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Transcriptome Complexity in Cardiac Development and Diseases:

An Expanding Universe Between Genome and Phenome

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Abstract

With the advancement of transcriptome profiling by micro-arrays and high-throughput RNA-sequencing, transcriptome complexity and its dynamics are revealed at different levels in cardiovascular development and diseases. In this review, we will highlight the recent progress in our knowledge of cardiovascular transcriptome complexity contributed by RNA splicing, RNA editing and noncoding RNAs. The emerging importance of many of these previously under-explored aspects of gene regulation in cardiovascular development and pathology will be discussed.

Keywords

Cardiovascular diseases; Genes; Molecular biology; Signal transduction

The mature heart develops through complex cellular differentiation involving morphological and functional changes during the embryonic and postnatal periods. Each step of this process, from lineage commitment to morphogenesis, is marked by distinct changes in gene expression profiles in both cardiomyocyte and non-myocyte components.[1] Earlier studies have revealed an elaborate transcriptional regulatory network driven by tissue-specific and temporally coordinated expression of transcription factors.[2,3] The importance of cardiac gene regulation is underscored by the critical contribution of transcriptional dysfunction to both congenital heart diseases and the pathogenesis of heart failure. In fact, many of the same transcriptional regulators involved in cardiac development also have important roles in cardiac hypertrophy, pathological remodeling and heart failure. Therefore, understanding cardiac transcriptome dynamics and regulation has been a major focus of research in the field of cardiac biology and cardiovascular medicine. However, much of our current knowledge of cardiac gene regulation is based on dynamic changes in the composition and expression level of mRNA. With recent advancement of RNA-sequencing technology, our view of transcriptome complexity has been expanded dramatically. Novel mRNA transcripts derived from alternative splicing and editing, and previously unrecognized species of

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noncoding RNAs have emerged as dominant features of the mammalian transcriptome, reshaping the fundamental concept of gene regulation, genome structure and genetic/epigenetic contribution to development and diseases. This review will highlight some of the recent progress and the new concepts emerging from studies of cardiac transcriptome regulation, with particular focus on RNA splicing and editing, as well as noncoding RNAs. Considering the fact that microRNA function and regulation have been discussed extensively by several excellent reviews,[4–6] we will not cover those issues except when it intercepts with the topics covered by this review.

Alternative RNA Splicing in Transcriptome Regulation

Regulatory Machinery of RNA Splicing

All multi-exon genes require coordinated splicing to generate mature RNA transcripts. Multiple mRNA species can be generated from a single gene through alternative RNA splicing events. Extensive profiling has revealed that alternative splicing is a major contributor to mRNA complexity in the mammalian transcriptome, affecting more than 94% of human transcripts.[7–9] At the global transcriptome level, alternative RNA splicing is a highly regulated process associated with physiological and pathological conditions,[10] including embryonic stem cell (ESC) differentiation and cancer development.[11–14] However, our knowledge of RNA splicing regulation and its role in cardiac development and diseases remains very limited, especially compared with the wealth of information about transcriptional regulation.

The constitutive RNA splicing event excises the intronic sequences according to pre-demarcated exon/intron boundaries. An alternative RNA splicing event, however, can use alternative 5′ or 3′ splice sites, leading to specific exon skipping or inclusion or intron retention[7,15,16] Although a basic spliceosome is responsible for constitutive RNA splicing, additional *trans*-acting factors and *cis*-acting sequence motifs are responsible for enhancing or repressing the alternative splicing events.[9] The RBP superfamily includes serine/arginine-rich (SR) proteins, neuro-oncological ventral antigen (Nova) proteins, heterogeneous nuclear ribonucleoproteins (hnRNPs) and RBFox proteins.[17–19] The tissue-specific and signal-regulated expression or activities of the RBPs are the key to coordinated mRNA splicing events[20] (Figure 1). More importantly, the RNA splicing machinery is an integral part of gene regulation, and its function has been implicated beyond RNA splicing events to effects on RNA transcription, quality control, transportation and other post-transcriptional processes.[21–25] However, very limited information is available on how these splicing factors are regulated in response to developmental or pathological cues to achieve coordinated RNA splicing in the heart.

