Molecular Therapy
Nucleic Acids
Review

# A contemporary review of snoRNAs in cardiovascular health: RNA modification and beyond

Noah Peter Jagielski,[1] Amit Kumar Rai,[1] K. Shanmugha Rajan,[2] Vatsal Mangal,[1] and Venkata Naga Srikanth Garikipati[1]

[1]Aging + Cardiovascular Discovery Center, Department of Cardiovascular Sciences, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA; [2]Department of Chemical and Structural Biology, Weizmann Institute, Rehovot 76100 001, Israel

**As cardiovascular diseases continue to be the leading cause of death worldwide, groundbreaking research is being conducted to mitigate their effects. This review looks into the potential of small nucleolar RNAs (snoRNAs) and the opportunity to use these molecular agents as therapeutic biomarkers for cardiovascular issues specific to the heart. Through an investigation of snoRNA biogenesis, functionality, and roles in cardiovascular diseases, this review relates our past and present knowledge of snoRNAs to the current scientific literature. Considering the initial discovery of snoRNAs and the studies thereafter analyzing the roles of snoRNAs in disease, we look forward to uncovering many other noncanonical functions that could lead researchers closer to finding preventive and curative solutions for cardiovascular diseases.**

## INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of death for both men and women in the United States, despite significant advancements in this area of study; studies show that approximately 48% of US adults older than 20 years of age suffer from some form of CVD.[1,2] Between 2007 and 2017, the total number of deaths caused by complications related to CVD increased by 21% when considering the entire global population. In 2017, stroke and ischemic heart disease emerged as the primary contributors to these deaths, accounting for 84.9% of the total.[3] There are many cellular mechanisms that are responsible for the onset of CVDs, such as genetic mutations and abnormalities present within signaling pathways.[4] These mutations may affect many components of the cardiovascular system, such as the muscular and vascular systems, as well as the electrical circuit of the heart.[4] Traditional therapies to treat and mitigate diseases of the CVD system include β-blockers, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, long-term weight loss, thrombolytic therapy, statins, aspirin, and nitrates.[5–7] Since the early 2000s, microRNAs (miRNAs) have been intensely studied in the scientific literature as therapeutic and biomarker agents in relation to CVD, but studies suggest that molecules beyond miRNAs may function as agents as well.[8] After years of searching for plausible treatments and identifying promising candidates to mitigate cardiovascular complications, small nucleolar RNAs (snoRNAs) may serve as a promising solution for patients suffering from CVD and heart-related issues.[8] If used as biomarkers and therapeutic targets, snoRNAs may provide hope for those currently suffering from CVD complications, as well as elongate the lives of those seeking to prevent the onset of heart failure. In a world where CVD rates continue to rise, there is a simultaneous need for an expansion in potential diagnostic and curative agents.[2]

Although the field is less extensive than that of other noncoding RNAs (ncRNAs), snoRNA research concerning the cardiovascular system is still in the preliminary stages but gaining increased attention within research. In recent years, the scientific literature has revealed that many ncRNAs share the likely ability to act as therapeutic targets or biomarkers for a wide variety of diseases, thereby challenging the longstanding assumption that the noncoding regions of RNA are mostly nonfunctional.[9,10] snoRNAs have been the subject of innovative ncRNA research concerning cancer-associated signaling pathways and pathway abnormalities in cells of the cardiovascular system.[11,12] Often, snoRNA expression is upregulated and results in high concentrations of snoRNAs that can be found in human organ cells, as well as human plasma, blood, and urine.[12] In general, snoRNAs are medium-size (60–300 nt) RNAs found in the nucleus of eukaryotic organisms and are classified by highly conserved sequences that are referred to as boxes.[13] These boxes are evolutionarily conserved across organisms, such as archaea, eukaryotes, and yeast, and the genetic code associated with these boxes has remained relatively unchanged through evolution, although degenerate boxes do exist.[14,15] It is important to note that the genomic organization of snoRNA coding sequences varies among eukaryotes due to the displacement and movement of these sequences over time.[14]

snoRNAs are not only known for their housekeeping and noncanonical functions, such as mRNA and tRNA methylation, chromatin

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**Correspondence:** Venkata Naga Srikanth Garikipati, MSc, PhD, Aging + Cardiovascular Discovery Center, Department of Cardiovascular Sciences, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA.
**E-mail:** venkata.naga.srikanth.garikipati@temple.edu

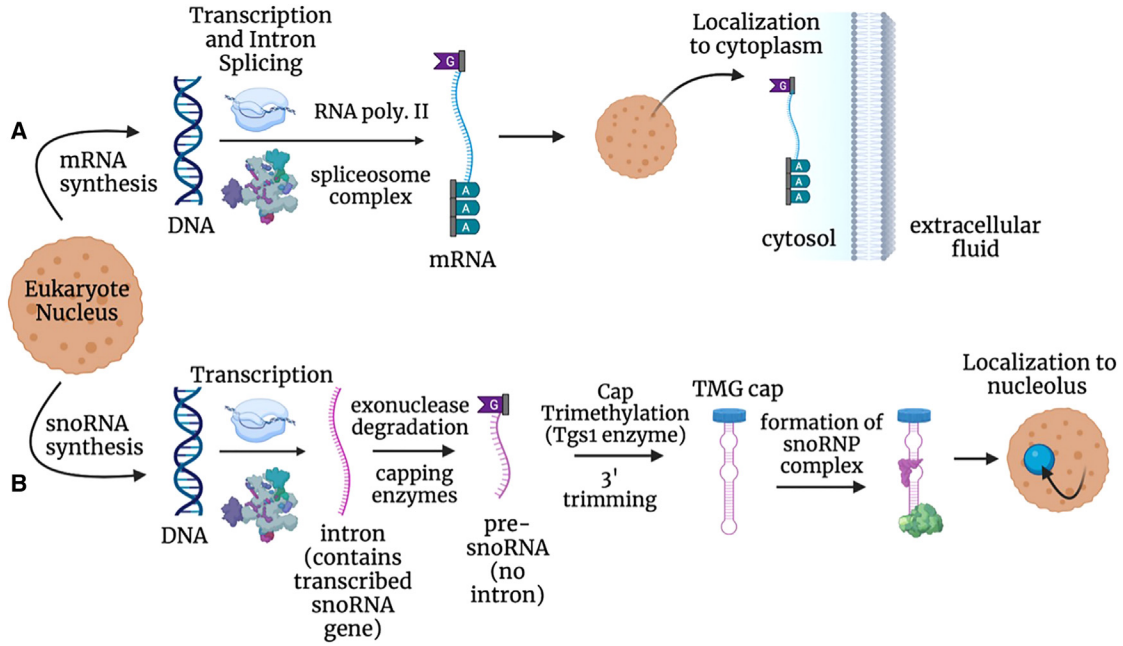


**Figure 1. The eukaryotic formation and localization of mRNA and snoRNA**

The eukaryotic formation and localization of mRNA (A) and snoRNA (B). The top portion of the figure corresponds to mRNA synthesis, in which the mRNA is transcribed from nuclear DNA using RNA polymerase II, the introns are removed by the spliceosome, the G cap and poly-A tail are added to the mRNA sequence, and the final mRNA is exported to the cellular cytoplasm. The bottom portion of the figure corresponds to snoRNA synthesis, in which nuclear DNA is transcribed using RNA polymerase II, resulting in mRNA (not pictured) and an intron sequence containing the snoRNA gene. This intron undergoes exonuclease degradation by the spliceosome and the addition of a 7-methylguanosine (G) cap. The snoRNA G cap is then converted into a TMG cap by the Tgs1 enzyme, followed by the 3′ trimming. The remaining components that make up the snoRNPs assemble and attach to the snoRNA molecule, forming the snoRNP complex that is relocated to the nucleolus.

