-protein complexes and as the mediators of the formation of nuclear domains that establish spatial control (Batista and Chang 2013). LincRNAs also have many functions in the cytoplasm; they can modulate mRNA stability, sequester miRNAs by functioning as decoys (miRNA sponges), or they can regulate the translation of target mRNAs (Batista and Chang 2013). For instance, a lincRNA named TUG1 was recently found to serve as a cytoplasmic miRNA sponge of PTEN, which is a master tumor suppressor in prostate cancer (Du et al. 2016). Therefore, loss of TUG1 expression in prostate cancer has been linked to its role in cancer advancement. This important finding shows that lincRNAs can have oncogenic or tumor-suppressive functions and that lincRNAs might be exploited for cancer therapy. LincRNAs can also localize to other subcellular compartments. In fact, global analysis of their localization shows that ribosomes are default destinations for many cytoplasmic lincRNAs, a finding whose biological significance is yet to be discovered (Carlevaro-Fita et al. 2016). Mitochondria are another destination for some nuclear-DNA-encoded lincRNAs. RMRP, which is a lincRNA encoded in the nucleus and transported to the mitochondria by RNA binding proteins, has recently been shown to play a crucial role in mitochondrial DNA replication and RNA processing (Noh et al. 2016). Recently, a thorough investigation by in situ hybridization of the subcellular localization of *Drosophila* lincRNAs showed that most lincRNAs have specific localizations during development (Wilk et al. 2016).

LincRNAs control gene expression

LincRNAs have the capacity to interact with various chromatin-modifying complexes to modulate the chromatin state (Rutenberg-schoenberg et al. 2016). This ability of lincRNAs to bind and recruit these complexes to chromatin can change the epigenetic landscape and thus control gene expression. The chromatin-modifying enzymes that interact with lincRNAs can be repressive, activating or in some cases have bivalent domains with both functions (Wang et al. 2011). The involvement of

lincRNAs in gene expression was revealed by RNA immunoprecipitation followed by RNA sequencing (RIP-Seq) experiments (Cloonan et al. 2008). The EZH2 component of PRC2, which deposits repressive H3K27me marks on chromatin, was shown to bind a number of lincRNAs, and this interaction was shown to be important for targeting PRC2 to specific gene loci for repression (Zhao et al. 2010). LincRNAs can also directly or indirectly interact with transcription factors to target RNA-bound chromatin-modifying enzymes to specific genomic sites. A good example of this mechanism was revealed by studies on the steroid receptor RNA activator (SRA) lincRNA that interacts with either activating TrxG or repressive PRC2 complexes as it controls various genes (Wongtrakoongate et al. 2015). SRA also interacts with NANOG, a master regulator transcription factor for pluripotent stem cells, to control the bivalent state of expression in various gene loci.

LincRNAs can act in *Cis* or in *Trans*

The mode of action of lincRNAs on gene expression can be either *cis*- or *trans*-acting. C*is*-acting lincRNAs affect the expression of genes located near their site of transcription on the same chromosome (see the function of lincRNA-p21 in Fig. 1 as an example). On the other hand, *trans*-acting lincRNAs can control gene expression at independent loci on other chromosomes. The HOX genes are distributed among four clusters (A-D) on different chromosomes. The HOX transcript antisense intergenic RNA (HOTAIR) transcript is a lincRNA expressed from the HOXC gene locus located on human chromosome 12. Even though HOTAIR is embedded within HOXC, its loss does not affect *HOXC* expression. RNAi silencing of the HOTAIR RNA and the targeted deletion of the HOTAIR gene locus result in the de-repression of the HOXD locus, which is located in chromosome 2 (Li et al. 2013; Rinn et al. 2007). HOTAIR interacts with the polycomb repressive complex 2 (PRC2) and lysine demethylase 1A (Lsd1) complexes to mark hundreds of target genes with inhibitory histone tags, such as methylation of histone H3 on lysine 27 (H3K27me) and demethylation of histone H3 on lysine 4 (H3K4). These results show that HOTAIR acts in *trans*.

HOTTIP (HOXA transcript at the distal tip) is another lincRNA transcribed from the HOXA gene locus located on human chromosome 7. Unlike the results observed for HOTAIR, siRNA knockdown of HOTTIP interferes with the expression of neighboring HOXA genes (Wang et al. 2011). HOTTIP interacts with the trithorax group (Trx/MLL) complex to mark HOXA genes with the activating histone modification, H3K4me3 (Wang et al. 2011). Thus, HOTTIP acts in *cis*.

Another lincRNA that acts in trans is lnc-IL7R, a lincRNA that overlaps with the 3′UTR of the interleukin-7 receptor gene (Cui et al. 2014). So far, few examples of lincRNAs in inflammation and immunity exist (Heward and Lindsay 2014). This lincRNA was identified as an LPS-induced RNA, which acts to recruit chromatin silencing complexes. As IL7R signaling is critical for the survival of T lymphocytes, this new complexity in the regulation of the IL7R gene locus is very exciting. Recently, we identified that a suppressor transcription factor, Gfi1, regulated IL7R gene expression in CD8 positive but not in CD4 positive T lymphocytes (Ligons et al. 2012). The presence of lincRNA-dependent regulation may yield clues to the complex and cell type-specific regulation of this gene.

