Am J Stem Cells 2024;13(2):59-74
www.AJSC.us /ISSN:2160-4150/AJSC0155224

Review Article

lncRNAs regulate cell stemness in physiology and pathology during differentiation and development

Jie Lu, Li Xu, Ying Wang, Bing Guan

Department of Otolaryngology Head and Neck Surgery, Northern Jiangsu People's Hospital Affiliated to Yangzhou University, Yangzhou, Jiangsu, China

Received January 2, 2024; Accepted March 6, 2024; Epub April 25, 2024; Published April 30, 2024

Abstract: Long non-coding RNA (lncRNA) are an important class of ubiquitous genes involved in diverse biological functions. lncRNAs, defined as noncoding RNAs with a length exceeding 200 nucleotides, are abundantly expressed throughout cells; however, their precise functions remain largely elusive. From embryonic stem cell proliferation and differentiation to cancer cell proliferation and invasion, lncRNAs play multifaceted regulatory roles across various cellular stages. Moreover, lncRNAs participate in the regulation of differentiation and regeneration during cellular development processes while also playing a pivotal role in maintaining and regulating cell stemness. In this article, we comprehensively review the current knowledge regarding lncRNAs in this field, discussing their biological functions and mechanisms underlying stemness regulation along with the factors implicated in these processes. We emphasize the growing evidence supporting the significance of lncRNAs in governing cell stemness while indicating that disruptions or mutations within them may serve as fundamental causes for certain developmental disorders.

Keywords: lncRNA, stemness, differentiation, regeneration, pluripotency

Introduction

lncRNAs are widely distributed in prokaryotic and eukaryotic genomes. Despite being one of the most conserved and rapidly evolving sequences in vertebrate genomes, non-protein-coding RNAs (ncRNAs) have long been considered to lack functionality. In the human genome, there are 5,446 lncRNA genes identified out of a total of approximately 24,000 full-length cDNAs [1]. When compared to protein-coding transcripts, lncRNAs are smaller in size, ranging from 200 nt to thousands of nt, with an average exon count of 2.9 [2]. They also possess longer introns and exhibit higher non-poly(A) ratios than protein-coding transcripts [3]. According to their position relative to protein-coding genes, lncRNAs can be categorized as antisense RNAs, lncRNAs, sense overlapping transcripts, sense intronic transcripts, and processed transcripts [3].

LncRNAs represent one of the regulatory components of the eukaryotic genome and play diverse functions that are essential for a variety of biological processes and for coordinating gene expression. Compared to microRNAs (miRNA), lncRNAs can fold into intricate secondary and higher structures, thereby providing a greater potential for target recognition [4]. lncRNAs play diverse roles in gene expression. The functional roles of lncRNAs can be summarized into four mechanisms: signaling, decoy, guidance, and scaffolding [5].

Signaling involves the spatiotemporal expression of gene regulation pathways. lncRNAs regulate transcription factors (TFs), including modulating TF activity, interacting with TFs to influence gene expression, and facilitating the nuclear translocation of TFs.

Decoy functions by guiding target factors to specific regions. As competitive endogenous RNAs (ceRNAs), lncRNAs act as decoys that can effectively consume miRNAs while competitively binding to target genes to regulate their expression.

Guidance recruits the target proteins to either cis- or trans-regulate the target genes. Upon

https://doi.org/10.62347/VHVU7361

signals of DNA damage, the proximal promoters of cyclin genes are transcribed into lncRNAs, which subsequently recruit RNA-binding proteins to modulate transcriptional processes [6]. lncRNAs bind to polynuclear restriction complex 2 (PRC2) and induce Histone H3 lysine-27 trimethylation, leading to the inhibition of gene transcription and trans-regulation of coding genes [7]. lncRNAs interact with chromatin-modifying proteins and recruit them to specific regions to inhibit the expression of adjacent loci, thus cis-regulating the neighboring genomic elements [8].

Scaffolding facilitates the aggregation of multiple proteins into functional complexes. lncRNAs serve as scaffold molecules that regulate protein delivery. The translation process is regulated through interactions between protein-coding genes and their homologous antisense transcripts, antisense ncRNA pairs with the mRNA bases to form RNA double strands that cover key cis-elements within mRNA, subsequently guiding the binding of mRNA isoforms with splicing regulatory proteins for alternative splicing [9]. The lncRNA Xist participates in chromosome dosage compensation by regulating X chromosome inactivation [10], forms RNA double-strands with the antisense transcript Tsix through annealing, and subsequently generate small interfering RNAs upon Dicer processing, thereby participating in repressive chromatin modifications [11].

Furthermore, lncRNA can actively contribute to the regulation of pluripotency in embryonic stem cells (ESC) through the targeted modulation of gene expression. Additionally, lncRNAs have been shown to play significant roles in various human diseases, including cancer, endocrine disorders, neurodegenerative and psychiatric conditions, cardiovascular diseases, hypertension, stroke, immune dysfunction, and autoimmune disorders [4].

This review provides a comprehensive summary of how lncRNAs can alter cell differentiation potential and inhibit or promote the formation of functionally mature channels by regulating the spatiotemporal expression of coding genes through the binding of specific TFs, priming targets, recruiting protein-regulated target genes, and recruiting protein-functional complexes. During tissue repair and regeneration, lncRNAs regulate the self-renewal capacity of cells and participate in cell differentiation. Moreover, during injury or disease, lncRNAs contribute to cellular activation and differentiation for effective tissue and organ repair and regeneration. In developmental contexts, cell stemness refers to the undifferentiated state observed in ESCs and induced pluripotent stem cells (iPSCs). lncRNAs can dynamically modulate cell stemness through gene expression regulation mechanisms such as epigenetic modifications and intricate signaling pathways that orchestrate tissue development events. Cancer initiation and progression are significantly influenced by alterations in cell stemness. Here we have discussed the involvement of lncRNA-regulated cancer cell stemness in the proliferation, migration, invasion, and metastasis of various tumo cells. Several examples are illustrated in **Table 1**.

lncRNA regulates cell differentiation

In biology, the regulation of cell differentiation is a pivotal research direction. Recent studies have revealed that certain epigenetic regulatory factors, such as histone modification and DNA methylation, can influence gene expression, thereby modulating the trajectory of cellular differentiation. Additionally, signal transduction pathways have been shown to play a crucial role in governing cell differentiation, and aberrant activation or inhibition of molecules within these pathways can result in abnormal cellular differentiation patterns. Remarkable progress has been made in the field of biotechnology in investigating the regulation of cell differentiation. For instance, researchers can precisely manipulate gene expression within cells using gene-editing technologies, thus elucidating the key factors that are involved in cellular differentiation. Certain previous studies have demonstrated that AAV-ie-K558R [12] and AAV-Net1 [13] promote trans-differentiation from supporting cells to hair cells within the mouse cochlea and knocking out Foxg1 in supporting cells facilitates their trans-differentiation into hair cells [14]. Recent research has highlighted the crucial role of lncRNAs in cellular differentiation. By employing various mechanisms, lncRNAs effectively coordinate and regulate cell differentiation.

lncRNAs are involved in the spatiotemporal expression of genes through signal transduc-

Table 1. Advances in lncRNAs targeting cell stemness

lncRNA	Disease	Mechanism	Stemness regulation	Reference
H19	Ulcerative colitis	Inhibiting p53 protein, microRNA 34a, and let-7	Promotes mucosal regeneration	[15]
	Esophageal cancer	Regulated STAT3 negatively regulated let-7c in esophageal cancer cell lines	Involves in esophageal cancer cell proliferation, migration and invasion, EMT and metastasis	[16]
	Gastric cancer	H19-derived miR-675 targets the tumor suppressor RUNX1	Regulates the gastric cancer cell proliferation phenotype	[17]
NEAT1	Myocardial ischemia/reperfusion injury	Negative regulation targeting miR-193a	Inhibits cell proliferation and increases apoptosis	[18]
CAREL	Myocardial infarction	Derepress the expression of Trp53inp1 and Itm2a, the target genes of miR-296	Reduces cardiomyocyte division and proliferation and blunt neonatal heart regeneration after injury	[19]
PCA3	Prostate cancer	Controlling the androgen receptor pro-survival signaling	Involves in prostate cancer cells survival pathways by controlling cell growth and viability	[20]
HOTAIR	Breast cancer	Recruits and binds the transcriptional co-repressor polynucleotide restriction complex 2 to specific target genes across the genome, resulting in H3K27 trimethylation and subsequent epigenetic silencing of metastasis suppressor genes	Mediates invasion and metastasis of breast cancer cells	[21]
	Pancreatic cancer		Involves in the pro-oncogenic function in pancreatic cancer	[22]
	Hepatocellular carcinoma		Increased risk of hepatocellular carcinoma recurrence and lymph node metastasis	[23]
	Human non-small cell lung cancer		Promotes aggressive behavior in human non-small cell lung cancer	[24]
UCA1	Bladder carcinoma	Modulates the expression of several genes involved in tumorigenic potential, drug resistance and embryonic development	Enhances the proliferative, migrative, invasive, and drug resistance behaviors of human bladder TCC cell line BLS-211	[25]
TUG1	Bladder cancer	Activating AnnexinA8 by sponging miR-140-3p	Promotes bladder cancer progression and metastasis	[26]

