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Trends in Molecular Medicine

Review

SnoRNAs in cardiovascular development, function, and disease

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Small nucleolar RNAs (snoRNAs) are emerging as important regulators of cardiovascular (patho)biology. Several roles of snoRNAs have recently been identified in heart development and congenital heart diseases, as well as their dynamic regulation in hypertrophic and dilated cardiomyopathies, coronary heart disease (CHD), myocardial infarction (MI), cardiac fibrosis, and heart failure. Furthermore, reports of changes in vesicular snoRNA expression and altered levels of circulating snoRNAs in response to cardiac stress suggest that snoRNAs also function in cardiac signaling and intercellular communication. In this review, we summarize and discuss key findings and outline the clinical potential of snoRNAs considering current challenges and gaps in the field of cardiovascular diseases (CVDs).

Highlights

Small nucleolar RNAs (snoRNAs) have extremely versatile functions. They regulate cardiac-relevant signaling pathways, oxidative and metabolic cellular stress, gene expression, and intercellular communication.

Global downregulation of snoRNA expression was uncovered in patients with congenital heart disease and resembles the expression profiles of developing hearts.

A broad snoRNA-tRNA-tRNA fragments (tRF) network regulates cellular transcriptome, translatome, and cardiovascular biology.

Cardioprotective effects of cortical bone stem cell-derived exosomes on fibroblast activation are largely mediated by snoRNAs.

An association between levels of circulating snoRNAs and myocardial infarction and heart failure has been found, indicating the potential of these snoRNAs as biomarkers.

SnoRNAs: emerging regulators of cardiovascular development and (patho)biology

CVDs are the leading cause of death worldwide, with 20 million lives lost each year [1]. Despite decades of research, the molecular and cellular mechanisms driving the development and progression of CVDs are not fully understood and their prevalence continues to increase. Over recent years, noncoding RNAs (ncRNAs) have emerged as important regulators of physiological and pathological cellular processes with roles in CVDs [2]. SnoRNAs are a rising class of ncRNAs with multifaceted functions, including rRNA processing and modifications, **ribosome** biogenesis, **heterogeneity** (see Glossary) and **specialization**, gene expression regulation, via a plethora of mechanisms (Figure 1 and Box 1).

Several recent discoveries have uncovered the fascinating functional versatility and significance of snoRNAs in cardiovascular (patho)biology, as reviewed in [3–5]. In this review, we summarize key findings and outline the pivotal roles of snoRNAs in cardiac development, metabolic regulation, exercise adaptation, and, most importantly, **cardiac remodeling** and CVDs (Table 1). We also discuss opportunities and challenges of translating snoRNA research into clinical practice. Ultimately, we hope to reinforce the growing interest in snoRNAs and fuel further snoRNA-related research in the cardiovascular field.

SnoRNAs in cardiac development and (dys)function

Here, we describe snoRNAs with reported functions in cellular signaling, metabolic regulation, as well as exercise and physiological stress, which are relevant for cardiac development, physiology, and pathology.

Early cardiac development

Cardiac development is a complex and well-orchestrated series of cellular events. **Epithelial–mesenchymal transition (EMT)** is a critical process during early cardiac development, tightly regulated by WNT, transforming growth factor-β (TGF-β), mitogen-activated protein kinase (MAPK), and other signaling pathways [6]. Importantly, data from the cancer field suggest that

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snoRNAs regulate these signaling pathways and EMT. For instance, SNORD1C and SNORD76 activate WNT signaling and promote β-catenin and transcription factor 7 (TCF7) expression in colorectal cancer (CRC) [7] and hepatocellular carcinoma (HCC) [8]. Furthermore, SNORA71A promoted proliferation and EMT induction in non-small cell lung cancer (NSCLC) by inducing phosphorylation of MEK and ERK1/2 in the MAPK/ERK pathway [9]. By contrast, SNORD113-1 negatively regulated the phosphorylation of ERK1/2 and SMAD2/3 in the MAPK/ERK and TGF-β pathways in HCC [10]. Nevertheless, because these data originate from cancer-related EMT research, careful interpretation of these results in the context of developmental EMT is necessary. SNORD113-6 (AF357425 in mouse) regulated pre-mRNA processing, splicing, and 2′-*O*-methylation of mRNAs of the integrin pathway in primary murine fibroblasts, thus controlling their splice variants and protein levels [11]. Interestingly, *af357425* knockout was lethal for *in vitro* cultured NIH 3T3 cells, indicating that SNORD113-6 is essential for cellular survival [11].

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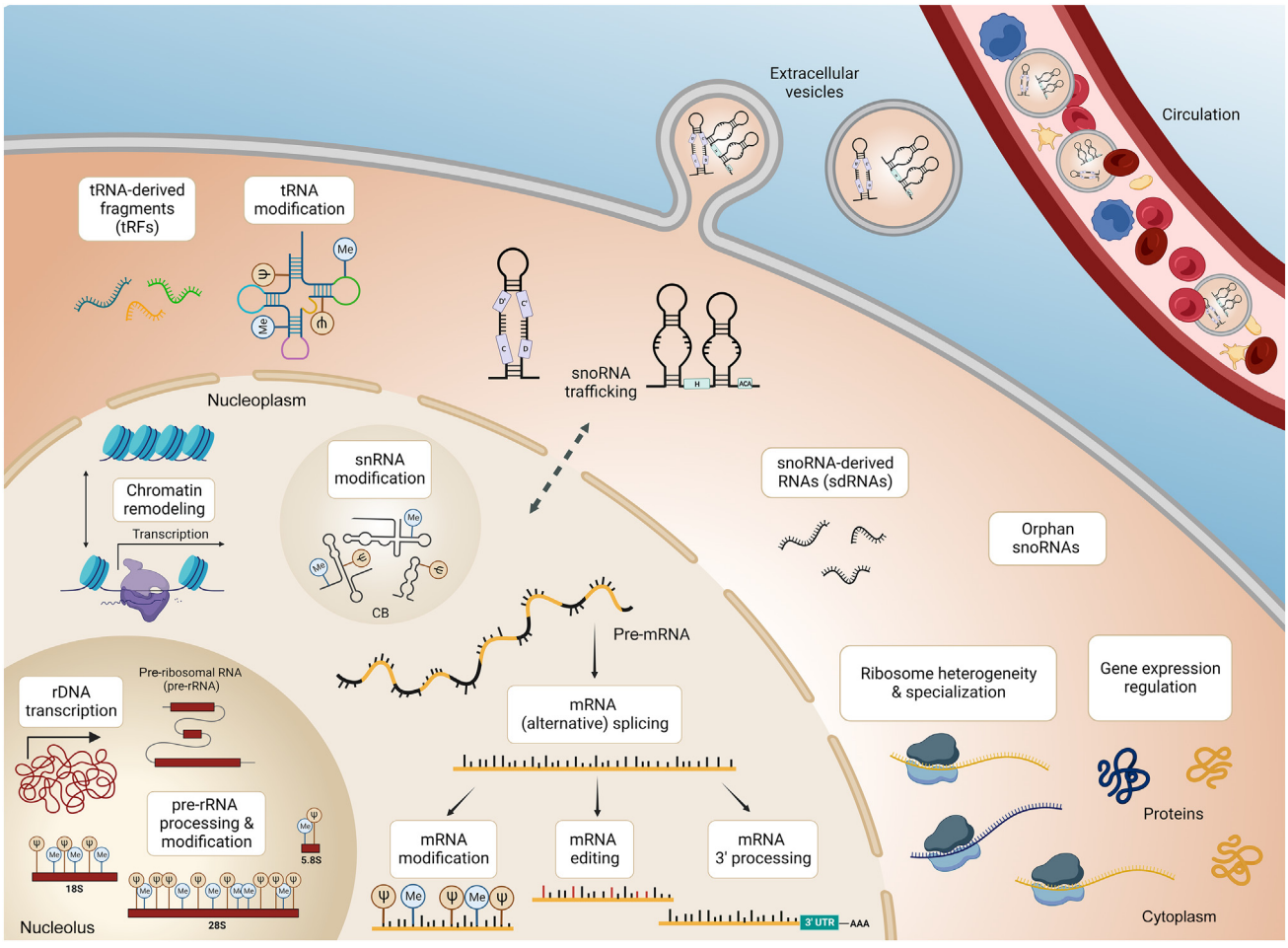

Figure 1. Functional versatility of small nucleolar RNAs (snoRNAs). SnoRNAs are present in the nucleolus, nucleoplasm, and cytoplasm of cells, but can also be packaged into extracellular vesicles (EVs) and released into extracellular space and circulation (see Box 2 in the main text). Their cytoplasmic abundance, as well as secretion by EVs, is dynamically regulated by various stress conditions, such as oxidative lipotoxic stress, or heat shock. Altogether, snoRNAs are important factors of ribosome biogenesis and heterogeneity as well as gene expression regulation (see Box 1 in the main text). A subset of snoRNAs, referred to as 'orphan', have unknown targets and their functions are yet to be elucidated. Created with BioRender.com. Abbreviations: CB, Cajal body; rDNA, ribosomal DNA; snRNA, small nuclear RNA; UTR, untranslated region.