The functions of lincRNAs

LincRNAs in stem cells and pluripotency

The lincRNAs that are associated with pluripotency are either expressed in embryonic stem cells (ESCs) or upregulated in induced pluripotent stem cells (iPSCs) after they are reprogrammed from somatic cells (Guttman et al. 2011). RNAseq and ChIPSeq analysis revealed that thousands of lincRNA genes harbor binding sites for at least one of the major pluripotency transcription factors (including Oct4, Sox2, Klf4, and c-Myc, among others) in their promoter regions. Moreover, many of these lincRNAs are expressed coordinately with these core components of the transcriptional network that controls pluripotency (Dinger et al. 2008; Yang et al. 2013). Transciptome data revealed that over 1000 lincRNAs are differentially expressed in a stage-specific manner during reprogramming (Hussein et al. 2014). Of these lincRNAs, many were found to be suppressors of lineage-specific markers. Furthermore, ChIP and RIP experiments showed that some lincRNAs were chromatin bound during the induction of pluripotency.

There is growing evidence for RNA-based control of pluripotency (Wright and Ciosk 2013). LincRNA-RoR (*R*egulator *o*f *R*eprogramming) is one of the important lincRNAs in both the iPSC and p53 pathways. It was the first lincRNA implicated in reprogramming and discovered to be upregulated in iPSC compared to ESCs by a microarray experiment (Loewer et al. 2010). The gene locus encoding LincRNA-RoR was shown to be occupied by the pluripotency transcription factors—OCT4, SOX2, and NANOG—indicating that lincRNA-RoR plays a role in iPSC generation. Indeed, in the same study, the knockdown of lincRNA-RoR was found to result in a significant decrease of iPSC formation. Conversely, the overexpression of lincRNA-RoR was found to result in an increase in the number of iPSC colonies. LincRNA-RoR may be inhibiting iPSC generation by suppressing the p53 pathway, through which it likely promotes cell survival during dedifferentiation (Loewer et al. 2010). Besides the p53 pathway, lincRNA-RoR also increases the

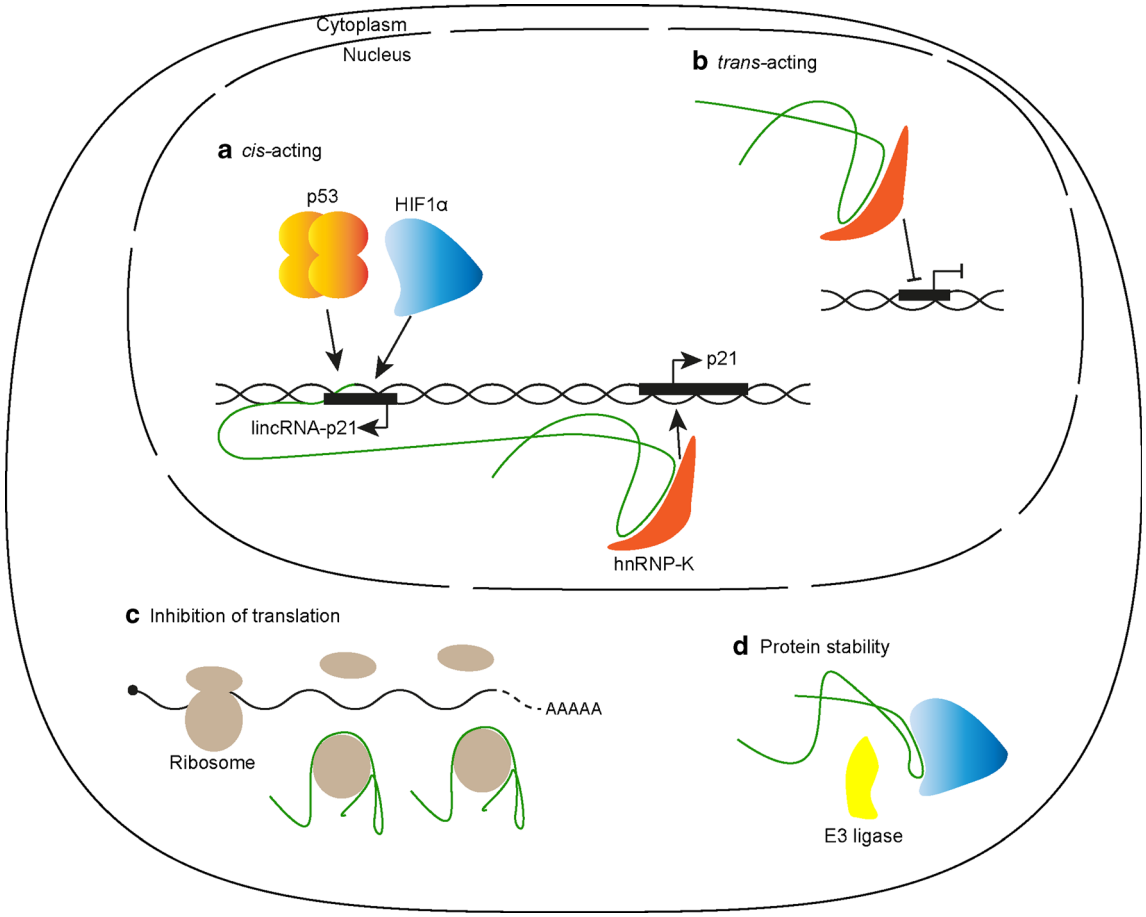

Fig. 1 The four functions of lincRNA-p21. LincRNAs perform a wide variety of functions in cellular processes, and lincRNA-p21 is an important example to these. **a** One of the first nuclear genes that get transcriptionally activated upon the induction of p53 after cellular stress is the gene that encodes the cell cycle inhibitor p21 (*black arrow facing right*). The p21 locus contains a recently identified lincRNA gene on the opposite strand of the p21 gene. This lincRNA gene promoter was also shown to be controlled by p53 and by HIF1α. HnRNP-K binds to the p21 lincRNA and the complex activates the p21 promoter. **b** In addition to this cis regulation, lincRNAs can act in trans. **c** LincRNAs can inhibit the translation of specific genes by competing with the translation machinery. **d** Alternatively, lincRNAs have also been shown to modify protein stability by interfering with the recruitment of cytoplasmic ubiquitinating enzymes