RNA Splicing Regulation in Cardiac Development and Diseases

Salomonis et al revealed that alternative RNA splicing is important for calcium signaling and cardiomyocyte differentiation from progenitor cells,[26,27] In another study, cross-talk between the microRNA regulatory network and alternative splicing is demonstrated to define transcriptome maturation during postnatal cardiac development in the mouse.[28] However, a comprehensive analysis of mRNA splicing in the heart during development has

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not been reported. Therefore, it would be interesting to perform RNA splicing analysis at different stages of cardiac development, a task becoming increasingly feasible with more sensitive high-throughput RNA-sequencing capabilities and more sophisticated bioinformatics tools.[29–31]

A number of cardiac-enriched RNA splicing regulators, including muscle-blind-like protein 1 (MBNL1), RBFox2, CUG-BPI and CUG-BP2, are highly expressed during early fetal heart development but decreased postnatally. In contrast, the cardiac expression of RBFox 1 is significantly induced only after birth.[32–33] On the other hand, the change in the expression of CUGBPI and CUGBP2 is directly regulated by miR-23a/b during cardiac development and this contributes to a significant number of developmentally associated splicing events in the heart.[28]

In additional to cardiomyocyte differentiation and development, the alternative splicing profiles in the heart are also tightly associated with the pathogenesis of heart failure. Global alternative splicing profiling has been done in the diseased heart, including cardiac hypertrophy and heart failure.[34,35] For example, an earlier study compared pressure overload-induced cardiac hypertrophy and heart failure in the mouse heart using deep RNA-sequencing and revealed a global change of alternative splicing in the failing murine heart.[35] More recently, Ames et al and other groups also identified a significant number of alternative splicing events during cardiac hypertrophy in rats.[34,36] For splicing regulators, it is suggested that the expression of PTB and ASF/SF2 is altered in the pressure overload-induced hypertrophic heart.[37] The transcriptome signature and RNA splicing events have also been profiled in human heart disease.[38,39] Based on a gene expression profiling analysis, a total of 17 splicing factors were found to be upregulated in the human failing heart, including RBM25. QK1, hnRNPA1 and Tra2a.[40]

Genetic inactivation of ASF/SF2 in cardiomyocytes causes a hypercontractile phenotype, in part because of aberrant splicing of the Ca^{2+}/calmodulin-dependent kinase II δ(CaMKIIδ) transcript.[41] Cardiac-specific ablation of SC35 also causes dilated cardiomyopathy, associated with mis-regulated splicing of cardiac ryanodine receptor 2 (RyR2).[42] Disruption of the MBNL1 gene in the mouse also leads to muscle, eye and splicing abnormities mimicking the phenotype of myotonic dystrophy.[43] Lastly, as a key regulator for alternative splicing of Titin mRNA, RBM20-deficient rats develop dilated cardiomyopathy.[44,45] Therefore, tissue-specific and coordinated RNA splicing events carried out by a well-orchestrated RNA splicing regulatory network are critical to normal cardiac development and physiology.

Stress Signaling in Cardiac RNA Splicing Regulation

Emerging evidence suggests that the RNA splicing machinery is also a common target of pathological signaling. In response to pressure overload, the expression level of dual-specificity tyrosine-phosphorylated and regulated kinase 1A (Dyrk1 A) is significantly upregulated, which represses the expression of the splicing factor ASF and downstream alternative splicing of CaMKIIδ.[46] In addition, angiotensin II (AngII) can also affect the expression of another splicing regulator (68-kDa Src) associated during mitosis (SAM68) in the heart.[47] SAM68, in turn, regulates the alternative splicing of both mTOR and SMN and

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plays a key role in spinal muscular atrophy.[48,49] In a recent report by el Mabrouk et al,[47] AngII was shown to stimulate the binding of SAM68 to a poly-U region through a PI3K-dependent pathway. Lastly, splicing factor hnRNP A1 forms a molecular complex with activated p38 in vivo, which is important for the proper intracellular localization of this splicing factor.[50]