Box 1. SnoRNA discovery, biogenesis, and classification

SnoRNAs were discovered during the 1990s in the nucleolus, where they guide rRNA modifications [2′-*O*-methylation (2′-*O*-me) and pseudouridylation (ψ)] and facilitate pre-rRNA processing [90]. Many new snoRNAs have since been found, with the human snoRNAome listing over 2000 snoRNAs [91]. SnoRNAs reside in Cajal bodies (small Cajal body-specific RNAs; scaRNAs) [92], nucleoplasm [13,93], cytoplasm [15,17], and extracellular vesicles (EVs) [18,35,62], where they fulfil functions beyond ribosome biogenesis (see Figure 1 in the main text). These include modifications of small nuclear RNAs (snRNAs) [92], tRNAs [12,13], mRNAs [50], 3′-mRNA processing [94], alternative mRNA splicing [95], mRNA editing [96], and chromatin structure maintenance [93]. Some snoRNAs are further processed into stable short fragments called snoRNA-derived RNAs (sdRNAs), which have miRNA-like features and gene-silencing potential [3]. Others regulate the synthesis of tRNA fragments (tRFs), a newly discovered group of short regulatory ncRNAs with roles in human diseases [12,13]. Notably, almost half of snoRNAs in the human snoRNAome have unknown functions and, hence, are termed 'orphan' snoRNAs [91].

Most snoRNAs in humans are encoded within intronic regions of protein-coding host genes [97], with many encoding ribosomal proteins and ribosome-related factors [98]. The biosynthesis of intron-encoded snoRNAs begins with transcription of the host gene by RNA polymerase II. The snoRNA-containing lariat is excised during splicing, linearized by a debranching enzyme, and trimmed by exonucleases. Pre-snoRNAs can also be excised from the intron by endonucleolytic cleavage via splicing-independent pathways. SnoRNP proteins bind to the pre-snoRNA co-transcriptionally to ensure correct delineation of the snoRNA terminus [4]. A subset of snoRNAs, including U3, U8, U13, and RNA component of mitochondrial RNA processing endoribonuclease (RMRP), are transcribed independently with their own intrinsic promoters by RNA polymerase III [97].

Specific conserved sequence motifs ('boxes') and secondary structures are used to classify snoRNAs into two subclasses; box C/D (SNORD) or box H/ACA snoRNAs (SNORA) (Figure IA) [99]. SnoRNAs associate with proteins to form small nucleolar ribonucleoprotein complexes (snoRNPs) [98] (Figure IB). Associations with alternative protein partners have been reported for some snoRNAs, supporting their non-canonical functions, such as RMRP [100] and SNORA73 [93].

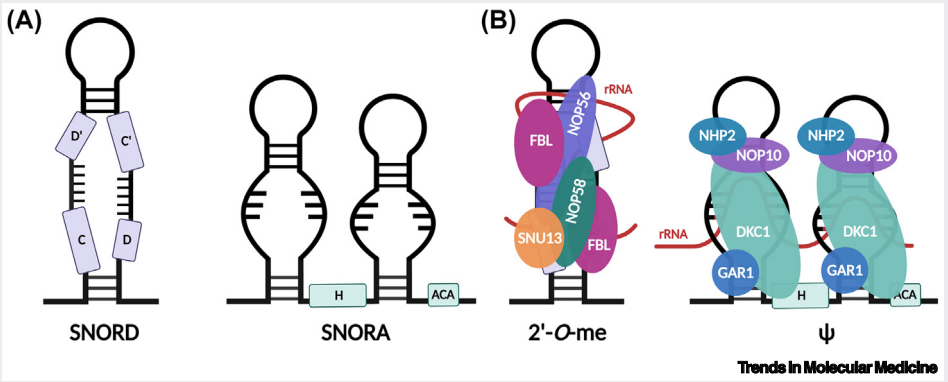

Figure I. Box C/D and H/ACA small nucleolar ribonucleoproteins (snoRNPs). (A) Secondary structure of SNORDs and SNORAs. (B) Architecture of the canonical box C/D and H/ACA snoRNP complexes. SnoRNAs guide the site specificity of the modification by base pairing with the target RNA, while their protein partners catalyze the modification. 2′-O-methyltransferase fibrillarin (FBL) catalyzes the 2′-hydroxy methylation of the RNA ribose and pseudouridine synthase 1 Dyskerin (DKC1) catalyzes the isomerization of the target uridine to pseudouridine. Created with BioRender.com.

SNORD113-6 also guided 2′-*O*-methylation of tRNA leucine anti-codon TAA [tRNALeu(TAA)]. This tRNA formed $tRF^{Leu\ 47–64}$ in primary murine and human fibroblasts and intact human arteries. Importantly, SNORD113-6-mediated 2′-*O*-methylation of tRNALeu(TAA) prevented its fragmentation and the formation of $tRF^{Leu\ 47–64}$ in vascular remodeling [12]. Similarly, SNORD97 in cooperation with SNORD133 (also known as SCARNA97) guide 2'-*O*-methylation of the wobble cytidine C34 of human elongator tRNAMet(CAT). This again prevents site-specific, stress-induced tRNAMet(CAT) cleavage and tRF processing [13]. The critical role of SNORD97/SNORD133 in guiding the 2'-*O*-methylation of tRNAs and regulating tRF formation was elaborated in another study [14], where in-depth investigation of the snoRNA–tRNA modification network showed that

Glossary

Antisense oligonucleotide (ASO): short, synthetic, single-stranded oligodeoxynucleotide, complementary to selected target RNA. By binding the target RNA, an ASO transiently regulates its expression by blocking translation, causing degradation, or regulating splicing.

ASO gapmers: specific class of ASOs with a mixed backbone of a central block of DNA and 3′, 5′-flanking blocks of RNA residues, which can be variously modified to increase stability and specificity. When bound to its target, the central block DNA and target RNA sequences form a DNA/RNA heteroduplex, which is recognized by RNase H and degraded.

Biomarker: characteristic of a molecule that can be objectively measured and evaluated as an indicator of normal or pathological biological processes, or pharmacological responses to a therapeutic intervention. Accuracy, precision, high sensitivity, and specificity are important characteristics of an ideal biomarker.

Cardiac remodeling: series of structural (size, hypertrophy, atrophy, wall thickness, heart shape, fibrosis, and scaring) and functional changes in the heart that occur in response to cardiac disease, injury, and damage.

Epithelial–mesenchymal transition (EMT): biological process during which epithelial cells lose their polarity and cell–cell adhesion and gain migratory and invasive properties to become mesenchymal stem cells.

High-throughput screening (HTS): drug discovery process using robotics and automation to rapidly test the biological or biochemical activity of a large number of chemical and/or biological molecules. It is extensively used in the pharmaceutical industry.

Hydrogel: water-insoluble cross-linked polymer networks forming microporous 3D structures. They can be used for local, controlled, and sustained release of therapeutics.