remodeling, and recruitment into exosomes for intercellular communication, but also for their large roles in many biological processes such as tumor cell proliferation, apoptosis, and migration within the human body.[16,17] The abundance or lack of snoRNAs present within cells has the potential to signify potential CVD issues, such as tetralogy of Fallot (TOF) and fetal myocardium, and even cellular stressors such as cancers.[18,19] For example, in patients diagnosed with non-small cell lung carcinoma, low levels of SNORD78 (C/D-box snoRNA 78) promote cell-cycle arrest, eventually leading to the apoptosis of cancerous cells.[20] However, snoRNA 113.2 was found to be upregulated in plasma samples in patients who underwent failed coronary bypass surgery compared to controls.[13] The over- and underexpression of snoRNAs in the genome alters cellular mechanisms and can ultimately be beneficial, neutral, or detrimental to the cell, organ, or organism.[21] When the expression of cellular snoRNA is altered, the probability of malfunctioning cellular machinery and pathways begins to increase.[22] This can negatively affect the cell, and assuming the phenomenon occurs in multiple cells, can lead to tissue and organ defects as seen in CVDs.

snoRNAs are also known to be involved in modifying tRNA, rRNA, small nuclear RNA (snRNA), and mRNA targets, thereby influencing the concentrations of such targets and the overall gene-to-protein pathway.[23] SNORDs guiding tRNA 2′-*O*-methylation (Nm) offer protection from endonucleases, thereby increasing tRNA functionality.[24] Regarding mRNA, snoRNAs have been found to encourage or inhibit mRNA 3′ processing, leading to more or fewer protein products, respectively.[24] Other ncRNAs, including snRNAs and rRNAs, are also subject to both pseudouridylation and 2′-*O*-ribose methylation.[24] Current investigations prove that snoRNAs, molecules that were discovered and initially believed to have limited functions, are capable of much more than previously thought.

## BIOGENESIS

Eukaryotic snoRNAs are typically encoded within the introns of both coding and noncoding genes, and some are transcribed by RNA polymerase II from distinct snoRNA loci in the genome.[12] Typically, most mammalian snoRNAs are generated through cotranscription with a host gene followed by splicing, branching of the intron lariat, and exonucleolytic digestion in the nucleoplasm. Compared to heterogeneous nuclear RNAs or pre-mRNAs that undergo intron excision and the addition of a 5′-m7G cap and 3′-poly-adenosine tail before association with the cytoplasm, most snoRNAs were shown to remain in the nucleus after transcription and modification.[25] In humans, this is accompanied by either maintaining the m7G cap or being transformed into a 2,2,7-trimethylguanosine (TMG) cap, along with the removal of the poly-A tail; these phenomena may explain why most snoRNAs are not exported outside of the nucleus (Figure 1).[26]

In addition, small nucleolar ribonucleoproteins (snoRNPs) play essential roles in the maturation of snoRNAs and nucleolar localization.[21] These proteins must be recruited to the nascent intronic snoRNA after being initiated cotranscriptionally.[21] SNORDs form complexes with four specific proteins—fibrillarin, nucleolar protein 56, nucleolar protein 58, and small nuclear ribonucleoprotein 13—to form C/D snoRNPs, the final product capable of performing Nm.[27,28] H/ACA-box snoRNA form a complex with dyskerin (Cbf5), Gar1, Nhp2, and Nop10 to guide pseudouridine.[29] To supplement the work of the snoRNPs, other auxiliary factors are used to efficiently assist in the process of maturing snoRNA and function. The Cajal bodies accept previously transported snoRNPs to undergo more processing and maturation, which is then followed by their final delivery to the nucleolus, where they remain.[30] However, like many cellular genetic processes, the snoRNP modification process is prone to abnormalities. Both C/D-box and H/ACA-box snoRNPs require an assembly chaperone system known as HSP90/R2TP, which is a complex found in yeast; the human homolog is composed of PIH1D1, RUVBL2, and other factors.[22] In humans, the depletion of RUVBL2 leads to a decrease in C/D-box and H/ACA-box snoRNPs and reduced trafficking of these complexes into the nucleolus.[22] In addition to SNPs that affect the genetic material coding for snoRNAs,[13] these molecules are affected by posttranscription processes involving chaperone systems and protein factors.[22] Our understanding of the complex nature of snoRNP formation has thus increased over the years, making this field of research much more multifaceted than scientists originally thought.

## FUNCTIONALITY

snoRNAs are split into two classes, C/D- and H/ACA-box, based on their sequence patterns and the corresponding enzyme complexes. The SNORDs are characterized by the sequence RUGAUGA (where R is any purine), which composes the C-box, and CUGA, which composes the D-box.[31,32] These sequence motifs recruit proteins that are vital to forming the Nm complexes, including fibrillarin in eukaryotes and NOP1 in yeast.[32] In addition, each snoRNA contains at least one antisense element that corresponds to and recruits a target RNA. The antisense element in each SNORD is 5′ to each D-box; specifically, the target RNA binds 5 nt immediately adjacent to the D-box.[32] Thus, SNORDs are used to bring together the methyltransferase complex with its target RNA to form an snoRNP, beginning the methylation modification process.[32]

In a similar method, H/ACA-box snoRNAs aid in the pseudouridylation of RNAs by recruiting a pseudouridine synthase enzyme, Cbf5 in eukaryotes and Cbf5 in yeast, to the target RNA.[31,32] The sequence motifs that characterize H/ACA-box snoRNAs are H-boxes, ANANNA (where N is any nucleotide), and ACA-boxes. The ACA box is 3 nt immediately adjacent to the 3′ end of the RNA.[31,32] A key difference between the two classes of snoRNAs is that H/ACA-box snoRNAs are somewhat longer than SNORDs and form two stem-loops instead of one.[24,32] Each stem-loop is used to form a pseudouridylation pocket; the pocket contains the antisense element used to recruit, position, and bind the target RNA, where the nucleotide targeted for pseudouridylation is localized 14–16 nt upstream from the H/ACA-boxes.[32] The target RNAs in this case are typically rRNAs and a few snRNAs.[32] Despite SNORDs and H/ACA-box snoRNAs behaving in very different ways, both molecules are associated with the modification of cellular targets.

snoRNAs are generally localized to the nucleolus of a cell, where they are known to function as guide molecules for site-specific modification of rRNA. snoRNAs play a major role in the biogenesis of eukaryotic ribosomes by guiding enzymes that cleave or modify rRNAs.[33] snoRNAs such as U3, U14, U22, U17, and snR10 participate in pre-rRNA cleavage during rRNA processing.[34] snoRNAs guide methyltransferase and pseudouridine synthetase to site-specific locations in rRNA cotranscriptionally.[32] Completely processed human rRNA contains approximately 91 Ψs and 106 Nm, indicating that both modifications are necessary for the functionality of rRNA and the maturation of ribosomal units.[32] It is likely that rRNA requires Nm because the methyl groups can both increase the stability of the rRNA and the strength of intramolecular bonds with ribosomal protein components,[32] whereas Ψs offer increased base pairing, base stacking, and also rigidify the sugar-phosphate backbone compared to uridine.[32]

## DETECTION OF snoRNAs

When using human and animal models as the foundation for research studies, snoRNA samples often come from tissues (*in vivo*) or cell lines (*in vitro*). One study revealed that snoRNAs can be detected *in vivo* from blood plasma derived from human patients.[35] Another recent study that looked at endometrial cancer in mice combined both designs, using *in vitro* methods to measure tumor cell proliferation and *in vivo* methods to detect tumor formation.[36] Furthermore, snoRNAs can be manipulated using knockin and knockdown technology, a method that involves introducing or suppressing the expression of specific snoRNAs in cells or organisms. This can be followed by quantitative RNA analysis such as northern blotting, qPCR, or RNA sequencing.[37–39] These procedures allow researchers to measure the expression of specific snoRNAs and detect changes in these levels over time.