Among the different stress-signaling molecules, the best characterized regulator for RNA splicing is protein kinase C (PKC). Its regulatory role in alternative splicing was first demonstrated for the alternative splicing of Bcl-x pre-mRNA, which generates 3 distinct isoforms, Bcl-xL and Bcl-xs.[51] Sarcoplasmic reticulum Ca^{2+}-ATPase1 (SERCA1) splicing is also regulated by PKC.[52] A potential underlying mechanism is through PKC-mediated modulation of the activity of an RNA splicing regulator, SC35. In the postnatal rat heart, nucleus-localized PKC can also phosphorylate and activate SC35, which is important for cardiac morphogenesis.[53,54] As demonstrated by Cataldi et al, hypoxia in the adult heart can modulate the phosphorylation level of SC35 through a PKC-dependent pathway that contributes to the hypoxia-induced expression of vascular endothelial growth factor (VEGF),[55] thus providing a RNA splicing dependent mechanism for hypoxia-induced neoangiogenesis (Figure 2). A recent study has demonstrated that in diabetic cardiomyopathy, a significant number of alternative splicing events switch back to an embryonic pattern. This phenotype is potentially regulated by PKCα/β via phosphorylating and up-regulating of 2 key cardiac splicing regulators: CELF1 and RBFox2.[56] All together, it appears that the RNA splicing machinery is an important downstream target of stress signaling in the heart. Considering the molecular complexity of the stress-signaling network and the RNA splicing machinery, there must be more interactions between alternative RNA splicing and stress signaling waiting to be explored.

Molecular Targets of Alternative RNA Splicing in the Heart

Alternative RNA splicing affects many genes in the heart. One example is SCN5A, which encodes the Na-channel α-subunit,[57,58] whereby its splicing variants are associated with heart failure, arrhythmia and Brugada syndrome in humans.[40,59–61] Its splicing is regulated at least in part by RBM25/LUC7L3.[40] Another cardiac gene subjected to alternative splicing is KCNQ1 which generates a truncated isoform with a dominant-negative function associated with cardiac arrhythmias in both human and rat hearts.[62,63] In addition, the aberrant splicing isoforms of cardiac troponin T (cTnT) has been identified in dilated cardiomyopathy.[64] Both in vitro and in vivo studies have revealed that cardiac-enriched splicing regulators, including CUGBP1, CUGBP2 and MBNL,[65–67] are responsible for cTnT alternative splicing at different exons. Titin has also been demonstrated to be regulated by alternative splicing during both cardiac development and disease. The inclusion level of the PEVK domain and the alternatively spliced IG domains increases in mature cardiomyocytes, generating the N2B-containing Titin isoform that is critical for cardiomyocytes' passive tension regulation. During early development or heart failure, however, the dominant isoform of Titin switches to the N2BA isoform.[68,69] This alternative splicing switch is a highly conserved splicing event across different species.[45,68,70] A recent study further demonstrated that the splicing regulator RBM20 is responsible for regulating

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Titin alternative splicing, and a mutation of RBM20 is associated with human dilated cardiomyopathy.[45,71]

The Bnip3L/Nix gene also produces an alternative splicing variant: sNix, which is localized in both the cytoplasm and the nucleus. On stimulation of tumor necrosis factor α (TNFα), sNix rapidly translocates to the nucleus where it activates cardiac gene expression through the nuclear factor κ-B (NF-κB) pathway.[72] Finally, transcription co-regulator PGC-1α is also subjected to alternative splicing regulation to generate a splicing variant: PGC-1α4. Instead of targeting classical PGC-1α downstream targets, this splicing variant induces expression of insulin-like growth factor and can further stimulate muscle hypertrophy both in vitro and in vivo.[73]

In summary, RNA splicing is a prevailing molecular event in cardiac transcriptoine programming and reprogramming during development and pathogenesis. It is an emerging area of research with the advances in high-throughput whole transcriptome profiling. The importance of RNA splicing in cardiac development and diseases is beginning to be recognized. However, our understanding of the molecular nature of the RNA splicing machinery in the heart, the regulatory mechanisms during development and pathogenesis, the molecular targets of RNA splicing as well as their functional impact remains very primitive at this time and need to be further explored in the future.