Locked nucleic acid (LNA) oligonucleotides: RNA oligonucleotides with an extra methylene group connecting the 2′ oxygen and 4′ carbon of the ribose ring, locking it in the 3′-endo conformation. This increases stability and improves specificity and base-pairing affinity.

Ribosome heterogeneity: describes the fact that not all ribosomes have the same composition as a result of rRNA

SNORD97/SNORD133 guide 2′-*O*-methylation of multiple tRNAs and that their loss reduced target tRNA levels and induced codon adaptation of the transcriptome and translatome. In a mouse embryonic stem cell model of development, loss of SNORD97/SNORD133 promoted differentiation to endoderm and mesoderm fates (including cardiomyocytes; CMs), without compromising pluripotency [14]. These fascinating data implicate snoRNAs as regulators of tRNA modification and tRF formation, both novel research areas in the field of gene expression regulation.

Metabolic regulation

Metabolic regulation is critical for cardiac (patho)physiology and numerous studies have implicated snoRNAs in the regulation of cellular metabolism. SnoRNAs encoded within the ribosomal protein L13a (*Rpl13a*) locus, SNORD32A, SNORD33, SNORD34, and SNORD35A, are involved in the lipotoxic and oxidative stress response *in vitro* and *in vivo* [15]. Homozygous *Rpl13a* snoRNA loss-of-function mice demonstrated reduced reactive oxygen species, diminished oxidative response, improved mitochondrial function, glucose tolerance, and enhanced insulin release upon glucose challenge compared with their wild-type (WT) littermates [16]. Furthermore, the subcellular localization of *Rpl13a*-derived snoRNAs was dynamically regulated by NADPH oxidase, and oxidative stress induced rapid cytosolic translocation of these snoRNAs, as shown in the embryonic CM cell line H9c2 [17]. Interestingly, *Rpl13a*-derived snoRNAs were also secreted into the circulation to perform 2′-*O*-methylation of their targets in distant tissues [18] (Box 2). In addition, SNORD60 and SNORA73 were implicated in the regulation of cholesterol trafficking and homeostasis [19,20]. Furthermore, both fatty acid oxidation and glycolysis were increased upon *SNORA73* deletion [21]. Loss of *SNORA73* also caused mTOR-mediated resistance to oxidative stress *in vitro* and ameliorated diet-induced lipotoxicity *in vivo* [21]. Mitochondrial dysfunction, oxidative stress, and lipotoxicity are important components of CVDs. Moreover, the mTOR pathway is a master regulator of cardiac metabolism, hypertrophy, and contractility [22]. Therefore, it is likely that snoRNAs with functions in metabolic regulation are involved in the pathobiology of CVDs.

Exercise and physiological stress

Exercise and physiological stress induce cellular and molecular (mal)adaptations of the cardiovascular system. Two recent papers investigated levels of circulating snoRNAs in endurance athletes compared with healthy volunteers with sedentary lifestyle and patients with peripheral artery disease (PAD). The first study measured levels of snoRNAs from the 14q32 locus and identified four snoRNAs, namely SNORD112, SNORD113-2, SNORD113-6, and SNORD114-1, which were significantly lower in endurance athletes compared with patients with PAD. Furthermore, SNORD114-1 was significantly upregulated in response to endurance exercise, gradually increasing throughout the recovery period in endurance athletes [23]. Interestingly, an association between elevated levels of circulating snoRNAs from the 14q32 DLK1-DIO3 locus and heart failure and MI was described recently [24]. A second study compared endurance athletes with age-, gender- and body mass index (BMI)-matched sedentary volunteers and found upregulated levels of circulating SNORD14E and SNORD4B, as well as transcripts related to translation and mitochondria function, in athletes [25]. SNORD14E regulated alternative splicing, as well as 2′-*O*-methylation and the stability of *FOXM1* mRNA [26]. FOXM1 is a transcription factor essential for not only heart embryonic development, but also age-induced cardiac hypertrophy and fibrosis [27]. Altogether, these findings suggest that snoRNAs have roles in cardiovascular (mal)adaptations in response to exercise.

Myotonic dystrophy type 1 (DM1) is a muscular disorder caused by a mutation in *DMPK* (encoding dystrophia myotonica protein kinase) and is characterized by loss of muscle functionality, hypotonia, and significant cardiac abnormalities [28]. A recent study compared expression profiles of skeletal

sequence variation, rRNA post-transcriptional modifications, incorporation of ribosomal protein paralogs, alterations in ribosomal protein stoichiometry, and ribosomal protein post-translational modifications. Ribosome heterogeneity has been documented at the level of different species, developmental stages, tissues within a single cell, and in disease or distinct growth conditions.

Ribosome specialization: concept in which ribosome heterogeneity confers functional ribosome specialization, contributing to translational control. The origin of the ribosome specialization theory dates back to the 1950s; however, the compelling evidence of functional specialization of ribosomes emerged relatively recently.

Small-molecule drugs (SMDs): molecules with low molecular weights (usually <1 kDa) that are typically administered orally for distribution throughout the body. Their small size, negligible charge, and hydrophobicity allow them to pass cell membranes to reach intracellular targets. They are usually easy and cheap to synthesize and modify.

Table 1. SnoRNAs with functions in cardiovascular development, function, and disease[a]

SnoRNA	Host gene	Target/mechanism of action	Disease/process	Direction	Model	Refs
SnoRNAs in cardiac development and function						
Early cardiac development						
SNORA71A	SNHG17	EMT, proliferation, MAPK/ERK pathway	NSCLC, pathway regulation	↑	Human NSCLC tissue, A549, PC9, NCI-H1299, NCI-H1975, H292, H460, H661 cells, mouse model	[9]
SNORD1C	SNHG16	TCF7, Wnt/β-catenin pathway	CRC, pathway regulation	↑	Human CRC, paracancerous tissues, SW620, SW480 cells	[7]
SNORD76 (U76)	GAS5	EMT, Wnt/β-catenin pathway	HCC, pathway regulation	↑	Primary human HCC tissue, Hep3B, SK-Hep1, Huh7, HepG2, and HCC-LM3 cells, mouse model	[8]
SNORD113-1	14q32	ERK1/2, SMAD2/3, MAPK/ERK, TGF-β pathway	HCC, pathway regulation	↓	Human HCC tissue	[10]
SNORD113-6	14q32	Pre-mRNA processing, splicing, 2′-*O*-methylation of mRNAs of integrin pathway	Gene expression regulation, pathway regulation	–	Primary murine and human fibroblasts	[11]
SNORD113-6	14q32	2′-*O*-methylation of tRNALeu(TAA), $tRF^{Leu\ 47–64}$	Gene expression regulation	–	Primary murine and human fibroblasts and intact human arteries	[12]
SNORD97	EIF4G2	2′-*O*-methylation of tRNAMet(CAT) and other tRNAs, tRFs	Gene expression regulation	–	HeLa, HAP, HEK293 cells, TC1 mES cells	[13,14]
SNORD133	LARP4	2′-*O*-methylation of tRNAMet(CAT) and other tRNAs, tRFs	Gene expression regulation	–	HeLa, HAP, HEK293 cells, TC1 mES cells	[13,14]
Metabolic regulation						
SNORD32A (U32A) SNORD33 (U33) SNORD34 (U34) SNORD35 (U35A)	RPL13	Oxidative stress response	Oxidative stress	–	H9c2 rat cardiomyoblasts	[17]
		Lipotoxic and oxidative stress response	Metabolic stress	↑ in LPS	CHO-K1, 6F2 cells, C2C12 myoblasts, mouse model of LPS-mediated oxidative stress	[15]
		Glucose metabolism, mitochondrial function, oxidative stress response	Glucose metabolism	–	Rpl13a-snoless$^{(-/-)}$ mice, their β cell mass, islets, fibroblasts from *Rpl13a*-snoless$^{(-/-)}$ embryos	[16]
		SnoRNA secretion via EVs, intracellular communication	Inflammation	↑ in LPS	Murine parabiosis model, bone marrow-derived macrophages and their EVs, human/murine serum EVs	[18]
SNORD60 (U60)	SNHG19	Cellular cholesterol trafficking	Cholesterol homeostasis	–	CHO-K1 cells, 3T3 mouse fibroblasts	[19]
SNORA73 (U17)	RCC1	Cholesterol trafficking via *HUMMR* mRNA	Cholesterol homeostasis	–	CHO-K1 cells	[20]
		Metabolic stress response via mTOR	Metabolic stress, hepatic steatosis	–	CHO, 2E4, NIH 3T3 cells, human skin fibroblasts, male C57BL/6J mice	[21]
Exercise and physiological stress						
SNORD4B	RPL23A	nd	Exercise, endurance	↑	Blood sample of well-trained young endurance-swimming athletes, sedentary controls	[25]
SNORD14E	HSPA8	nd	Exercise, endurance	↑	Blood sample of well-trained young endurance-swimming athletes, sedentary controls	[25]
		Alternative splicing, 2′-*O*-methylation of *FOXM1* mRNA	Gene expression regulation	–	HEC-1B cells	[26]