efficiency of pluripotency by titrating down the levels of miR-145, which is a known micro-RNA that targets the pluripotency regulators OCT4, SOX2, and KLF4 (Wang et al. 2013; Xu et al. 2009).

The ability of lincRNAs to regulate the amount of available miRNAs is called decoy activity, in which they act as "sponges" for the miRNAs that normally target mRNAs (Rigoutsos and Furnari 2010; Zhang et al. 2014). So, lincRNA-RoR and its partners could be fine-tuning the activity of miRNAs. Thus, lincRNAs likely set important thresholds for cell lineage identity and fate through their interactions with miRNAs and transcription factors.

LincRNAs in development

LincRNAs have been implicated in differentiation and in developmental processes due to their interaction with chromatin-modifying complexes. In particular, the central nervous system, with a vast range of neuronal cell types, has the most complex and large number of noncoding RNAs (Fatica and Bozzoni 2014). The number of lincRNAs expressed in the brain has been linked to evolutionary complexity. Comparative transcriptome analysis of primate brains indicates that there are many human-specific lincRNAs (Xu et al. 2010). Apart from the nervous system, well-defined roles have been documented for Braveheart (Bvht) and fetal-lethal noncoding developmental regulatory RNA (Fendrr) lincRNAs in the development of cardiomyocytes, the heart, and the body wall (Grote et al. 2013; Klattenhoff et al. 2013).

The *Kcnq1ot1* (*Kcnq1 overlapping transcript 1*) lincRNA is a paternally expressed, imprinted RNA (Mancini-DiNardo et al. 2003). This lincRNA gene is very closely situated to the *Kcnq1* gene which encodes a potassium transporter which when mutated can result in cardiac arthymias of the long QT type that can cause sudden cardiac arrest (Kapplinger et al. 2009). *Kcnq1ot1* silences surrounding genes by recruiting

repressive methylation marks. The link between polymorphisms in the *Kcnq1ot1* gene and long QT syndrome has not been studied but may yield interesting paternally inherited phenotypes that may impact the diagnosis and treatment of this serious disease.

Probably, the best-known lincRNA is the X-inactive-specific transcript (*Xist*) RNA encoded by mammalian X chromosomes. This RNA does not encode any known proteins; yet, it functions to silence one of the two chromosomes of female cells for dosage compensation between XX females and XY males. *Xist* RNA functions by competing with *Tsix* RNA, recruits histone modifiers, and results in the methylation and inactivation of the X chromosome (Gendrel and Heard 2014). The *Xist* RNA, encoded by 8 exons, is 17 kb long and plays a nuclear rather than a cytoplasmic function. Polycomb group proteins bind to this transcript and exclusively coat the inactive X chromosome (Yildirim et al. 2013). At the onset of X chromosome inactivation, in the early embryo, *Tsix* RNA, another lincRNAs, encoded by the opposite DNA strand of the *Xist* locus is also expressed and inhibits the *Xist* RNA on the active X chromosome. Interestingly, *Tsix* RNA has been found in mice but not in humans. The formation of inactive (Xi) and active (Xa) chromosomes during embryonic development is a process controlled by these (and potentially other) lincRNAs.

LincRNAs in cancer

There are large numbers of lincRNAs annotated in the human genome that participate in cancer-related biological processes. Therefore, it is not surprising that their mutations, dysregulation, or aberrant expression cause disease. Genome-wide association studies show that a large number of single-nucleotide polymorphisms (SNPs) in the human genome reside within intergenic or intronic regions, indicating that they may effect lincRNA function (Hindorff et al. 2009). One well-studied lincRNA that has been associated with cancer is HOTAIR. As mentioned above, it recruits the PRC2 complex to deposit inhibitory H3K27me marks. The dysregulation of HOTAIR in breast cancer cells results in the genome-wide retargeting of PRC2, creating a new pattern of gene expression more similar to that of embryonic fibroblasts (Gupta et al. 2010). This redirection influences breast cancer progression by increasing cancer invasiveness and metastasis.