## INHIBITION OF snoRNAs IN THE LABORATORY THROUGH MOLECULAR TECHNIQUES

There has also been research that has focused on the inhibition of snoRNA, which has been both successful and futile. When using antisense nucleotides that match the target RNA sequence and bind to snoRNAs, no one was successful in reducing the binding properties of snoRNAs to RNA counterparts.[40] However, both CRISPR and gene knockout proved successful in binding to snoRNAs, preventing the formation of the snoRNA/target RNA complexes.[40] The gene silencing oligonucleotides 3Gas proved less effective when used on snoRNAs compared to miRNAs, possibly because of the complex circular structure of snoRNAs compared to miRNAs.[13] Considering all of the studies, the molecular inhibition of snoRNAs proves successful depending on the specific method used. This knowledge is essential when conducting experiments that aim to silence snoRNAs and limit their effects on molecular targets.

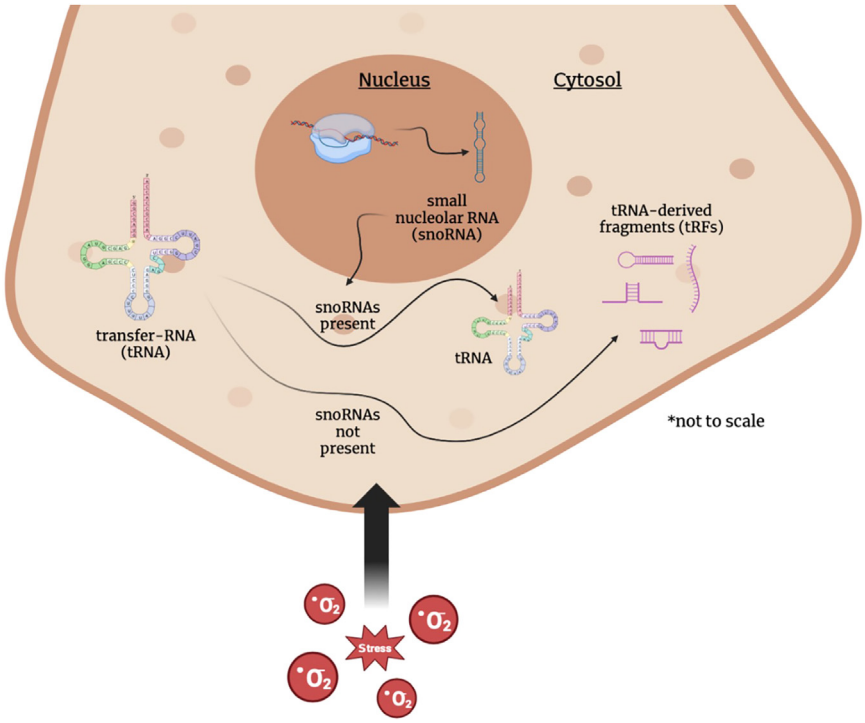


**Figure 2. The breakdown of tRNA in a eukaryotic cell**
When cells are exposed to stress hypoxia, tRNA molecules degrade into tRFs, molecules that can be detrimental to cellular health (bottom row). However, in the presence of snoRNAs which are transcribed from the genome, this degradation process appears to be limited, thus reducing the number of tRFs and maintaining the number of tRNA species (top row).

## THE EFFECTS OF snoRNAs ON tRNA AND tRFs

Recently, it has been discovered that snoRNAs play a huge role as inhibitors of tRNA processing. Reduced levels of tRNA and increased levels of tRNA-derived fragments (tRFs) have been detected in cells exposed to stress hypoxia.[41] When fragmented, tRFs perform different functions and have been shown to inhibit angiogenesis; promote vascular remodeling; and regulate endothelial, skeletal, and cardiac muscle.[41] To discover the effects of snoRNAs on tRFs, internal mammary arteries were harvested from patients with coronary artery disease.[41] When analyzing the biopsied umbilical arterial fibroblast cells, the expression of SNORD113-6 was much higher than controls.[41] More specifically, SNORD113-6 and the murine equivalent, AF357425, were believed to methylate $tRNA^{Leu}$(TAA) and prevent the fragmentation of its original structure into smaller tRFs, based on the high-to-low ratio of $tRNA^{Leu}$(TAA) to $tRF^{Leu47-64}$ (Figure 2).[41]

In addition to cardiovascular functions, when using knockout snoRNA mouse islet pancreatic cells, the four RpL13a locus snoRNAs have been found to modify mitochondrial metabolism, decrease oxygen in the mitochondrial matrix, increase cellular resistance to oxidative stress, regulate lipid-induced oxidative stress, and increase pancreatic islet insulin secretion in response to glucose.[42,43] Furthermore, in response to inflammatory signals, murine macrophage cells were found to release the RpL13a snoRNAs into circulation, which allowed for increased cell-to-cell long-distance communication.[25] These studies reveal that one specific snoRNA may contribute to multiple cellular processes, whether or not those are related to CVDs. The following sections discuss snoRNAs and CVDs.

## snoRNA EXPRESSION AT VARYING LOCI AND THEIR CARDIOVASCULAR IMPACTS

The loci for snoRNAs are not specific to one region of the genome; rather, the loci are located on multiple chromosomes. On chromosome 14 lies the DLK1-DIO3 locus, which harbors 41 C/D-box orphan snoRNAs for whom RNA targets are known.[40] Originally thought to be orphan snoRNAs, recent studies suggest that human snoRNA SNORD113-6 acts upon fibrillarin and integrin proteins via Nm in human fibroblasts.[40] The inhibition of SNORD113-6 resulted in reduced Nm of mRNA targets associated with the fibrillarin and integrin pathways.[40] These are proteins whose signaling pathways promote cell-to-cell fibroblast communication, allow fibroblasts to interact with the extracellular matrix, and are vital for cardiovascular remodeling.[40] When this remodeling system is not functioning properly, CVD can result. Therefore, the study reveals two possibilities: the effect of snoRNAs on integrin efficiency and, consequently, the potential for snoRNAs to disrupt cardiovascular system remodeling.[40]

## ROLE OF scaRNAs IN CARDIOVASCULAR DISEASE

Regarding cardiovascular disease, present studies suggest that snoRNAs have functions associated with either cardiovascular developmental deficits, adult heart diseases, or both. Essentially, abnormal snoRNA levels can be seen in atypical growing hearts or completely developed adult hearts that eventually experience disease. Whether certain snoRNAs serve as molecular motivators for the disease itself or are simply affected by the disease is an topic of great scientific debate. To explore this question, it is necessary to discuss how snoRNAs relate to cellular processes and cardiovascular disease.