Table 1. (continued)

SnoRNA	Host gene	Target/mechanism of action	Disease/process	Direction	Model	Refs
SNORD112, SNORD113-2, -6, SNORD114-1	14q32	nd	Exercise, endurance	↓	Blood of endurance athletes and patients with PAD	[23]
55 DE snoRNAs		na	DM1	↑	Skeletal muscle biopsy of patients with DM1	[29]
snoRNAs in CVDs						
Congenital heart diseases						
135 DE snoRNAs		na	TOF		Right ventricular myocardium from infants with TOF, normally developing infants, fetal heart samples	[32]
23 DE snoRNAs		na	HCM	↓	*Drosophila melanogaster* model of feline HCM	[34]
SNORA3B, SNORA20, SNORD6, SNORD18A, SNORD42A, SNORD43, SNORD58C, SNORD60, SNORD101, RMRP, SNORD116-25, -29		na	HCM	–	hiPSC-CMs (c.*ACTC1*^{G301A}) and isogenic control	[35]
SNORD73A	RPS3A	nd	HCM	↑	EVs of hiPSC-CMs (c.*ACTC1*^{G301A}), 2-Hz stimulation	[35]
SNORD96A	RACK1	nd	HCM	↑	EVs of hiPSC-CMs (c.*ACTC1*^{G301A}), 2-Hz stimulation	[35]
RMRP	RMRP	miR-1 sponging	HCM	↑	HCM myocardial tissue, pulmonary embolism-induced primary human CMs	[38]
35 DE snoRNAs (notably SNORD14C)		na	DCM	–	Myocardium tissue of patients with DCM	[39]
CHD and MI						
SNORD112, SNORD113, SNORD114	14q32	SNPs	Heart failure	↑	Blood samples from PROSPER study	[24]
SNORD113-2, -9	14q32	nd	Heart failure	↑	VSM tissue taken after CABG, naïve VSM tissue	[24]
SNORD112, SNORD113-2, -6, -8, -9, SNORD114-1	14q32	nd	Heart failure	↑	VSM tissue taken after CABG, tissues from explanted end-stage failing human hearts	[24]
SNORD113-2	14q32	nd	MI	↑	Plasma from patients with ST-elevation MI	[24]
SNORD113-2, SNORD114-1	14q32	Platelet activation	PAD	↑	Plasma from PAD patients with critical ischemia	[12,41]
SNORD113-6	14q32	nd	CAD, oxidative stress	↑	Primary human umbilical arterial fibroblasts and murine fibroblasts; *ex vivo* cultured internal mammary arteries from patients with CAD	[12]
24 DE snoRNAs		na	Ischemic cardiomyopathy	–	Myocardial tissue from patients with ischemic cardiomyopathy	[39]
SNORD116-18	SNHG14	na	Ischemic cardiomyopathy, ejection fraction	–	Myocardial tissue from patients with ischemic cardiomyopathy	[39]
RMRP	RMRP	miR-128-1-5p/Gadd45g axis	Atherosclerosis	↑	Human vascular smooth muscle cells treated in rat atherosclerotic coronary artery tissue	[43]
	RMRP	miR-214-5p/p53 axis	Hypoxia-induced acute MI	↑	H9c2 cells	[42]

(continued on next page)

Table 1. (continued)

SnoRNA	Host gene	Target/mechanism of action	Disease/process	Direction	Model	Refs
Cardiac fibrosis						
RMRP	RMRP	miR-613 sponging	Cardiac fibrosis	↑	Primary rat CFs and CMs	[47]
SNORA20, SNORA24, SNORA30, SNORD42A, SNORD50A, SNORD59A, SNORD61, SNORD71, SNORD114-9, -26, SNORD116-26,-22		na	Cardiac fibrosis	↑	NHCFs treated with TGF-β	[49]
SNORA1, SNORA14, SNORA14B, SNORA32, SNORA40, SNORA55, SNORA61, SNORA62, SNORA65, SNORA71B, SNORA72, SNORD1C, SNORD11D, SNORD12, SNORD15C, SNORD21, SNORD27, SNORD29, SNORD34, SNORD44, SNORD45A, SNORD55, SNORD58A, SNORD60, SNORD87, SNORD99, SNORD113-6, SNORD116-2		na	Cardiac fibrosis	↓	NHCFs treated with TGF-β and hCBSC-dEXOs	[49]
SNORD32A (U32A)	RPL13	2′-*O*-methylation of *Pxdn* mRNA	Gene expression regulation	–	293T cells, *Rpl13a*-snoless$^{(-/-)}$ mice	[50]
SNORD51 (U51)	EEF1B2	2′-*O*-methylation of *Pxdn* mRNA	Gene expression regulation	–	293T cells, *Rpl13a*-snoless$^{(-/-)}$ mice	[50]

[a]Abbreviations: LPS, lipopolysaccharide; mES, mouse embryonic stem cell; na, not applicable; nd, not determined; PROSPER, PROspective study of pravastatin in the elderly at risk; ↑/↓, increase/decrease in disease condition (compared with controls); –, directionality of the regulation not determined or not applicable.

muscle of patients with DM1 before and after exercise (12-week cycling regime). The authors identified 55 differentially expressed snoRNAs in DM1 samples pre-exercise compared with controls (53 upregulated and two downregulated). Importantly, among the top 50 upregulated genes, 28 were snoRNAs (SNORA2C, SNORA7B, SNOR/A20, SNORA23, SNORA37, SNORA38, SNORA38B, SNORA49, SNORA54, SNORA55, SNORA63D, SNORA71A, SNORA72, SNORA80A, SNORA80B, SNORA2B, SNORD3C, SNORD8, SNORD9, SNORD13, SNORD94, and seven SNORD116 transcripts). Furthermore, the authors observed a significant reduction in the levels of snoRNAs following the 12-week training regime. SnoRNA expression positively correlated with metrics of muscle mass, strength, and function, and these correlations were strengthened following training. These data imply that snoRNAs have important roles in DM1 biology and could be utilized as **biomarkers** for DM1 disease progression and severity [29].

SnoRNAs in cardiovascular diseases

Congenital heart diseases

Approximately 1% of the global population suffers from congenital heart diseases, wherein the heart develops abnormally, causing morphological and/or physiological impairments [30]. Here, we summarize evidence implicating snoRNAs in the pathobiology of congenital CVDs (Figure 2).

Tetralogy of Fallot

Tetralogy of Fallot (TOF) is characterized by a ventricular septal defect accompanied by pulmonary stenosis and ventricular hypertrophy [31]. An analysis of snoRNA expression in the right ventricular myocardium from infants with TOF and healthy infants, as well as fetal myocardium samples [32],

Box 2. SnoRNAs in extracellular vesicles

Extracellular vesicles (EVs) are a heterogeneous group of secreted membranous vesicles facilitating intercellular communication and (patho)physiological processes throughout the body. Based on their size, content, and biogenesis and release pathways, EVs are subcategorized into three main subtypes: exosomes, microvesicles, and apoptotic bodies. EV cargoes comprise proteins, lipids, DNA, and various RNA species, including snoRNAs, metabolites, and whole organelles. Importantly, the processes of EV production, loading, and release by the parental cell are cell type and condition specific [101,102].