Constitutive activation of the Notch signaling pathway by dominant mutations in the Notch-1 gene is the most common genetic defect in T-acute lymphoblastic leukemia (T-ALL) (Weng et al. 2004). A Notch-regulated lincRNA, LUNAR, was shown to mediate T-ALL growth (Trimarchi et al. 2014). There are a growing number of studies that implicate the presence of SNPs in lincRNA genes to a person's susceptibility to cancer (Ling et al. 2015). Another significant lincRNA linked to cancer is metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), which is indicated in many cancer types (lung, breast, colon, and hepatocarcinoma) (Zhang et al. 2014). Normally, MALAT1 interacts with mRNA splicing factors in nuclear speckles and participates in alternative splicing (Gutschner et al. 2013). Some breast cancers have mutations in the splicing factor-binding sites of MALAT1 which results in an alteration of the splicing pattern (Ellis et al. 2012). Like protein-coding genes, lincRNAs may also function as oncogenes and tumor suppressor genes that impact tumorigenesis. There is a great need for large-scale gain-of-function (GOF) and loss-of-function (LOF) studies followed by in vivo characterization. These studies will reveal the functions and demonstrate the importance of lincRNAs in development, disease, and other cellular processes. Therefore, like protein-coding genes and miRNAs, lincRNAs may also be used as cancer biomarkers and will soon be targets of chemotherapeutic drug development efforts (Huarte 2015; Ling et al. 2013; Yarmishyn and Kurochkin 2015). Thus, the tissue and cell type specific expression patterns of lincRNAs makes them ideal candidates for targeted therapy (Schmitz et al. 2016).

LincRNAs in DNA damage

DNA damage, induced by treating mouse embryonic fibroblasts (MEFs) with the genotoxic drug doxorubicin (DOX), significantly upregulated the expression of 39 lincRNAs in a p53-dependent manner (Guttman et al. 2009). The promoters of these lincRNAs contained p53 *cis*-regulatory binding elements, suggesting that at least some of these targets are controlled by p53 in the presence of DNA damage. Another study identified 49 lincRNAs, upregulated after DNA damage in MEFs that are dependent on the presence of p53 (Huarte et al. 2010). A more recent study combining RNASeq expression analysis and ChIPSeq binding site analysis on MEFs and human fibroblast found similar numbers of lincRNA genes regulated by DOX treatment (21 upregulated and 4 downregulated lincRNA upon DOX treatment in MEF and 22 upregulated and 1 downregulated in human fibroblasts) (Younger et al. 2015). These experiments show that lincRNAs are regulated during the DNA damage response and at least some of these are maintained by p53. It is important to note that the two studies were performed using microarrays assessing a limited lincRNA repertoire and that more recent RNASeq experiments will likely yield larger lists of damage-induced lincRNAs.

MEG3, a maternally expressed and imprinted gene, was recently shown to activate the p53 tumor suppressor (Zhou et al. 2007, 2012). The MEG3 lincRNA is expressed ubiquitously, and its expression is lost in many human tumors and cell lines. Another lincRNA that plays a role in the DNA damage response is lncRNA-JADE, which is induced after DNA damage, mediates histone H4 acetylation, and is

implicated in breast cancer progression (Wan et al. 2013). LncRNA NEAT1 was recently discovered to be a component of p53-dependent paraspeckles in the nucleus (Adriaens et al. 2016). While the biological significance of paraspeckle formation is not clear, NEAT1 silencing sensitizes cells to DNA damage-induced death and impaired skin tumorigenesis.

LincRNA-p21 (also known as Trp53cor1) was first identified as a DNA damage-induced and p53-regulated gene (Huarte et al. 2010). It resides 15 kb upstream of the *Cdkn1a* gene, which encodes the critical cell cycle regulator p21 protein. LincRNA-p21 is situated in the opposite orientation from the *Cdkn1a* gene and has its own distinct promoter. LincRNAs in general perform a wide variety of functions in cellular processes and carry out these roles by themselves or along with other regulatory factors. Because lincRNA-p21 has been documented as a prime example to this diversity of function, we included a schematic representation of its several different functions in a cell (Fig. 1). LincRNA-p21 gene expression is controlled by p53, and in an apparent feedback loop, lincRNA-p21 suppresses many targets of the p53 pathway to regulate p53 responses, specifically the induction of apoptosis (Fig. 1a). The mechanism behind the transcriptional repression activity of lincRNA-p21 on the p53 response is dependent on the physical interaction of the lincRNA-p21 with hnRNP-K, a known member of a repressor complex regulating the p53 pathway (Fig. 1b) (Huarte et al. 2010; Kim et al. 2008). A recent study demonstrated that lincRNA-p21 actually acts in *cis* only on the *p21* gene locus to activate *p21* expression rather than acting in *trans* on multiple genes across the genome (Dimitrova et al. 2014). Indeed, hnRNP-K is also reported to be a coactivator for p53-mediated expression of *p21* (Moumen et al. 2005). These studies implicate lincRNA-p21 in cell proliferation. Consistent with this function, lincRNA-p21 negatively affects reprogramming efficiency, likely through *p21*-dependent inhibition of cell proliferation (Dimitrova et al. 2014).

In addition to the *cis* and *trans* activities of lincRNA-p21 on gene expression control, it was also found to be a posttranscriptional inhibitor of translation, where it interacts with several target mRNAs through base-pair interactions causing ribosome drop-off and reduction in polysome sizes (Fig. 1c) (Yoon et al. 2012). Finally, lincRNA-p21 was also identified to be a stabilizer of proteins. As such, it can interrupt the association between HIF-1a and a ubiquitin E3 ligase and interfering with the ubiquitination of this protein (Fig. 1d) (Yang et al. 2014). It is noteworthy to mention that the expression of lincRNA-p21 is controlled by HIF-1a, establishing a positive feedback loop. In summary, lincRNA-p21 has been documented in a number of studies to have pleitropic roles during multiple steps of gene expression control. These various functions of lincRNA-21 highlight the very complex and versatile functions of lincRNAs in cellular processes.