Small Cajal body–specific RNAs (scaRNAs), a specific type of snoRNA predominantly in the Cajal body of the nucleus, differ from snoRNAs in terms of location, structure, and sequence; snoRNAs originate in the nucleolus and contain one C/D- or H/ACA-box, whereas scaRNAs contain either two of one domain or one of each domain.[20,44] What drives localization to the nucleolus for snoRNAs and the Cajal bodies for scaRNAs is a sequence known as the Cajal body box (CAB) motif.[45] The absence of the CAB motif in snoRNAs prevents these molecules from localizing into other parts of the

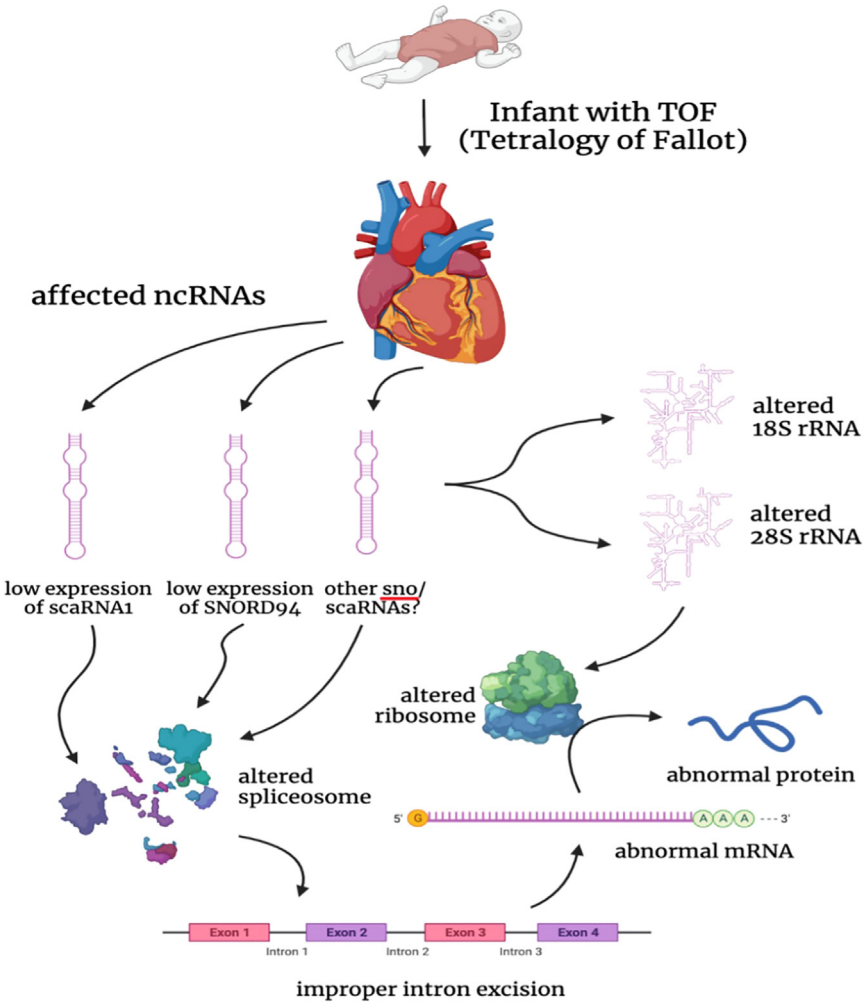


**Figure 3. Hearts of infants with TOF can have an abnormal hole located between the right and left ventricles, resulting in the pumping of deoxygenated blood through the aorta and into the body**

In the affected heart, it is believed that the underexpression of scaRNA1, SNORD94, and possibly other undiscovered sno-/scaRNAs may disrupt several molecular processes, such as splicing, transcription, and translation, as well as alter cellular products, such as spliceosomes, mRNA, rRNA, ribosomes, and proteins.

nucleus. Interestingly, human telomerase RNA contains an H/ACA-box and CAB motif similar to scaRNAs, suggesting that human telomerase is not only structurally related to scaRNAs but also localized to the same portion of the nucleus.[46,47] This realization has led scientists to discovering that certain scaRNAs engage in telomere lengthening and, ultimately, DNA preservation.[46] When scaRNA expression is defective, improper snRNA/scaRNA complex formation occurs, followed by improper gene splicing by the spliceosome; this phenomenon is demonstrated in infants with TOF.[48] Hence, it appears that these molecules can be associated with cellular processes that trigger cardiovascular issues.[49]

## ROLE OF snoRNAs IN TOF

In a pilot study analyzing biopsied cells in the right ventricle of infants with TOF, 12 scaRNAs were found to be expressed in low concentrations compared to controls.[49] The expression of snoRNAs in myocardium from infants living with TOF is similar to the expression patterns in fetal myocardium; this is comparable to infant myocardium without TOF, because expression levels are usually much higher.[49] The mechanism by which the expression of myocardium snoRNAs increases as the fetus develops is an area that requires further exploration. Two studies proposed that the downregulation of snoRNAs in TOF predominantly affects 4 targets, including the U2 and U6 snRNA of the spliceosome complex, as well as the 18S and 28S rRNAs (Figure 3).[48,49] When an insufficient amount of scaRNA is expressed by the cell, as is the case for individuals with TOF, improper snRNA modification and defective gene splicing can result.[49] This may give rise to irregular proteins that are necessary for cell function and cardiovascular survival, which may explain the phenotypical symptoms of TOF. Thus, the reduced expression levels of scaRNAs in TOF and other potential diseases may serve as indicators for disease onset and prevention. A more recent study revealed that cardiomyocyte tissue from infants with TOF contained very low levels of scaRNA1 (Figure 3). Consequently, pseudouridylation of the U2 snRNA occurred much less frequently, thereby lowering spliceosome fidelity and interrupting the proper formation of mRNA.[50] Another study demonstrated that SNORD94, a specific scaRNA, guides Cm62 in U6 snRNA and alters overall spliceosome function.[51] Whether scaRNA expression in TOF can be mechanistically induced to alleviate TOF symptoms deserves further attention.

## GENETIC MUTATIONS OF snoRNAs AND CVD

In a similar manner to traditional DNA and mRNA, the genetic material encoding for snoRNAs is also prone to genetic mutations. Researchers in an experiment that sought to determine the effects of SNPs in genes that encode for specific snoRNAs looked at possible SNPs at the 14q32 locus.[13] This locus was previously found to be home to a large gene cluster encoding for multiple miRNAs, and the same was found to be true for snoRNAs.[13] Some of the SNPs present at the 14q32 locus included rs2145501, rs2145504, rs2295657, and rs10133948, out of the total of 51 SNPs that resulted in abnormal snoRNA products.[13] Of these 51 SNPs, 17 were found to be associated with cardiovascular endpoints and 14 were associated with heart failure in the subjects.[13] Thus, in patients with failing hearts, snoRNAs

**Table 1. The chromosome location and locus of specific snoRNAs, as well as the potential cellular effects of those snoRNAs**

| Specific snoRNA(s) | Human or murine, chromosome location, loci | Consequential heart disease and tissue effects | Upregulated or downregulated | Specific cellular mechanism | Specific cell line and/or tissue type | References |
|---|---|---|---|---|---|---|
| SNORD113, -114, -116 | Human, 14, 14q32 (DLK-DIO3) | Prader-Willi syndrome, acute stroke, vascular remodeling peripheral arterial disease, blood coagulation | Upregulated | Targets mRNAs associated with the integrin signaling pathway, binds via Nm to tRNA leucine anti-codon to prevent formation of tRFs, and guides fibrillarin Nm of mRNA targets | Primary human umbilical arterial fibroblasts | Nossent et al.[11]; Håkansson et al.[13]; van Ingen et al.[40]; van Ingen et al.[41]; Falaleeva et al.[66] |
| MBII-52 (SNORD 115) | Murine, 7 and 15, 15q11-13 | Prader-Willi syndrome | Upregulated | Targets alternative exon regions of serotonin receptor 2C pre-mRNA via Nm | Transgenic deletion of Parder-Willi (TgPW) murine model, murine brain tissues | Håkansson et al.[13]; Kishore et al.[67]; Soeno et al.[68] |
| DQ267100, DQ267101, DQ267102, AF357355, AF357359, AF357426, AF357425 | Murine, 12, 12F11 | Vascular remodeling, neovascularization, restenosis, and atherosclerosis | Upregulated | Direct Nm via fibrillarin on noncanonical RNA targets | Primary murine fibroblasts | Håkansson et al.[13]; van Ingen et al.[41] |
| SNORD94 | Human, 2, 2p11.2 | Congenital heart defects | Upregulated | Targets a cytosine on spliceosomal RNA subunit U6 via Nm | Human infant right ventricle tissue, primary neonatal cardiomyocytes | Ogren et al.[51] |
| SNORD96A, SNORD73A | Human, 5, 5q35 and 4, 4q31, respectively | Hypertrophic cardiomyopathy | Upregulated | Regulate alternative splicing via Nm | hi-PSC-CMs | James et al.[55] |

specific to the 14q32 locus were found to be higher in concentration in correlation with nucleotide-specific SNPs.