Cardiac cell populations (CMs, fibroblasts, endothelial cells, smooth muscle cells, and progenitors) release significant amounts of EVs. These facilitate short- and long-range communication, thus regulating cardiovascular homeostasis, interorgan crosstalk, systemic metabolic fitness, and pathological cardiac remodeling [56,102]. Cardiac-derived EVs are enriched in ncRNA, including snoRNAs, and their profile changes following cardiac injury [58,59,103], in genetic heart diseases [35] or inflammatory conditions [18,104]. Indeed, a parabiosis model suggested that inflammation stimulates the secretion of *Rpl13a* snoRNAs SNORD32a, SNORD33, SNORD34, and SNORD35a into the circulation, via which they travel to distant tissues to direct 2′-*O*-methylation of target rRNAs [18].

EVs are emerging as promising targets for diagnosis and treatment of CVDs. The EV content reflects the condition of the parental cell; therefore, measuring snoRNA signatures of circulating cardiac-derived EVs could provide insights into the state of the cardiovascular system (for additional details, see the section 'SnoRNAs as diagnostic tools' in the main text).

identified 135 differentially expressed snoRNAs in TOF samples compared with healthy controls (126 downregulated and nine upregulated). Interestingly, there was a marked similarity in snoRNA expression profiles between fetal and TOF samples, further corroborating roles of snoRNAs in heart development and function. Exon array analysis revealed significant differential splicing of genes with functions in heart development, suggesting that snoRNAs might regulate cardiac gene splicing during heart development and in TOF [32].

Inherited cardiomyopathies

Inherited cardiomyopathies, such as hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), contribute to a significant portion of congenital heart disease cases. HCM is characterized by hypertrophic expansion of the myocardium and diastolic dysfunction, whereas DCM is characterized by thinning of the left ventricle resulting in systolic dysfunction [33].

Introducing feline HCM-associated mutations of the gene encoding myosin binding protein C3 (*MYBPC3*) in *Drosophila melanogaster* caused cardiovascular defects and decreased cardiovascular endurance in mutant flies. Transcriptome analysis revealed a cluster of 23 snoRNAs, levels of which decreased in exercised mutant female flies [34].

A link between HCM and snoRNA expression was also explored in another study that utilized human-induced pluripotent stem cell (hiPSC)-CMs generated from patients with an HCM-causing mutation (c.*ACTC1*^{G301A}) and isogenic controls. Twelve snoRNAs were found to be differentially expressed in HCM hiPSC-CMs compared with WT controls, specifically: SNORA3B, SNORA20, SNORD6, SNORD18A, SNORD42A, SNORD43, SNORD58C, SNORD60, SNORD101, SNORD116-25, SNORD116-29, and RNA component of mitochondrial RNA processing endoribonuclease (RMRP). Along with snoRNAs, many differentially expressed mRNAs were identified in this comparison, mostly transcripts associated with metabolic regulation [35]. Interestingly, the HCM-regulated snoRNAs SNORD60 and SNORD116 were previously linked to cholesterol trafficking and regulation of metabolic processes [19,36,37]. Thus, snoRNAs might contribute to HCM pathogenesis through metabolic modulation. Furthermore, when subjected to increased contractile workload, HCM hiPSC-CMs released extracellular vesicles (EVs) with a higher abundance of SNORD3A and SNORA12 (compared with unstimulated HCM hiPSC-CMs), and increased levels of SNORD96A and SNORD73A (compared with stimulated WT hiPSC-CMs) [35]. These data suggest snoRNAs as mediators of cardiac stress in HCM. Notably, contractile stimulation did not induced alterations in

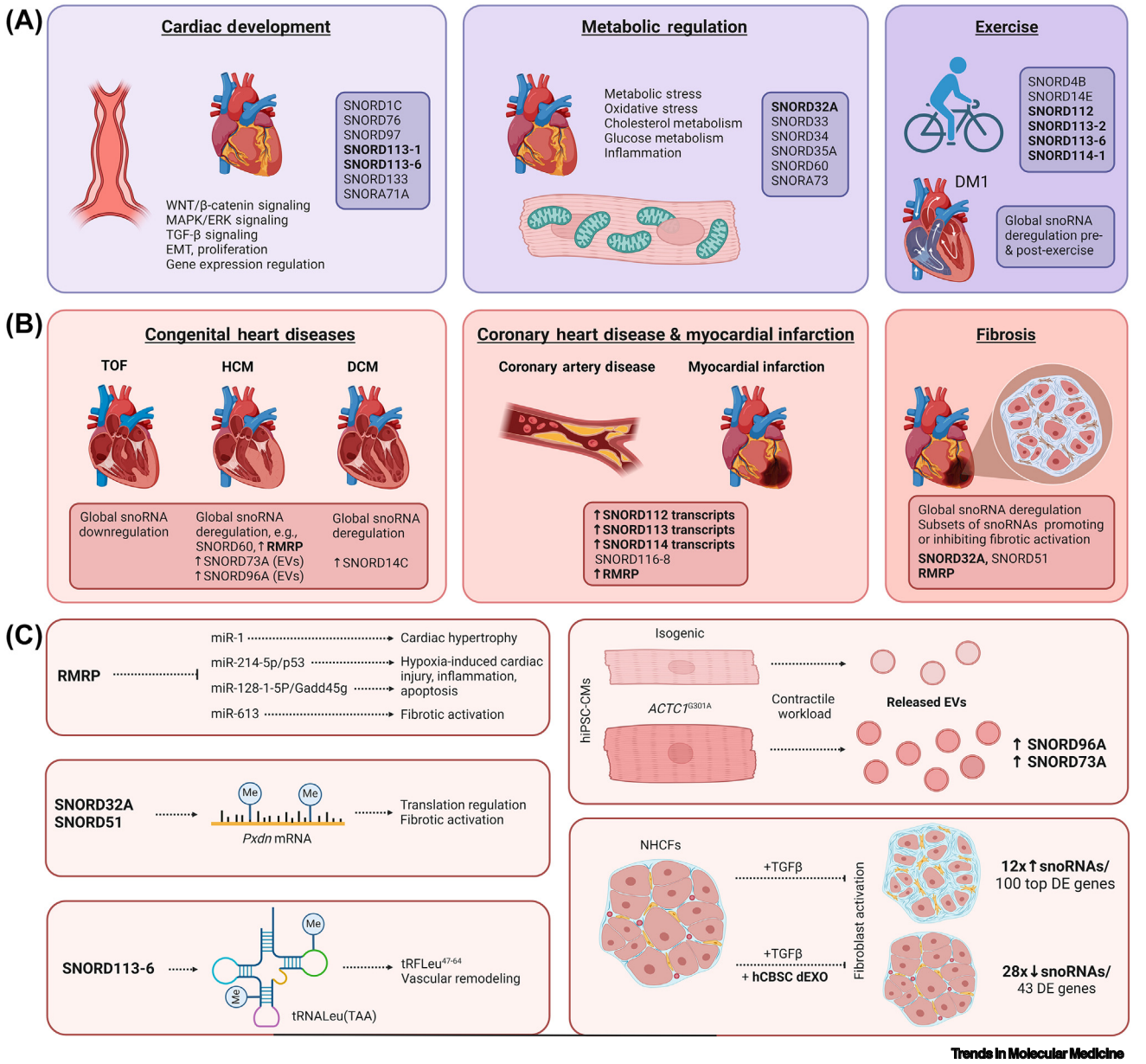

Figure 2. Small nucleolar RNAs (snoRNAs) in cardiovascular development, function, and disease. Overview of the most important findings for snoRNAs in (A) cardiac development and function and (B) cardiovascular diseases (snoRNAs with overlapping functions are in bold). (C) The underlying mechanisms of actions. Abbreviations: *ACTC1*^{G301A}, HCM-causing mutation; DCM, dilated cardiomyopathy; DE, significantly differentially expressed; EMT, epithelial–mesenchymal transition; EV, extracellular vesicle; hCBSC-dEXOs, human cortical bone stem cell-derived exosomes; HCM, hypertrophic cardiomyopathy; hiPSC-CMs, human-induced pluripotent stem cell cardiomyocytes; MAPK, mitogen-activated protein kinase; NHCFs, normal human cardiac fibroblasts; Pxdn, peroxidasin; RMRP, RNA component of mitochondrial RNA processing endoribonuclease; TGF, transforming growth factor; TOF, Tetralogy of Fallot. Created with BioRender.com.

the snoRNA cargo in WT hiPSC-CMs EVs. Interestingly, while both control and mutant CMs increased their overall EV secretion in response to stress, the increment was significantly higher in HCM hiPSC-CMs. Another snoRNA with a link to HCM is RMRP, which was upregulated in human cardiac hypertrophic tissue and phenylephrine-induced hypertrophic CMs, sponging hypertrophy-inhibiting miR-1 and promoting cardiac hypertrophy [38].

