Small Nucleolar RNAs:
Players in Human Development and Disease

Writing assignment
23 January 2023

Utrecht University

Princess máxima center
pediatric oncology

Author

Noud Verstappen, BSc
Molecular and Cellular Life Sciences
Utrecht University

Examiners

Supervisor / First examiner
Sebastiaan van Heesch, PhD
Princess Máxima Center for Pediatric Oncology

Second examiner
Jens Bunt, PhD
Princess Máxima Center for Pediatric Oncology

Abstract

Throughout the late 20th century, small nucleolar RNAs (snoRNAs) were identified as a novel class of non-coding RNA. SnoRNAs comprise three main classes based on conserved sequence motifs: C/D box, H/ACA box and Cajal body-associated snoRNAs. Their canonical function is to provide specificity to discrete ribonucleoprotein complexes, which post-transcriptionally modify ribosomal RNAs and spliceosomal small nuclear RNAs. It has become increasingly clear that snoRNAs can adopt unexpected cellular functions, ranging from the regulation of chromatin states to cell-cell communication. In addition, snoRNAs are intimately involved in cellular programs critical to development, as revealed by studies from model organisms. In this writing assignment, the ever-expanding repertoire of snoRNA biology will be reviewed with an ultimate focus on developmental disease.

Layman's summary

According to the now old-fashioned central dogma of biology, DNA is transcribed into messenger RNA which is then translated into protein by ribosomes. However, it has been discovered that a wide range of RNA species do not code for proteins, but which are still able to exert cellular functions. One type of these so-called non-coding RNAs (ncRNAs) are the small nucleolar RNAs (snoRNAs), which function together with protein complexes to modify RNAs associated with ribosomes. In addition to these functions, a growing body of literature has revealed unexpected cellular roles for snoRNAs, for example in regulating the accessibility of DNA to facilitate gene transcription, or by acting as messengers between cells. Importantly, studies implicating snoRNAs in cell division and cellular specialization suggest that they may have roles in development. Here, this expanding repertoire of snoRNA biology will be discussed with an ultimate emphasis on developmental diseases.

Table of contents

Scope

The aim of this writing assignment is to describe the roles of small nucleolar RNAs (snoRNAs) in human development and childhood disease, ultimately discussing their putative roles in childhood cancers. First, a broad introduction to snoRNAs will be provided– what are their origins, and what are their (non-canonical) functions? Following this, their roles in cellular processes related to development and cancer will be discussed, which will – for the first time – include insights from model organisms. Finally, the possible roles of snoRNAs in developmental diseases will be discussed.

1. What are snoRNAs?

1.1 History of small nucleolar RNAs – from U-RNA to directors of post-transcriptional modifications

The discovery of the non-coding RNA (ncRNA) world has been transformative to how we approach biological problems. Dating back to the late 1950's, the low-molecular weight ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) were the first ncRNAs to be identified, later to be dubbed 'housekeeping ncRNAs'[1–4]. By the late 1960's, sedimentation and electrophoretic analyses of nuclear preparations had made it clear that in addition to rRNAs and tRNAs, the nucleus harbors a variety of other low-molecular weight RNAs that were potentially functional as well[5–12]. These pioneering studies, primarily led by Harris Busch, revealed several interesting properties: the low-molecular weight RNAs were found to be associated with rRNAs (1), their uracil content was relatively high (2), they contained – like rRNA – methylated bases (3), and lastly, some were localized to the nucleolus (4). Because of their high uracil content, these low-molecular weight RNAs were coined U-RNAs, and initial electrophoretic mobility and base composition experiments were able to resolve six distinct U-RNAs (U1-U6)[12]. But it was only one U-RNA in particular – the nucleolar U3 RNA – that would lay the groundwork for research into the ncRNAs that became known as the snoRNAs.

In the early 1970's, U3 was hypothesized to contribute to ribosome biogenesis through transient binding to the 28S rRNA, as it was found associated with mature 28S rRNA in the nucleolus but not with 28S rRNA bound to preribosomal particles[13–15]. In the same decade, immunoprecipitation experiments demonstrated that small nuclear RNAs

(snRNAs) reside within discrete ribonucleoprotein particles (RNPs) – complexes formed between RNA and RNA-binding proteins). However, whether this was the case for the U3 snoRNA remained elusive until the discovery of anti-U3 RNP antibodies in 1985[16,17]. In the 1990's, studies in yeast, mouse and *Xenopus* finally elucidated that the U3 small nucleolar RNP (snoRNP) assists in multiple pre-rRNA cleavage events, contributing to 18S rRNA maturation[18–24]. Interestingly, it also became apparent that this is not the sole function of snoRNPs. Hints for additional functions of snoRNPs had in fact emerged as early as 1985 when immunoprecipitation studies found a 34 kDa protein in the U3 snoRNP, identified as fibrillarin (FBL) shortly after[16,25]. FBL was also found within two novel human snoRNPs, U8 and U13, and it was later suggested that two conserved box sequences, C (GAUGA) and D (UCUGA), are important for FBL binding[26–28]. Accordingly, these snoRNAs were now classified as C/D box snoRNAs. In 1991 it was demonstrated that impairment of the yeast FBL homolog Nop1p results in decreased 2′-O-methylation of rRNA, which strongly suggested a role for snoRNAs/snoRNPs in this process[29]. Yet, it was not until 1996 that C/D box snoRNAs were ascribed the additional function of guiding 2′-O-Methylation of pre-rRNA through direct base-pairing interactions[30]. In the following year a new class of snoRNA/snoRNP was defined, the H/ACA box snoRNAs, which harbor the conserved H (ANANNA) and ACA box sequences[31]. Their function was quickly elucidated to be that of guiding pseudouridylation of rRNAs (conversion of the uracil nucleoside into a pseudouridine)[32,33]. The growing number of snoRNAs with identified rRNA targets quickly revealed that guiding the post-transcriptional modification of rRNAs is the canonical function of snoRNAs rather than assisting in pre-rRNA cleavage[30,34,35]. And thus, by the end of the 1990's, snoRNAs had established themselves as multi-class guiders of post-transcriptional rRNA modifications. Over the course of the last two decades, snoRNAs have become a well-characterized species of ncRNA whose associated protein factors, functions and potential involvement in disease have been widely studied[36–38]. Before expanding on the functions of C/D and H/ACA box snoRNAs, their characteristics and biogenesis will be considered briefly.

1.2 Composing the functional snoRNP unit

1.2.1 Characteristics of snoRNAs/snoRNPs

SnoRNAs are an intermediately sized ncRNA species varying in length from 50 to 200 nucleotides[37,39]. As was alluded to in the previous chapter, two main classes of snoRNA exist based on conserved sequence motifs: C/D and H/ACA box snoRNAs (*SNORD*s and *SNORA*s, respectively). A third less represented class are the Cajal body-associated snoRNAs (scaRNAs), which may possess C/D, H/ACA or both box motifs in addition to a Cajal-body retention motif (CAB box, UGAG)[37]. So far, about 380 C/D box and 180 H/ACA box snoRNAs have been identified, although less stringent bioinformatics approaches have suggested that the human genome hosts over 2,000 snoRNAs[37,40]. More recently, non-canonical snoRNAs have been identified as well, including snoRNA-derived piwi-interacting RNAs (piRNAs) and micro-RNAs (miRNAs). These are collectively known as snoRNA-derived RNAs (sdRNAs)[41–43].

C/D box snoRNAs typically adopt a stem-loop-stem structure. In addition to C/D box motifs, C/D box snoRNAs may possess C' and D' box motifs (Fig. 1). The C/D box motifs are highly conserved, and by being brought in proximity of each other by the terminal stem, they form a functionally important K-turn structure through imperfect base pairing. In contrast, the loosely conserved C' and D' boxes often do not form such structure[44]. The regions between the C/D' and C'/D boxes offer between 7 to 21 nucleotides of complementarity to target RNAs, and methylation of ribose moieties takes place exactly 5 nucleotides upstream of the D and/or D' boxes (Fig. 1)[37]. Additional base-pairing of the C/D box snoRNA with sequences outside of the complementary target region may enhance 2′-O-Methylation, as has been shown for rRNAs[45]. H/ACA box snoRNAs form a hairpin-hinge-hairpin-tail secondary structure (Fig. 1). The H box is located in the hinge region while the ACA box is located in the tail, 3 nucleotides upstream of the 3′ terminus[46]. Within each hairpin, a 9 to 13 nucleotide internal loop forms a complementary binding site for target RNAs, also termed the 'pseudouridylation pocket'[44].

The core protein components of the human C/D and H/ACA box snoRNPs have been completely mapped, but structural and functional information has been largely derived from studies on Archaeal snoRNPs[47]. C/D box snoRNPs comprise NOP58, NOP56, SNU13 and the methyltransferase FBL. It is currently thought that C/D box snoRNPs have a

pseudo-dimeric structure with two FBL and SNU13 moieties[46]. The NOP proteins interact with FBL to provide RNA-binding specificity, while SNU13 is an important factor in C/D snoRNP biogenesis. H/ACA snoRNPs comprise NOP10, GAR1, NHP2 and the pseudouridine synthase Dyskerin (DKC1). H/ACA snoRNPs are thought to exist as a dimeric structure with one set of proteins covering each of the two H/ACA snoRNA hairpins. Although GAR1 is not required for H/ACA snoRNP stability, all core proteins are essential for enzymatic activity of the H/ACA RNP[44].