RNA Editing in Cardiac Development and Disease

RNA Editing Machinery

RNA editing refers to post-transcriptional sequence alterations in mature transcripts different from their genomic sequences.[74] The types of RNA editing identified so far include nucleotide insertions, deletions and exchanges. RNA editing can result in amino acid substitutions, alternative splicing and changes in gene expression levels, leading to an expansion of transcriptome complexity.[18,75] RNA editing has been suggested to play important roles during multiple biological processes and diseases, including cancer and the immune response.[76] By targeting RNA editing, novel therapeutic strategies can be formulated to treat genetic disorders.[77,78] With newly developed high-throughput sequencing method and bioinformatics tools, RNA editing is now recognized as a significant phenomena in transcriptome programming during tissue regeneration, including of the heart. Currently, at least 2 major proteins are implicated in RNA editing for the mammalian transcriptome. The ADARs (adenosine deaminases) can convert adenosines to inosines in double-stranded or specific structured regions of RNA.[79,80] Another involves deaminating cytidine to uridine, which is carried out by the APOBEC family of cytidine deaminases.[81–84] At least 2 members of the APBEC family, APOBEC1 and APOBEC2, have C (cytidine) to U (uridine) editing capability. Although APOBEC1 is ubiquitously expressed among multiple tissues, APOBEC2 is a cardiac- and skeletal muscle-specific RNA editing enzyme.[85]

RNA Editing of Cardiac Genes

The mRNA expression level of ADAR1 is significantly increased in the developing heart and limbs, correlating with increased proliferation and cellular remodeling.[86] In a parallel

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study, RNA editing events were found to be significantly increased in children with cyanotic congenital heart disease and it is suggested that A-to-I RNA editing in the MED 13 gene was significantly higher among the cyanotic patients and the RNA level of ADAR2 was reduced.[87] Furthermore, both the expression level and alternative splicing of ADARs are tightly regulated in the heart. ADAR2 is the key enzyme responsible for Q/R site editing in the GluR-B transcript, and ADAR2 itself is regulated by alternative splicing. Recently, a novel alternative splicing variant including part of the ADAR2 intron 7 was found to be highly expressed in heart and skeletal muscle, but not in the brain. The inclusion of this novel exon generates stop codons that potentially can affect the total ADAR2 expression.[88]

One of the key substrates for APOBEC 1 is the translational repressor, NAT1. APOBEC1 deaminates specific cytidine bases in NAT1 to uridine, changing a codon for glutamine into a premature stop codon. In the embryonic heart, NAT1 expression level is high in both atrial and ventricular myocytes based on immunohistochemical staining analysis; however, western blotting showed that the different isoforms of NAT1 generated by RNA editing have distinct expression levels during cardiac development.[89] Interestingly, during cardiac hypertrophy, the mRNA level of NAT1 is significantly increased without an associated increase at the total protein level. However, western blot analysis confirms the presence of different protein species because of RNA editing, providing a possible link between NAT1 RNA editing regulation and cardiac hypertrophy.[90] Another important substrate for APOBEC1 is apolipoprotein B (ApoB). ApoB mRNA editing involves converting a single C into U at the codon of glutamine 2153, resulting in an in-frame stop codon (UAA) and a truncated protein. The RNA editing of ApoB mRNA is tissue-specific and developmentally regulated.[91] In the heart, it is suggested that the regulatory role of APOBEC1 in ApoB editing involves another chaperone regulator, Bcl2-associated athanogene-4 (BAG-4). BAG-4 is predominantly expressed in the heart and brain, and by interacting with both Hsp90 and APOBEC1. BAG-4 functions as a negative regulator for APOBEC1-mediated RNA editing by shuttling APOBEC1 from the nucleus to the cytoplasm.[92] ApoB RNA editing could also be regulated by PKC.[93] Further, it is suggested that, at least in vitro, increasing the extracellular calcium concentration or depleting SR calcium stores is sufficient to change the level of mRNA editing of ApoB.[94] Lastly, the cardiac-enriched splicing regulator, CUGBP2, can also regulate ApoB mRNA editing. According to Anant et al,[95] CUGBP2 forms a complex with APOBEC1 together with another factor, ACF, CUGBP2 binds to the AU-rich sequence located upstream of the edited cytidine in ApoB RNA and inhibits the RNA editing process.