In a recent preliminary report, researchers investigated snoRNA regulation in DCM. They performed ncRNA-sequencing analysis of DCM and control heart tissue and identified 35 snoRNAs differentially expressed in DCM. In particular, SNORD14C was upregulated in DCM tissues, with more than a twofold increase compared with controls [39].

Coronary heart disease and myocardial infarction

Coronary artery disease (CAD) is one of the most common forms of heart disease, caused by reduced blood flow to the heart tissue due to blood vessel impairment. This often results in MI as the myocardium is starved of crucial oxygen and nutrients [40].

Several studies linked snoRNAs to CAD and MI. A genome-wide association analysis of the 14q32 region encoding a cluster of SNORD112, SNORD113, and SNORD114 transcripts in 5244 participants showed that single nucleotide polymorphisms (SNPs) in this region were associated with heart failure [24]. In this study, researchers also measured a moderate increase in the expression of SNORD113 transcripts in venous tissue from failed coronary arterial–venous bypass grafts (CABG) compared with naïve vena saphena magna (VSM) tissues, and a marked increase in levels of SNORD112, SNORD113, and SNORD114 transcripts in tissues from explanted end-stage failing human hearts compared with CABG. SNORD113-2 was also upregulated in the plasma of patients with ST-elevation MI and decreased with treatment [24].

In line with these results, another study found an association between elevated plasma levels of SNORD113-2 and SNORD114-1 and platelet activation, an important factor in MI pathogenesis and prognosis [41]. Hypoxia and oxidative stress also increased SNORD113-6 levels in *in vitro*-cultured primary human umbilical arterial fibroblasts and murine fibroblasts and in *ex vivo*-cultured internal mammary arteries from patients with CAD [12]. Analysis of myocardial tissue from patients with ischemic cardiomyopathy and controls revealed the differential expression of 24 snoRNAs. Furthermore, levels of SNORD116-18 were associated with cardiac ejection fraction, which is a widely used prognostic factor for adverse cardiovascular events [39]. Taken together, 14q32 snoRNAs appear to be involved in several aspects of hypoxia-induced heart injury and failure. Thus, their biomarker and therapeutic potential should be investigated in more depth.

Another snoRNA with functions in CAD is RMRP. RMRP was increased in hypoxia-treated H9c2 cells, human vascular smooth muscle cells treated with oxidized low-density lipoprotein (ox-LDL), and rat atherosclerotic coronary artery tissue. Its downregulation attenuated hypoxia-induced injury, as well as inflammation and apoptosis. RMRP inhibited several miRNAs, and the RMRP/miR-214-5p/p53 and RMRP/miR-128-1-5P/Gadd45g regulatory axes promoting cardiac injury were identified [42,43]. MiR-214-5p and miR-128-1-5p have cardioprotective functions [44,45] and, thus, patients with myocardial ischemia and reperfusion injury might benefit from RMRP silencing.

Cardiac fibrosis

In response to cardiac injury, the myocardium often undergoes fibrotic remodeling characterized by the activation of cardiac fibroblasts (CFs) into myofibroblasts and increased synthesis and deposition of extracellular matrix (ECM). Although physiological ECM remodeling is an important process of tissue regeneration, it can also develop into pathological cardiac fibrosis and ultimately lead to heart failure [46].

One of the first snoRNAs implicated in cardiac fibrosis was RMRP, levels of which were elevated in a rat cardiac fibrosis model following aortic banding, where it promoted fibrotic activation

through inhibition of miR-613 [47]. Interestingly, this miRNA inhibits canonical Wnt signaling [48], a crucial pathway of early cardiac development.

A recent study used adult normal human CFs (NHCFs), activated by TGF-β and treated with human cortical bone stem cell-derived exosomes (hCBSC-dEXOs) [49]. First, TGF-β treatment alone induced profound changes in gene expression, notably with 12 of the top 100 differentially expressed genes being snoRNAs, implying their role in promoting fibroblast activation. By contrast, NHCF treatment with TGF-β and hCBSC-dEXOs reduced fibrotic activation. Among 43 significantly differentially expressed genes, 28 were snoRNAs, all downregulated in hCBSC-dEXOs and TGF-β-treated NHCFs. Interestingly, there was no overlap between snoRNAs regulated by TGF-β alone and those regulated by TGF-β in combination with hCBSC-dEXOs. Finally, the treatment of NHCFs with hCBSC-dEXOs induced only minimal expression changes in the absence of TGF-β. This supports the notion that hCBSC-dEXOs and the effects they have on fibroblast expression profiles (including snoRNAs) are specifically linked to the process of fibrotic activation.

Two other snoRNAs were implicated in translation regulation of fibrosis-relevant mRNA, namely SNORD32A and SNORD51, which guide 2′-*O*-methylation of peroxidasin (*Pxdn)* mRNA [50]. PXDN is important for ECM formation and, while its mRNA is expressed in various tissues, PXDN protein abundance is highest in the heart, vasculature, and circulation. Furthermore, PXDN is upregulated during fibrotic activation [51], ischemia/reperfusion, and hypoxia/reoxygenation [52]. 2′-*O*-methylation of *Pxdn* mRNA by SNORD32A and SNORD51 inhibited its translation in mouse hearts and 293T cells [50]. Thus, targeting these snoRNAs might be beneficial for attenuating fibrotic activation.

Biomedical potential of SnoRNAs

SnoRNAs as diagnostic tools

Considerable amounts of snoRNAs are present in the circulation, protected from RNases by binding to high-density lipoproteins or encapsulation in EVs [53] (Box 2). Cardiac cell populations utilize EVs for intercellular communication [54–57] and significantly contribute to the pool of circulating EVs, as demonstrated in a study that used transgenic mice with GFP-expressing CMs [58]. In response to injury or stress, cardiac cells increase EV production and alter snoRNA EV cargo [35,58,59]. Furthermore, levels of specific snoRNAs were increased in patients with PAD with critical ischemia [23,41], or during MI [24] and, thus, are potential CVD biomarkers (Figure 3A).

Extracellular snoRNAs can be reliably detected in biofluids, including blood, plasma, and serum [60]. The clinical potential of circulating snoRNAs has been recognized in other fields, including oncology [61] and neurology [62]. Nevertheless, there are technical and biological challenges associated with the detection and quantification of circulating snoRNAs related to the bias that can be introduced at the levels of RNA isolation, library preparation, sequencing, and bioinformatic analysis. This is due to the short length of snoRNAs, secondary structures, and lack of poly(A) tails. However, this issue is well recognized and novel small RNA-sensitive RNA-sequencing pipelines and bioinformatic tools have been developed recently [63–66].

SnoRNA-targeting tools

Great advances in the field of nucleic acid therapeutics (NATs), **small-molecule drugs (SMDs)**, and gene therapy have been made over the past decade. Originally developed to target proteins and mRNAs, these methods are also useful for targeting small ncRNAs (Figure 3B). In fact, several agents targeting or mimicking ncRNAs to treat human diseases have entered clinical trials [67,68].