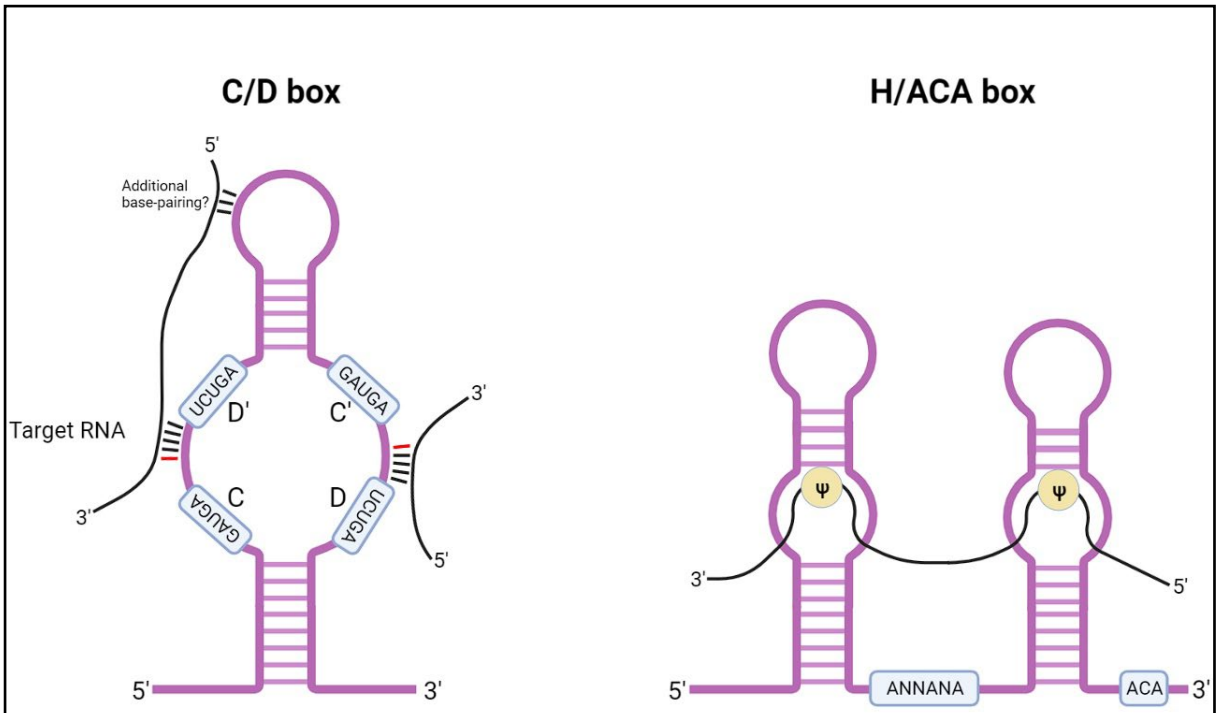

Figure 1 Structure of C/D and H/ACA box snoRNAs. The red nucleotide in the C/D box snoRNA indicates the fifth nucleotide upstream of the D/D' boxes where target 2'-O-Methylation is catalyzed by FBL. The Ψ symbol depicts pseudouridylation. Note that scaRNAs, while not shown here, may adopt hybrid C/D-H/ACA box structures. Drawing is not to scale. Figure was generated using BioRender.

1.2.2 SnoRNA/snoRNP biogenesis

SnoRNP biogenesis is a complex, multi-step process in which over 25 assembly, localization and RNA processing factors are involved, as reviewed in[46,48]. The genomic organization of snoRNAs is an important factor for which snoRNA processing and snoRNP assembly steps take place[48,49]. Human snoRNAs have a relatively uniform genomic organization, with 90% of snoRNAs being encoded as individual units that are co-

transcribed by RNA polymerase II from so-called host-genes (HGs)[50]. Consequently, snoRNP biogenesis follows the same general principles for nearly all human snoRNAs. Still, snoRNP biogenesis differs slightly between C/D and H/ACA box snoRNAs. HG transcription yields a pre-mRNA that acts as a snoRNA precursor and forms a pre-snoRNP that protects the snoRNA precursor from excessive exonucleolytic degradation[46]. The formation of pre-snoRNPs is coupled to splicing for C/D box snoRNAs, and to RNA polymerase II transcription for H/ACA box snoRNAs[46]. In both cases pre-snoRNP formation is mediated by the R2TP chaperone complex[46]. Following intronic debranching, snoRNA precursors are exonucleolytically trimmed before being transported to the Cajal body[46,51]. During this transport the stripping of pre-snoRNPs of assembly factors results in mature C/D box snoRNPs, whereas H/ACA box snoRNPs require the final addition of GAR1 in the Cajal body[46]. Finally, with the exception of scaRNAs, the mature snoRNPs are shuttled from the Cajal body to the nucleolus by NOP140[46].

2. Functions of snoRNAs

2.1 Why modify?

Currently, the canonical function of snoRNAs is to guide the post-transcriptional modification of rRNAs. Most of the post-transcriptional rRNA modifications guided by snoRNAs are highly conserved and concentrated in functionally relevant regions of the ribosome, including the tRNA binding sites and the interaction surfaces of the ribosomal subunits[52,53]. This suggests an importance for proper post-transcriptional rRNA modification for normal ribosomal functioning in healthy cells. Indeed, loss of global pseudouridylation by inactivating DKC1 is lethal in yeast, fruit flies and mice[54–56]. Alternatively, loss of individual C/D box snoRNAs in yeast leads to growth defects upon adaptive stress compared to wild-type cells[57]. Of note, humans have more snoRNA-related rRNA modification sites than yeast and *Escherichia coli*[52]. Thus, the complexity of the post-transcriptional rRNA landscape scales with increasing organismal complexity, which may reflect an adaptive mechanism for translational fine tuning required to support the wide variety of cell identities in the human body. Interestingly, this translational fine tuning is one of the underlying ideas of the specialized ribosome hypothesis, which states that alterations in the post-transcriptional rRNA modification landscape or in the stoichiometry between ribosomal proteins contributes to a heterogeneous ribosome

pool[58]. This may ultimately contribute to disease states. For example, the rRNA modification landscape may be skewed in such a way so that one ribosomal species favors translation of oncogenic transcripts, while another ribosomal species disfavors translation of tumor suppressive transcripts. Although dysregulation of single rRNA modification sites has not yet been linked to a role in disease, multiple reports have demonstrated that global alterations to 2′-O-Methylation and pseudouridylation can impair translational fidelity and induce changes in the preference for IRES-mediated translation, potentially contributing to oncogenesis (reviewed in)[59].

In addition to guiding rRNA modifications, sca/snoRNAs guide the post-transcriptional modification of spliceosomal snRNAs[60]. Currently, there are 35 snoRNAs – primarily scaRNAs – with sequences complementary to snRNAs, although experimental validation is required for some of these targets[60]. As with rRNAs, the post-transcriptional modifications cluster in functionally relevant regions, and are likely to contribute to spliceosomal function by mediating snRNA secondary structure formation and stability, spliceosome assembly and pre-mRNA base pairing[60,61].

Finally, a select set of snoRNAs – often transcribed from independent promoters – are involved in rRNA maturation by guiding cleavage events and assisting in rRNA folding[36,47]. Importantly, snoRNA-guided modification at specific rRNA sites is thought to be required to facilitate these cleavage and folding events[36,47].

Thus, depending on the affected snoRNAs, deregulation of individual or multiple snoRNAs can have negative consequences for cellular functioning by affecting rRNA biogenesis, translation or splicing.

2.2 Venturing beyond the nucleolus

Many snoRNAs have yet unknown targets or functions and are termed ‘orphan’ snoRNAs[62]. Nonetheless, research over the last two decades has increasingly shown that snoRNAs have novel functions and targets spanning beyond those mentioned so far. For example, in addition to rRNAs, tRNAs have been found to be subject to snoRNA-guided modifications as well, and it has been suggested that mRNAs undergo the same process[63–65]. Alternatively, two C/D box snoRNAs in yeast have been shown to guide 18S rRNA acetylation by the cytidine acetyltransferase Kre33[66]. This suggests the existence of non-

canonical snoRNPs, since C/D and H/ACA box snoRNAs respectively associate with FBL and DKC1, neither of which have known acetyltransferase functions[62].

Many of the proposed non-canonical snoRNA functions are of a regulatory kind, although the precise mechanisms are sometimes unclear. For instance, snoRNAs can function as a pool for the generation of snoRNA-derived miRNAs, and this has been shown to provide a level of indirect regulation for Sortin-Nexin 27 expression in the case of the human U3 snoRNA[41]. SNORD50A has been shown to regulate mRNA 3' processing by modulating FIP1 interactions within the 3′ polyadenylation complex[67]. SNORD27 has been demonstrated to regulate alternative splicing of several genes, including the E2F7 transcription factor, likely by competing with spliceosomal snRNAs[68]. In addition, SNORD115 has been shown to mediate alternative splicing of serotonin receptor 5-HT_{2c} (*HTR2C*) pre-mRNA[69–71]. Interestingly, one study suggested that this may regulate the expression of miRNAs hosted by the *HTR2C* gene[72]. Experiments in yeast have shown that snoRNAs can modulate ribosomal activity in a stress-dependent manner through direct binding to ribosome-associated RNAs[73]. *In vitro* analyses of *Drosophila melanogaster* (fruit fly) chromatin extracts and cultured cells have demonstrated that snoRNAs, together with Decondensation factor 31 (Df31), contribute to an open chromatin state[74]. Studies of murine snoRNAs encoded by the *Rpl13a* gene have demonstrated regulatory roles for snoRNAs in metabolic pathways through yet unknown cytoplasmic mechanisms. These snoRNAs have been shown to regulate lipotoxic and oxidative stress and glucose metabolism in a manner that is independent of rRNA modification[75–77]. Furthermore, two other snoRNAs, snoRNA U60 and U17, have been shown to regulate intracellular cholesterol trafficking; for U60 the mechanism was only shown to be independent of rRNA methylation, while U17 was shown to regulate intracellular cholesterol trafficking by affecting HUMMR mRNA stability through direct binding[78,79]. Recently, snoRNAs have been proposed to indirectly self-regulate their expression through active export of snoRNAs in the form of lariats to the cytosol[80]. Indirect self-regulation has also been proposed to occur through yet unknown compensatory mechanisms between the C/D and H/ACA box snoRNAs, exemplified by the finding that decreased H/ACA snoRNA expression upon DKC1 ablation in mouse livers leads to increased C/D box snoRNA expression[81]. Intriguingly, circulating snoRNAs in extracellular vesicles have been shown to direct 2′-O-methylation in distant liver and intestinal tissue in mice, demonstrating a role for snoRNAs as messengers in cell-cell communication[82]. Lastly, snoRNAs are able to

directly interact with cytosolic proteins, as has been shown for K-RAS and the TRIM21-GMPS complex[83,84]. What is especially interesting is that some of the snoRNAs implicated in these non-canonical roles do have known rRNA target sites, demonstrating that one snoRNA can simultaneously operate canonically and non-canonically in the cell[67,68,75–78]. The above studies show that snoRNAs have more diverse functional modes in the cell than initially anticipated.