In summary, RNA editing is a newly recognized phenomena in cardiac transcriptome programming and reprogramming. Much of the data so far offers only a correlative indication of its relevance in cardiac development and pathological process. APOBEC1 knockout mice have an abnormal lipoprotein profile because of abnormal editing of ApoB RNA, and APOBEC2 knockout leads to muscle-type switch and myopathy.[96,97] While genetic evidence for other RNA editing enzymes is still lacking, the functional significance of RNA editing in cardiac development and diseases remains to be established.

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Long Noncoding RNA in Cardiac Development and Disease

Genesis of Long Noncoding RNA

Although the definition of long noncoding RNA (lncRNA) continues to evolve, the generally accepted hallmarks include: (1) longer than 200 nucleotides, (2) no functional open reading frames, and (3) poor conservation at the sequence level.[98] Although the concept of lncRNA was established decades ago, few studies have explored their functional significance beyond X chromosome inactivation and imprinting based on the early discovery of Xist and H19.[99,100] With deep RNA-sequencing and recently developed lncRNA-sensitive microarray technologies, much more information is now available about the expression profiles and biochemical properties of the lncRNAs.[101,102] The identification, annotation and predication of lncRNAs are further facilitated by recent developments in bioinformatics analysis tools,[103] including lncRNAMap and NONCODEv4.[104–106] In contrast to the previous notion that low copies of lncRNA may represent “transcriptional noise” because of aberrant transcriptional initiation,[107] it is increasingly recognized that lncRNAs actually are expressed in a developmental and cell type-specific manner.[108,109] The lncRNAs species are generally classified into 4 categories depending on their relationship to neighboring coding transcripts, including (a) overlapping with annotated gene bodies with transcription initiating from either exons or introns from the sense or (b) antisense strands, (c) lying within the cis-regulatory regions of genes, and (d) lying in the intergenic regions.[110,111] Although lncRNAs are widely detected, it remains controversial to what extent their expressions are functional in development and human diseases.

LncRNAs in Cardiac Development

The expression of lncRNAs shows distinct profiles in different tissues (eg, brown adipose tissue vs. skeletal muscle)[112] and in different cell types (eg, erythroblasts, megakaryocytes and mega-karyocyte-erythroid precursors).[113] Although the sequence conservation of lncRNAs is relatively low compared with mRNAs and miRNAs, multiple evidence suggests that lncRNAs can also be highly conserved at the functional region. In addition to mammals, lncRNAs are also widely expressed and tightly regulated in zebrafish,[114,115] pigs and tetrapods,[116–118] suggesting a potentially highly conserved function among some lncRNA species. The critical role of lncRNAs in development was reported for neurogenesis, involving a large screening approach demonstrating that inactivation of lncRNAs can block human ESC differentiation into mature neurons.[119]

In the heart, microarray and deep RNA-sequencing analyses have revealed a large number of lncRNAs with expression profiles associated with both cardiac development and disease[120–122] (Table). In a recent study,[121] a total of 1,237 lncRNAs were found to have different expression levels during development, with a particular potential effect on developmental processes, metabolic processes and mTOR signaling pathways, according to gene ontology (GO) analysis of neighboring mRNAs. The first lncRNA identified to play a critical role in cardiovascular lineage commitment was Braveheart (Bvht).[123–126] This 590-nt transcript was identified during screening of lncRNAs potentially important for ESC cardiac lineage commitment. Another lncRNA that also controls lineage commitment and cell differentiation in cardiomyocytes is Fendrr. [127,128] As more lncRNAs are discovered in

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the heart, their functional role in cardiac development will be expanded beyond gene expression to morphogenesis and growth regulation.