tion, thereby regulating differentiation. Four neuronal lncRNAs have been identified: RMST, lncRNA_N1, lncRNA_N2, and lncRNA_N3 [27]. RMST and lncRNA_N1 were specifically expressed in the brain. lncRNA_N3 interacts with SUZ12 to participate in epigenetic gene silencing. lncRNA_N2 maintains the cytoplasmic levels of miR-125B and LET7, promoting the neurogenesis of neural progenitor cells [27]. The transcript of metastasis-associated lung adenocarcinoma transcript 1 (Malat1) is a highly conserved 8.7 kb RNA molecule. Malat1 acts as a downstream target of myogenic proteins and potently regulates skeletal muscle growth and development [28]. Myostatin downregulates Malat1 expression in skeletal muscle and inhibits Malat1 transcripts levels in proliferating primary human myoblasts, whereas it has been shown that myostatin does not impact the expression of Malat1 in proliferating mouse C2C12 myoblasts [28]. The ANCR gene, located on human chromosome 4, produces a single 855-bp RNA transcript known as LncRNA NR_024031 or anti-differentiation ncRNA (ANCR) [29]. Within the epidermal tissue progenitor-containing compartment, ANCR prevents the expression of differentiation-related genes to maintain an undifferentiated state. Deletion of ANCR disrupts the normal expression pattern of TFs (such as CEBPA [30], GRHL3 [31], and HOPX [32]) involved in epidermal differentiation. The lncRNA RNCR2 exhibits high expression levels in differentiating mouse retinal cells and is predominantly localized within the nucleus [33]. Knockout of the RNCR2 gene and ectopic nuclear export of RNCR2 RNA results in an augmented population of amniotic cells and Müller glial cells while concomitantly reducing the number of photoreceptor cells [33].

lncRNAs participate in cell differentiation by directing target factors to specific regions to function as decoys. Muscle-specific long noncoding RNA (lnc-MD1) expression is localized in the cytoplasm and is induced during muscle cell differentiation [34]. Functioning as a ceRNA, lnc-MD1 competes with miR-133 and miR-135 for binding, thereby regulating the distribution of these miRNAs to their targets and facilitating post-transcriptional gene regulation [35, 36]. lnc-MD1 functions as a competitive endogenous RNA (ceRNA) by binding to miR-133 and miR-135, thereby competing for their mRNA targets such as MAML1 and MEF2C, and plays a crucial role in regulating the timing of myocyte differentiation [34]. lncRNA H19, a well-known maternally expressed and paternally imprinted gene [37], does not encode proteins, but rather encodes a 2.3 kb long H19 ncRNA. H19 was found to promote the differentiation of human mesenchymal stem cells (hMSCs) into osteoblasts by targeting miR-141 and miR-22, thereby activating the Wnt/β-catenin pathway and facilitating hMSCs osteoblastic differentiation [38]. H19 acts as a ceRNA for two miRNAs, miR-141 and miR-22. H19 and its exon 1 encode two evolutionarily conserved miRNAs [39]: miR-675-3p and miR-675-5-p, which are involved in myoblast differentiation, skeletal muscle satellite cell differentiation, and skeletal muscle regeneration. MiR-675-3p and miR-675-5-p exert their promyogenic effects by targeting Smad1 and Smad5 (TFs mediating the anti-differentiation effect of the BMP pathway), and the DNA replication initiation factor Cdc6 [40]. Sirtuin 1 (Sirt1) is an NAD-dependent deacetylase involved in myogenesis [41]. During C2C12 cell myogenic differentiation, Sirt1 and Sirt1-AS exhibit decreased expression. It has been shown that, miR-34a targeting Sirt1 gradually causes its accumulation [41]. MiR-34a reduces the abundance of Sirt1 mRNA by binding to its target site within the 3'UTR region. Overexpression of the lncRNA Sirt1-AS leads to increased levels of the corresponding protein, thereby counteracting the miR-34a-mediated downregulation of SIRTUIN 1 [41]. PU.1, an Ets family TF, has been shown to plays a crucial role in lymphocyte differentiation in the bone marrow [42]. lncRNA PU.1-as acts as an antisense transcript for PU.1. lncRNA PU.1-as forms a double-chain complex with PU.1 mRNA, mRNA/AS lncRNA, which hinders the translation of PU.1 mRNA [42].

lncRNAs recruit target proteins to cis- or trans-regulate target genes, thereby affecting cell differentiation. The lncRNA ANCR is involved in the regulation of osteoblast differentiation of mesenchymal stem cells through its interaction with the Runx2 gene [43]. Specifically, ANCR physically interacts with EZH2 at its 3' end within the 305-nt region. EZH2 inhibits gene expression by catalyzing histone H3 lysine 27 methylation in the promoter regions of the target genes [44]. EZH2 specifically mediates his-

tone H3 lysine 27 methylation within the promoter region of Runx2, consequently impeding its regulatory function exerted by Runx2 on osteoblast differentiation [43]. The lncRNA H19 and its encoded miR-675 promote osteoblastic differentiation of human bone marrow mesenchymal stem cells, and miR-675 is involved in regulating H19's osteogenic effect [45]. The H19/miR-675 axis negatively regulates TGF-b1 expression in hMSCs, leading to the down-regulation of TGF-b-phosphorylated Smad3 and subsequently attenuating the recruitment of Smad3 to HDAC4/5 [46]. This reduces the binding affinity between HDAC4/5 and the runt-related TF 2 (Runx2) DNA sequence [47]. HDAC4/5 activity-dependent TGF-b1 inhibits the osteogenic differentiation of hMSCs; however, decreased expression of histone deacetylase HDAC4/5 due to TGF-b1 downregulation impedes hMSCs' commitment towards an osteoblastic lineage. Upregulation of H19 expression stimulated endogenous TGF-b1 expression, thereby inducing tendon differentiation and repair [48]. H19 promotes tendon differentiation by targeting miR-29b-3p, which downregulates the expression of key tendon marker genes, including SCX, MKX, EGR1, TNMD, FMOD, and DCN. Specifically, miR-29b-3p binds to TGF-b1 within its coding region and targets COL1A1 in skin fibroblasts post-transcriptionally. lncRNA SRA transcripts are present in distinct ribonucleoprotein complexes and they exhibit selective interactions with steroid receptors, thereby activating their transcriptional activity [49]. The noncoding RNA steroid receptor activator (SRA) functions as a coactivator of MyoD during skeletal muscle differentiation [50]. SRA acts as a coactivator specifically for MyoD-E12, promoting the conversion of MyoD-induced mouse fibroblasts into squamous myocytes [50]. The yin-yang 1 (YY1)-associated muscle lncRNA (Yam-1) is a muscle-specific lncRNA expressed in the nucleus and cytoplasm with a transcript length of 923 nt. Yam-1 has been shown to function as an anti-myogenic factor during in vivo muscle regeneration. It interacts with the TF YY1 in the promoter region and cis-regulates miR-715 to inhibit Wnt7b levels [51]. In additionally, Yam-2 promotes early stage myogenic differentiation. The bone morphogenetic proteins (BMP)/OP-responsive gene (BORG) represents a commonly targeted gene by BMPs. In the C2C12 mouse myoblast cell line, transfection with antisense oligonucleotides targeting BORG partially inhibited BMP-induced alkaline phosphatase activity, thereby impeding the differentiation of C2C12 cells into osteoblasts [52]. Nkx2.2 is a pivotal TF involved in the differentiation of neural stem cells into oligodendrocytes [53]. lncRNA Nkx2.2AS acts as an endogenous antisense transcript of Nkx2.2 gene, comprising a 4.3 kb cDNA that exhibits specific cytoplasmic expression patterns [54]. While positively regulating the mRNA level of Nkx2.2AS, overexpression of this lncRNA also leads to the upregulation of the corresponding Nkx2.2 mRNA levels within neural stem cells and subsequently induces their differentiation into oligodendrocyte lineages [54]. The lncRNA Evf-2 is transcribed from a highly conserved sequence located between the two Dlx-5/6 genes during neuronal differentiation in the brain [55]. Evf-2 interacts with the homeodomain protein Dlx-2 and selectively enhances the activity of the Dlx-5/6 enhancer region through a targeted mechanism specific for homeodomain binding [55].