2.3 SnoRNAs in control of stemness, cell proliferation and differentiation

Since this writing assignment will focus on the snoRNA biology of human development and childhood disease, an introduction to the snoRNA biology of stemness, proliferation, differentiation, and development is in place.

It is becoming increasingly clear that snoRNAs contribute to stemness – the property of stem cells to self-renew and remain in an undifferentiated state[85]. For example, 63 snoRNAs have been found differentially expressed in senescent human bone marrow mesenchymal stem cells (hBSMCs) compared to low passage hBMSCs. This may indicate that some snoRNAs have a function in maintaining stemness[86]. Indeed, overexpression of SNORA7A has been shown to enhance the self-renewal capability of primary human umbilical cord mesenchymal stem cells (hUMSCs) in a snoRNP-dependent manner[87]. Accordingly, overexpression of SNORA7A in hUMSCs was shown to inhibit osteogenic differentiation whereas knockdown of SNORA7A was shown to promote osteogenic differentiation. These findings are consistent with a previous report which demonstrated that H/ACA snoRNPs can act as transcriptional co-activators of stemness-related genes[88]. Studies of the human ortholog of the *D. melanogaster* snoRNA *jouvence*, *h-jou*, have demonstrated that *h-jou* overexpression leads to increased proliferation of primary human vein umbilical cord stem cells (USCs)[89]. Conversely, small-interfering RNA - mediated knockdown of *h-jou* was shown to decrease the proliferation of USCs. Further transcriptomic analyses indicated that *h-jou* overexpression was correlated with an increased expression of dedifferentiation-associated genes in HCT116 cancer cells. These experiments therefore suggest that *h-jou* may contribute to stemness by inhibiting differentiation and by enhancing self-renewal. Interestingly, small ncRNA sequencing has revealed that snoRNAs are among the most highly expressed ncRNAs present in extracellular vesicles secreted by human pluripotent stem cells, hinting at a role for

snoRNAs in maintaining the stem cell niche or in lineage commitment[90]. Extracellular vesicles containing snoRNAs have been found in *D. melanogaster* as well[91]. Since *D. melanogaster* and humans diverged approximately 700 million years ago, this may suggest a conserved role for snoRNAs in cell-cell communication[92].

Other studies have focused on profiling snoRNA expression during cellular differentiation and proliferation. For instance, using an optimized RNA-seq analysis pipeline for the detection of small ncRNAs, Warner *et al*. showed that snoRNAs exhibit developmentally regulated expression patterns during hematopoiesis[93]. Accordingly, it has been demonstrated that snoRNAs are differentially expressed in macrophages undergoing terminal differentiation into polarized subtypes[94]. SnoRNAs are also differentially expressed during the differentiation of human induced pluripotent stem cells into hepatocyte-like cells[95]. Several reports have highlighted the potential role of snoRNAs in neuronal differentiation. For example, Skreka *et al*. have shown that eight C/D box snoRNAs are differentially expressed during differentiation of mouse embryonic stem cells (mESCs) into neuronal cells, which notably also includes a proliferative phase[96]. Another study focused on differential H/ACA snoRNA expression using two independent RNA-expression methods (small RNA-seq and NanoString nCounter), and similarly revealed that H/ACA snoRNAs are differentially expressed in mESCs undergoing retinoic-acid -induced neuronal differentiation. Interestingly, the study by Skreka *et al*. reported a ~100-fold change in expression of SNORD115 during neuronal differentiation, which is consistent with a more recent study demonstrating that SNORD115 expression increases during neuronal differentiation and is associated with changes in alternative splicing of serotonin receptor 5-HT_{2c} pre-mRNA[71]. Lastly, the expression of SNORD126 has been shown to increase in primary human adipose-derived stem cells differentiating into pre-adipocytes[97]. Additionally, overexpression of SNORD126 in mouse pre-adipocyte 3T3-L1 cells was shown to stimulate mitotic clonal expansion and differentiation.

These examples demonstrate that snoRNAs are involved in the development of cell identity. Further investigations of snoRNAs in stemness, proliferation and differentiation are required to obtain a better functional and mechanistic understanding of the snoRNA biology underlying these processes.

2.3.1 Lessons from flies, frogs, fish, and mice

Since developmental studies in model organisms essentially represent a model for cells collectively committing to differentiation and proliferation, such studies can also indirectly inform on the importance of snoRNAs in these processes. Of note, compared to other model organisms such as yeast and plants, *D. melanogaster* snoRNAs have a genomic organization that is highly similar to that of human snoRNAs[50]. Studies on snoRNAs in model organisms have mostly focused on levels of snoRNA expression and rRNA modifications during development, specifically 2′-O-methylation. For example, ribose methylation sequencing (Ribo-MethSeq) in *Xaenopus laevis* (frog) has shown that specific rRNA residues are differentially methylated during embryonal development, likely as a result of fluctuating snoRNA expression levels[98]. Furthermore, Ribo-MethSeq in *Danerio rerio* (zebrafish) has shown that 7 rRNA sites become increasingly methylated during development compared to 82 sites showing an invariable methylation pattern. This correlated with the expression of the 7 corresponding snoRNAs[99]. A comprehensive study in mouse employed Ribo-MethSeq to compare rRNA methylation levels between developing and adult tissues[100]. Interestingly, this revealed three distinct rRNA methylation patterns during brain development: increased, slightly increased, or decreased methylation. Additionally, rRNA methylation levels were generally lower in the developing tissues than in adult tissues, where residues are fully or close to fully methylated. The study further focused on SNORD78, which is encoded by the *gas5* gene, and demonstrated that decreasing SNORD78 expression during brain development correlates with decreasing methylation of its corresponding rRNA site. Lastly, snoRNAs have been shown to be dynamically expressed during development from embryo to adult in *D. melanogaster* and the nematode *Caenorhabditis elegans*, further pointing towards a role for snoRNAs in developmental processes such as differentiation[101,102]. Additional studies on snoRNA-guided pseudouridylation will be necessary to complement our current knowledge on the rRNA modification landscape during development. This has been made possible by the development of sequencing methods that allow transcriptome-wide assessment of pseudouridylated RNA moieties[64,103,104].

Functional studies have investigated changes in development or proliferation upon snoRNA perturbation. For example, knockdown of the U26, U44 or U78 C/D box snoRNAs in *D. rerio* has been demonstrated to result in severe developmental defects and

embryonic lethality[105]. Of note, knockdown of these snoRNAs was shown to lead to reduced rRNA methylation levels at the corresponding sites. In addition, excision of 16 snoRNAs encoded within the *Uhg1* locus in *D. melanogaster* was shown to result in delays in larval development[106]. Furthermore, deletion of the recently identified *D. melanogaster* snoRNA *jouvence* has been shown to increase the proliferation of enterocytes in young flies, which could be rescued by re-expressing the *jouvence* snoRNA[107]. Although undemonstrative of developmental changes, one study has demonstrated that the H/ACA box snoRNA U17 regulates intracellular cholesterol trafficking *in vivo* in post-natal developing mouse ovaries, affecting levels of pregnenolone and progesterone which in turn are important for ovarian development[79]. Related to a role for snoRNAs in female sexual development, three SNORD12 family members encoded in the *Zfas1* gene have been shown to be differentially expressed in developing mouse mammary gland tissue[108]. Moreover, the SNORD12 family members were differentially expressed in HC11 murine mammary epithelial cells stimulated for proliferation and differentiation.

Importantly, regulation of snoRNA expression during developmental processes appears to be tightly controlled. For instance, chromatin immunoprecipitation experiments in *D. melanogaster* have shown that the *Myc* proto-oncogene, an essential transcription factor during vertebrate development, directly regulates many snoRNAs encoded in translation-associated HGs[106]. Alternatively, snoRNA expression can be uncoupled from HG expression during development, for example through alternative splicing or by differences in structural stability between snoRNA family members[100,101,108,109]. Such regulatory mechanisms further strengthen the notion that snoRNAs play important roles during developmental processes.

The discussed studies in model organisms, together with the studies in the human context, collectively indicate that deregulated expression of snoRNAs may negatively affect stemness, proliferation and differentiation, promoting malignant phenotypes. In addition, model organisms provide powerful systems to directly study stemness, differentiation and proliferation and should be harnessed to leverage our understanding of human snoRNA biology.

3. SnoRNAs in human developmental disorders

It has become clear from the above studies in model organisms that snoRNAs appear to have critical functions in development. If and how snoRNAs contribute to developmental disorders will be the focus of this chapter. First, non-malignant developmental disorders will be discussed. Since we have also seen that snoRNAs are implicated in cellular programs commonly affected in cancers (i.e., stemness, differentiation, proliferation), pediatric malignancies will be considered in the second and last part of this chapter.

3.1 SnoRNAs in non-malignant developmental disorders

For this writing assignment, developmental disorders will be defined as diseases where defects in development result in health deficits that present at birth or that may start to appear during infancy. In essence, development starts at the moment a female oocyte is fertilized by male sperm and thus when two sets of chromosomes combine. In this regard, certain hereditary disorders can also be regarded as developmental disorders. The best-studied non-malignant developmental disorders in terms of snoRNA biology are Dyskeratosis Congenita (DC) and Prader-Willi syndrome.