Plant lincRNAs

Just like the mammals, plants also have many different kinds of lincRNAs regulating their genes, creating an additional layer of complexity in the regulation of their genomes. While only 4 years ago, there were roughly 6000 identified lincRNAs in *Arabidopsis thaliana* transcriptomes, a more systematic study increased this number to around 37,000 (Liu et al. 2012; Wang et al. 2014). COLDAIR and COOLAIR lincRNAs were identified to take part in the process of vernalization, the period of cold necessary for the induction of flowering (Heo and Sung 2011; Swiezewski et al. 2009). These lincRNAs function by recruiting PCR2 to deposit the repressive H3K27me3 marks on the flowering locus C (FLC) to induce stable silencing during the vernalization period. The knockdown of COLDAIR leads to improper flowering—due to a deficiency in the PRC2-mediated repression of FLC.

In the plant world, identified and characterized lincRNAs are not restricted only to the model organism *A. thaliana*. LincRNAs have been documented in many other plant species including but not limited to maize, rice, cotton, canola, tomato, sunflower, *Medicago truncatula*, *Populus tomentosa*, and *Populus trichocarpa* (Chen et al. 2016; Flórez-Zapata et al. 2016; Joshi et al. 2016; Lu et al. 2016). Recently, a number of genome-wide identification studies were conducted under various conditions, which resulted in the annotation of numerous plant lincRNAs that function in a wide variety of cellular processes associated with metabolism. Drought stress in cotton (*Gossypium hirsutum L.*) was found to deregulate the expression of more than 10,000 lincRNA genes, which may be involved in controlling plant hormone pathways (Lu et al. 2016). Canola plants (*Brassica napus*) changed the expression pattern of about 1000 lincRNAs when infected with *Sclerotinia sclerotiorum*, a fungus which causes Sclerotinia stem rot, a disease affecting canola production worldwide (Joshi et al. 2016). In *Populus tomentosa*, a common model tree for woody plants, nitrogen deficiency resulted in an alteration of the expression of more than 100 lincRNA genes, while gibberellin hormone treatment led to changes in the expression of about 400 lincRNA genes (Chen et al. 2016; Tian et al. 2016). These and other results suggest that many lincRNAs have important functions in plant responses to biotic and abiotic stresses. Genome-wide identification studies revealed a vast number of different lincRNAs from unrelated plants under various conditions, and these exploratory studies are a valuable resource for accumulating databases of novel transcripts. However, in order to better understand the mechanism behind all these gene expression changes and map their roles in plant responses, functional characterization studies must be performed for at least selected lincRNAs.

Given that lincRNAs are emerging as master regulators of many biological functions both in animals and in plants, it is

also essential to adapt newly developed cutting-edge techniques in molecular biology to fully characterize lincRNAs. These include the recently very popular genome editing technologies such as CRISPR-Cas9. CRISPR-Cas9 has been used in recent studies for studying the functions of noncoding RNAs in human cells. For instance, Ho et al. (2015) edited the genomic loci of three different lincRNAs and successfully knocked down the expression of these genes. Similar studies in plant molecular biology are still in the planning stages (Basak and Nithin 2015). This creates a great opportunity to adapt CRISPR-Cas9 genome editing tools for plant lincRNA genes to characterize the novel and vital roles of these enigmatic RNAs.

Resources

A number of databases are dedicated for lincRNAs. One of the most comprehensive lincRNA databases is NONCODE. This list contains more than 485,000 lincRNA transcripts from 16 different species, all retrieved from the literature and other public databases (Xie et al. 2014). Other specialized databases include the Human Body Map (Broad Institute) and LNCipedia. GENECODE lists about 16,000 human long noncoding RNAs (Harrow et al. 2012). Two early datasets that documented the functions and classes of lincRNAs were assembled after RNASeq experiments (Cabili et al. 2011; Fritah et al. 2014). An excellent resource for all things related to lincRNAs is the Web site http://www.lncrnablog.com. Here, one can find links to recent papers related to lincRNAs and links to sequence databases. Finally, an excellent recent review summarized the available Web servers and database resources for lincRNA predictions (Ching et al. 2015).

Conclusion

Even though the RNA world hypothesis is widely accepted as a critical step in the evolution of life on earth, our protein-centric viewpoint on molecular biology has long relegated the role of RNAs to be simple messengers between genetic material and protein function. The recent revolution in noncoding RNA identification indicates that there may be as many lincRNA coding genes in our genome as there are protein coding genes. These discoveries have opened up new vistas for RNA function, ranging from the control of gene expression, epigenetic mechanisms, and scaffold formation for signal transduction. Not surprisingly, viruses use lincRNAs for many modes of biological regulation (Tycowski et al. 2015). Can most of epigenetics be explained by the function of lincRNAs? The "junk DNA" that we all learned from our high school teachers was not junk after all! Perhaps, here lies an RNA world hidden from our very eyes since the beginning of life itself.