LncRNAs in Cardiovascular Diseases

The expression profiles of IncRNAs have been demonstrated to be dynamically regulated under different disease conditions.[129–131] In a study by Song et al of human ventricular septal defects (VSD), more than 1,500 IncRNAs showed altered expression associated with the disease state.[132] Moreover, changes in IncRNA expression are significantly associated with the expression of their neighboring genes, suggesting a possible cis-regulatory relationship between IncRNAs and coding mRNAs. A more recent study comparing ischemic and dilated human failing heart samples pre- and post-LVAD (left ventricular assisted device) therapy with non-failing human heart samples using deep RNA-sequencing analysis also revealed a significant number of IncRNAs with dysregulated expression in the diseased hearts and some of these IncRNAs were normalized in post-LVAD hearts.[133] More interestingly, the IncRNA expression profiles in the human failing hearts were found to be a more significant indicator for the different etiologies than the mRNA profiles, suggesting a potential contribution to the different etiologies of heart failure. In addition, IncRNAs detected in the plasma of a mouse heart failure model were also significantly associated with heart failure, and thus may serve as independent biomarkers of the diseases.[120]

Taken together, the dynamic expression of IncRNAs during cardiac development and disease suggests a potential link between them and cardiac pathology (Table). Beyond these correlative analyses, however, the direct contribution of IncRNAs to cardiovascular diseases is only beginning to be revealed.

Molecular Mechanisms of LncRNA-Mediated Regulation

As IncRNA species proliferate, the variations in the underlying mechanisms have also expanded to involve almost every aspect of gene regulation. By binding directly to the promoter region of coding genes, or facilitating transcription factor or miR binding, the IncRNAs are able to activate/silence expression in both a *cis* and *trans* manner.[134–137]

The IncRNAs can function as a functional sponge for miRNAs to interfere global gene expression.[138] A recently identified IncRNA, cardiac hypertrophy related factor (CHRF),[139] is one of the IncRNAs showing expression changes in AngII-treated NRVM. This 1843-nt IncRNA plays a critical role in the cardiomyocytes' hypertrophic response by interacting directly with miR-489, which in turn regulates Myd88 expression, leading to the activation of NF-κB pathway.

Finally, IncRNAs can act as a scaffold for the histone modification complex, antagonizing the localization and regulatory function of the SWI/SNF chromatin-modifying complex or directly interacting with the polycomb repressive complex 2 (PRC2) and the TrxG/MII complex in order to modify the status of histone methylation and acetylation.[127,140,141] This is one of the most important mechanisms for IncRNA regulation of cell differentiation.[140] In the heart, the IncRNA, Bvht, acts as a molecular scaffold[140,142] by interacting with SUZ12, a core subunit of PRC2, and together the complex regulates the promoter activity of genes

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critical for the cardiac lineage, including MesP1, Gata6, Hand1 and Nkx2.5 by changing the histone lysine methylation status. In contrast, Fendrr regulates downstream target expression either in -*cis* by binding to Foxf1 promoter or in -*trans* by binding to the Pitx2 promoter.

The IncRNAs are a newly recognized transcriptome component with an emerging significance in development and diseases. [143,144] Studies of IncRNAs in the heart have just begun and many questions about IncRNA function, regulation and underlying mechanisms are yet to be addressed (Figure 3). More investigations are needed to demonstrate the diagnostic and therapeutic value of IncRNAs in heart diseases.

Small Nucleolar RNAs and Cardiovascular Disease

During RNA metabolism, another noncoding RNA species present in the transcriptome is the small nucleolar RNAs (snoRNAs). These are a family of noncoding small RNAs playing important roles in guiding the modification of other noncoding RNAs, including ribosomal, small nuclear and transfer RNAs.[145–147] Although originally considered as a biological byproducts of alternative splicing, the dynamic expression profiles of snoRNAs suggest they could have functional effects in disease, including cancer.[148,149]

The expression of snoRNAs associated with cardiac diseases has been demonstrated at the whole transcriptome level. By comparing right ventricular tissue samples from 16 infants with nonsyndromic tetralogy of Fallot (TOF) and 8 normal samples, more than 100 snoRNA expression profiles were identified to be significantly changed. Interestingly, it should be noted that the expression profiles of snoRNAs in the infants with TOF resembled the expression profiles observed in the fetal myocardium.[150] To date, this is the first evidence of a global change in snoRNA expression associated with cardiovascular disease. As regulation of snoRNA expression in the heart is still poorly studied and the molecular mechanisms for snoRNA functions in cardiovascular diseases remain elusive. snoRNAs in the cardiovascular system should be an interesting area for future investigations.