3.1.1 Dyskeratosis Congenita

DC is a hereditary congenital disorder in which patients typically present with oral lesions, abnormal skin pigmentation and dysplastic finger- and toenail growth[110]. More importantly, DC patients are predisposed to develop liver and pulmonary disease, solid tumors, hematological malignancies and bone marrow failure[110]. Approximately 12% of patients do not make it past age 20, with bone marrow failure still being the primary cause of death[110,111]. The X-linked form of DC (X-DC) is primarily caused by missense mutations in the coding sequence of *DKC1*, which alongside its role as the pseudouridine synthase in the H/ACA snoRNP, is part of the telomerase H/ACA RNP through association with the H/ACA motif in human telomerase RNA component (TERC)[112–114]. The autosomal recessive variant of DC is associated with mutations in other H/ACA snoRNP components that are also part of the telomerase H/ACA RNP, including NOP10 and NHP2[115,116]. Mutations in the telomerase H/ACA RNP components lead to reduced telomerase activity, assembly and stability, which contribute to the premature shortening of telomeres thought to underlie the DC phenotype[117–120]. The fact that H/ACA snoRNP components are

mutated in DC suggests that in addition to impairing telomerase function, these mutations may contribute to the DC phenotype by affecting rRNA pseudouridylation through impaired H/ACA snoRNP functioning. Indeed, further supporting this hypothesis is the observation that mutations affecting the catalytic pseudouridylation domain of DKC1 result in a more severe phenotype in a subtype of DC called the Hoyeraal-Hreidarsson (HH) syndrome[111]. Although the precise functions of snoRNAs in (X-)DC remain elusive, many efforts have been made to elucidate the contribution of snoRNAs and post-transcriptional rRNA modifications to DC.

From 1999 to 2011, four studies reported on the link between DKC1 mutations, snoRNA expression, and rRNA pseudouridylation in patient X-DC cells. Collectively these studies showed that snoRNA expression and rRNA pseudouridylation are not altered in patient X-DC cells, although the number of snoRNAs analyzed was limited to three to four snoRNAs[114,119,121,122]. However, later studies in mice contrasted these findings. For example, it was shown that a hypomorphic *Dkc1* allele phenocopies human X-DC symptoms in early mouse generations and results in a 10-40% reduction in pseudouridylation of 18S and 28S rRNA in primary lymphocytes. In contrast, telomere lengths remained unchanged, suggesting that the phenotype was due to decreased rRNA pseudouridylation[123]. However, it was unclear whether the observed decrease in rRNA pseudouridylation was due to decreased Dkc1 catalytic activity, altered H/ACA snoRNA expression, or both. Of note, mice form an interesting model to study the role of snoRNAs and rRNA modifications in X-DC. They have significantly longer telomeres than humans and as a result are not affected by telomerase defects until after several generations. Therefore, any X-DC symptoms observed in earlier generations are more likely to be the result of defects in snoRNA expression and rRNA modifications[123–125]. Further mouse studies by Mason and colleagues continued to shed light on the role of snoRNAs in X-DC. Using mouse embryonic stem (ES) cells harboring the human A353V or G402E mutations in Dkc1, they demonstrated that the expression of 10 H/ACA snoRNAs was variably affected depending on the Dkc1 mutation[120]. In addition, both mutations resulted in decreased total pseudouridylation of 18S and 28S rRNA. Furthermore, it was shown that lysates from A353V mutant ES cells displayed a reduced capacity to pseudouridylate a 28S rRNA target site corresponding to one of the snoRNAs that was downregulated in these cells. Interestingly, while both the A353V and G402E mutations affected snoRNA expression and rRNA pseudouridylation, telomere lengths and telomerase function were

affected only in the A353V mutant. This would imply that in G402E mutant mice, DC symptoms can arise due to defects in rRNA pseudouridylation and snoRNA expression independent of telomere defects, given the G402E mutation is associated with clinical symptoms in humans[112]. However, it was later shown that the G402E mutation does not recapitulate X-DC symptoms in mice, possibly due to structural differences between the human and mouse Dkc1 enzymes[124]. Mason and colleagues attempted to generate an X-DC model with mice harboring a Dkc1 mutant lacking exon 15 (Dkc1Δ15)[126]. Although this mutation also failed to phenocopy the disease, they observed that ES cells derived from these mice showed a decreased expression of two out of the six snoRNAs examined. Analysis of the distribution of wild type versus mutant cells in the hematopoietic tissues of heterozygous female mice revealed that the mutant cells had a proliferative disadvantage. While the authors reported that this phenotype was dependent on the association of Dkc1Δ15 with active telomerase, no controls were included to exclude the contribution of aberrant snoRNA expression or rRNA pseudouridylation to this phenotype.

Thus, to summarize the mouse studies on snoRNAs in X-DC: first, attempts to phenocopy X-DC in mice by introducing human DKC1 mutations have not yet been successful, which makes it challenging to study the exact contribution of snoRNAs to X-DC. Second, the studies in mice contrast the earlier studies done with patient X-DC cells, demonstrating that cells with DKC1 mutations do in fact display altered levels of snoRNA expression and rRNA pseudouridylation defects. Indeed, these findings have been further corroborated in three studies using human X-DC cells and which employed more sensitive methods for the detection of rRNA pseudouridylation differences.

In one study, Taoka *et al*. mapped the complete landscape of rRNA modifications in the human ribosome[52]. Using quantitative mass spectrometry, they measured the relative abundance of rRNA pseudouridylation in primary cells from 7- to 19-year-old X-DC patients with four distinct DKC1 mutations (delL37, T66A, A353V or A386T) and compared it to unaffected cells from relatives. In all four mutational backgrounds two sites in the 28S rRNA were found to have decreased pseudouridylation levels. However, the expression of the corresponding snoRNAs was not investigated. Indeed, one shortfall of the X-DC studies discussed so far is that although reductions in rRNA pseudouridylation levels have been reported in cells with mutations in DKC1, it is unknown whether this is

due to altered activity of DKC1, decreased H/ACA snoRNA expression or both. A study by Bellodi *et al*. provided more insight on this front by analyzing a larger panel of snoRNAs and by quantifying pseudouridylation levels at corresponding 18S rRNA sites using mass spectrometry[127]. In primary cells from X-DC patients with four distinct mutational backgrounds (delL37, T66A, A386T, or c.-141C>G), it was demonstrated that the expression of 27 H/ACA snoRNAs was generally decreased, and for 12 of these snoRNAs a reduction in 18S rRNA pseudouridylation was observed at their corresponding target sites. Notably, two scaRNAs that are involved in the post-transcriptional modification of spliceosomal snRNAs were consistently downregulated across three mutational backgrounds and two different tissues. This suggests that snRNAs with aberrant post-transcriptional modification levels may also contribute to X-DC.

While the precise roles of snoRNAs in the X-linked form of DC remain unclear, a recent landmark study by Nachmani *et al*. proposes a mechanistic explanation for the contribution of C/D box snoRNAs to a form of DC characterized by *NPM1* mutations[128]. It was shown that mutant NPM1, as part of a C/D box snoRNP complex, has a reduced capacity to bind C/D box snoRNAs leading to reduced 2'-O-Methylation in 28S rRNA at five specific sites. This was shown to impair ribosomal function, ultimately resulting in hematopoietic deficiencies characteristic of DC. Importantly, NPM1 mutant mice perfectly recapitulated DC symptoms. Interestingly, this study also highlighted the possibility of non-canonical C/D box snoRNP complexes. Thus, aberrant post-transcriptional rRNA modifications guided by snoRNAs appear to be common players in both X-linked and non -X-linked forms of DC.

3.1.2 Prader-Willi syndrome

A well-characterized developmental disease in terms of snoRNA biology is Prader-Willi syndrome (PWS). PWS is a congenital hereditary disorder in which affected infants present with a wide range of symptoms throughout development, including intellectual disabilities, behavioral problems, hypogonadism, reduced growth hormone levels, decreased muscle tone, and a high risk of developing obesity[129]. At the genetic level, around 65-75% of PWS cases are characterized by (micro)deletions in the paternal 15q11-q13 locus[130]. Among other genes, this locus harbors the *SNURF-SNRPN* gene, which hosts six individually encoded C/D box snoRNAs as well as two C/D box snoRNA clusters,

SNORD116 (29 copies) and SNORD115 (48 copies)[131]. Interestingly, the expression of these snoRNAs is the highest in the brain when compared across 20 human tissues, which favors a model where loss of these snoRNAs contributes to the mental impairments seen in PWS patients[132]. Indeed, genetic analyses of patients with microdeletions affecting specific snoRNAs in 15q11-q13 has already provided clues for the significance of snoRNAs in PWS. For example, SNORD115 has already been excluded as a candidate gene contributing to PWS, as familial analysis has demonstrated that loss of the SNORD115 cluster alone does not result in PWS[133,134]. This is in contrast to what other studies have suggested, as SNORD115 and its processing products were shown to regulate alternative splicing of 5-HTC2 serotonin receptor pre-mRNA and serotonergic deregulation has been implicated in the PWS pathology[135–137]. However, it was later shown that alternative splicing of 5-HT2C pre-mRNA is not altered in C57L/6J mice lacking a paternal SNORD115 allele[138]. Moreover, loss of SNORD115 alone in mice does not cause any PWS symptoms, thus confirming the initial findings in humans[138]. Thus, SNORD115 involvement in PWS is highly unlikely. It should be noted that PWS mouse models are reliable for studying the contribution of each gene from the paternal 15q11-q13 locus. This is exemplified by the fact that mice lacking paternal expression of the '*PWS* region' of genes in 15q11-q13, which include the snoRNAs, suffer from postnatal PWS symptoms similar to humans, although loss of the locus in mice is associated with lethality approximately one week after birth[139].

The snoRNA cluster upstream of SNORD115, SNORD116, holds more promise to be an important contributor to PWS. Several studies from families and in mice have provided strong evidence that SNORD116 snoRNAs are major, if not causative, factors contributing to PWS. The most conclusive of these reports are three case studies of patients with microdeletions involving the paternal SNORD116 allele[140–142]. These patients displayed many of the major PWS symptoms, including decreased muscle tone, obesity and hypogonadism. Unsurprisingly, then, the role of SNORD116 in PWS has also been extensively investigated in mice (reviewed in)[143]. Key examples are two independent mouse models, *Snord116del* and *PWScr*$^{m+/p-}$, where the paternal SNORD116 allele was deleted without affecting the expression of the surrounding genes[144,145]. Indeed, *Snord116del* mice showed a phenotype that was in good agreement with that of PWS in humans, while the *PWScr*$^{m+/p-}$ model resulted only in severe growth retardation. How SNORD116 could contribute to PWS remained elusive up until 2021, as there had been no

known targets sites for the snoRNAs of this cluster. Through a phylogenetic analysis of SNORD116 sequences across 16 mammalian species, Baldini *et al*. revealed the mRNAs of Neuroligin 3 (Nlgn3), Diacylglycerol kinase kappa (Dgkk) and Round spermatid basic protein 1 like (Rsbn1l) as potential targets for SNORD116 snoRNAs[131]. Knocking down SNORD116 in HeLa S3 cells was shown to increase the mRNA expression of these genes. Interestingly, increased exon 3 inclusion was also observed in the case of *Nlgn3*, which was further confirmed in a vector-based splicing assay. Thus, a possible function for SNORD116 in PWS could be that loss of SNORD116 contributes to aberrant mRNA expression of not one but multiple targets, and that it increases exclusion of exon 3 of *Nlgn3*. It will be interesting to see if this hypothesis holds true in *Snord116del* and *PWScr*$^{m+/p}$ PWS mouse models.