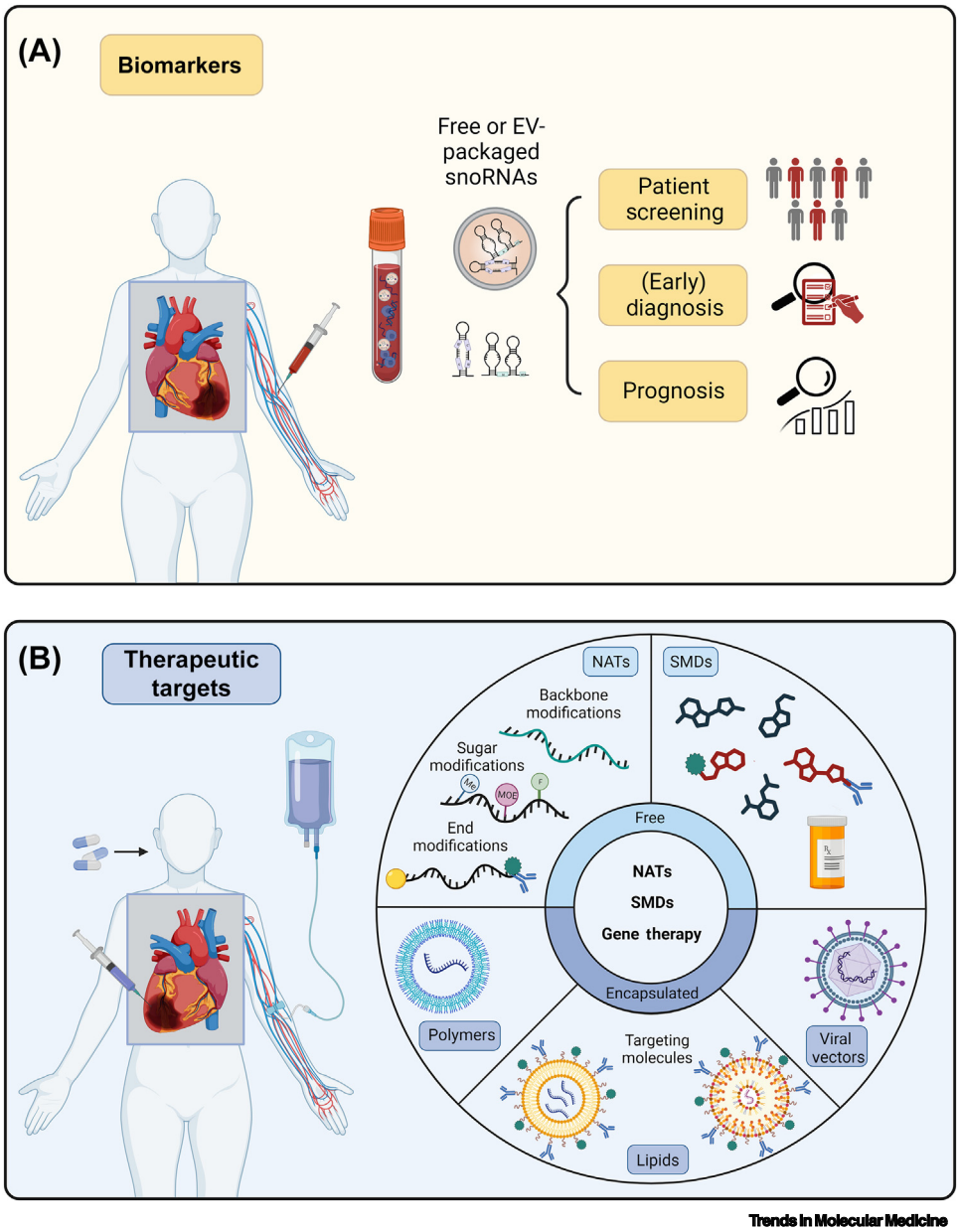

Figure 3. Biomedical potential of small nucleolar RNAs (snoRNAs). (A) SnoRNAs are emerging as promising biomarkers for cardiovascular diseases (CVDs). They are present in the circulation, either as free molecules or encapsulated in extracellular vesicles (EVs). Cardiac cells contribute significantly to the pool of circulating snoRNAs, and measuring levels of specific cardiac-derived circulating snoRNAs could provide insights into the state of the cardiovascular system and serve as screening, diagnostic, or prognostic biomarkers. (B) SnoRNAs can be targeted using nucleic acid therapeutics (NATs), small-molecule drugs (SMDs), and gene therapy to treat CVDs. Antisense oligonucleotides (ASOs), ASO gapmers, and locked nucleic acids (LNAs) are examples of the most efficient NATs for snoRNA targeting. Furthermore, various formulations and modifications of the backbone and sugar groups of NATs [e.g., adding 2 -*O*-methoxyethyl (MOE) or trifluoromethyl (F) group], as well as conjugations with uptake-enhancing ligands [cholesteryl, 3′ or 5′ TEG, or N-acetylgalactosamine (GalNAc)] or targeting molecules, or encapsulation within polymer or lipid-based nanoparticles and viral vectors have been developed to improve delivery, cellular uptake, specificity, and stability of these therapeutics. Created with BioRender.com.

Clinician's corner

Considerable levels of small nucleolar RNAs (snoRNAs) are present in the circulation. Studies have shown an association between plasma levels of specific snoRNAs and myocardial infarction (MI) and peripheral arterial disease (PAD). Thus, circulating snoRNAs could serve as biomarkers for cardiovascular diseases (CVDs).

SNPs in the 14q32 region encoding a cluster of SNORD112, SNORD113, and SNORD114 transcripts are associated with heart failure. Analysis of venous tissue from failed coronary artery bypass graft (CABG), end-stage failing human hearts, internal mammary arteries from patients with coronary artery disease (CAD), and hypoxic human umbilical arterial fibroblasts uncovered deregulated expression of these snoRNAs, suggesting their roles in molecular and cellular processes leading to heart failure.

RMRP (RNA component of mitochondrial RNA processing endoribonuclease) expression is increased in cardiac hypertrophy and CAD. It negatively regulates several cardioprotective miRNAs, including miR-128-1-5P, miR-214-5p/p53, and miR-1. *In vitro* knockdown of RMRP attenuated hypoxia-induced injury, inflammation, apoptosis, and hypertrophy of cardiac cells. These data suggest that patients with myocardial ischemia/reperfusion injury might benefit from RMRP silencing.

In a murine model of ischemia/reperfusion, an injection of cortical bone stem cells derived exosomes (CBSC-dEXOs) immediately on reperfusion reduced infarct size. Cardioprotective properties of CBSC-dEXOs were recapitulated in an *in vitro* experiment using human cardiac fibroblasts (CFs). This was accompanied by a profound change in the snoRNA expression profile, suggesting that snoRNAs mediate cardioprotective functions of CBSC-dEXOs.

As active regulators of cellular processes in heart (patho)biology, snoRNAs are emerging as promising therapeutic targets for cardiovascular disorders. The repertoire of therapeutics for targeted snoRNA modulation continues to grow and improve. In addition, new

approaches to target cardiac cells, or even more specifically, injured or stressed cardiac cells, have been developed, making the clinical use of snoRNAs increasingly feasible.

The most commonly used strategy to target snoRNAs is NATs, specifically **antisense oligonucleotides (ASOs)**, **ASO gapmers**, and **locked nucleic acid (LNA) oligonucleotides**. Importantly, NAT efficacy, efficiency, specificity, and stability have been significantly improved over the past decade by adjustments in NATs, conjugations with uptake-enhancing ligands, or encapsulation within lipid- or polymer-based nanoparticles (NPs) [69]. SnoRNA-targeting ASOs and LNAs have demonstrated promising results in preclinical cancer models [70], and we expect to see reports of the successful use of these tools in the cardiovascular field in the near future.

Compared with NATs, SMDs offer several advantages, including good solubility, bioavailability and metabolic stability, a considerable variety of compounds, and relatively low costs related to their synthesis. Over the years, extensive and diverse libraries of SMDs have been built [71], allowing for fast and relatively cheap **high-throughput screening (HTS)**. Furthermore, *in silico* libraries offer a cheaper and more accessible alternative. These libraries are primarily built to assess SMD–protein interactions and, therefore, they need to be optimized for snoRNAs. Fortunately, snoRNAs have diverse secondary and tertiary structures and motifs providing a plethora of potential SMD-binding sites. Furthermore, by targeting snoRNA biogenesis pathways or its protein partners, SMD could influence snoRNA levels and functions even without directly binding to them. While no HTS focusing on snoRNAs has been attempted so far, successful miRNA-focused screens demonstrated the viability of this approach [72].