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References

Adriaens C, Standaert L, Barra J, Latil M, Verfaillie A, Kalev P, Boeckx B, Wijnhoven PWG, Radaelli E, Vermi W et al (2016) p53 induces formation of NEAT1 lncRNA-containing paraspeckles that modulate replication stress response and chemosensitivity. Nat Med 22: 861–868

Basak J, Nithin C (2015) Targeting Non-coding RNAs in plants with the CRISPR-Cas technology is a challenge yet worth accepting. Front Plant Sci 6:1001

Batista PJ, Chang HY (2013) Review long noncoding RNAs : cellular address codes in development and disease. Cell 152:1298–1307

Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, Rinn JL (2011) Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev 25:1915–1927

Carlevaro-Fita, J., Rahim, A., Vardy, L.A., Johnson, R., Guigó, R., Vardy, L.A., and Johnson, R (2016) Cytoplasmic long noncoding RNAs are frequently bound to and degraded at ribosomes in human cells. RNA 1–16

Chen M, Wang C, Bao H, Chen H, Wang Y (2016) Genome-wide identification and characterization of novel lncRNAs in Populus under nitrogen deficiency. Mol Genet Genomics 291:1663–1680

Ching T, Masaki J, Weirather J, Garmire LX (2015) Non-coding yet non-trivial: a review on the computational genomics of lincRNAs. BioData Min 8:44

Cloonan N, Forrest ARR, Kolle G, Gardiner BBA, Faulkner GJ, Brown MK, Taylor DF, Steptoe AL, Wani S, Bethel G et al (2008) Stem cell transcriptome profiling via massive-scale mRNA sequencing. Nat Methods 5:613–619

Cui H, Xie N, Tan Z, Banerjee S, Thannickal VJ, Abraham E, Liu G (2014) The human long noncoding RNA lnc-IL7R regulates the inflammatory response. Eur J Immunol 44:2085–2095

Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG et al (2012) The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 22: 1775–1789

Dimitrova N, Zamudio JR, Jong RM, Soukup D, Resnick R, Sarma K, Ward AJ, Raj A, Lee JT, Sharp PA et al (2014) LincRNA-p21 activates p21 in cis to promote polycomb target gene expression and to enforce the G1/S checkpoint. Mol Cell 54:777–790

Dinger ME, Amaral PP, Mercer TR, Pang KC, Bruce SJ, Gardiner BB, Askarian-Amiri ME, Ru K, Soldà G, Simons C et al (2008) Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. Genome Res 18:1433–1445

Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F et al (2012) Landscape of transcription in human cells. Nature 489:101–108

Du Z, Sun T, Hacisuleyman E, Fei T, Wang X, Brown M, Rinn JL, Lee MG-S, Chen Y, Kantoff PW et al (2016) Integrative analyses reveal a long noncoding RNA-mediated sponge regulatory network in prostate cancer. Nat Commun 7:10982

Ellis MJ, Ding L, Shen D, Luo J, Suman VJ, Wallis JW, Van Tine BA, Hoog J, Goiffon RJ, Goldstein TC et al (2012) Whole-genome

analysis informs breast cancer response to aromatase inhibition. Nature 486:353–360

Fatica A, Bozzoni I (2014) Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet 15:7–21

Flórez-Zapata, N.M.V., Reyes-Valdés, M.H., and Martínez, O (2016) Long non-coding RNAs are major contributors to transcriptome changes in sunflower meiocytes with different recombination rates. BMC Genomics 1–16

Fritah S, Niclou SP, Azuaje F (2014) Databases for lncRNAs: a comparative evaluation of emerging tools. RNA 20:1655–1665

Gendrel A-V, Heard E (2014) Noncoding RNAs and epigenetic mechanisms during X-chromosome inactivation. Annu Rev Cell Dev Biol 30:561–580

Grote P, Wittler L, Hendrix D, Koch F, Währisch S, Beisaw A, Macura K, Bläss G, Kellis M, Werber M et al (2013) The tissue-specific lncRNA fendrr is an essential regulator of heart and body wall development in the mouse. Dev Cell 24:206–214

Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai M-C, Hung T, Argani P, Rinn JL et al (2010) Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 464:1071–1076

Gutschner T, Hämmerle M, Diederichs S (2013) MALAT1 – a paradigm for long noncoding RNA function in cancer. J Mol Med (Berl) 91: 791–801

Guttman M, Rinn JL (2012) Modular regulatory principles of large non-coding RNAs. Nature 482:339–346

Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP et al (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 458:223–227

Guttman M, Donaghey J, Carey BW, Garber M, Grenier JK, Munson G, Young G, Lucas AB, Ach R, Bruhn L et al (2011) lincRNAs act in the circuitry controlling pluripotency and differentiation. Nature 477:295–300

Harrow J, Frankish A, Gonzalez JM, Tapanari E, Diekhans M, Kokocinski F, Aken BL, Barrell D, Zadissa A, Searle S et al (2012) GENCODE: the reference human genome annotation for The ENCODE Project. Genome Res 22:1760–1774

Heo JB, Sung S (2011) Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. Science 331:76–79

Heward JA, Lindsay MA (2014) Long non-coding RNAs in the regulation of the immune response. Trends Immunol 35:408–419

Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, Manolio TA (2009) Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci U S A 106:9362–9367

Ho T-T, Zhou N, Huang J, Koirala P, Xu M, Fung R, Wu F, Mo Y-Y (2015) Targeting non-coding RNAs with the CRISPR/Cas9 system in human cell lines. Nucleic Acids Res 43:e17

Huarte M (2015) The emerging role of lncRNAs in cancer. Nat Med 21: 1253–1261

Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, Kenzelmann-Broz D, Khalil AM, Zuk O, Amit I, Rabani M et al (2010) A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. Cell 142:409–419

Hussein SMI, Puri MC, Tonge PD, Benevento M, Corso AJ, Clancy JL, Mosbergen R, Li M, Lee D-S, Cloonan N et al (2014) Genome-wide characterization of the routes to pluripotency. Nature 516:198–206