In summary, the aforementioned studies have made it abundantly clear that the SNORD116 cluster, but not the SNORD115 cluster, is a major contributor to PWS.

3.2 Pediatric cancer as a developmental disorder

Since cancers can arise as early as infancy (<1 year), it is not hard to imagine that mutations causing or predisposing infants and children to cancer already accumulate *in utero*. In this sense, childhood malignancies could be understood as developmental disorders. There is considerable evidence supporting this view (reviewed extensively by Marshall *et al*.)[146]. The most notable examples of pediatric cancers that are thought to have origins during *in utero* development are neuroblastoma, B-lineage acute lymphoblastic leukemia (B-ALL), medulloblastoma (cancer of the cerebellum) and myeloid leukemia in Down syndrome patients[146]. Convincing evidence comes from studies on pediatric leukemias where genetic tracing of leukemic cells in monozygotic twins can reveal clonal origins of mutations[147]. For example, by using whole genome sequencing of two twin pairs with concordant ALL, Ma *et al*. showed that twin pairs had a number of shared 'first hit' leukemic mutations, including the prevalent *ETV6-RUNX1* fusion[148]. These were distinct from the 'secondary hit' mutations identified in each individual twin. Tracing fusion genes provides a good measure for clonality since the breakpoints are highly variable in unrelated patients despite a functionally similar fusion gene product[147]. However, it should be noted that such studies still rest on the assumption that leukemic mutations shared between twins arise *in utero* and are not obtained

through germline transmission from the parent. Another notable example are the retinoblastomas, where *RB* gene inactivation required for tumorigenesis is thought to occur during retinal progenitor cell proliferation, which only occurs during the fetal stages of development[149]. Consistent with this model, retinoblastomas have been diagnosed in premature twin babies[150]. These examples show that childhood malignancies can be considered as developmental diseases.

3.3 SnoRNAs in pediatric cancer

In 2017, a seminal systematic pan-cancer analysis of 10.000 cancer samples revealed that snoRNA expression is deregulated in 31 cancer types[151]. Although some functional insights had already been obtained with regards to the involvement of snoRNAs in various cancer types, this study revealed that deregulated snoRNA biology may play a more ubiquitous role in cancers than initially anticipated[151]. Indeed, there is currently a growing body of literature on the expression and functional characterization of snoRNAs in cancers. Wu *et al*. systematically reviewed all snoRNA-associated phenotypes observed in functional and mechanistical studies across 14 cancer types and found that deregulated snoRNA expression is functionally associated with nine cancer biology themes, including self-renewal, proliferation and migration[152]. In spite of this surge in snoRNA studies, there have been only few studies specifically aimed at investigating the roles of snoRNAs in pediatric cancers, and the 2017 pan-cancer snoRNA expression analysis did not stratify snoRNA expression profiles by age[151]. Of note, the distinction between adult and pediatric cancer is a necessary one, given how different the mutational backgrounds are; pediatric cancers are characterized by an estimated 14-fold lower mutational burden than cancers affecting adults, and therefore the underlying pathology should be regarded separate from adults[153].

Currently there are three studies that have directly investigated snoRNAs in pediatric cancers. In one study, gene regulatory network analysis of expression profiling cohorts of high-risk pediatric and adult acute myeloid leukemias (AML) revealed that three RNA binding proteins are upregulated and interact with 103 upregulated RNA targets, three of which were snoRNAs[154]. However, this was only found in the adult high-risk AML cohort. This finding highlights that even on the ncRNA level, pediatric and adult forms of AML are likely to be molecularly distinct[154]. Another study performed a genome-wide small ncRNA

profiling screen of 14 pediatric high-grade gliomas (brain tumors) and identified 118 upregulated snoRNAs and 39 snoRNAs that were downregulated[155]. Interestingly, the latter group included 36 snoRNAs from the SNORD115 cluster, which showed distinct expression profiles depending on *H3F3A* and *TP53* mutational status. Finally, deep RNA sequencing of 20 neuroblastoma (a childhood brain cancer) samples with or without *MYCN* amplification showed that, surprisingly, snoRNAs were the second-most abundant transcript class next to mRNAs[156]. In addition, snoRNAs were among the differentially regulated genes that could stratify the neuroblastoma samples by *MYCN* amplification status and, except for one sample, by *MYCN*-associated survival. Furthermore, three snoRNAs (SNORA76, SNORD6, SNORD77) were identified as potential *MYCN* targets, suggesting a yet unknown regulatory axis between *MYCN* and these snoRNAs[156].

4. Conclusion

SnoRNAs are a class of small ncRNA molecules whose canonical role as guiders of post-transcriptional rRNA modifications became apparent throughout the late 20th century. Intriguingly, it has become clear that snoRNAs may adopt roles in the cell that span beyond the modification of rRNAs, ranging from the regulation of alternative splicing to mediating open chromatin states, and, even further, cell-cell communication. Such alternative modes of snoRNA functioning should be considered in future investigations of snoRNAs. Moreover, there may be diseases related to these non-canonical functions where snoRNAs have still been poorly investigated. For example, the findings that snoRNAs can regulate intracellular cholesterol trafficking and glucose metabolism suggest that snoRNAs may have a role in certain metabolic disorders, such as type 2 diabetes[75,78,79,157].

The discussed studies in model organisms have indicated that snoRNAs are tightly involved in organismal development, suggesting that deregulation of snoRNAs can contribute to human developmental disorders. Indeed, snoRNAs are among the main molecular targets of investigation in the childhood developmental disorders DC and PWS. Loss of SNORD116 is likely the primary factor underlying PWS, whereas the role of deregulated snoRNAs in DC appears more accessory. It has also become clear that snoRNAs are involved in the cellular programs commonly affected in cancer, including stemness, differentiation, and proliferation. However, despite the fact that snoRNAs have been broadly investigated in cancers affecting adults, research aimed at investigating the

role of snoRNAs in pediatric cancers is still at an immature stage. Differences in the molecular pathology between pediatric and adult cancers call for a separate approach to investigate this group of patients, which will hopefully pave the way to the discovery of novel therapeutic targets.

References

1. Hall, B. D. & Doty, P. The preparation and physical chemical properties of ribonucleic acid from microsomal particles. *J. Mol. Biol.* **1**, 111–126 (1959).
2. Hoagland, M. B., Stephenson, M. L., Scott, J. F., Hecht, L. I. & Zamecnik, P. C. A SOLUBLE RIBONUCLEIC ACID INTERMEDIATE IN PROTEIN SYNTHESIS. *J. Biol. Chem.* **231**, 241–257 (1958).
3. Littauer, U. Z. & Eisenberg, H. Ribonucleic acid from Escherichia coli preparation, characterization and physical properties. *Biochim. Biophys. Acta* **32**, 320–337 (1959).
4. Timasheff, S. N., Brown, R. A., Colter, J. S. & Davies, M. The molecular weight of ribonucleic acid prepared from ascites-tumor cells. *Biochim. Biophys. Acta* **27**, 662–663 (1958).
5. Higashi, K., Shankar, K., Adams, H. R. & Busch, H. Utilization of the Citric Acid Procedure and Zonal Ultracentrifugation for Mass Isolation of Nuclear RNA from Walker 256 Carcinosarcoma. *Cancer Res.* **26**, 1582–1590 (1966).
6. Peacock, A. C. & Dingman, C. W. Resolution of Multiple Ribonucleic Acid Species by Polyacrylamide Gel Electrophoresis *. *Biochemistry* **6**, 1818–1827 (1967).
7. Nakamura, T., Prestayko, A. W. & Busch, H. Studies on Nucleolar 4 to 6 S Ribonucleic Acid of Novikoff Hepatoma Cells. *J. Biol. Chem.* **243**, 1368–1375 (1968).
8. Muramatsu, M., Hodnett, J. L. & Busch, H. Base Composition of Fractions of Nuclear and Nucleolar Ribonucleic Acid Obtained by Sedimentation and Chromatography. *J. Biol. Chem.* **241**, 1544–1550 (1966).
9. Pene, J. J., Knight, E. & Darnell, J. E. Characterization of a new low molecular weight RNA in HeLa cell ribosomes. *J. Mol. Biol.* **33**, 609–623 (1968).
10. Prestayko, A. W. & Busch, H. Low molecular weight RNA of the chromatin fraction from Novikoff hepatoma and rat liver nuclei. *Biochim. Biophys. Acta BBA - Nucleic Acids Protein Synth.* **169**, 327–337 (1968).
11. Hodnett, J. L. & Busch, H. Isolation and Characterization of Uridylic Acid-rich 7 S Ribonucleic Acid of Rat Liver Nuclei. *J. Biol. Chem.* **243**, 6334–6342 (1968).
12. Weinberg, R. A. & Penman, S. Small molecular weight monodisperse nuclear RNA. *J. Mol. Biol.* **38**, 289–304 (1968).
13. Prestayko, A. W., Tonato, M. & Busch, H. Low molecular weight RNA associated with 28 s nucleolar RNA. *J. Mol. Biol.* **47**, 505–515 (1970).
14. Prestayko, A. W., Tonato, M., Lewis, B. C. & Busch, H. Heterogeneity of Nucleolar U3 Ribonucleic Acid of the Novikoff Hepatoma. *J. Biol. Chem.* **246**, 182–187 (1971).
15. Busch, H. *et al.* Low-Molecular-Weight Nuclear RNAs. *Perspect. Biol. Med.* **15**, 117–139 (1971).
16. Lischwe, M. A. *et al.* Purification and partial characterization of a nucleolar scleroderma antigen (Mr = 34,000; pI, 8.5) rich in NG,NG-dimethylarginine. *J. Biol. Chem.* **260**, 14304–14310 (1985).
17. Lerner, M. R. & Steitz, J. A. Snurps and scyrps. *Cell* **25**, 298–300 (1981).
18. Susan Kass, Kazimierz Tyc, Joan A. Steitz, & Barbars Sollner-Webb. The U3 Small Nucleolar Ribonucleoprotein Functions in the First Step of Preribosomal RNA Processing. *Cell* **60**, 897–908 (1990).
19. Mougey, E. B. & Pape, L. K. A U3 Small Nuclear Ribonucleoprotein-Requiring Processing Event in the 5' External Transcribed Spacer of Xenopus. *MOL CELL BIOL* **13**, 9 (1993).
20. Savino, R. & Gerbi, S. A. In vivo disruption of Xenopus U3 snRNA affects ribosomal RNA processing. *EMBO J.* **9**, 2299–2308 (1990).