## ROLE OF snoRNAs IN FAILING HUMAN HEARTS

Failing hearts were found to upregulate the expression of the DLK-DIO3 locus, increasing the number of resultant orphan snoRNAs in cardiovascular tissue.[13] Considering the present literature, a great amount of research can still be done regarding potential stressors or conditions that result in the inhibition of human SNORD113-6 and orphan snoRNAs, as well as how this inhibition directly affects processes of the cardiovascular system (Table 1). In addition to the DLK1-DIO3 and 14q32 loci, the 12F1 locus on chromosome 12 encodes for a tandemly repeated array of multiple SNORDs.[52] In a study that modeled arterial-venous bypass failure, the levels of six different snoRNAs transcribed from the 12F1 locus increased significantly, with snoRNA concentrations peaking at 7 days postsurgery and steadily decreasing and leveling out almost 1 month.[13] This suggests that bypass surgery, as well as related stresses to the heart, may increase the transcription of 12F1 snoRNAs and possibly other snoRNAs located throughout the genome.

## ADDITIONAL CELLULAR TARGETS OF snoRNAs AND THEIR CARDIOVASCULAR EFFECTS

There appear to be many cardiovascular-related proteins that are affected by snoRNA involvement in the mRNA-to-protein translation process. Peroxidasin homolog is a protein encoded by the PXDN gene, which is found most abundantly in cardiac tissue.[53] The mRNA produced from the PXDN gene is believed to be targeted by two SNORDs, U32A and U51.[53] Homozygous recessive (knockout) and heterozygous cells for genes encoding for U32A and U51 were shown to have elevated levels of peroxidasin protein, meaning that Nm modification of PXDN mRNA prevents translation into the protein form.[53] In U32A and U51 knockout cells, the consequential lack of Nm modification and increased PDXN mRNA resulted in increased cardiac peroxidase activity.[53] Thus, snoRNAs appear to inhibit cardiac peroxidase, an enzyme that is responsible for regulating oxidative stress in the heart. It seems that as snoRNA concentrations increase, specifically those of U32A and U51, the capability of the heart to combat oxidative stress decreases.

Regarding heart size and development, snoRNAs appear to play a regulatory role as the heart matures.[54] The RpL13a locus, which produces the respective SNORDs U32a, U33, U34, and U35a, performs Nm modification on targets.[54] In a study that used genetic knockout of all 4 snoRNAs in mice, as well as antisense oligonucleotide to ineffectively render the 4 snoRNAs in H9c2 rat cardiomyoblast cells, the consequential lack of effective snoRNA led to developmentally smaller hearts.[54] The snoRNAs were found to perform Nm on mRNA targets that produce the mammalian target of rapamycin protein, thereby suggesting that U32a, U33, U34, and U35a operate on the level of translation.[54] Therefore, snoRNAs appear essential to the developmental processes of the heart and the composite tissues.

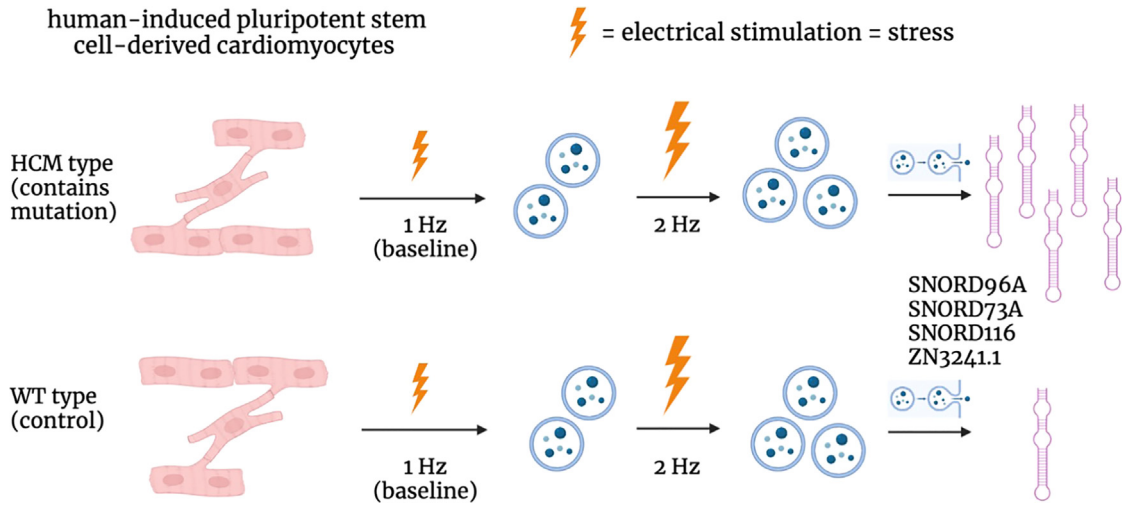


**Figure 4. Varying concentrations of snoRNAs found in the EVs released by HCMs and WT hiPSC-CM cells when exposed to electrical stimulation**

The top and bottom rows display HCM and WT cells, respectively, when exposed to electrical stimulation to simulate hypertrophic cardiomyopathy stress. Both cell populations release the same number of EVs, but the concentration of snoRNAs is much higher in the HCM type. These snoRNAs include SNORD96A, SNORD73A, SNORD116, and ZN3241.1.

## ROLE OF snoRNAs ON HYPERTROPHIC CARDIOMYOPATHY

Hypertrophic cardiomyopathy is a devastating heart condition characterized by an increased left ventricle wall thickness and altered cardiac output of the heart, as well as distorted blood flow between the pulmonary veins and aorta. In a study that used two groups of human-induced pluripotent stem cell–derived cardiomyocytes (hiPSCMs), human cardiac myocytes (HCMs) and wild type (WT), the HCM group contained the c.ACTC1$^{G301}$ mutation characteristic of hypertrophic cardiomyopathy; the mutation was absent from the WT group.[55] When subject to a 2-Hz and a 1-Hz electrical stimulation as a baseline to replicate hypertrophic cardiomyopathy stress, hiPSCMs released extracellular vesicles (EVs) containing varying concentrations of snoRNAs.[55] When analyzing the EVs released during the 2-Hz stimulation, 10 specific snoRNAs, including SNORD96A, SNORD73A, SNORD116, and ZN3241.1, were found to be statistically higher in concentration in the HCM group than in the WT group (Figure 4).[55] This finding suggests that certain snoRNAs are upregulated and overexpressed in cardiomyocytes in stressful conditions, and more specifically, cardiomyocytes containing the mutation for hypertrophic cardiomyopathy.[55] However, the exact molecular pathophysiology of these 10 specific snoRNAs and how their molecular interactions contribute to the development of CVDs remain unclear.