lncRNAs can act as scaffolds for aggregating multiple proteins to form functional complexes that regulate differentiation. The nuclear localization of Men epsilon (ε) and Men beta (β) was significantly upregulated at paraspeckles during myoblast differentiation, which resulted in a 3.2-4.9 fold increase in expression levels [56]. lncRNA Men ε, also known as Neat1, is derived from mouse chromosome 19qA and spans approximately 3.2 kb in length. lncRNA MEN β represents an unspliced transcript spanning around 23 kb, with its 3' end being subjected to RNase H digestion resulting in a short poly(A)-enriched segment formation [57]. The tRNA-like structure generated by MEN β/MEN β locus transcript through RNase P action can also undergo subsequent cleavage mediated by RNase Z enzyme activity. lncRNA MEN ε/β exhibits with Non-POU-domain-containing, octamer binding protein (also known as p54/nrb) complex crucial for initial establishment and maintenance of stable paraspeckles architecture [56]. The transcript size of the nuclear factor of activated T cells (NFAT) non-coding inhibitor (NRON) gene ranges from 0.8-3.7 kb [58]. NFAT, a calcium-sensitive TF, plays a crucial role in T cell receptor-mediated immune responses by regulating immune cell differentiation. The direct interaction between the lncRNA NRON and importin-beta 1 modulates NFAT

activity. Furthermore, lncRNA NRON specifically regulates the nuclear trafficking of NFAT, thereby controlling its transcriptional activity [58].

lncRNA regulate cell regeneration and tissue development

Recently, significant advances have been made in biological science regarding the regulation of cell regeneration. Through the investigations of cell surface receptors, signaling pathway molecules, and TFs, researchers have been able to elucidate the intricate interactions and regulatory networks of multiple signaling pathways involved in cellular differentiation processes. Researchers have been able to identify key cells with regenerative potential during embryonic development, offering novel insights into tissue and organ regeneration. For instance, Lgr5-positive progenitors in mammals can regenerate into hair cells [59] and researchers have enhanced this regeneration process by overexpressing Rps14 [60]. Additionally, some studies have demonstrated that AAV-IE-mediated upregulation of Espin can optimize stereocilia development in Atoh1-induced cochlear hair cells [61]. lncRNAs have a wide range of important functions in cellular regeneration and tissue development. It regulates these processes through various mechanisms, including chromatin remodeling, transcriptional and translational regulation, and cell cycle modulation.

Intestinal mucosa regeneration

H19 LncRNA promotes the proliferation and epithelial regeneration of intestinal epithelial cells (IEC) by inhibiting p53 protein, microRNA 34a, and let-7 [15]. Upon the IECS injury and inflammation, interleukin-22 (IL-22) induces the expression of H19 lncRNA. IL-22 induces the expression of H19 lncRNAs in IECs via cyclic adenosine monophosphate-independent activation of protein kinase A and signal transducer and activator of transcription 3 (STAT3) signal transduction. Inflammation triggers the IL-22 signaling cascade, where H19 lncRNA acts as a downstream target involved in the crucial processes of intestinal epithelial healing and repair. Studies have demonstrated that the binding between H19 lncRNA and let-7 miRNA reduces their bioavailability [62]. Inflammation-induced H19 lncRNA potentially competes with growth-inhibitory miRNAs in vivo by binding to Mir34a and let-7, thereby attenuating their negative regulation of the IECs, whilst promoting MYCN expression (a positive regulator of epithelial cell proliferation) [15]. Furthermore, inflammation-induced H19 lncRNA interacts with p53 to dampen its activity, reducing the growth inhibition of the IECs while enhancing FOXM1 expression (a growth-promoting factor), thus facilitating regeneration of the intestinal mucosa [15].

Liver regeneration

lncRNA-uc008aun exhibits differential expression during liver regeneration and displays high nucleotide homology with human sequences, leading to its designation as lncRNA-long noncoding RNA associated with liver regeneration (LALR1) [63]. During the early stage of liver regeneration in mice, lncRNA-LALR1 facilitated hepatocyte proliferation and cell cycle progression, thereby enhancing liver regenerative capacity [63]. Mechanistically, lncRNA-LALR1 recruited CCCTC-binding factor to the AXIN1 promoter region, resulting in Axin1 downregulation. Moreover, lncRNA-LALR1 inhibited β-Catenin protein phosphorylation, promoting active β-Catenin translocation into the nucleus and significantly increasing nuclear β-Catenin levels.

Cardiac regeneration

Adult mammalian hearts lose their regenerative capacity following ischemic injury. Cardiac regeneration-associated lncRNA (CAREL) is significantly upregulated during neonatal heart development in mice [19]. CAREL acts as a ceRNA for miR-296, which inhibits CAREL expression by targeting the 3' untranslated region of Trp53inp1 and Itm2a genes. The lncRNA CAREL regulates postnatal and adult cardiac injury-induced cardiomyocyte (CM) proliferation and cardiac regeneration through the CAREL-miR-296-Trp53inp1/Itm2a signaling pathway [19].

The lncRNA NONHSAG042100.1 exhibited >12-fold upregulation in fetal heart and was designated as an endogenous cardiac regeneration-associated regulator (ECRAR) [64]. Significant enrichment of H3K4me3 and H3K36me3 indicates that ECRAR is an active chromatin state in the fetal heart. ECRAR participated in cell cycle regulation by promoting DNA synthesis,

mitosis, and cell division in rat CMs [64]. The TF E2F1 targets the promoter region of ECRAR and upregulates its transcription. Increased levels of ECRAR bind to extracellular signal-regulated kinases 1 and 2 (ERK1/2) leading to them being phosphorylated. Activated ERK1/2 induces cyclin D1 expression through multiple mechanisms that further activate E2F1, establishing an E2F1-ECRAR-ERK1/2 positive feedback loop for the continuous promotion of CM proliferation [64].

The upregulated lncRNA NONHSAG007671 in adult cardiac tissue was designated as a CM regeneration-related lncRNA (CRRL) with a full-length of 562 bp [65]. CRRL negatively regulates endogenous CM proliferation. CRRL acts as a competitive endogenous RNA (ceRNA) for miR-199a-3p, exerting inhibitory effects on its ability to upregulate the expression of Hopx, a key negative regulator of embryonic CM proliferation [65].

Lung epithelial cell regeneration

Foxa2-adjacent lncRNA (Falcor) is downstream of Foxa2 and its expression patterns exhibit remarkable similarities throughout mouse development. The TF Foxa2 binds to chromatin and plays a crucial role in early embryonic development. Although Falcor is dispensable for normal development, its absence may attenuate the epithelial response to injury [66]. Falcor positively regulates the expression of Foxa2, while Foxa2 negatively regulates Falcor expression by directly binding to the Falcor promoter. In this regulatory feedback loop between Foxa2 and Falcor, Falcor finely tunes the expression of Foxa2, maintains airway epithelial cell homeostasis, and facilitates lung epithelial cell regeneration [66]. The absence of Falcor in the lung disrupts the specific regulation mediated by the Foxa2-Falcor feedback loop, leading to chronic inflammatory changes and impaired epithelial cell regeneration following airway injury, ultimately resulting in defective repair [66].

Otocyst development

The non-coding RNA Meg3/Gtl2 gene is implicated in early stage growth, otocysts proliferation, and late-stage specific cell differentiation within the inner ear [67]. During mouse embryonic development, lncRNA Meg3/Gtl2 is expressed in the otocyst region from the one-cell stage onwards. In highly proliferative otocyst, Meg3/Gtl2 RNA expression is localized in the cytoplasm. As development progressed, there was a sharp increase in Meg3/Gtl2 expression levels. By E14.5, Meg3/Gtl2 expression was restricted to the epithelial cells of the cochlear canaliculus. In the later stages of development, Meg3/Gtl2 predominantly localizes to the cochlear canaliculus, where it becomes nuclear and is closely associated with neurogenesis, epithelial canalization, and myogenesis. At this stage, Meg3/Gtl2 RNA is expressed in the Great Epithelial Ridge region and contributes to inner hair cell differentiation [67].