21. Hughes, J. M. X. Functional Base-pairing Interaction Between Highly Conserved Elements of U3 Small Nucleolar RNA and the Small Ribosomal Subunit RNA. *J. Mol. Biol.* **259**, 645–654 (1996).
22. Hughes, J. M. & Ares, M. Depletion of U3 small nucleolar RNA inhibits cleavage in the 5′ external transcribed spacer of yeast pre-ribosomal RNA and impairs formation of 18S ribosomal RNA. *EMBO J.* **10**, 4231–4239 (1991).
23. Beltrame, M. & Tollervey, D. Identification and functional analysis of two U3 binding sites on yeast pre-ribosomal RNA. *EMBO J.* **11**, 1531–1542 (1992).
24. Beltrame, M., Henry, Y. & Tollervey, D. Mutational analysis of an essential binding site for the U3 snoRNA in the 5' external transcribed spacer of yeast pre-rRNA. *Nucleid Acids Res.* **22**, 4057–4065 (1994).
25. R.L. Ochs, M.A. Lischwe, W.H. Spohn, & H. Busch. Fibrillarin: a new protein of the nucleolus identified by autoimmune sera. *Biol. Cell* **54**, 123–133 (1985).
26. Guyang M. Huang, Artur Jarmolowski, Joachim C.R. Struck, & Maurille J. Fournier. Accumulation of U14 Small Nuclear RNA in *Saccharomyces cerevisiae* Requires Box C, Box D, and a 5', 3' Terminal Stem. *Mol. Cell. Biol.* **12**, 4456–4463 (1992).
27. Baserga, S. J., Yang, X. D. & Steitz, J. A. An intact Box C sequence in the U3 snRNA is required for binding of fibrillarin, the protein common to the major family of nucleolar snRNPs. *EMBO J.* **10**, 2645–2651 (1991).
28. Tyc, K. & Steitz, J. A. U3, U8 and U13 comprise a new class of mammalian snRNPs localized in the cell nucleolus. *EMBO J.* **8**, 3113–3119 (1989).
29. Tollervey, D., Lehtonen, H., Carmo-Fonseca, M. & Hurt, E. C. The small nucleolar RNP protein NOP1 (fibrillarin) is required for pre-rRNA processing in yeast. *EMBO J.* **10**, 573–583 (1991).
30. Kiss-László, Z., Henry, Y., Bachellerie, J.-P., Caizergues-Ferrer, M. & Kiss, T. Site-Specific Ribose Methylation of Preribosomal RNA: A Novel Function for Small Nucleolar RNAs. *Cell* **85**, 1077–1088 (1996).
31. Ganot, P., Caizergues-Ferrer, M. & Kiss, T. The family of box ACA small nucleolar RNAs is defined by an evolutionarily conserved secondary structure and ubiquitous sequence elements essential for RNA accumulation. *Genes Dev.* **11**, 941–956 (1997).
32. Ganot, P., Bortolin, M.-L. & Kiss, T. Site-Specific Pseudouridine Formation in Preribosomal RNA Is Guided by Small Nucleolar RNAs. *Cell* **89**, 799–809 (1997).
33. Jingwei Ni, Amy L. Tien, & Maurille J. Fournier. Small Nucleolar RNAs Direct Site-Specific Synthesis of Pseudouridine in Ribosomal RNA. *Cell* **89**, 565–573 (1997).
34. Nicoloso, M., Qu, L.-H., Michot, B. & Bachellerie, J.-P. Intron-encoded, Antisense Small Nucleolar RNAs: The Characterization of Nine Novel Species Points to Their Direct Role as Guides for the 2′-O-ribose Methylation of rRNAs. *J. Mol. Biol.* **260**, 178–195 (1996).
35. Lowe, T. M. & Eddy, S. R. A Computational Screen for Methylation Guide snoRNAs in Yeast. *Science* **283**, 1168–1171 (1999).
36. Ojha, S., Malla, S. & Lyons, S. M. snoRNPs: Functions in Ribosome Biogenesis. *Biomolecules* **10**, 783 (2020).
37. Jorjani, H. *et al.* An updated human snoRNAome. *Nucleic Acids Res.* **44**, 5068–5082 (2016).
38. Dupuis-Sandoval, F., Poirier, M. & Scott, M. S. The emerging landscape of small nucleolar RNAs in cell biology. *Wiley Interdiscip. Rev. RNA* **6**, 381–397 (2015).
39. Bergeron, D. *et al.* SnoRNA copy regulation affects family size, genomic location and family abundance levels. *BMC Genomics* **22**, 414 (2021).
40. Bouchard-Bourelle, P. *et al.* snoDB: an interactive database of human snoRNA sequences, abundance and interactions. *Nucleic Acids Res.* **48**, D220–D225 (2020).
41. Lemus-Diaz, N., Ferreira, R. R., Bohnsack, K. E., Gruber, J. & Bohnsack, M. T. The

human box C/D snoRNA U3 is a miRNA source and miR-U3 regulates expression of sortin nexin 27. *Nucleic Acids Res.* **48**, 8074–8089 (2020).
42. Zhong, F. *et al.* A SnoRNA-derived piRNA interacts with human interleukin-4 pre-mRNA and induces its decay in nuclear exosomes. *Nucleic Acids Res.* gkv954 (2015) doi:10.1093/nar/gkv954.
43. Światowy, W. & Jagodziński, P. P. Molecules derived from tRNA and snoRNA: Entering the degradome pool. *Biomed. Pharmacother.* **108**, 36–42 (2018).
44. Reichow, S. L., Hamma, T., Ferre-D'Amare, A. R. & Varani, G. The structure and function of small nucleolar ribonucleoproteins. *Nucleic Acids Res.* **35**, 1452–1464 (2007).
45. van Nues, R. W. *et al.* Box C/D snoRNP catalysed methylation is aided by additional pre-rRNA base-pairing: Extended snoRNA-rRNA base-pairing. *EMBO J.* **30**, 2420–2430 (2011).
46. Massenet, S., Bertrand, E. & Verheggen, C. Assembly and trafficking of box C/D and H/ACA snoRNPs. *RNA Biol.* **14**, 680–692 (2017).
47. Watkins, N. J. & Bohnsack, M. T. The box C/D and H/ACA snoRNPs: key players in the modification, processing and the dynamic folding of ribosomal RNA: Box C/D and H/ACA snoRNPs. *Wiley Interdiscip. Rev. RNA* **3**, 397–414 (2012).
48. Baldini, L., Charpentier, B. & Labialle, S. Emerging Data on the Diversity of Molecular Mechanisms Involving C/D snoRNAs. *Non-Coding RNA* **7**, 30 (2021).
49. Kufel, J. & Grzechnik, P. Small Nucleolar RNAs Tell a Different Tale. *Trends Genet.* **35**, 104–117 (2019).
50. Dieci, G., Preti, M. & Montanini, B. Eukaryotic snoRNAs: A paradigm for gene expression flexibility. *Genomics* **94**, 83–88 (2009).
51. Berndt, H. *et al.* Maturation of mammalian H/ACA box snoRNAs: PAPD5-dependent adenylation and PARN-dependent trimming. *RNA* **18**, 958–972 (2012).
52. Taoka, M. *et al.* Landscape of the complete RNA chemical modifications in the human 80S ribosome. *Nucleic Acids Res.* **46**, 9289–9298 (2018).
53. Garus, A. & Autexier, C. Dyskerin: an essential pseudouridine synthase with multifaceted roles in ribosome biogenesis, splicing, and telomere maintenance. *RNA* **27**, 1441–1458 (2021).
54. Giordano, E., Peluso, I., Senger, S. & Furia, M. minifly, A Drosophila Gene Required for Ribosome Biogenesis. *J. Cell Biol.* **144**, 1123–1133 (1999).
55. Jiang, W., Middleton, K., Yoon, H.-J., Fouquet, C. & Carbon, J. An Essential Yeast Protein, CBF5p, Binds In Vitro to Centromeres and Microtubules. *MOL CELL BIOL* **13**, (1993).
56. He, J. *et al.* Targeted disruption of Dkc1, the gene mutated in X-linked dyskeratosis congenita, causes embryonic lethality in mice. *Oncogene* **21**, 7740–7744 (2002).
57. Esguerra, J., Warringer, J. & Blomberg, A. Functional importance of individual rRNA 2′- *O* -ribose methylations revealed by high-resolution phenotyping. *RNA* **14**, 649–656 (2008).
58. Norris, K., Hopes, T. & Aspden, J. L. Ribosome heterogeneity and specialization in development. *WIREs RNA* **12**, (2021).
59. Pelletier, J., Thomas, G. & Volarević, S. Ribosome biogenesis in cancer: new players and therapeutic avenues. *Nat. Rev. Cancer* **18**, 51–63 (2018).
60. Bohnsack, M. T. & Sloan, K. E. Modifications in small nuclear RNAs and their roles in spliceosome assembly and function. *Biol. Chem.* **399**, 1265–1276 (2018).
61. Karijolich, J. & Yu, Y.-T. Spliceosomal snRNA modifications and their function. *RNA Biol.* **7**, 192–204 (2010).
62. Bratkovič, T., Božič, J. & Rogelj, B. Functional diversity of small nucleolar RNAs. *Nucleic Acids Res.* **48**, 1627–1651 (2020).
63. Vitali, P. & Kiss, T. Cooperative 2′-O-methylation of the wobble cytidine of human