Conclusions

Gene regulation has been a central issue in modern molecular biology, and understanding the underlying mechanisms and functional roles is at the core of cardiac research, both in fundamental understanding for cardiac development and in translational investigation of disease mechanisms. With the rapid expansion of transcriptome complexity, the functional paths from genome to phenome have become even more complicated. The emerging roles of RNA splicing, RNA editing, IncRNAs and snoRNAs in the heart add additional intricacy to the regulatory network of cardiac gene expression, and also reveal more ways of potential perturbation in response to pathological stressors (Figure 4). Although much of the work remains to be accomplished, current progress has demonstrated the potential of these new insights of cardiac transcriptome regulation for diagnostic and therapeutic applications. Revealing and understanding cardiac transcriptome complexity should be an important goal for future efforts.

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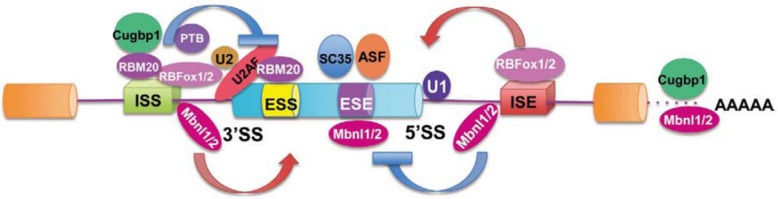

Figure 1.

Illustration of key splicing regulators in heart. A representative alternatively spliced exon with ISS (intron splicing silence), ESS (exon splicing silence), ESE (exon splicing enhancer) and ISE (intron splicing enhancer) is shown. *Trans-acting* splicing regulators that have been implicated in cardiac gene regulation include: CUGBP1/2, MBNL1/2, RBFox-1/2, RBM20, PTB, SC35, ASF. Constitutive RNA splicing factors illustrated include U1/2, U2AF. Arrows indicate enhancing alternative splicing, and block indicates repressing alternative splicing of the neighboring exon.

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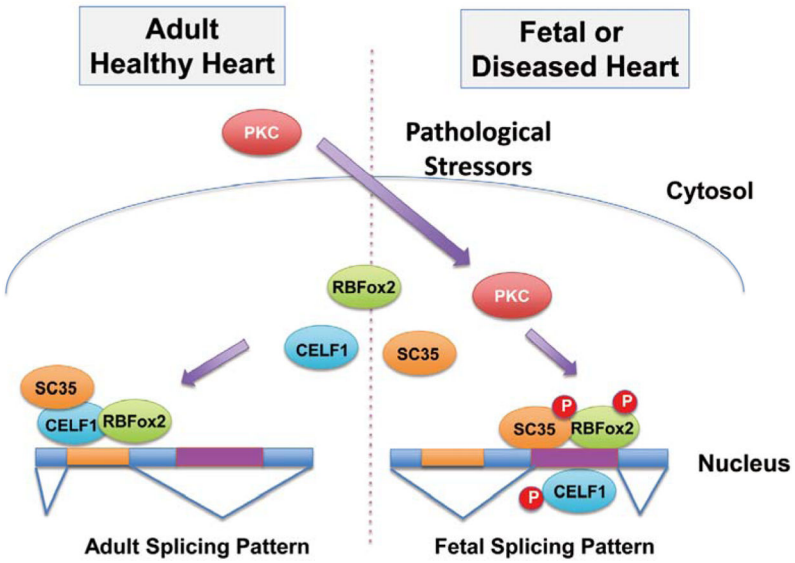

Figure 2.

PKC-mediated regulation of alternative splicing in cardiac development and diseases. PKC, protein kinase C.