Mammary gland development

The full-length pregnancy-induced ncRNA (PINC) is 6.3 kb, with a transcript size ranging from 1.2-4.8 kb. PINC is a developmentally regulated ncRNA that is abundantly expressed during mouse embryogenesis, whereas its high expression in adult mouse tissues is predominantly restricted to the mammary glands and testis [68]. PINC expression is induces during pregnancy and hormonal stimulation of the mammary glands. PINC-1.6 transcript participates in cell cycle regulation by reducing its expression to promote S-phase entry.

Embryonic development

The lncRNA UCA1 was isolated from the human bladder transitional cell carcinoma cell line BLZ-211 and yield a full-length cDNA sequence of 1442 bp [25]. Expression of UCA1 initiates at an early-stage post-fertilization and persists throughout embryogenesis. UCA1 exhibits robust transcriptional activity in the placenta, embryo, and various fetal tissues during development [25]. Furthermore, UCA1 downregulates methyl-CpG binding domain protein 3 [69], a pivotal player in mouse embryogenesis.

lncRNA regulates ESC pluripotency and participates in embryonic development

The regulation of stem cell pluripotency has always been a focal point in life sciences. Researchers have identified a plethora of pivotal factors involved in regulating this process, including signaling pathways, TFs, and epigenetic regulators. The Wnt, Notch, and FGF signal-

ing pathways have been shown to exert a significant influence on stem cell pluripotency regulation. Researchers have discovered that structurally tunable reduced substrates preserves the pluripotency of mouse ESCs [70], and that two-dimensional Ti3C2TxMXene combined with electrical stimulation enhances the proliferation of neural stem cells [71]. Further investigation of ESC TFs and their lncRNA targets have the potential to enhance our understanding of stem cell genomic regulatory networks, thereby facilitating rational therapeutic interventions based on the regulatory capacity of ESC lncRNAs.

Mouse embryonic stem cells

Using a mouse ESCs model, researchers found that lncRNAs maintain ES cell pluripotency and regulate their differentiation, and used dynamic expression patterns to define three different types of EB differentiation stages: pluripotency, primitive streak formation, and mesodermal differentiation. During the pluripotency stage ncRNA expression is regulated by TFs that maintain pluripotency. At the primitive streak formation stage, ncRNA is highly correlated with the expression profiles of Brachyury (T) and Evx1 (a hallmark of EB differentiation into blastomeres is the expression of genes such as Evx1 and Brachyury (T) [72-74]), and at the mesodermal differentiation stage, ncRNA is highly correlated with the expression of hemoglobin genes and is enriched with pluripotent RNAs [75]. Mammalian trithorax protein (MLL1) expression is progressively up-regulated during EB differentiation and regulates the Hox locus as well as several other developmental targets in human and mouse cells [76, 77]. The spliced Hoxb5/6as and Evx1as transcripts bind to MLL1 at the H3K4me3 site and are involved in directing MLL1 activity.

The transcriptional regulators Oct4 and Nanog, along with their regulated lncRNAs AK028326 and AK141205, play a crucial role in governing the pluripotency and differentiation of mouse embryonic stem cells (mESCs) [78], by targeting AK028326 via binding to its 5' end, thereby activating the expression of AK028326 transcripts. The down-regulation of Oct4 significantly reduces the expression of AK028326. Conversely, targeted knockout of AK028326 led to decreased Oct4 mRNA levels, while maintaining its overall stability through an autofeedback loop between Oct4 and AK028326. This loop regulates mESC pluripotency and differentiation [78]. AK141205 is an lncRNA that is suppressed by Nanog and promotes differentiation. The lncRNAs, AK028326 and AK141205 are directly controlled by the key TFs Oct4 and Nanog, respectively, and can modulate their own transcription to promote differentiation and dedifferentiation in disturbed mESCs while regulating pluripotency.

Human embryonic stem cells

Several specific lncRNAs participate in maintaining pluripotency in hESCs through physical interactions with SUZ12, a component of the SOX2 and PRC2 complex. The lncRNA_ES1 (AK056826), lncRNA_ES2 (EF565083), and lncRNA_ES3 (BC026300) are exclusively expressed in undifferentiated hESCs and iPSCs, and serve as direct downstream targets of the pluripotency regulators OCT4 and NANOG [27]. Both lncRNA_ES1 and lncRNA_ES2 form complexes with SOX2 to regulate the maintenance of pluripotency. Acting as a modular scaffold, lncRNA_ES1 facilitates the assembly of SUZ12 and inhibitory PRC2 complex [79], thereby silencing neural targets regulated by SOX2 within pluripotent hESCs.

In self-renewing hESCs, lncRNA-RoR interacts with mature miR-145 through its function as a miRNA sponge, thereby negatively regulating the post-transcriptional expression of miR-145 and inhibiting the degradation of core TFs Oct4, Sox2, and Nanog transcripts [80]. Core TFs such as Oct4, Sox2, and Nanog, play key roles in the transcriptional network by promoting the expression of ESC-specific genes and repressing differentiation [81]. Oct4 or Nanog deletion downregulates lncRNA-RoR expression in hESCs. During self-renewal, Oct4, Nanog, and Sox2 were found within the promoter region of lncRNA-RoR, but not during differentiation. The expression levels of lncRNA-RoR and core TFs changed synchronously in hESC, with an initial change in lncRNA-RoR expression. lncRNA-RoR positively regulates the expression of Oct4, Nanog, and Sox2, whereas these core TFs directly regulate the transcription of lncRNA-RoR, forming a regulatory feedback loop [80]. Three miRNAs, miR-145, miR-181, and miR-99 participates in regulating hESCs self-renewal

[82, 83]. As crucial ceRNA molecules in hESCs, lncRNAs interact with these miRNAs to modulate the maintenance and differentiation processes through the core TF network.

iPSCs reprogramming

Numerous lncRNAs are associated with cellular pluripotency. The expression of lncRNAs observed in fibroblasts is suppressed upon achieving pluripotency, whereas distinct lncRNAs are activated during cellular reprogramming. This consistent enrichment pattern of lncRNAs persisted across iPSCs generated by reprogramming various primary cell types. Three lncRNAs enriched iPSCs, namely, lncRNA-SFMBT2, lncRNA-VLDLR, and lncRNA-ST8SIA3, were found to bind to the core TFs Oct4, Sox2, and Nanog near the promoter region [84]. The expression of iPSC-enriched lncRNAs depends on pluripotent TFs. lncRNA-ST8SIA3 expression is essential for iPSC derivation, deletion of lncRNA-ST8SIA3 inhibits the reprogramming process of iPSCs, whereas overexpression promotes their generation during reprogramming. Furthermore, its deletion activates cellular stress pathways, such as the p53 response, oxidative stress response, DNA damage-inducing factor, and cell death pathway [84].

lncRNA regulates tumor cell stemness

lncRNAs participate in the regulation of stemness, thereby contributing to the proliferation, migration, invasion, and metastasis of diverse tumor cells.

HOX antisense intergenic RNA (HOTAIR), a lncRNA spanning 2158 kb, is located within the HOXC locus [85]. Its mechanism involves recruiting PRC2 to specific target genes across the genome, resulting in H3K27 trimethylation and subsequent epigenetic silencing of metastasis suppressor genes [7]. HOTAIR expression is significantly upregulated in primary breast cancer and breast cancer metastases [86]. HOTAIR facilitates the invasion capability of breast cancer cells by targeting CHST15 (GalNAc4S-6ST), a chondroitin sulfate transferase, leading to chondroitin sulfate expression upregulation in glycosaminoglycan metabolism on the cell surface. Simultaneously, the HOTAIR-CHST15 axis sustains the stemness of cancer cells and promotes tumorigenesis and metastasis of breast cancer cells to lung xenografts [86]. H19 was initially characterized as a tumor suppressor factor [87]. H19 serves as a major downstream target of c-Myc and activated by c-Myc to directly bind to E-boxes proximal to the imprinting control region, thereby facilitating the recruitment of histone acetylation to the promoter region of H19 and subsequently promoting its transcriptional activation [88]. H19 is involved in essential functions during the normal development of mammary glands and in pathological mechanisms [89]. In breast adenocarcinoma, there is an overall upregulation of H19 gene expression compared to healthy tissues [90]. Overexpression of H19 is significantly correlate with tumor malignancy and the presence of estrogen and progesterone receptors [90].