## MODIFICATION OF snoRNA AND rRNA AND THE POSSIBILITY OF SPECIALIZED RIBOSOMES IN CVD

Once thought to be static, stoichiometric analysis of rRNA modification in different cell types, including cancer cells, have suggested that these RNA modifications are dynamic and could generate ribosomes of different functions.[56] Quantitative analysis of rRNA Nm sites in RNA derived from different developmental stages of the heart using RiboMeth-seq revealed that the stoichiometry of 4 Nms on small subunit rRNA (Um354, Gm436, Gm867, Cm1272) was increased and 1 Nm in large subunit rRNA (Gm4593) was decreased in the adult heart.[57] Interestingly, the study correlated the level of the cognate SNORD78 to the level of Gm4593; alternative splicing of the snoRNA host gene and growth arrest–specific 5 (Gas5) was proposed to be the mechanism by which the SNORD78, and thereby Gm4593, are regulated.[57] Similarly, analysis of snoRNA in TOF patients suggested that 99 snoRNAs guiding RNA modification on rRNA are downregulated; this includes 119 modifications on large subunit rRNA and 80 modifications on small subunit rRNA.[48] This analysis suggests that almost all snoRNAs mediating rRNA modification are downregulated in TOF patients. Recent evidence suggests that the loss of even 1 Nm modification rRNA (Cm174 on small subunit rRNA) could affect the ability of ribosomes to translate mRNAs with AT- and GC-rich codons.[58] It is thus intriguing to determine whether ribosomes from TOF patients or other CVD patients are functionally different from those of normal humans.

## ROLE OF snoRNAs IN DIAMOND BLACKFAN ANEMIA

Concurrently, it is known that patients with ribosomopathies such as Diamond Blackfan anemia have mutations or deletions in ribosomal proteins, thus affecting the function of ribosomes.[59] Further quantitative studies measuring rRNA modification and ribosomal proteins are warranted to challenge the static function of ribosomes in cardiac systems.

## FUTURE PERSPECTIVES

Since the publication of the first snoRNA paper in 1983, research using snRNAs and scaRNAs has gained momentum in the scientific

community and is expanding across several research disciplines, from cancer development to CVDs.[12,60] Specific to this review, the abnormal expression of sno-/scaRNA molecules in human and animal models can be measured and studied in the context of many CVDs; current findings reveal the correlation between such molecules and CVDs such as TOF, coronary heart disease, and doxorubicin cardiotoxicity.[8] Future studies are likely to explore the intricacies of these existing findings while also suggesting ulterior correlations to additional diseases. On another note, regarding treatment for patients currently suffering from CVDs, whether artificial or harvested snoRNAs can be clinically administered to patients to reduce cardiovascular symptoms (such as in TOF) remains unclear, because there could be unintentional consequences to cellular and organ tissues. To investigate this, whole tissues and organs from animal and human models could be studied to ethically deliver applicable results to vulnerable human populations. Regarding snoRNAs as targeting and curative agents, the delivery of snoRNAs into CVD-affected tissues begs several questions: How do we stabilize snoRNAs before delivery to prevent RNA degradation and maximize successful cellular uptake? Are there synergistic effects between snoRNAs and other therapeutic agents that may enhance stability or mitigate degradation, and how can combination therapies be optimized? What preclinical models and methodologies are suitable for evaluating the stability of snoRNAs *in vivo*, especially within the complex environment of CVD-affected tissues? At this stage in our scientific endeavors, snoRNAs need to be continually studied if we intend to successfully use these molecules as preventive disease biomarkers and curative therapeutic agents. Once the aforementioned questions begin to be answered through the implementation of successful basic and clinical studies, we will be one step closer to treating those who are suffering with CVD complications in the near future.

Considering the abnormal expression of snoRNAs in human disease models, when exactly in the pathophysiology time line do these molecules exhibit irregular levels? Does the disease trigger atypical snoRNA expression, or is the reverse scenario true, in which abnormal snoRNA levels are seen before CVD onset? In addition, does the chronology of pathophysiology remain the same for all CVDs, or does it vary depending on the disease? Current studies only partially answer these questions, signifying the need to dedicate more scientific efforts and develop more complex instrumentation to measure this phenomenon. In addition, orphan sno-/scaRNAs deserve further attention because many undiscovered targets may help in elucidating how exactly these molecules operate mechanistically, as well as the extent to which sno-/scaRNAs operate in the human genome.

Alternatively, the study of snoRNA-mediated RNA modification can also guide researchers to design and deliver RNA targets into cardiac cells. The invention of technologies such as RiboMeth-seq, HydraPsi-seq, and Oxford-nanopore to quantitatively measure Nm and Y RNA modification could fuel the discovery of how various RNA modifications alter and may affect the outcome of CVDs.[61–63] Interestingly, the use of modified nucleotides such as Ψs has gained significant interest in developing mRNA vaccines for the recent coronavirus disease 2019 outbreak.[64] Previous studies have also successfully explored such technologies to deliver mRNA in cardiac cells—for example, delivery of mRNA carrying $N^1$-methylpseudouridine-5-triphosphate enhanced translation in cardiac cells and tissues *in vivo*.[65]

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## AUTHOR CONTRIBUTIONS

N.P.J., A.K.R., and V.M. wrote the initial manuscript and created the figures. N.P.J., A.K.R., and K.S.R. revised the manuscript. V.N.S.G. conceptualized and revised the manuscript. All of the authors who contributed to the article have approved the submitted version.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

## REFERENCES

1. Virani, S.S., Alonso, A., Aparicio, H.J., Benjamin, E.J., Bittencourt, M.S., Callaway, C.W., Carson, A.P., Chamberlain, A.M., Cheng, S., Delling, F.N., et al. (2021). Heart disease and stroke statistics—2021 update: a report from the American Heart Association. Circulation *143*, e254–e743.
2. Tsao, C.W., Aday, A.W., Almarzooq, Z.I., Alonso, A., Beaton, A.Z., Bittencourt, M.S., Boehme, A.K., Buxton, A.E., Carson, A.P., Commodore-Mensah, Y., et al. (2022). Heart disease and stroke statistics—2022 update: a report from the American Heart Association. Circulation *145*, e153–e639.
3. GBD 2017 Causes of Death Collaborators, Abate, D., Abate, K.H., Abay, S.M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., and Abdelalim, A. (2018). Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet *392*, 1736–1788.
4. Kathiresan, S., and Srivastava, D. (2012). Genetics of human cardiovascular disease. Cell *148*, 1242–1257.
5. Xu, M., and Song, J. (2021). Targeted therapy in cardiovascular disease: A precision therapy era. Front. Pharmacol. *12*, 623674.
6. Zomer, E., Gurusamy, K., Leach, R., Trimmer, C., Lobstein, T., Morris, S., James, W.P.T., and Finer, N. (2016). Interventions that cause weight loss and the impact on cardiovascular risk factors: a systematic review and meta-analysis. Obes. Rev. *17*, 1001–1011.
7. Leong, D.P., Joseph, P.G., McKee, M., Anand, S.S., Teo, K.K., Schwalm, J.-D., and Yusuf, S. (2017). Reducing the global burden of cardiovascular disease, part 2: prevention and treatment of cardiovascular disease. Circ. Res. *121*, 695–710.
8. Das, S., Shah, R., Dimmeler, S., Freedman, J.E., Holley, C., Lee, J.-M., Moore, K., Musunuru, K., Wang, D.-Z., Xiao, J., et al. (2020). Noncoding RNAs in cardiovascular disease: current knowledge, tools and technologies for investigation, and future directions: a scientific statement from the american heart association. Circ. Genom. Precis. Med. *13*, e000062.
9. Havstad, J.C., and Palazzo, A.F. (2022). Not functional yet a difference maker: junk DNA as a case study. Biol. Philos. *37*, 29.
10. Gloss, B.S., and Dinger, M.E. (2018). Realizing the significance of noncoding functionality in clinical genomics. Exp. Mol. Med. *50*, 1–8.

