

Review Article

Recent Progresses of Long Noncoding RNA

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Abstract: Long noncoding RNAs (lncRNAs) are a class of regulatory RNA molecules that are no shorter than 200 nucleotides in length with rare protein encoding capacity. It is widely accepted nowadays that lncRNAs play important roles in tumorigenesis and related events. Recently, some comprehensive investigations and major advances of lncRNAs have also been made in other fields including cardiovascular and central nervous system. Here, we will overview the recent progress of lncRNAs, particularly in those fields other than cancer biology, from which big breakthroughs or milestone works have been made.

Keywords: Long Noncoding RNAs, Cardiovascular System, Nervous System, Imprinting

1. Introduction

Long noncoding RNAs (lncRNAs) are regulatory RNA molecules that are no shorter than 200 nucleotides in length with rare protein encoding capacity. On one hand, lncRNAs share many common features with mRNAs. Therefore, lncRNAs are also called mRNA-like ncRNAs. On the other hand, lncRNAs possess several special characters that are distinct from mRNAs. One most recognized feature is that compared with protein-encoding genes, lncRNAs show more significant spatial and temporal tissue-specific expression pattern in normal cells or during development, suggesting specific roles of lncRNAs. Indeed, recent work demonstrated that the expression and dysregulation of lncRNAs also displayed stronger cancer type specificity than protein-coding genes [1].

lncRNAs play important roles in almost all the tested physiological and pathophysiological cellular events. Perhaps, imprinting and dosage compensation related lncRNAs represent the most understood function of lncRNA, which were the first investigated subclass of lncRNA even long before the definition of lncRNA being widely accepted and continue to give us surprising findings. Many lncRNAs have been found to play roles in transcriptional regulation in extremely diverse mechanisms, including but not limited to epigenetic regulation (e. g. HOTAIR [2]), allosterically regulating enzymatic activity (e. g. ncRNACCND1 [3]) and

chromosome interaction (e. g. Firre [4]), thus affect cell proliferation and differentiation and result in biological phenomena in development or disease. Moreover, with the accumulating knowledge and advanced genetic modification techniques, lncRNA *in vivo* study has made a big breakthrough in recent years. Here, we will review the biological functions of lncRNAs, primarily focusing on imprinting, cardiovascular and central nervous system in which big breakthrough and milestone works have been recently made.

2. Surprising Discovery of Imprinting-Related lncRNA Generating Novel Genetic Modification Tools

The study of lncRNAs can be traced back to two imprinting related lncRNAs, H19 and Xist, discovered by Shirley M. Tilghman group in 1990 and Janet R. Lee group in 1991 respectively [5, 6]. Shortly after the identification, several independent groups including Tilghman and Lee groups have generated genetic mouse models for H19 and Xist. Xist was successfully and consistently shown to be crucial in X chromosome inactivation [7, 8]. In contrast, the first three H19 genetic knockout mouse models exhibited trivial phenotypes in body weight and growth [9-11]. Lately on, by using tumor mouse model, H19 was found to play some roles in tumor

initiation [12].

Very recently, Jinsong Li group from Shanghai Institutes for Biological Sciences, China established a full-term development system of semi-cloned (SC) embryos [13]. A critical step is that differentially DNA methylated regions (DMR) of *H19* and *Gtl2* [13] have to be deleted to generate mouse androgenetic haploid stem cells (AG-haESCs) without functional *H19* and *Gtl2* (DKO-haESCs). Mouse AG-haESCs can support full-term development of SC embryos upon injection into MII (metaphase II) oocytes and generate alive SC pups. However the efficiency of live-born SC animals was relatively low (around 4.5% of the transferred embryos in mouse and 1% in rat), and approximately 50% of SC mice displayed a growth-retarded phenotype or died shortly after birth [14]. The advantages of DKO-haESCs include that: (1) this system is independent of Caesarean section which is required for WT AG-haESCs, (2) this system produces high efficient production rate with live and normal sized SC mice. Therefore, this system provide useful laboratory tools in genetic modification.