Joshi RK, Megha S, Basu U, Rahman MH, Kav NNV (2016) Genome wide identification and functional prediction of long non-coding RNAs responsive to sclerotinia sclerotiorum infection in brassica napus. PLoS One 11:e0158784

Kapplinger JD, Tester DJ, Salisbury BA, Carr JL, Harris-Kerr C, Pollevick GD, Wilde AAM, Ackerman MJ (2009) Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test. Heart Rhythm 6:1297–1303

Kim K, Choi J, Heo K, Kim H, Levens D, Kohno K, Johnson EM, Brock HW, An W (2008) Isolation and characterization of a novel H1.2 complex that acts as a repressor of p53-mediated transcription. J Biol Chem 283:9113–9126

Klattenhoff CA, Scheuermann JC, Surface LE, Bradley RK, Fields PA, Steinhauser ML, Ding H, Butty VL, Torrey L, Haas S et al (2013) Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. Cell 152:570–583

Li L, Liu B, Wapinski OL, Tsai M-C, Qu K, Zhang J, Carlson JC, Lin M, Fang F, Gupta RA et al (2013) Targeted disruption of Hotair leads to homeotic transformation and gene derepression. Cell Rep 5:3–12

Ligons DL, Tuncer C, Linowes BA, Akcay IM, Kurtulus S, Deniz E, Atasever Arslan B, Cevik SI, Keller HR, Luckey MA et al (2012) CD8 lineage-specific regulation of interleukin-7 receptor expression by the transcriptional repressor Gfi1. J Biol Chem 287:34386–34399

Ling H, Fabbri M, Calin GA (2013) MicroRNAs and other non-coding RNAs as targets for anticancer drug development. Nat Rev Drug Discov 12:847–865

Ling H, Vincent K, Pichler M, Fodde R, Berindan-Neagoe I, Slack FJ, Calin GA (2015) Junk DNA and the long non-coding RNA twist in cancer genetics. Oncogene 34:5003–5011

Liu J, Jung C, Xu J, Wang H, Deng S, Bernad L, Arenas-Huertero C, Chua N-H (2012) Genome-wide analysis uncovers regulation of long intergenic noncoding RNAs in arabidopsis. Plant Cell 24: 4333–4345

Loewer S, Cabili MN, Guttman M, Loh Y-H, Thomas K, Park IH, Garber M, Curran M, Onder T, Agarwal S et al (2010) Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells. Nat Genet 42:1113–1117

Lu X, Chen X, Mu M, Wang J, Wang X, Wang D, Yin Z, Fan W, Wang S, Guo L et al (2016) Genome-wide analysis of long noncoding RNAs and their responses to drought stress in cotton (gossypium hirsutum L.). PLoS One 11:e0156723

Mancini-DiNardo D, Steele SJS, Ingram RS, Tilghman SM (2003) A differentially methylated region within the gene Kcnq1 functions as an imprinted promoter and silencer. Hum Mol Genet 12:283–294

Moumen A, Masterson P, O'Connor MJ, Jackson SP (2005) hnRNP K: an HDM2 target and transcriptional coactivator of p53 in response to DNA damage. Cell 123:1065–1078

Ng S-Y, Johnson R, Stanton LW (2012) Human long non-coding RNAs promote pluripotency and neuronal differentiation by association with chromatin modifiers and transcription factors. EMBO J 31: 522–533

Noh, J.H., Kim, K.M., Abdelmohsen, K., Yoon, J., Panda, A.C., Munk, R., Kim, J., Curtis, J., Moad, C.A., Wohler, C.M., et al (2016) HuR and GRSF1 modulate the nuclear export and mitochondrial localization of the lncRNA RMRP. Genes Dev 1–16

Pang KC, Frith MC, Mattick JS (2006) Rapid evolution of noncoding RNAs: lack of conservation does not mean lack of function. Trends Genet 22:1–5

Pollard KS, Salama SR, Lambert N, Lambot M-A, Coppens S, Pedersen JS, Katzman S, King B, Onodera C, Siepel A et al (2006) An RNA gene expressed during cortical development evolved rapidly in humans. Nature 443:167–172

Ponting CP, Oliver PL, Reik W (2009) Evolution and functions of long noncoding RNAs. Cell 136:629–641

Rigoutsos I, Furnari F (2010) Gene-expression forum: decoy for microRNAs. Nature 465:1016–1017

Rinn JL, Chang HY (2012) Genome regulation by long noncoding RNAs. Annu Rev Biochem 81:145–166

Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E et al (2007) Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 129:1311–1323

Ruiz-Orera J, Messeguer X, Subirana JA, Alba MM (2014) Long non-coding RNAs as a source of new peptides. Elife 3:1–24

Rutenberg-schoenberg, M., Sexton, A.N., and Simon, M.D (2016) The Properties of Long Noncoding RNAs That Regulate Chromatin 1–26.