11. Nossent, A.Y., Ektefaie, N., Wojta, J., Eichelberger, B., Kopp, C., Panzer, S., and Gremmel, T. (2019). Plasma levels of snoRNAs are associated with platelet activation in patients with peripheral artery disease. Int. J. Mol. Sci. *20*, 5975.

12. Wajahat, M., Bracken, C.P., and Orang, A. (2021). Emerging functions for snoRNAs and snoRNA-derived fragments. Int. J. Mol. Sci. *22*, 10193.

13. Håkansson, K.E.J., Goossens, E.A.C., Trompet, S., Van Ingen, E., De Vries, M.R., van der Kwast, R.V.C.T., Ripa, R.S., Kastrup, J., Hohensinner, P.J., Kaun, C., et al. (2019). Genetic associations and regulation of expression indicate an independent role for 14q32 snoRNAs in human cardiovascular disease. Cardiovasc. Res. *115*, 1519–1532.

14. Dieci, G., Preti, M., and Montanini, B. (2009). Eukaryotic snoRNAs: a paradigm for gene expression flexibility. Genomics *94*, 83–88.

15. Gardner, P.P., Bateman, A., and Poole, A.M. (2010). SnoPatrol: how many snoRNA genes are there? J. Biol. *9*, 4.

16. Baral, D., Wu, L., Katwal, G., Yan, X., Wang, Y., and Ye, Q. (2018). Clinical significance and biological roles of small nucleolar RNAs in hepatocellular carcinoma. Biomed. Rep. *8*, 319–324.

17. Bergeron, D., Fafard-Couture, É., and Scott, M.S. (2020). Small nucleolar RNAs: continuing identification of novel members and increasing diversity of their molecular mechanisms of action. Biochem. Soc. Trans. *48*, 645–656.

18. van der Werf, J., Chin, C.V., and Fleming, N.I. (2021). SnoRNA in cancer progression, metastasis and immunotherapy response. Biology *10*, 809.

19. Rajan, K.S., Ramasamy, S., George-William, J.N., and Rajendhran, J. (2017). Emerging cardiac non-coding landscape: The importance of meta-analysis. Biochimie *133*, 87–94.

20. Huang, Z.-h., Du, Y.-p., Wen, J.-t., Lu, B.-f., and Zhao, Y. (2022). snoRNAs: functions and mechanisms in biological processes, and roles in tumor pathophysiology. Cell Death Dis. *8*, 259.

21. Liang, J., Wen, J., Huang, Z., Chen, X.-p., Zhang, B.-x., and Chu, L. (2019). Small nucleolar RNAs: insight into their function in cancer. Front. Oncol. *9*, 587.

22. Ojha, S., Malla, S., and Lyons, S.M. (2020). snoRNPs: Functions in Ribosome Biogenesis. Biomolecules *10*, 783.

23. Shi, J., Zhou, T., and Chen, Q. (2022). Exploring the expanding universe of small RNAs. Nat. Cell Biol. *24*, 415–423.

24. Bratkovič, T., Božič, J., and Rogelj, B. (2020). Functional diversity of small nucleolar RNAs. Nucleic Acids Res. *48*, 1627–1651.

25. Rimer, J.M., Lee, J., Holley, C.L., Crowder, R.J., Chen, D.L., Hanson, P.I., Ory, D.S., and Schaffer, J.E. (2018). Long-range function of secreted small nucleolar RNAs that direct 2′-O-methylation. J. Biol. Chem. *293*, 13284–13296.

26. Kufel, J., and Grzechnik, P. (2019). Small nucleolar RNAs tell a different tale. Trends Genet. *35*, 104–117.

27. Baldini, L., Charpentier, B., and Labialle, S. (2021). Emerging data on the diversity of molecular mechanisms involving C/D snoRNAs. Noncoding. RNA *7*, 30.

28. Quinternet, M., Chagot, M.-E., Rothé, B., Tiotiu, D., Charpentier, B., and Manival, X. (2016). Structural features of the box C/D snoRNP Pre-assembly process are conserved through species. Structure *24*, 1693–1706.

29. Lin, P., Mobasher, M.E., Hakakian, Y., Kakarla, V., Naseem, A.F., Ziai, H., and Alawi, F. (2015). Differential requirements for H/ACA ribonucleoprotein components in cell proliferation and response to DNA damage. Histochem. Cell Biol. *144*, 543–558.

30. Deryusheva, S., and Gall, J.G. (2019). scaRNAs and snoRNAs: Are they limited to specific classes of substrate RNAs? RNA *25*, 17–22.

31. Lin, L.-M., Pan, Q., Sun, Y.-M., and Wang, W.-T. (2021). Small nucleolar RNA is potential as a novel player in leukemogenesis and clinical application. Blood Sci. *3*, 122–131.

32. Holley, C.L., and Topkara, V.K. (2011). An introduction to small non-coding RNAs: miRNA and snoRNA. Cardiovasc. Drugs Ther. *25*, 151–159.

33. Bachellerie, J.-P., Cavaillé, J., and Hüttenhofer, A. (2002). The expanding snoRNA world. Biochimie *84*, 775–790.

34. Hokii, Y., Sasano, Y., Sato, M., Sakamoto, H., Sakata, K., Shingai, R., Taneda, A., Oka, S., Himeno, H., Muto, A., et al. (2010). A small nucleolar RNA functions in rRNA processing in Caenorhabditis elegans. Nucleic Acids Res. *38*, 5909–5918.

35. Freedman, J.E., Gerstein, M., Mick, E., Rozowsky, J., Levy, D., Kitchen, R., Das, S., Shah, R., Danielson, K., Beaulieu, L., et al. (2016). Diverse human extracellular RNAs are widely detected in human plasma. Nat. Commun. *7*, 11106.

36. Bao, H.-j., Chen, X., Liu, X., Wu, W., Li, Q.-h., Xian, J.-y., Zhao, Y., and Chen, S. (2022). Box C/D snoRNA SNORD89 influences the occurrence and development of endometrial cancer through 2'-O-methylation modification of Bim. Cell Death Dis. *8*, 309.

37. Jorjani, H., Kehr, S., Jedlinski, D.J., Gumienny, R., Hertel, J., Stadler, P.F., Zavolan, M., and Gruber, A.R. (2016). An updated human snoRNAome. Nucleic Acids Res. *44*, 5068–5082.

38. Qu, L.H., Meng, Q., Zhou, H., and Chen, Y.Q. (2001). Identification of 10 novel snoRNA gene clusters from Arabidopsis thaliana. Nucleic Acids Res. *29*, 1623–1630.

39. Ro, S., Park, C., Jin, J., Sanders, K.M., and Yan, W. (2006). A PCR-based method for detection and quantification of small RNAs. Biochem. Biophys. Res. Commun. *351*, 756–763.

40. van Ingen, E., van den Homberg, D.A.L., van der Bent, M.L., Mei, H., Papac-Milicevic, N., Kremer, V., Boon, R.A., Quax, P.H.A., Wojta, J., and Nossent, A.Y. (2022). C/D box snoRNA SNORD113-6/AF357425 plays a dual role in integrin signalling and arterial fibroblast function via pre-mRNA processing and 2′ O-ribose methylation. Hum. Mol. Genet. *31*, 1051–1066.

41. van Ingen, E., Engbers, P.A.M., Woudenberg, T., van der Bent, M.L., Mei, H., Wojta, J., Quax, P.H.A., and Nossent, A.Y. (2022). C/D box snoRNA SNORD113-6 guides 2′-O-methylation and protects against site-specific fragmentation of tRNALeu (TAA) in vascular remodeling. Mol. Ther. Nucleic Acids *30*, 162–172.