Targeted drug delivery

The discovery of targeting ligands (e.g., peptides, antibodies, aptamers, inhibitors, or small molecules) has significantly improved drug delivery. For example, intravenous and intramuscular administration of myostatin-targeting siRNAs conjugated with anti-CD71 Fab′ fragments, silenced myosin in murine hearts and skeletal muscle [73]. Other groups utilized the fact that CMs overexpress specific receptors and molecules in response to stress, such as ischemia. An angiotensin II type 1 receptor (AT_{1R}) peptide-anchored nanovector loaded with an miRNA-1 inhibitor was injected intravenously and accumulated in ischemic myocardium, where it attenuated CM apoptosis following MI in mice [74]. Researchers developed NPs with α4β1 integrin to mimic the macrophage membrane. Following intravenous administration, α4β1 integrin-NPs demonstrated significant affinity toward CMs challenged by hypoxia and reperfusion via interaction with vascular cell adhesion molecule-1 (VCAM-1) on the cell surface [75]. The endocardium of the failing heart could be targeted using an atrial natriuretic, as evidenced in a rat MI model [76]. In an interesting study, researchers developed magnetic NPs with antibodies binding exosomal CD63 antigen and myosin light chain markers present on the surface of injured CMs. When tested in rabbit and rat MI models, CD63-expressing exosomes captured from the circulation accumulated in the infarcted tissue upon the application of a local magnetic field. The acidic pH of injured cardiac tissue resulted in the cleavage of the hydrazine bond and the local release of captured exosomes. As a result, the authors observed improved left ventricle ejection fraction, angiogenesis, and a reduction in infarct size [77]. This demonstrated that manipulation of endogenous exosome biodistribution could be used to treat CVDs.

Drug or gene therapy-engrafted polymers represent another promising avenue for sustained local drug delivery. Injectable, pH-responsive or protease-sensitive **hydrogels** releasing oligonucleotides targeting miRNAs showed great potential in porcine and mouse models of CVDs, particularly MI, as it is associated with reduced pH and locally increased proteolytic activity [78–80].

Gene editing using lipid NPs delivering plasmid DNA (pDNA) was successfully used to target CMs *in vitro* and *in vivo*. GFP-encoding pDNA lipid NPs formulated and optimized for delivery to CMs

were administered intravenously to mice. This resulted in a twofold increase in GFP expression in heart tissue, without any significant immunogenicity or negative effects on cardiac functions [81]. Vectors and plasmids could be further specialized using cardiac-specific promoters, or promoters activated in stressed or diseased CMs.

Altogether, these data suggest that targeted delivery of snoRNA-targeting therapeutics to the cardiovascular tissues and cells is feasible. This can be achieved by conjugations with anti-CD71 Fab′ fragments, AT1R, α4β1 integrin, or atrial natriuretic peptides. Conjugated NPs in particular are showing great potential in targeted delivery of ncRNA therapeutics in malignant diseases in preclinical and clinical trials [82,83]. This is due to their reduced toxicity, improved stability and biocompatibility, enhanced permeability and retention effect, as well as precise targeting. Furthermore, the use of local injections of pH-responsive and protease-sensitive hydrogels loaded with snoRNA therapeutics or drug-eluting stents and balloons has also been reported [84].

Concluding remarks

Discovered relatively recently in the cardiovascular field, snoRNAs are emerging as important and multifaceted regulators of cardiovascular (patho)biology. In this review, we examined the most compelling and striking examples of the roles of snoRNAs in cardiac development, function, and diseases, and considered their diagnostic and therapeutic implications (see Clinician's corner). We also highlighted technical advances in snoRNA biomarker research, developments in small ncRNA-driven therapeutics, and innovative approaches for targeted cardiac therapy. A future objective is to decipher the underlying mechanisms of how individual snoRNAs regulate cardiovascular (patho)biology. This will be challenging, primarily due to the incredible functional versatility of snoRNAs, which spans from regulation of gene expression and translation on a global level by guiding ribosome biogenesis, to translation fine-tuning via ribosome heterogeneity and specialization, tRNA and mRNA modifications, mRNA splicing, editing, 3′ processing, polyA, as well as formation of tRFs and snoRNA-derived RNAs (sdRNAs). Research into snoRNA-mediated ribosome heterogeneity and specialization is particularly active at the moment due to the development of RNA sequencing-based methods for rRNA 2′-*O*-methylation and pseudouridylation (ψ) profiling. Many of the identified cardiac-regulated snoRNAs are canonical snoRNAs guiding rRNA modifications. Ribosome heterogeneity and specialization are increasingly recognized for their roles in the development and pathobiology of human diseases, such as cancer, dyskeratosis congenita, or osteoarthritis [85–87]. To the best of our knowledge, no study on ribosome heterogeneity in the cardiovascular context has been published so far, although we might expect one soon. Investigations of the rRNA modification landscape and ribosome functionality would be particularly interesting in the fibrotic activation model, as the majority of snoRNAs identified in both conditions (NHFC + TGF-β and NHFC + TGF-β + hCBSC-dEXOs) were canonical snoRNAs. Importantly, the connection between differential rRNA modification (specifically 2′-*O*-methylation of 5.8S-U14 guided by SNORD71) and increased translation efficiency of fibrotic *COL1A1* mRNA has been described in chondrocytes in the context of osteoarthritis [87]. In addition to techniques analyzing functions of canonical snoRNAs, high-throughput methods to analyze tRNA abundance and modifications, tRFs, sdRNAs, mRNA processing/splicing variants, and polyA are progressing, becoming increasingly available and prevalent. Notably however, we are missing a high-throughput method for global mRNA modification profiling, which would greatly support the uncovering of noncanonical functions of snoRNAs.

EVs represent another fascinating avenue of future cardiovascular research, as apparent from two key publications, which demonstrated that EVs and snoRNAs mediate intercellular communication under stress in HCM and regulate fibrotic activation. Nevertheless, the EV field is still developing, and several challenges remain to be addressed. These include standardization of

Outstanding questions

How do snoRNA profiles change across different CVDs? Are there specific snoRNAs that act as primary drivers or inhibitors of different CVDs? What is their mechanism of action?

Do canonical snoRNAs induce ribosome heterogeneity in cardiac cells? What are the consequences of such ribosome heterogeneity for ribosome function? Do ribosomes and translation regulation have roles in CVDs?

Could circulating snoRNAs serve as indicators of overall cardiovascular health and improve current screening, diagnostic, and prognostic CVD biomarkers? How stable are circulating snoRNAs and what are the best methodologies for their detection and quantification in clinical settings?

Can cardiac-derived EVs in the circulation be isolated to measure their snoRNA profiles? *In vitro* models indicate that snoRNA profiles of cardiac-derived EVs change under stress conditions: can we measure such changes in captured circulating cardiac-derived EVs?

Can snoRNAs be targeted to treat CVDs? Which approach(es) result in the most targeted and effective result? What are the potential risks and side effects?

methods to produce, isolate, and characterize EVs focusing on achieving maximal purity, homogeneity, and integrity. A relative novelty of the EV field drives accelerated publications but comes at the cost of limited reproducibility. The International Society for Extracellular Vesicles addresses these issues and guides the standardization of EV protocols [88], including blood draw procedure, general sample handling, timeliness, and sample processing [89]. In addition, tests to optimize the storage conditions ensuring stability and biological activity of EVs, as well as careful evaluation of EV safety profiles, will be paramount for their future clinical use. Although many questions remain, particularly regarding the mechanism of action of individual snoRNAs in CVDs (see Outstanding questions), there is accumulating evidence that snoRNAs present a valuable, yet relatively unexplored, subject in cardiovascular research.

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Declaration of interests

The authors declare no competing interests.

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