elongator tRNA ^{Met} (CAT) by a nucleolar and a Cajal body-specific box C/D RNP. *Genes Dev.* **33**, 741–746 (2019).
64. Schwartz, S. *et al.* Transcriptome-wide Mapping Reveals Widespread Dynamic-Regulated Pseudouridylation of ncRNA and mRNA. *Cell* **159**, 148–162 (2014).
65. Carlile, T. M. *et al.* Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. *Nature* **515**, 143–146 (2014).
66. Sharma, S. *et al.* Specialized box C/D snoRNPs act as antisense guides to target RNA base acetylation. *PLOS Genet.* **13**, e1006804 (2017).
67. Huang, C. *et al.* A snoRNA modulates mRNA 3′ end processing and regulates the expression of a subset of mRNAs. *Nucleic Acids Res.* **45**, 8647–8660 (2017).
68. Falaleeva, M. *et al.* Dual function of C/D box small nucleolar RNAs in rRNA modification and alternative pre-mRNA splicing. *Proc. Natl. Acad. Sci.* **113**, (2016).
69. Cavaillé, J. *et al.* Identification of brain-specific and imprinted small nucleolar RNA genes exhibiting an unusual genomic organization. *Proc. Natl. Acad. Sci.* **97**, 14311–14316 (2000).
70. Kishore, S. & Stamm, S. The snoRNA HBII-52 Regulates Alternative Splicing of the Serotonin Receptor 2C. *Science* **311**, 230–232 (2006).
71. Bratkovič, T., Modic, M., Camargo Ortega, G., Drukker, M. & Rogelj, B. Neuronal differentiation induces SNORD115 expression and is accompanied by post-transcriptional changes of serotonin receptor 2c mRNA. *Sci. Rep.* **8**, 5101 (2018).
72. Zhang, Z. *et al.* The 5′ untranslated region of the serotonin receptor 2C pre-mRNA generates miRNAs and is expressed in non-neuronal cells. *Exp. Brain Res.* **230**, 387–394 (2013).
73. Mleczko, A. M. *et al.* Levels of sdRNAs in cytoplasm and their association with ribosomes are dependent upon stress conditions but independent from snoRNA expression. *Sci. Rep.* **9**, 18397 (2019).
74. Schubert, T. & Längst, G. Changes in higher order structures of chromatin by RNP complexes. *RNA Biol.* **10**, 175–179 (2013).
75. Lee, J. *et al.* Rpl13a small nucleolar RNAs regulate systemic glucose metabolism. *J. Clin. Invest.* **126**, 4616–4625 (2016).
76. Michel, C. I. *et al.* Small Nucleolar RNAs U32a, U33, and U35a Are Critical Mediators of Metabolic Stress. *Cell Metab.* **14**, 33–44 (2011).
77. Holley, C. L. *et al.* Cytosolic Accumulation of Small Nucleolar RNAs (snoRNAs) Is Dynamically Regulated by NADPH Oxidase. *J. Biol. Chem.* **290**, 11741–11748 (2015).
78. Brandis, K. A. *et al.* Box C/D Small Nucleolar RNA (snoRNA) U60 Regulates Intracellular Cholesterol Trafficking. *J. Biol. Chem.* **288**, 35703–35713 (2013).
79. Jinn, S. *et al.* snoRNA U17 Regulates Cellular Cholesterol Trafficking. *Cell Metab.* **21**, 855–867 (2015).
80. Talross, G. J. S., Deryusheva, S. & Gall, J. G. Stable lariats bearing a snoRNA (slb-snoRNA) in eukaryotic cells: A level of regulation for guide RNAs. *Proc. Natl. Acad. Sci.* **118**, e2114156118 (2021).
81. Ge, J., Crosby, S. D., Heinz, M. E., Bessler, M. & Mason, P. J. SnoRNA microarray analysis reveals changes in H/ACA and C/D RNA levels caused by dyskerin ablation in mouse liver. *Biochem. J.* **429**, 33–41 (2010).
82. Rimer, J. M. *et al.* Long-range function of secreted small nucleolar RNAs that direct 2′-O-methylation. *J. Biol. Chem.* **293**, 13284–13296 (2018).
83. Siprashvili, Z. *et al.* The noncoding RNAs SNORD50A and SNORD50B bind K-Ras and are recurrently deleted in human cancer. *Nat. Genet.* **48**, 53–58 (2016).
84. Su, X. *et al.* The noncoding RNAs SNORD50A and SNORD50B-mediated TRIM21-GMPS interaction promotes the growth of p53 wild-type breast cancers by degrading p53.

Cell Death Differ. **28**, 2450–2464 (2021).
85. Aponte, P. M. & Caicedo, A. Stemness in Cancer: Stem Cells, Cancer Stem Cells, and Their Microenvironment. *Stem Cells Int.* **2017**, 1–17 (2017).
86. Xiao, F. *et al.* Small Noncoding RNAome Changes During Human Bone Marrow Mesenchymal Stem Cells Senescence In Vitro. *Front. Endocrinol.* **13**, 808223 (2022).
87. Zhang, Y. *et al.* H/ACA Box Small Nucleolar RNA 7A Promotes the Self-Renewal of Human Umbilical Cord Mesenchymal Stem Cells. *Stem Cells* **35**, 222–235 (2017).
88. Fong, Y. W., Ho, J. J., Inouye, C. & Tjian, R. The dyskerin ribonucleoprotein complex as an OCT4/SOX2 coactivator in embryonic stem cells. *eLife* **3**, e03573 (2014).
89. El-Khoury, F., Bignon, J. & Martin, J.-R. jouvence, a new human snoRNA involved in the control of cell proliferation. *BMC Genomics* **21**, 817 (2020).
90. Kaur, S. *et al.* Small non-coding RNA landscape of extracellular vesicles from human stem cells. *Sci. Rep.* **8**, 15503 (2018).
91. Lefebvre, F. A. *et al.* Comparative transcriptomic analysis of human and Drosophila extracellular vesicles. *Sci. Rep.* **6**, 27680 (2016).
92. Shih, J., Hodge, R. & Andrade-Navarro, M. A. Comparison of inter- and intraspecies variation in humans and fruit flies. *Genomics Data* **3**, 49–54 (2015).
93. Warner, W. A. *et al.* Expression profiling of snoRNAs in normal hematopoiesis and AML. *Blood Adv.* **2**, 151–163 (2018).
94. Ma, D. *et al.* Changes in the Small Noncoding RNAome During M1 and M2 Macrophage Polarization. *Front. Immunol.* **13**, 799733 (2022).
95. Skrzypczyk, A. *et al.* Noncoding RNA Transcripts during Differentiation of Induced Pluripotent Stem Cells into Hepatocytes. *Stem Cells Int.* **2018**, 1–15 (2018).
96. Skreka, K. *et al.* Identification of differentially expressed non-coding RNAs in embryonic stem cell neural differentiation. *Nucleic Acids Res.* **40**, 6001–6015 (2012).
97. He, Y. *et al.* A small nucleolar RNA, SNORD126, promotes adipogenesis in cells and rats by activating the PI3K–AKT pathway. *J. Cell. Physiol.* **236**, 3001–3014 (2021).
98. Delhermite, J. *et al.* Systematic mapping of rRNA 2'-O methylation during frog development and involvement of the methyltransferase Fibrillarin in eye and craniofacial development in Xenopus laevis. *PLOS Genet.* **18**, e1010012 (2022).
99. Ramachandran, S. *et al.* The shift from early to late types of ribosomes in zebrafish development involves changes at a subset of rRNA 2′- *O* -Me sites. *RNA* **26**, 1919–1934 (2020).
100. Hebras, J., Krogh, N., Marty, V., Nielsen, H. & Cavaillé, J. Developmental changes of rRNA ribose methylations in the mouse. *RNA Biol.* **17**, 150–164 (2020).
101. Angrisani, A., Tafer, H., Stadler, P. F. & Furia, M. Developmentally regulated expression and expression strategies of Drosophila snoRNAs. *Insect Biochem. Mol. Biol.* **61**, 69–78 (2015).
102. Deng, W. *et al.* Organization of the *Caenorhabditis elegans* small non-coding transcriptome: Genomic features, biogenesis, and expression. *Genome Res.* **16**, 20–29 (2006).
103. Carlile, T. M., Rojas-Duran, M. F. & Gilbert, W. V. Pseudo-Seq. *Methods Enzymol.* **560**, 219–245 (2015).
104. Begik, O. *et al.* Quantitative profiling of pseudouridylation dynamics in native RNAs with nanopore sequencing. *Nat. Biotechnol.* **39**, 1278–1291 (2021).
105. Higa-Nakamine, S. *et al.* Loss of ribosomal RNA modification causes developmental defects in zebrafish. *Nucleic Acids Res.* **40**, 391–398 (2012).
106. Herter, E. K. *et al.* snoRNAs are a novel class of biologically relevant Myc targets. *BMC Biol.* **13**, 25 (2015).
107. Soulé, S., Mellottée, L., Arab, A., Chen, C. & Martin, J.-R. Jouvence a small nucleolar RNA required in the gut extends lifespan in Drosophila. *Nat. Commun.* **11**, 987 (2020).