HOTAIR is highly expressed in pancreatic cancer cells, with higher expression observed in advanced tumors [22]. In pancreatic cancer cells, HOTAIR interacts with polycomb repressive complex 2, comprising EZH2, SUZ12, and EED proteins to enhance the trimethylation of histone H3 at lysine 27, thereby synergistically inhibiting the growth inhibitory/apoptotic gene GDF15 alongside PRC2 [22].

MALAT-1 plays a crucial role in cancer development by promoting cell proliferation and inhibiting apoptosis [91]. Its expression is notably high in the lung, pancreatic, and non-small cell lung cancers. Moreover, MALAT-1 expression is several times higher in metastatic adenocarcinoma than in non-metastatic adenocarcinoma and serves as a strong predictor of poor prognosis in early stage disease [92]. In proto-oncogene-induced mouse hepatocellular carcinoma (HCC) nodules and human HCC samples, MALAT-1 expression was significantly increased compared to that in unaffected liver tissue [93]. HOTAIR is significantly overexpressed in tumor samples obtained from patients with HCC. Elevated HOTAIR expression is associated with an increased risk of HCC recurrence and lymph node metastasis following hepatectomy, and directly correlates with long-term patient survival [23]. Deletion of HOTAIR leads to downregulation of matrix metalloproteinase-9 and vascular endothelial growth factor proteins, which are crucial players in tumor progression [23].

HOTAIR is highly expressed in human non-small cell lung cancer and promotes aggressive

behavior. Increased HOTAIR expression is frequently observed in patients with advanced lung cancer, lymph node metastasis, lymphatic vessel invasion, and shorter disease-free intervals. Moreover, compared with primary cancer tissues, brain metastases display significantly elevated levels of HOTAIR expression [24]. There is general upregulation of HOTAIR and type I collagen (Col-1) expression in human non-small cell lung cancer. The upregulation of Col-1 regulates the promoter activity of HOTAIR and triggers its transcriptional activation [94]. In the pathogenesis of Wilms' tumor, the imprinted gene H19 located at 11p15 exhibits loss of imprinting (LOI), and in the context of lung cancer development, hypomethylation in the promoter region of H19 leads to LOI and subsequent overexpression of H19 [95]. The expression is significantly higher in H19 lung cancer tissues than in adjacent normal tissues [96]. MiR-200a expression decreases significantly with increasing tissue malignancy. Acting as a sponge RNA molecule, H19 sequesters miR-200a and inhibits its function, consequently upregulating the expression of miR-200a target genes, ZEB1 and ZEB2, while promoting epithelial mesenchymal transition (EMT) [96].

The lncRNA Taurine Up-regulated Gene 1 (TUG1) is a spliced polyadenylated transcript approximately 6.7 kb in length, which is upregulated in developing retinal cells upon taurine stimulation to actively participate in photoreceptor formation [97]. It can be induced by p53 to interact with PRC2 and exert inhibitory effects on specific genes involved in cell cycle regulation [98]. TUG1 is upregulated in bladder cancer and promotes its pathogenesis [99], whereas miR-140-3p is downregulated and inhibits tumor function. TUG1 acts as a sponge for miR-140-3p to inhibit Annexin A8 3'UTR targeting, thereby promoting progression and metastasis of bladder cancer. The expression of lncRNA UCA1 is elevated in bladder cancer tissues and promotes tumor invasion, indicating its potential as a carcinoembryonic gene [25]. Its spatiotemporal expression pattern resembles that of ncRNA H19 [100]. Moreover, UCA1 regulated the proliferation of BLS 211 cells, enhanced sustained tumor growth, and augmenteds tumorigenicity in nude mice [25]. In osteosarcoma, lncRNA TUSC7 (LOC285194) and LINC00901 (BC040587) are located within frequently altered genomic regions associated with osteosarcoma. TUSC7 deletion enhances the proliferation of normal osteoblasts by regulating apoptosis and cell cycle-related genes [101]. Both TUG1 and one of its transcript variants (n377360) exhibit significantly elevated expression levels in osteosarcoma tissues compared to adjacent normal tissues [102].

H19, STAT3, and EZH2 are highly expressed in malignant esophageal cancer (EC) cell lines. H19 knockout suppresses cell proliferation, migration, invasion, EMT, and metastasis in EC cell lines, and induces apoptosis [16]. Functionally acting as a ceRNA, H19 exerts its inhibitory role by sequestering let-7c in EC cell lines, thereby upregulating the expression of STAT3 and its downstream molecules, including EZH2, SOX4, and β-catenin.

In gastric cancer cells, miR-675 acts as a negative regulator by targeting the tumor suppressor gene runt-domain TF 1 (RUNX1), thereby mediating H19-induced progression of gastric cancer [17]. The proliferation of gastric cancer cells is regulated by the H19/miR-675/RUNX1 signaling pathway.

lncRNA loc285194, also known as LSAMP antisense RNA 3, is a tumor suppressor transcriptionally regulated by the p53 protein. Located at osteo3q13.31 and spanning >2 kb in length [101], loc285194 is downregulated in colon cancer. In this context, p53 directly targets the putative p53 response element located upstream of loc285194 to inhibit tumor cell growth. Mechanistically, loc285194 exerts its tumor-suppressive function through specific inhibition of miR-211 activity via an RNA-induced silencing complex [103].

The expression of H19 varies in normal, hyperplastic, and cancerous endometria. In the normal endometrium, H19 is exclusively expressed in the stromal cells. Within the stromal cell population, the frequency of H19 expression decreased with increasing differentiation levels. The lowest frequency of H19 expression was observed in cancerous endometrial stromal cells [104]. The expression of H19 decreases significantly with the progression of cervical cancer, which can be attributed to excessive methylation of the H19 promoter, leading to frequent down-regulation [105].

Conclusion

lncRNAs play a pivotal role in cellular development, encompassing the regulation of cell differentiation and regeneration through modulation of gene expression. They are also crucial for maintaining and regulating cellular stemness, facilitating self-renewal, and preserving the undifferentiated state to ensure tissue and organ development and repair. The regulatory mechanisms employed by lncRNAs involve various processes, including binding to chromatin to form chromatin loops that influence chromatin structure and histone modifications; interaction with TFs or other RNA molecules to affect their function and stability; and modulation of cell cycle progression, apoptosis, and other related pathways during cell differentiation and regeneration. Moreover, lncRNAs have been shown to significantly contribute to the maintenance of cellular stemness by promoting self-renewal and inhibiting differentiation, whereas they can enhance stemness by facilitating differentiation induction and self-renewal promotion. In cancer cells, lncRNAs specifically regulate stemness through diverse mechanisms, such as modulating key processes, including cell cycle progression, fate determination, and apoptosis. lncRNA can also influence metabolic activities and signaling pathways. Therefore, a comprehensive understanding of the intricate mechanism underlying the regulation of cancer cell stemness by lncRNAs will provide valuable insights into cancer initiation and progression, and offer novel perspectives for effective therapeutic strategies.

Acknowledgements

This study was funded by Yangzhou Social Development Project, No. YZ2023114.

Disclosure of conflict of interest

None.

Address correspondence to: Bing Guan, Department of Otolaryngology Head and Neck Surgery, Northern Jiangsu People's Hospital Affiliated to Yangzhou University, Yangzhou 225001, Jiangsu, China. E-mail: aliceguan0685@sina.com

References

[1] Jia H, Osak M, Bogu GK, Stanton LW, Johnson R and Lipovich L. Genome-wide computational identification and manual annotation of human long noncoding RNA genes. RNA 2010; 16: 1478-1487.

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

[3] Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J and Guigó R. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 2012; 22: 1775-1789.

[4] Harries LW. Long non-coding RNAs and human disease. Biochem Soc Trans 2012; 40: 902-906.

[5] Wang KC and Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell 2011; 43: 904-914.

[6] Wang X, Arai S, Song X, Reichart D, Du K, Pascual G, Tempst P, Rosenfeld MG, Glass CK and Kurokawa R. Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription. Nature 2008; 454: 126-130.

[7] Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E and Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 2007; 129: 1311-1323.

[8] Umlauf D, Fraser P and Nagano T. The role of long non-coding RNAs in chromatin structure and gene regulation: variations on a theme. Biol Chem 2008; 389: 323-331.