Schmitz SU, Grote P, Herrmann BG (2016) Mechanisms of long noncoding RNA function in development and disease. Cell Mol Life Sci 73: 1–19

St. Laurent G, Wahlestedt C, Kapranov P (2015) The Landscape of long noncoding RNA classification. Trends Genet 31:239–251

Swiezewski S, Liu F, Magusin A, Dean C (2009) Cold-induced silencing by long antisense transcripts of an Arabidopsis Polycomb target. Nature 462:799–802

Tian J, Song Y, Du Q, Yang X, Ci D, Chen J, Xie J, Li B, Zhang D (2016) Population genomic analysis of gibberellin-responsive long non-coding RNAs in *Populus*. J Exp Bot 67:2467–2482

Trimarchi T, Bilal E, Ntziachristos P, Fabbri G, Dalla-Favera R, Tsirigos A, Aifantis I (2014) Genome-wide mapping and characterization of Notch-regulated long noncoding RNAs in acute leukemia. Cell 158: 593–606

Tycowski KT, Guo YE, Lee N, Moss WN, Vallery TK, Xie M, Steitz JA (2015) Viral noncoding RNAs: more surprises. Genes Dev 29:567–584

Wan G, Hu X, Liu Y, Han C, Sood AK, Calin GA, Zhang X, Lu X (2013) A novel non-coding RNA lncRNA-JADE connects DNA damage signalling to histone H4 acetylation. EMBO J 32:2833–2847

Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, Lajoie BR, Protacio A, Flynn RA, Gupta RA et al (2011) A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. Nature 472:120–124

Wang Y, Xu Z, Jiang J, Xu C, Kang J, Xiao L, Wu M, Xiong J, Guo X, Liu H (2013) Endogenous miRNA sponge lincRNA-RoR regulates Oct4, nanog, and Sox2 in human embryonic stem cell self-renewal. Dev Cell 25:69–80

Wang H, Chung PJ, Liu J, Jang IC, Kean MJ, Xu J, Chua NH (2014) Genome-wide identification of long noncoding natural antisense transcripts and their responses to light in Arabidopsis. Genome Res 24:444–453

Weng AP, Ferrando AA, Lee W, Morris JP, Silverman LB, Sanchez-Irizarry C, Blacklow SC, Look AT, Aster JC (2004) Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 306:269–271

Wilk R, Hu J, Blotsky D, Krause HM (2016) Diverse and pervasive subcellular distributions for both coding and long noncoding RNAs. Genes Dev 30:594–609

Wongtrakoongate P, Riddick G, Fucharoen S, Felsenfeld G (2015) Association of the long non-coding RNA steroid receptor RNA activator (SRA) with TrxG and PRC2 complexes. PLoS Genet 11: e1005615

Wright JE, Ciosk R (2013) RNA-based regulation of pluripotency. Trends Genet 29:99–107

Xie C, Yuan J, Li H, Li M, Zhao G, Bu D, Zhu W, Wu W, Chen R, Zhao Y (2014) NONCODEv4: exploring the world of long non-coding RNA genes. Nucleic Acids Res 42:98–103

Xu N, Papagiannakopoulos T, Pan G, Thomson JA, Kosik KS (2009) MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. Cell 137:647–658

Xu AG, He L, Li Z, Xu Y, Li M, Fu X, Yan Z, Yuan Y, Menzel C, Li N et al (2010) Intergenic and repeat transcription in human, chimpanzee and macaque brains measured by RNA-Seq. PLoS Comput Biol 6: e1000843

Yan X, Hu Z, Feng Y, Hu X, Yuan J, Zhao SD, Zhang Y, Yang L, Shan W, He Q et al (2015) Comprehensive genomic characterization of long non-coding RNAs across human cancers. Cancer Cell 28:529–540

Yang J-H, Li J-H, Jiang S, Zhou H, Qu L-H (2013) ChIPBase: a database for decoding the transcriptional regulation of long non-coding RNA and microRNA genes from ChIP-Seq data. Nucleic Acids Res 41: D177–D187

Yang F, Zhang H, Mei Y, Wu M (2014) Reciprocal regulation of HIF-1α and LincRNA-p21 modulates the warburg effect. Mol Cell 53:88–100

Yarmishyn AA, Kurochkin IV (2015) Long non-coding RNAs: a potential novel class of cancer biomarkers. Front Genet 6:1–10

Yildirim E, Kirby JE, Brown DE, Mercier FE, Sadreyev RI, Scadden DT, Lee JT (2013) Xist RNA is a potent suppressor of hematologic cancer in mice. Cell 152:727–742

Yoon JH, Abdelmohsen K, Srikantan S, Yang X, Martindale JL, De S, Huarte M, Zhan M, Becker KG, Gorospe M (2012) LincRNA-p21 suppresses target mRNA translation. Mol Cell 47:648–655

Younger ST, Kenzelmann-Broz D, Jung H, Attardi LD, Rinn JL (2015) Integrative genomic analysis reveals widespread enhancer regulation by p53 in response to DNA damage. Nucleic Acids Res 43: 4447–4462

Zhang, A., Xu, M., and Mo, Y (2014) Role of the lncRNA− p53 regulatory network in cancer. J. Mol. Cell Biol 1–34

Zhao J, Ohsumi TK, Kung JT, Ogawa Y, Grau DJ, Sarma K, Song JJ, Kingston RE, Borowsky M, Lee JT (2010) Genome-wide identification of polycomb-associated RNAs by RIP-seq. Mol Cell 40:939–953

Zhou Y, Zhong Y, Wang Y, Zhang X, Batista DL, Gejman R, Ansell PJ, Zhao J, Weng C, Klibanski A (2007) Activation of p53 by MEG3 non-coding RNA. J Biol Chem 282:24731–24742

Zhou Y, Zhang X, Klibanski A (2012) MEG3 noncoding RNA: a tumor suppressor. J Mol Endocrinol 48:R45–R53