108. Askarian-Amiri, M. E. *et al.* SNORD-host RNA *Zfas1* is a regulator of mammary development and a potential marker for breast cancer. *RNA* **17**, 878–891 (2011).
109. Lykke-Andersen, S. *et al.* Human nonsense-mediated RNA decay initiates widely by endonucleolysis and targets snoRNA host genes. *Genes Dev.* **28**, 2498–2517 (2014).
110. Alter, B. P., Giri, N., Savage, S. A. & Rosenberg, P. S. Cancer in the National Cancer Institute inherited bone marrow failure syndrome cohort after fifteen years of follow-up. *Haematologica* **103**, 30–39 (2018).
111. Dokal, I. Dyskeratosis congenita in all its forms: Review. *Br. J. Haematol.* **110**, 768–779 (2000).
112. Heiss, N. S. *et al.* X-Iinked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. *Nat. Genet.* **19**, 32–38 (1998).
113. Knight, S. W. *et al.* X-Linked Dyskeratosis Congenita Is Predominantly Caused by Missense Mutations in the DKC1 Gene. *Am. J. Hum. Genet.* **65**, 50–58 (1999).
114. Mitchell, J. R., Wood, E. & Collins, K. A telomerase component is defective in the human disease dyskeratosis congenita. *Nature* **402**, 551–555 (1999).
115. Walne, A. J. *et al.* Genetic heterogeneity in autosomal recessive dyskeratosis congenita with one subtype due to mutations in the telomerase-associated protein NOP10. *Hum. Mol. Genet.* **16**, 1619–1629 (2007).
116. Vulliamy, T. *et al.* Mutations in the telomerase component NHP2 cause the premature ageing syndrome dyskeratosis congenita. *Proc. Natl. Acad. Sci.* **105**, 8073–8078 (2008).
117. Trahan, C., Martel, C. & Dragon, F. Effects of dyskeratosis congenita mutations in dyskerin, NHP2 and NOP10 on assembly of H/ACA pre-RNPs. *Hum. Mol. Genet.* **19**, 825–836 (2010).
118. Niewisch, M. R. & Savage, S. A. An update on the biology and management of dyskeratosis congenita and related telomere biology disorders. *Expert Rev. Hematol.* **12**, 1037–1052 (2019).
119. Parry, E. M. *et al.* Decreased dyskerin levels as a mechanism of telomere shortening in X-linked dyskeratosis congenita. *J. Med. Genet.* **48**, 327–333 (2011).
120. Mochizuki, Y., He, J., Kulkarni, S., Bessler, M. & Mason, P. J. Mouse dyskerin mutations affect accumulation of telomerase RNA and small nucleolar RNA, telomerase activity, and ribosomal RNA processing. *Proc. Natl. Acad. Sci.* **101**, 10756–10761 (2004).
121. Wong, JudyM. Y., Kyasa, MouhammedJ., Hutchins, L. & Collins, K. Telomerase RNA deficiency in peripheral blood mononuclear cells in X-linked dyskeratosis congenita. *Hum. Genet.* **115**, (2004).
122. Wong, J. M. Y. & Collins, K. Telomerase RNA level limits telomere maintenance in X-linked dyskeratosis congenita. *Genes Dev.* **20**, 2848–2858 (2006).
123. Ruggero, D. *et al.* Dyskeratosis Congenita and Cancer in Mice Deficient in Ribosomal RNA Modification. *Science* **299**, 259–262 (2003).
124. He, J. *et al.* Variable expression of *Dkc1* mutations in mice. *genesis* **47**, 366–373 (2009).
125. Blasco, M. A. *et al.* Telomere Shortening and Tumor Formation by Mouse Cells Lacking Telomerase RNA. *Cell* **91**, 25–34 (1997).
126. Gu, B.-W., Bessler, M. & Mason, P. J. A pathogenic dyskerin mutation impairs proliferation and activates a DNA damage response independent of telomere length in mice. *Proc. Natl. Acad. Sci.* **105**, 10173–10178 (2008).
127. Bellodi, C. *et al.* H/ACA Small RNA Dysfunctions in Disease Reveal Key Roles for Noncoding RNA Modifications in Hematopoietic Stem Cell Differentiation. *Cell Rep.* **3**, 1493–1502 (2013).
128. Nachmani, D. *et al.* Germline NPM1 mutations lead to altered rRNA 2′-O-methylation and cause dyskeratosis congenita. *Nat. Genet.* **51**, 1518–1529 (2019).

129. Angulo, M. A., Butler, M. G. & Cataletto, M. E. Prader-Willi syndrome: a review of clinical, genetic, and endocrine findings. *J. Endocrinol. Invest.* **38**, 1249–1263 (2015).
130. Cassidy, S. B., Schwartz, S., Miller, J. L. & Driscoll, D. J. Prader-Willi syndrome. *Genet. Med.* **14**, 10–26 (2012).
131. Baldini, L., Robert, A., Charpentier, B. & Labialle, S. Phylogenetic and Molecular Analyses Identify SNORD116 Targets Involved in the Prader–Willi Syndrome. *Mol. Biol. Evol.* **39**, msab348 (2022).
132. Galiveti, C. R., Raabe, C. A., Konthur, Z. & Rozhdestvensky, T. S. Differential regulation of non-protein coding RNAs from Prader-Willi Syndrome locus. *Sci. Rep.* **4**, 6445 (2014).
133. Runte, M., Varon, R., Horn, D., Horsthemke, B. & Buiting, K. Exclusion of the C/D box snoRNA gene cluster HBII-52 from a major role in Prader?Willi syndrome. *Hum. Genet.* **116**, 228–230 (2005).
134. Bürger, J., Horn, D., Tönnies, H., Neitzel, H. & Reis, A. Familial interstitial 570 kbp deletion of the *UBE3A* gene region causing Angelman syndrome but not Prader-Willi syndrome: Familial *UBE3A* Gene Deletion. *Am. J. Med. Genet.* **111**, 233–237 (2002).
135. Kishore, S. *et al.* The snoRNA MBII-52 (SNORD 115) is processed into smaller RNAs and regulates alternative splicing. *Hum. Mol. Genet.* **19**, 1153–1164 (2010).
136. Morabito, M. V. *et al.* Mice with altered serotonin 2C receptor RNA editing display characteristics of Prader–Willi syndrome. *Neurobiol. Dis.* **39**, 169–180 (2010).
137. Doe, C. M. *et al.* Loss of the imprinted snoRNA mbii-52 leads to increased 5htr2c pre-RNA editing and altered 5HT2CR-mediated behaviour. *Hum. Mol. Genet.* **18**, 2140–2148 (2009).
138. Hebras, J. *et al.* Reassessment of the involvement of Snord115 in the serotonin 2c receptor pathway in a genetically relevant mouse model. *eLife* **9**, e60862 (2020).
139. Stefan, M. *et al.* Hormonal and Metabolic Defects in a Prader-Willi Syndrome Mouse Model with Neonatal Failure to Thrive. *Endocrinology* **146**, 4377–4385 (2005).
140. Duker, A. L. *et al.* Paternally inherited microdeletion at 15q11.2 confirms a significant role for the SNORD116 C/D box snoRNA cluster in Prader–Willi syndrome. *Eur. J. Hum. Genet.* **18**, 1196–1201 (2010).
141. Sahoo, T. *et al.* Prader-Willi phenotype caused by paternal deficiency for the HBII-85 C/D box small nucleolar RNA cluster. *Nat. Genet.* **40**, 719–721 (2008).
142. de Smith, A. J. *et al.* A deletion of the HBII-85 class of small nucleolar RNAs (snoRNAs) is associated with hyperphagia, obesity and hypogonadism. *Hum. Mol. Genet.* **18**, 3257–3265 (2009).
143. Kummerfeld, D.-M. *et al.* A Comprehensive Review of Genetically Engineered Mouse Models for Prader-Willi Syndrome Research. *Int. J. Mol. Sci.* **22**, 3613 (2021).
144. Ding, F. *et al.* SnoRNA Snord116 (Pwcr1/MBII-85) Deletion Causes Growth Deficiency and Hyperphagia in Mice. *PLoS ONE* **3**, e1709 (2008).
145. Skryabin, B. V. *et al.* Deletion of the MBII-85 snoRNA Gene Cluster in Mice Results in Postnatal Growth Retardation. *PLoS Genet.* **3**, e235 (2007).
146. Marshall, G. M. *et al.* The prenatal origins of cancer. *Nat. Rev. Cancer* **14**, 277–289 (2014).
147. Cazzola, A. *et al.* Prenatal Origin of Pediatric Leukemia: Lessons From Hematopoietic Development. *Front. Cell Dev. Biol.* **8**, 618164 (2021).
148. Ma, Y. *et al.* Developmental timing of mutations revealed by whole-genome sequencing of twins with acute lymphoblastic leukemia. *Proc. Natl. Acad. Sci.* **110**, 7429–7433 (2013).
149. Federico, S., Brennan, R. & Dyer, M. A. Childhood Cancer and Developmental Biology. *Curr. Top. Dev. Biol.* **94**, 1–13 (2011).

150. Abramson, D. H. Rapid Growth of Retinoblastoma in a Premature Twin. *Arch. Ophthalmol.* **120**, 1232 (2002).
151. Gong, J. *et al.* A Pan-cancer Analysis of the Expression and Clinical Relevance of Small Nucleolar RNAs in Human Cancer. *Cell Rep.* **21**, 1968–1981 (2017).
152. Wu, F. *et al.* The Potential Role of Small Nucleolar RNAs in Cancers – An Evidence Map. *Int. J. Gen. Med.* **Volume 15**, 3851–3864 (2022).
153. Gröbner, S. N. *et al.* The landscape of genomic alterations across childhood cancers. *Nature* **555**, 321–327 (2018).
154. Liu, Z., Spiegelman, V. S. & Wang, H. Distinct noncoding RNAs and RNA binding proteins associated with high-risk pediatric and adult acute myeloid leukemia detected by regulatory network analysis. *Cancer Rep.* **5**, (2022).
155. Jha, P. *et al.* Genome-wide small noncoding RNA profiling of pediatric high-grade gliomas reveals deregulation of several miRNAs, identifies downregulation of snoRNA cluster HBII-52 and delineates H3F3A and TP53 mutant-specific miRNAs and snoRNAs: Small noncoding RNA profiles in pediatric HGG. *Int. J. Cancer* **137**, 2343–2353 (2015).
156. Schramm, A. *et al.* Next-generation RNA sequencing reveals differential expression of MYCN target genes and suggests the mTOR pathway as a promising therapy target in *MYCN*-amplified neuroblastoma. *Int. J. Cancer* **132**, E106–E115 (2013).
157. Alipoor, B. *et al.* Long non-coding RNAs in metabolic disorders: pathogenetic relevance and potential biomarkers and therapeutic targets. *J. Endocrinol. Invest.* **44**, 2015–2041 (2021).