3. LncRNA in Cardiovascular System

Fendrr (Fetal-lethal noncoding developmental regulatory RNA) is a musculus intergenic lncRNA located at chromosome 8, which is about 2.4kb long and composed of 6-7 exons [15, 16]. *Foxfla*, which shares one bidirectional promoter with *Fendrr*, is an mRNA gene located at 1.3kb downstream of *Fendrr*. Through RNA *in situ* hybridization experiment, Herrmann's group found that mice *Fendrr* specifically expressed in the caudal lateral plate mesoderm (caudal LPM) [16]. This area is a key part for the heart and body wall development. Further, Herrmann group inserted a stop codon ahead of Exon1 and obtained the gene silenced mice *Fendrr*^{3xpA/3xpA}. Due to the heart and body wall abnormalities, these mice died at embryo E13.75 days. To exclude *Foxfla*'s effect, Herrmann group transduced a *Fendrr* BAC clone in which *Foxfla* was inactivated into *Fendrr*^{3xpA/3xpA} mice ES cell. The normally developed mice by RNA rescue experiment of *Fendrr* proved that the phenotype of *Fendrr*^{3xpA/3xpA} mice was caused by lacking of *Fendrr* lncRNA. This elegant *in vivo* work from Herrmann group for the first time proved the phenotype by deleting a lncRNA-containing area is indeed an result of the affected lncRNA, not its neighboring mRNA gene. Using a different strategy, Sauvageau *et al.* [15] entirely deleted *Fendrr* gene loci replaced with lacZ reporter, and then found ventricular septal defect in mice, supporting Herrmann group's finding that *Fendrr* is a crucial noncoding RNA molecule in development. This hysteretic heart malformation was different from the rapidly formed malformation caused by general transcription factors regulating target genes inactivation.

Braveheart represents a lncRNA involved in myocardial differentiation [17]. When Braveheart expressed abnormally, mouse embryo stem cells failed to differentiate into myocardial cells, smooth muscle cells and endothelial cells, indicating that Braveheart was essential for transformation

from embryo plate mesoderm to myocardial cells. Braveheart interacts with SUZ12, a subunit of PRC2, to regulate target genes including mesoderm posterior1 (*Mesp1*, cardiovascular progenitor cell) and other heart-specific genes, such as *gata4*, *Hand2*, and *Nkx2.5*.

SRA (Steroid receptor RNA activator) is a multifunctional lncRNA molecule and has around 20 transcripts with the higher expression level in heart and skeleton muscle. During myogenic differentiation, SRA acts as a transcriptional coactivator for myogenic differentiation (*MyoD*) [18]. The genome locus expressing SRA also give rise to a protein encoding gene, named as SRAP, which can bind to SRA and counteract SRA's action [19]. Moreover, studies showed that decrease of SRA1 resulted in myocardial contractile dysfunction [20].

Tie-1 (Tyrosine kinase containing immunoglobulin and epidermal growth factor homology domain-1) is a tyrosine kinase receptor in cell surface, which can combine with angioprotein and be essential for vascular development. Keguo Li *et al.* [21] found tie-1 AS lncRNA could bind to tie-1 and regulated expression level of Tie-1 in zebrafish, mouse and human. Moreover, injection of tie-1 AS lncRNA in zebrafish embryos resulted in endothelial junction defects.

In addition, other lncRNAs such as uPAR [22] and β MHC [23] also participate in pathogenesis of cardiac disease. Together, these studies demonstrate crucial roles of lncRNAs in heart development and cardiac disease (Figure 1).

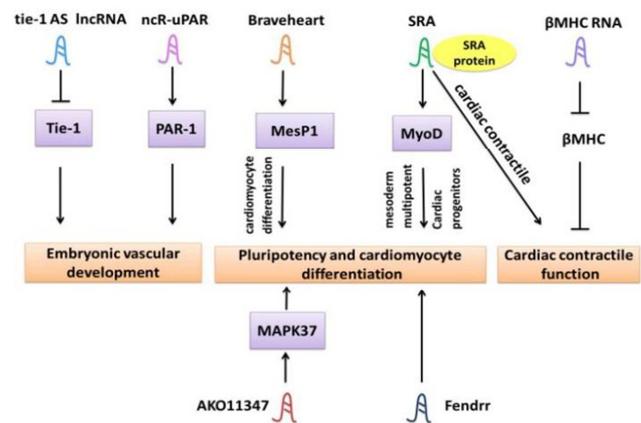


Fig. 1. The role of lncRNA in cardiovascular system.