[9] He Y, Vogelstein B, Velculescu VE, Papadopoulos N and Kinzler KW. The antisense transcriptomes of human cells. Science 2008; 322: 1855-1857.

[10] Payer B and Lee JT. X chromosome dosage compensation: how mammals keep the balance. Annu Rev Genet 2008; 42: 733-772.

[11] Ogawa Y, Sun BK and Lee JT. Intersection of the RNA interference and X-inactivation pathways. Science 2008; 320: 1336-1341.

[12] Tao Y, Liu X, Yang L, Chu C, Tan F, Yu Z, Ke J, Li X, Zheng X, Zhao X, Qi J, Lin CP, Chai R, Zhong G and Wu H. AAV-ie-K558R mediated cochlear gene therapy and hair cell regeneration. Signal Transduct Target Ther 2022; 7: 109.

[13] Zhang L, Fang Y, Tan F, Guo F, Zhang Z, Li N, Sun Q, Qi J and Chai R. AAV-Net1 facilitates the trans-differentiation of supporting cells into hair cells in the murine cochlea. Cell Mol Life Sci 2023; 80: 86.

[14] Zhang S, Zhang Y, Dong Y, Guo L, Zhang Z, Shao B, Qi J, Zhou H, Zhu W, Yan X, Hong G, Zhang L, Zhang X, Tang M, Zhao C, Gao X and Chai R. Knockdown of Foxg1 in supporting cells increases the trans-differentiation of supporting cells into hair cells in the neonatal mouse cochlea. Cell Mol Life Sci 2020; 77: 1401-1419.

[15] Geng H, Bu HF, Liu F, Wu L, Pfeifer K, Chou PM, Wang X, Sun J, Lu L, Pandey A, Bartolomei MS, De Plaen IG, Wang P, Yu J, Qian J and Tan XD. In Inflamed Intestinal Tissues and Epithelial Cells, Interleukin 22 Signaling Increases Expression of H19 Long Noncoding RNA, Which Promotes Mucosal Regeneration. Gastroenterology 2018; 155: 144-155.

[16] Chen MJ, Deng J, Chen C, Hu W, Yuan YC and Xia ZK. LncRNA H19 promotes epithelial mesenchymal transition and metastasis of esophageal cancer via STAT3/EZH2 axis. Int J Biochem Cell Biol 2019; 113: 27-36.

[17] Zhuang M, Gao W, Xu J, Wang P and Shu Y. The long non-coding RNA H19-derived miR-675 modulates human gastric cancer cell proliferation by targeting tumor suppressor RUNX1. Biochem Biophys Res Commun 2014; 448: 315-322.

[18] Ren L, Chen S, Liu W, Hou P, Sun W and Yan H. Downregulation of long non-coding RNA nuclear enriched abundant transcript 1 promotes cell proliferation and inhibits cell apoptosis by targeting miR-193a in myocardial ischemia/reperfusion injury. BMC Cardiovasc Disord 2019; 19: 192.

[19] Cai B, Ma W, Ding F, Zhang L, Huang Q, Wang X, Hua B, Xu J, Li J, Bi C, Guo S, Yang F, Han Z, Li Y, Yan G, Yu Y, Bao Z, Yu M, Li F, Tian Y, Pan Z and Yang B. The Long Noncoding RNA CAREL Controls Cardiac Regeneration. J Am Coll Cardiol 2018; 72: 534-550.

[20] Ferreira LB, Palumbo A, de Mello KD, Sternberg C, Caetano MS, de Oliveira FL, Neves AF, Nasciutti LE, Goulart LR and Gimba ER. PCA3 noncoding RNA is involved in the control of prostate-cancer cell survival and modulates androgen receptor signaling. BMC Cancer 2012; 12: 507.

[21] Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S and Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010; 464: 1071-1076.

[22] Kim K, Jutooru I, Chadalapaka G, Johnson G, Frank J, Burghardt R, Kim S and Safe S. HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. Oncogene 2013; 32: 1616-1625.

[23] Geng YJ, Xie SL, Li Q, Ma J and Wang GY. Large intervening non-coding RNA HOTAIR is associated with hepatocellular carcinoma progression. J Int Med Res 2011; 39: 2119-2128.

[24] Nakagawa T, Endo H, Yokoyama M, Abe J, Tamai K, Tanaka N, Sato I, Takahashi S, Kondo T and Satoh K. Large noncoding RNA HOTAIR enhances aggressive biological behavior and is associated with short disease-free survival in human non-small cell lung cancer. Biochem Biophys Res Commun 2013; 436: 319-324.

[25] Wang F, Li X, Xie X, Zhao L and Chen W. UCA1, a non-protein-coding RNA up-regulated in bladder carcinoma and embryo, influencing cell growth and promoting invasion. FEBS Lett 2008; 582: 1919-1927.

[26] Yuan JB, Gu L, Chen L, Yin Y and Fan BY. Annexin A8 regulated by lncRNA-TUG1/miR-140-3p axis promotes bladder cancer progression and metastasis. Mol Ther Oncolytics 2021; 22: 36-51.

[27] Ng SY, Johnson R and Stanton LW. Human long non-coding RNAs promote pluripotency and neuronal differentiation by association with chromatin modifiers and transcription factors. EMBO J 2012; 31: 522-533.

[28] Watts R, Johnsen VL, Shearer J and Hittel DS. Myostatin-induced inhibition of the long noncoding RNA Malat1 is associated with decreased myogenesis. Am J Physiol Cell Physiol 2013; 304: C995-1001.

[29] Kretz M, Webster DE, Flockhart RJ, Lee CS, Zehnder A, Lopez-Pajares V, Qu K, Zheng GX, Chow J, Kim GE, Rinn JL, Chang HY, Siprashvili Z and Khavari PA. Suppression of progenitor differentiation requires the long noncoding RNA ANCR. Genes Dev 2012; 26: 338-343.

[30] Yu Z, Lin KK, Bhandari A, Spencer JA, Xu X, Wang N, Lu Z, Gill GN, Roop DR, Wertz P and Andersen B. The Grainyhead-like epithelial transactivator Get-1/Grhl3 regulates epidermal terminal differentiation and interacts functionally with LMO4. Dev Biol 2006; 299: 122-136.

[31] Lopez RG, Garcia-Silva S, Moore SJ, Bereshchenko O, Martinez-Cruz AB, Ermakova O, Kurz E, Paramio JM and Nerlov C. C/EBPalpha and beta couple interfollicular keratinocyte proliferation arrest to commitment and terminal differentiation. Nat Cell Biol 2009; 11: 1181-1190.

[32] Yang JM, Sim SM, Kim HY and Park GT. Expression of the homeobox gene, HOPX, is modulated by cell differentiation in human keratinocytes and is involved in the expression of differentiation markers. Eur J Cell Biol 2010; 89: 537-546.

[33] Rapicavoli NA, Poth EM and Blackshaw S. The long noncoding RNA RNCR2 directs mouse