42. Lee, J., Harris, A.N., Holley, C.L., Mahadevan, J., Pyles, K.D., Lavagnino, Z., Scherrer, D.E., Fujiwara, H., Sidhu, R., Zhang, J., et al. (2016). Rpl13a small nucleolar RNAs regulate systemic glucose metabolism. J. Clin. Invest. *126*, 4616–4625.

43. Michel, C.I., Holley, C.L., Scruggs, B.S., Sidhu, R., Brookheart, R.T., Listenberger, L.L., Behlke, M.A., Ory, D.S., and Schaffer, J.E. (2011). Small nucleolar RNAs U32a, U33, and U35a are critical mediators of metabolic stress. Cell Metabol. *14*, 33–44.

44. Bergstrand, S., O'Brien, E.M., Coucoravas, C., Hrossova, D., Peirasmaki, D., Schmidli, S., Dhanjal, S., Pederiva, C., Siggens, L., Mortusewicz, O., et al. (2022). Small Cajal body-associated RNA 2 (scaRNA2) regulates DNA repair pathway choice by inhibiting DNA-PK. Nat. Commun. *13*, 1015.

45. Venteicher, A.S., and Artandi, S.E. (2009). TCAB1: driving telomerase to Cajal bodies. Cell Cycle *8*, 1329–1331.

46. Fu, D., and Collins, K. (2006). Human telomerase and Cajal body ribonucleoproteins share a unique specificity of Sm protein association. Genes Dev. *20*, 531–536.

47. Zhang, Q., Kim, N.-K., and Feigon, J. (2011). Architecture of human telomerase RNA. Proc. Natl. Acad. Sci. USA *108*, 20325–20332.

48. O'Brien, J.E., Jr., Kibiryeva, N., Zhou, X.-G., Marshall, J.A., Lofland, G.K., Artman, M., Chen, J., and Bittel, D.C. (2012). Noncoding RNA expression in myocardium from infants with tetralogy of Fallot. Circ. Cardiovasc. Genet. *5*, 279–286.

49. Cao, T., Rajasingh, S., Samanta, S., Dawn, B., Bittel, D.C., and Rajasingh, J. (2018). Biology and clinical relevance of noncoding sno/scaRNAs. Trends Cardiovasc. Med. *28*, 81–90.

50. Nagasawa, C.K., Kibiryeva, N., Marshall, J., O'Brien, J.E., and Bittel, D.C. (2020). scaRNA1 levels alter pseudouridylation in spliceosomal RNA U2 affecting alternative mRNA splicing and embryonic development. Pediatr. Cardiol. *41*, 341–349.

51. Ogren, A., Kibiryeva, N., Marshall, J., O'Brien, J.E., Jr., and Bittel, D.C. (2019). Snord94 expression level alters methylation at C62 in snRNA U6. PLoS One *14*, e0226035.

52. Dill, T.L., and Naya, F.J. (2018). A hearty dose of noncoding RNAs: the imprinted DLK1-DIO3 locus in cardiac development and disease. J. Cardiovasc. Dev. Dis. *5*, 37.

53. Elliott, B.A., Ho, H.-T., Ranganathan, S.V., Vangaveti, S., Ilkayeva, O., Abou Assi, H., Choi, A.K., Agris, P.F., and Holley, C.L. (2019). Modification of messenger RNA by 2′-O-methylation regulates gene expression in vivo. Nat. Commun. *10*, 3401.

54. Ho, H.T.T., and Holley, C.L. (2020). Physiologic role of Rpl13a SnoRNAs in cell size regulation. In Suppl_1 (Am Heart Assoc), p. A378.

55. James, V., Nizamudeen, Z.A., Lea, D., Dottorini, T., Holmes, T.L., Johnson, B.B., Arkill, K.P., Denning, C., and Smith, J.G.W. (2021). Transcriptomic analysis of

cardiomyocyte extracellular vesicles in hypertrophic cardiomyopathy reveals differential snoRNA cargo. Stem Cell. Dev. *30*, 1215–1227.

56. Motorin, Y., Quinternet, M., Rhalloussi, W., and Marchand, V. (2021). Constitutive and variable 2'-O-methylation (Nm) in human ribosomal RNA. RNA Biol. *18*, 88–97.

57. Hebras, J., Krogh, N., Marty, V., Nielsen, H., and Cavaillé, J. (2020). Developmental changes of rRNA ribose methylations in the mouse. RNA Biol. *17*, 150–164.

58. Jansson, M.D., Häfner, S.J., Altinel, K., Tehler, D., Krogh, N., Jakobsen, E., Andersen, J.V., Andersen, K.L., Schoof, E.M., Ménard, P., et al. (2021). Regulation of translation by site-specific ribosomal RNA methylation. Nat. Struct. Mol. Biol. *28*, 889–899.

59. Farley-Barnes, K.I., Ogawa, L.M., and Baserga, S.J. (2019). Ribosomopathies: old concepts, new controversies. Trends Genet. *35*, 754–767.

60. Volpe, A.L., Taggart, M., McStay, B., and Bird, A. (1983). DNaseI-hypersensitive sites at promoter-like sequences in the spacer of Xenopus laevis and Xenopus borealis ribosomal DNA. Nucleic Acids Res. *11*, 5361–5380.

61. Rajan, K.S., Zhu, Y., Adler, K., Doniger, T., Cohen-Chalamish, S., Srivastava, A., Shalev-Benami, M., Matzov, D., Unger, R., Tschudi, C., et al. (2020). The large repertoire of 2'-O-methylation guided by C/D snoRNAs on Trypanosoma brucei rRNA. RNA Biol. *17*, 1018–1039.

62. Rajan, K.S., Adler, K., Doniger, T., Cohen-Chalamish, S., Aharon-Hefetz, N., Aryal, S., Pilpel, Y., Tschudi, C., Unger, R., and Michaeli, S. (2022). Identification and functional implications of pseudouridine RNA modification on small noncoding RNAs in the mammalian pathogen Trypanosoma brucei. J. Biol. Chem. *298*, 102141.

63. Tavakoli, S., Nabizadeh, M., Makhamreh, A., Gamper, H., McCormick, C.A., Rezapour, N.K., Hou, Y.-M., Wanunu, M., and Rouhanifard, S.H. (2023). Semi-quantitative detection of pseudouridine modifications and type I/II hypermodifications in human mRNAs using direct long-read sequencing. Nat. Commun. *14*, 334.

64. Morais, P., Adachi, H., and Yu, Y.-T. (2021). The critical contribution of pseudouridine to mRNA COVID-19 vaccines. Front. Cell Dev. Biol. *9*, 789427.

65. Sultana, T., Zamborlini, A., Cristofari, G., and Lesage, P. (2017). Integration site selection by retroviruses and transposable elements in eukaryotes. Nat. Rev. Genet. *18*, 292–308.

66. Falaleeva, M., Surface, J., Shen, M., de la Grange, P., and Stamm, S. (2015). SNORD116 and SNORD115 change expression of multiple genes and modify each other's activity. Gene *572*, 266–273.

67. Kishore, S., Khanna, A., Zhang, Z., Hui, J., Balwierz, P.J., Stefan, M., Beach, C., Nicholls, R.D., Zavolan, M., and Stamm, S. (2010). The snoRNA MBII-52 (SNORD 115) is processed into smaller RNAs and regulates alternative splicing. Hum. Mol. Genet. *19*, 1153–1164.

68. Soeno, Y., Taya, Y., Stasyk, T., Huber, L.A., Aoba, T., and Hüttenhofer, A. (2010). Identification of novel ribonucleo-protein complexes from the brain-specific snoRNA MBII-52. RNA *16*, 1293–1300.