4. LncRNAs in the Central Nervous System

The nervous system is the most elaborate biological system. Understanding their molecular mechanisms is a big challenge and a subject interested by a lot of scientists. Noncoding sequences associated with neural genes exhibit prominent signatures of positive selection and accelerated evolution, which provide new avenues to link genetic and phenotypic changes to the evolution of the brain. The flexible and complex functions of lncRNAs coincide with the diversity and elaborate nature of central nervous system, making lncRNAs ideal candidates to explain the rapid evolution of central

nervous system or providing breakthrough for insight into the molecular mechanisms of central nervous system development and neuropsychiatric diseases [24, 25] (Table 1).

On the last day of the year 2013, John L. Rinn's group from Harvard University published their 18 lncRNAs knockout work on *Elife* [15]. In this work, they first performed systematic and comprehensive analysis on genome-wide musculus lncRNAs, including sequence, genomic position, relationship with mRNA and other noncoding RNA genes and expression pattern. Then, the selected 18 lncRNAs that suitable for genetic modification based on their stringent criterion were knocked out by entire gene deletion. Surprisingly, many of these selected lncRNAs showed a favor expression in nervous system. For the five lncRNA knockout mice with dramatic developmental defects, two of them (*Fendrr* and *Linc-Brn1b*) showed defect in central nervous system. Other two lncRNAs, *spasm*^{-/-} and *linc-p21*^{-/-} appeared abnormal behavior, which might due to abnormality in nervous system.

Table 1. Dysregulated lncRNAs in central nervous system diseases.

LncRNA	Biological Function	References
<i>Fendrr</i>	Take part in the development process in central nervous system	[15]
<i>Linc-Brn1b</i>	Take part in the development process of cerebral cortex	[15]
eRNA	Associated with autism spectrum disorders	[26]
BACE1-AS	Increase BACE1 mRNA stability via post-transcriptional feed-forward mechanism in AD	[27]
BC200	A likely modulator in AD	[28, 29]
Sox2OT	Biomarker of neurodegeneration in AD, PD	[30]
1810014B01Rik	Biomarker of neurodegeneration in AD, PD	[30]
NAT-Rad18	Transcription regulation of Rad18 to promote apoptosis in AD	[31]
17A	Impair GABAB signaling pathway by decreasing GABA B R2 transcription in AD	[32]
GDNFOS	Modulate the expression of endogenous GDNF in human brain in AD	[33]

Enhancer RNA (eRNA) is a special subset of lncRNAs found to be highly enriched in brain. It is now well accepted that eRNA contributes to the process of enhancer-regulated transcription. Recent work identified 103 human brain region specific enhancer regions expressing eRNA (rBEE) and showed that rBEE were enriched for genetic variants associated with autism spectrum disorders [26].

Alzheimer's disease (AD) is the most common worldwide neurodegenerative disorder and has no effective cure method. Many brain expressing lncRNAs are found to be associated with AD. For example, BACE1-AS is a lncRNA molecule expressing from the antisense strand of BACE1, a crucial extracellular cleavage enzyme for APP (amyloid precursor protein) and often upregulated in AD pathophysiology. BACE1-AS can promote BACE1 by forming an RNA duplex

with BACE1, thus stabilizing BACE1 mRNA and driving a positive post-transcriptional regulation of BACE1 [27]. BC200 is a primate brain specific lncRNA and a likely modulator for AD because the expression level of BC200 is found to be markedly upregulated or downregulated in AD patient brains [28, 29]. The possible scenarios to explain the difference may be caused by different sample location of brain or the severity of disease. Sox2OT and 1810014B01Rik are another two potential lncRNA biomarkers for neurodegenerative disease when an unbiased study was performed by comparing the regulated genes from microarray analysis in normal or anti-NGF AD11 transgenic mouse model [30]. Other AD-related lncRNAs include NAT-Rd18 [31], 17A [32], and GDNFOS [33].

5. Prospect

Although the study of lncRNAs has been extensively carried on for a decade or so, due to the relatively small portion of studied lncRNA, lncRNAs are still a vast group of mysterious molecules to us. Because the size of lncRNAs are longer than 200 nucleotides, they can form specific secondary structure. Upon stimulation, lncRNAs can rapidly response and generate new activities via structural change. We speculate here that this feature, together with the vast number of lncRNA, provides a highly diverse repertoire in our cell to fit the complex physiological and pathological need just like VDJ rearrangement in lymphocytes. We think this group of "dark matter" in cell will eventually help us to understand how the complexity of cellular event are fine controlled.

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