retinal cell specification. BMC Dev Biol 2010; 10: 49.
[34] Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, Tramontano A and Bozzoni I. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell 2011; 147: 358-369.
[35] Salmena L, Poliseno L, Tay Y, Kats L and Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 2011; 146: 353-358.
[36] Karreth FA, Tay Y, Perna D, Ala U, Tan SM, Rust AG, DeNicola G, Webster KA, Weiss D, Perez-Mancera PA, Krauthammer M, Halaban R, Provero P, Adams DJ, Tuveson DA and Pandolfi PP. In vivo identification of tumor-suppressive PTEN ceRNAs in an oncogenic BRAF-induced mouse model of melanoma. Cell 2011; 147: 382-395.
[37] Zhang Y and Tycko B. Monoallelic expression of the human H19 gene. Nat Genet 1992; 1: 40-44.
[38] Liang WC, Fu WM, Wang YB, Sun YX, Xu LL, Wong CW, Chan KM, Li G, Waye MM and Zhang JF. H19 activates Wnt signaling and promotes osteoblast differentiation by functioning as a competing endogenous RNA. Sci Rep 2016; 6: 20121.
[39] Cai X and Cullen BR. The imprinted H19 noncoding RNA is a primary microRNA precursor. Rna 2007; 13: 313-316.
[40] Dey BK, Pfeifer K and Dutta A. The H19 long noncoding RNA gives rise to microRNAs miR-675-3p and miR-675-5p to promote skeletal muscle differentiation and regeneration. Genes Dev 2014; 28: 491-501.
[41] Wang Y, Pang WJ, Wei N, Xiong Y, Wu WJ, Zhao CZ, Shen QW and Yang GS. Identification, stability and expression of Sirt1 antisense long non-coding RNA. Gene 2014; 539: 117-124.
[42] Pang WJ, Lin LG, Xiong Y, Wei N, Wang Y, Shen QW and Yang GS. Knockdown of PU.1 AS lncRNA inhibits adipogenesis through enhancing PU.1 mRNA translation. J Cell Biochem 2013; 114: 2500-2512.
[43] Zhu L and Xu PC. Downregulated LncRNA-ANCR promotes osteoblast differentiation by targeting EZH2 and regulating Runx2 expression. Biochem Biophys Res Commun 2013; 432: 612-617.
[44] Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS and Zhang Y. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 2002; 298: 1039-1043.
[45] Huang Y, Zheng Y, Jia L and Li W. Long Noncoding RNA H19 Promotes Osteoblast Differentiation Via TGF-β1/Smad3/HDAC Signaling Pathway by Deriving miR-675. Stem Cells 2015; 33: 3481-3492.
[46] Derynck R and Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 2003; 425: 577-584.
[47] Kang JS, Alliston T, Delston R and Derynck R. Repression of Runx2 function by TGF-beta through recruitment of class II histone deacetylases by Smad3. EMBO J 2005; 24: 2543-2555.
[48] Lu YF, Liu Y, Fu WM, Xu J, Wang B, Sun YX, Wu TY, Xu LL, Chan KM, Zhang JF and Li G. Long noncoding RNA H19 accelerates tenogenic differentiation and promotes tendon healing through targeting miR-29b-3p and activating TGF-β1 signaling. FASEM J 2017; 31: 954-964.
[49] Lanz RB, McKenna NJ, Onate SA, Albrecht U, Wong J, Tsai SY, Tsai MJ and O'Malley BW. A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex. Cell 1999; 97: 17-27.
[50] Caretti G, Schiltz RL, Dilworth FJ, Di Padova M, Zhao P, Ogryzko V, Fuller-Pace FV, Hoffman EP, Tapscott SJ and Sartorelli V. The RNA helicases p68/p72 and the noncoding RNA SRA are coregulators of MyoD and skeletal muscle differentiation. Dev Cell 2006; 11: 547-560.
[51] Lu L, Sun K, Chen X, Zhao Y, Wang L, Zhou L, Sun H and Wang H. Genome-wide survey by ChIP-seq reveals YY1 regulation of lincRNAs in skeletal myogenesis. EMBO J 2013; 32: 2575-2588.
[52] Takeda K, Ichijo H, Fujii M, Mochida Y, Saitoh M, Nishitoh H, Sampath TK and Miyazono K. Identification of a novel bone morphogenetic protein-responsive gene that may function as a noncoding RNA. J Biol Chem 1998; 273: 17079-17085.
[53] Guillemot F. Cell fate specification in the mammalian telencephalon. Prog Neurobiol 2007; 83: 37-52.
[54] Tochitani S and Hayashizaki Y. Nkx2.2 antisense RNA overexpression enhanced oligodendrocytic differentiation. Biochem Biophys Res Commun 2008; 372: 691-696.
[55] Feng J, Bi C, Clark BS, Mady R, Shah P and Kohtz JD. The Evf-2 noncoding RNA is transcribed from the Dlx-5/6 ultraconserved region and functions as a Dlx-2 transcriptional coactivator. Genes Dev 2006; 20: 1470-1484.
[56] Sunwoo H, Dinger ME, Wilusz JE, Amaral PP, Mattick JS and Spector DL. MEN epsilon/beta nuclear-retained non-coding RNAs are up-regulated upon muscle differentiation and are essential components of paraspeckles. Genome Res 2009; 19: 347-359.
[57] Wilusz JE, Freier SM and Spector DL. 3' end processing of a long nuclear-retained noncoding RNA yields a tRNA-like cytoplasmic RNA. Cell 2008; 135: 919-932.

[58] Willingham AT, Orth AP, Batalov S, Peters EC, Wen BG, Aza-Blanc P, Hogenesch JB and Schultz PG. A strategy for probing the function of noncoding RNAs finds a repressor of NFAT. Science 2005; 309: 1570-1573.
[59] Wang T, Chai R, Kim GS, Pham N, Jansson L, Nguyen DH, Kuo B, May LA, Zuo J, Cunningham LL and Cheng AG. Lgr5+ cells regenerate hair cells via proliferation and direct transdifferentiation in damaged neonatal mouse utricle. Nat Commun 2015; 6: 6613.
[60] Xu C, Qi J, Hu X, Zhang L, Sun Q, Li N, Chen X, Guo F, Wu P, Shi Y and Chai R. Rps14 upregulation promotes inner ear progenitor proliferation and hair cell regeneration in the neonatal mouse cochlea. Cell Prolif 2023; 56: e13458.
[61] Yang X, Qi J, Zhang L, Tan F, Huang H, Xu C, Cui Y, Chai R and Wu P. The role of Espin in the stereocilia regeneration and protection in Atoh1-overexpressed cochlear epithelium. Cell Prolif 2023; 56: e13483.
[62] Kallen AN, Zhou XB, Xu J, Qiao C, Ma J, Yan L, Lu L, Liu C, Yi JS, Zhang H, Min W, Bennett AM, Gregory RI, Ding Y and Huang Y. The imprinted H19 lncRNA antagonizes let-7 microRNAs. Mol Cell 2013; 52: 101-112.
[63] Xu D, Yang F, Yuan JH, Zhang L, Bi HS, Zhou CC, Liu F, Wang F and Sun SH. Long noncoding RNAs associated with liver regeneration 1 accelerates hepatocyte proliferation during liver regeneration by activating Wnt/β-catenin signaling. Hepatology 2013; 58: 739-751.
[64] Chen Y, Li X, Li B, Wang H, Li M, Huang S, Sun Y, Chen G, Si X, Huang C, Liao W, Liao Y and Bin J. Long Non-coding RNA ECRAR Triggers Postnatal Myocardial Regeneration by Activating ERK1/2 Signaling. Mol Ther 2019; 27: 29-45.
[65] Chen G, Li H, Li X, Li B, Zhong L, Huang S, Zheng H, Li M, Jin G, Liao W, Liao Y, Chen Y and Bin J. Loss of long non-coding RNA CRRL promotes cardiomyocyte regeneration and improves cardiac repair by functioning as a competing endogenous RNA. J Mol Cell Cardiol 2018; 122: 152-164.
[66] Swarr DT, Herriges M, Li S, Morley M, Fernandes S, Sridharan A, Zhou S, Garcia BA, Stewart K and Morrisey EE. The long noncoding RNA Falcor regulates Foxa2 expression to maintain lung epithelial homeostasis and promote regeneration. Genes Dev 2019; 33: 656-668.
[67] Manji SS, Sørensen BS, Klockars T, Lam T, Hutchison W and Dahl HH. Molecular characterization and expression of maternally expressed gene 3 (Meg3/Gtl2) RNA in the mouse inner ear. J Neurosci Res 2006; 83: 181-190.
[68] Ginger MR, Shore AN, Contreras A, Rijnkels M, Miller J, Gonzalez-Rimbau MF and Rosen JM. A noncoding RNA is a potential marker of cell fate during mammary gland development. Proc Natl Acad Sci U S A 2006; 103: 5781-5786.
[69] Ruddock-D'Cruz NT, Xue J, Wilson KJ, Heffernan C, Prashadkumar S, Cooney MA, Sanchez-Partida LG, French AJ and Holland MK. Dynamic changes in the localization of five members of the methyl binding domain (MBD) gene family during murine and bovine preimplantation embryo development. Mol Reprod Dev 2008; 75: 48-59.
[70] Zhao J, Tang M, Cao J, Ye D, Guo X, Xi J, Zhou Y, Xia Y, Qiao J, Chai R, Yang X and Kang J. Structurally Tunable Reduced Graphene Oxide Substrate Maintains Mouse Embryonic Stem Cell Pluripotency. Adv Sci (Weinh) 2019; 6: 1802136.
[71] Guo R, Xiao M, Zhao W, Zhou S, Hu Y, Liao M, Wang S, Yang X, Chai R and Tang M. 2D Ti(3)C(2)T(x)MXene couples electrical stimulation to promote proliferation and neural differentiation of neural stem cells. Acta Biomater 2022; 139: 105-117.
[72] Robertson SM, Kennedy M, Shannon JM and Keller G. A transitional stage in the commitment of mesoderm to hematopoiesis requiring the transcription factor SCL/tal-1. Development 2000; 127: 2447-2459.
[73] Hirst CE, Ng ES, Azzola L, Voss AK, Thomas T, Stanley EG and Elefanty AG. Transcriptional profiling of mouse and human ES cells identifies SLAIN1, a novel stem cell gene. Dev Biol 2006; 293: 90-103.
[74] Bruce SJ, Gardiner BB, Burke LJ, Gongora MM, Grimmond SM and Perkins AC. Dynamic transcription programs during ES cell differentiation towards mesoderm in serum versus serum-freeBMP4 culture. BMC Genomics 2007; 8: 365.
[75] Dinger ME, Amaral PP, Mercer TR, Pang KC, Bruce SJ, Gardiner BB, Askarian-Amiri ME, Ru K, Soldà G, Simons C, Sunkin SM, Crowe ML, Grimmond SM, Perkins AC and Mattick JS. Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. Genome Res 2008; 18: 1433-1445.
[76] Guenther MG, Jenner RG, Chevalier B, Nakamura T, Croce CM, Canaani E and Young RA. Global and Hox-specific roles for the MLL1 methyltransferase. Proc Natl Acad Sci U S A 2005; 102: 8603-8608.
[77] Milne TA, Dou Y, Martin ME, Brock HW, Roeder RG and Hess JL. MLL associates specifically with a subset of transcriptionally active target genes. Proc Natl Acad Sci U S A 2005; 102: 14765-14770.
[78] Sheik Mohamed J, Gaughwin PM, Lim B, Robson P and Lipovich L. Conserved long noncoding RNAs transcriptionally regulated by Oct4