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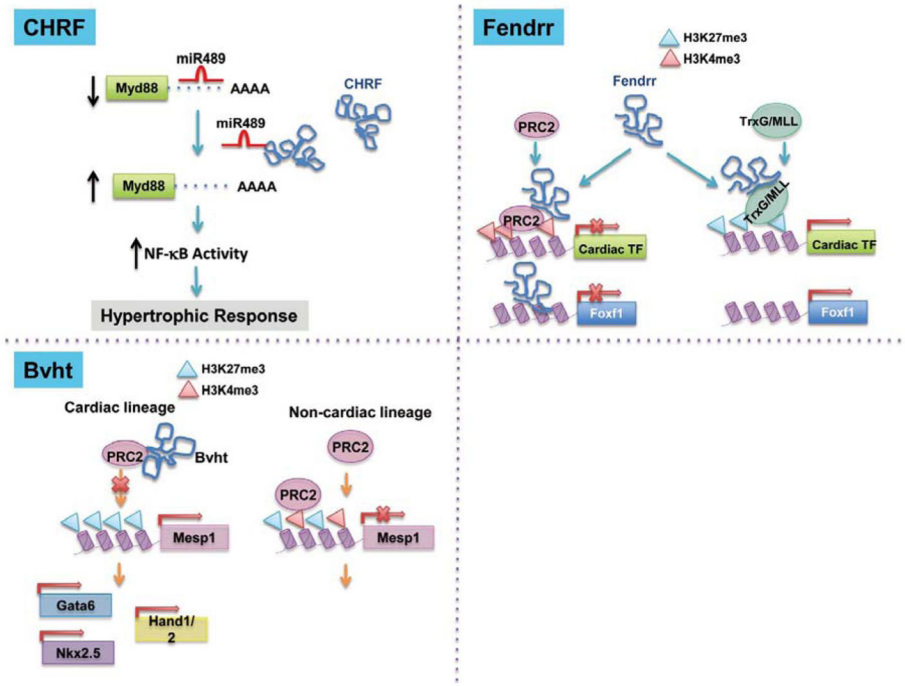

Figure 3.
Molecular mechanisms of known long noncoding RNA functions in the heart.

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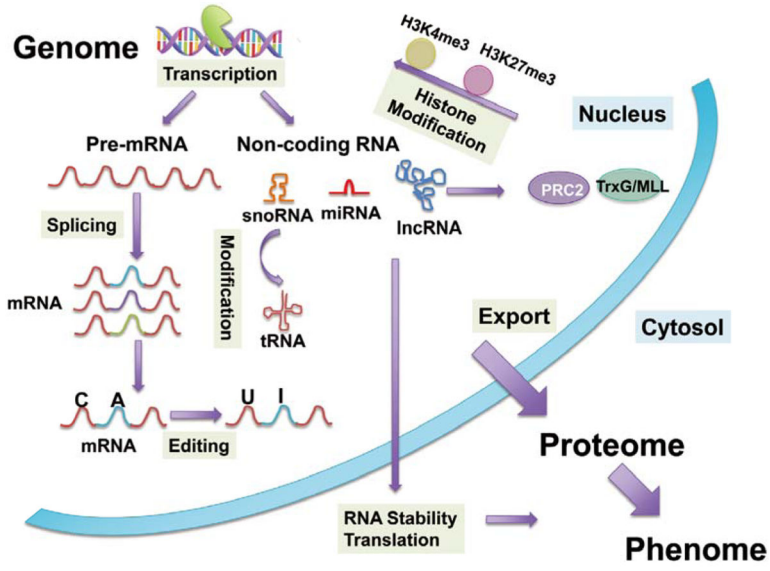

Figure 4.

Transcriptome programming contributed by RNA splicing, editing and noncoding RNA-mediated gene regulation.

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Table

List of LncRNAs implicated in Cardiovascular Development and Diseases

Category	IncRNAs	Reference
Upregulated IncRNAs during cardiac development	Bvht	Klattenhoff et al[124]
	Kcnq1	Korostowski et al[122]
	DT903035	Zhu et al[121]
	BC049716	
	AK085135	
	AK013988	
	Uc008hzy.1	
	Uc008mey.1	
	MM9LINCRNAEXON10678	
	NR_029457	
Downregulated IncRNAs during cardiac development	Fendrr	Grote et al[127,128]
	AK045554	Zhu et al[121]
	AK050713	
	Uc007xf.1	
	BC024929	
	AK008015	
	AK019733	
	Gm16133	
Upregulated IncRNAs in diseased heart	CHRF	Wang et al[139]
	N339730	Song et al[132]
	N408065	Yang et al[133]
	KCONS_0000467	Liu et al[103]
	N406465	
Downregulated IncRNAs in diseased heart	N383233	Song et al[132]
	N407211	Yang et al[133]
	N339159	Liu et al[103]

IncRNA, long noncoding RNA.