and Nanog modulate pluripotency in mouse embryonic stem cells. RNA 2010; 16: 324-337.
[79] Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E and Chang HY. Long noncoding RNA as modular scaffold of histone modification complexes. Science 2010; 329: 689-693.
[80] Wang Y, Xu Z, Jiang J, Xu C, Kang J, Xiao L, Wu M, Xiong J, Guo X and Liu H. Endogenous miRNA sponge lincRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. Dev Cell 2013; 25: 69-80.
[81] Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, Wong E, Orlov YL, Zhang W, Jiang J, Loh YH, Yeo HC, Yeo ZX, Narang V, Govindarajan KR, Leong B, Shahab A, Ruan Y, Bourque G, Sung WK, Clarke ND, Wei CL and Ng HH. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell 2008; 133: 1106-1117.
[82] Kane NM, Howard L, Descamps B, Meloni M, McClure J, Lu R, McCahill A, Breen C, Mackenzie RM, Delles C, Mountford JC, Milligan G, Emanueli C and Baker AH. Role of microRNAs 99b, 181a, and 181b in the differentiation of human embryonic stem cells to vascular endothelial cells. Stem Cells 2012; 30: 643-654.
[83] Xu N, Papagiannakopoulos T, Pan G, Thomson JA and Kosik KS. MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. Cell 2009; 137: 647-658.
[84] Loewer S, Cabili MN, Guttman M, Loh YH, Thomas K, Park IH, Garber M, Curran M, Onder T, Agarwal S, Manos PD, Datta S, Lander ES, Schlaeger TM, Daley GQ and Rinn JL. Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells. Nat Genet 2010; 42: 1113-1117.
[85] Woo CJ and Kingston RE. HOTAIR lifts noncoding RNAs to new levels. Cell 2007; 129: 1257-1259.
[86] Liu LC, Wang YL, Lin PL, Zhang X, Cheng WC, Liu SH, Chen CJ, Hung Y, Jan CI, Chang LC, Qi X, Hsieh-Wilson LC and Wang SC. Long noncoding RNA HOTAIR promotes invasion of breast cancer cells through chondroitin sulfotransferase CHST15. Int J Cancer 2019; 145: 2478-2487.
[87] Hao Y, Crenshaw T, Moulton T, Newcomb E and Tycko B. Tumour-suppressor activity of H19 RNA. Nature 1993; 365: 764-767.
[88] Barsyte-Lovejoy D, Lau SK, Boutros PC, Khosravi F, Jurisica I, Andrulis IL, Tsao MS and Penn LZ. The c-Myc oncogene directly induces the H19 noncoding RNA by allele-specific binding to potentiate tumorigenesis. Cancer Res 2006; 66: 5330-5337.
[89] Yballe CM, Vu TH and Hoffman AR. Imprinting and expression of insulin-like growth factor-II and H19 in normal breast tissue and breast tumor. J Clin Endocrinol Metab 1996; 81: 1607-1612.
[90] Adriaenssens E, Dumont L, Lottin S, Bolle D, Leprêtre A, Delobelle A, Bouali F, Dugimont T, Coll J and Curgy JJ. H19 overexpression in breast adenocarcinoma stromal cells is associated with tumor values and steroid receptor status but independent of p53 and Ki-67 expression. Am J Pathol 1998; 153: 1597-1607.
[91] Ren D, Li H, Li R, Sun J, Guo P, Han H, Yang Y and Li J. Novel insight into MALAT-1 in cancer: Therapeutic targets and clinical applications. Oncol Lett 2016; 11: 1621-1630.
[92] Ji P, Diederichs S, Wang W, Böing S, Metzger R, Schneider PM, Tidow N, Brandt B, Buerger H, Bulk E, Thomas M, Berdel WE, Serve H and Müller-Tidow C. MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene 2003; 22: 8031-8041.
[93] Lin R, Maeda S, Liu C, Karin M and Edgington TS. A large noncoding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas. Oncogene 2007; 26: 851-858.
[94] Zhuang Y, Wang X, Nguyen HT, Zhuo Y, Cui X, Fewell C, Flemington EK and Shan B. Induction of long intergenic non-coding RNA HOTAIR in lung cancer cells by type I collagen. J Hematol Oncol 2013; 6: 35.
[95] Kondo M, Suzuki H, Ueda R, Osada H, Takagi K, Takahashi T and Takahashi T. Frequent loss of imprinting of the H19 gene is often associated with its overexpression in human lung cancers. Oncogene 1995; 10: 1193-1198.
[96] Zhao Y, Feng C, Li Y, Ma Y and Cai R. LncRNA H19 promotes lung cancer proliferation and metastasis by inhibiting miR-200a function. Mol Cell Biochem 2019; 460: 1-8.
[97] Young TL, Matsuda T and Cepko CL. The noncoding RNA taurine upregulated gene 1 is required for differentiation of the murine retina. Curr Biol 2005; 15: 501-512.
[98] Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, Regev A, Lander ES and Rinn JL. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci U S A 2009; 106: 11667-11672.
[99] Han Y, Liu Y, Gui Y and Cai Z. Long intergenic non-coding RNA TUG1 is overexpressed in urothelial carcinoma of the bladder. J Surg Oncol 2013; 107: 555-559.

[100] Looijenga LH, Verkerk AJ, De Groot N, Hochberg AA and Oosterhuis JW. H19 in normal development and neoplasia. Mol Reprod Dev 1997; 46: 419-439.
[101] Pasic I, Shlien A, Durbin AD, Stavropoulos DJ, Baskin B, Ray PN, Novokmet A and Malkin D. Recurrent focal copy-number changes and loss of heterozygosity implicate two noncoding RNAs and one tumor suppressor gene at chromosome 3q13.31 in osteosarcoma. Cancer Res 2010; 70: 160-171.
[102] Zhang Q, Geng PL, Yin P, Wang XL, Jia JP and Yao J. Down-regulation of long non-coding RNA TUG1 inhibits osteosarcoma cell proliferation and promotes apoptosis. Asian Pac J Cancer Prev 2013; 14: 2311-2315.
[103] Liu Q, Huang J, Zhou N, Zhang Z, Zhang A, Lu Z, Wu F and Mo YY. LncRNA loc285194 is a p53-regulated tumor suppressor. Nucleic Acids Res 2013; 41: 4976-4987.
[104] Tanos V, Ariel I, Prus D, De-Groot N and Hochberg A. H19 and IGF2 gene expression in human normal, hyperplastic, and malignant endometrium. Int J Gynecol Cancer 2004; 14: 521-525.
[105] Roychowdhury A, Samadder S, Das P, Mazumder DI, Chatterjee A, Addya S, Mondal R, Roy A, Roychoudhury S and Panda CK. Deregulation of H19 is associated with cervical carcinoma. Genomics 2020; 112: 961-970.