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Review

When small RNAs become smaller: emerging functions of snoRNAs and their derivatives

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Small nucleolar RNAs (snoRNAs) are molecules located in the cell nucleolus and in Cajal bodies. Many scientific reports show that snoRNAs are not only responsible for modifications of other RNAs but also fulfill multiple other functions such as metabolic stress regulation or modulation of alternative splicing. Full-length snoRNAs as well as small RNAs derived from snoRNAs have been implied in human diseases such as cancer or Prader–Willi Syndrome. In this review we describe emerging, non-canonical roles of snoRNAs and their derivatives with the emphasis on their role in human diseases.

Key words: small RNAs, snoRNAs, sdRNAs, microRNAs, regulatory RNAs

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INTRODUCTION

Small nucleolar RNAs (snoRNAs) are one of the most ancient and evolutionarily conserved non-protein coding RNAs. They have been identified in Eukaryotes, Archaea and one in the Epstein-Barr virus (Hutzinger *et al.,* 2009). There are two classes of snoRNAs (C/D and H/ACA box) that function as ribonucleoprotein (RNP) complexes to guide enzymatic modifications of other RNAs, mainly ribosomal RNAs (rRNAs). Posttranscriptional rRNA modifications are common for all domains of life; there are three basic types: base methylation, ribose methylation and pseudouridylation.

C/D box snoRNAs take their name from the conserved sequence elements that they contain, known as C/C' (RUGAUGA, R=purine) and D/D' (CUGA) boxes, located near the 5' and 3' ends of the snoRNA, respectively. C/D box snoRNAs form a ribonucleoprotein complex with 2'-O-methyltransferase fibrillarin and such snoRNP complex methylates the target RNAs. A conserved region of 10–21 nucleotides (nt) upstream of the D and/or D' box is complementary to the methylation site of the target RNA and enables the snoRNA to form an RNA duplex with the RNA. H/ACA box snoRNAs in turn form complexes with the pseudouridine synthase dyskerin and perform pseudouridylation. Small nucleolar RNAs from this class are built by two stems that form a pseudouridinilation pocket and two single stranded regions that enclose the H (ANANNA) and ACA elements.

Many studies have investigated snoRNA-guided modifications. As a result, some features characterizing the functional snoRNA-target site interactions have been inferred and are used in the computational prediction of snoRNA targets (e.g. sequence complementarity between putative target RNAs and the antisense elements or the recognition loops within the snoRNA) as well as biochemical identifications of snoRNA targets (e.g. based on CLASH: crosslinking, ligation, and sequencing of hybrids). However, there are numerous snoRNAs for which no target RNAs have been identified so far. They are called "orphan snoRNAs". In multiple cases, orphan snoRNAs possess the guide sequences, however, they are not complementary to other canonical RNAs targeted by snoRNAs.

Small nucleolar RNAs were initially discovered in the nucleolus and were thought to exclusively target ribosomal RNAs inside this subnuclear compartment. However, years of research on the snoRNA biology have revealed a tremendous number of unexpected discoveries that shed a new light on their functions in processes different than modifications of other RNAs. Small nucleolar RNAs may take part in the regulation of metabolic stress responses, development of some diseases or disorders, like cancer or the Prader–Willi syndrome. Moreover, there is a growing number of evidence that mature snoRNAs undergo further processing into stable shorter fragments, known as snoRNA-derived RNAs (sdRNAs) (Falaleeva & Stamm, 2013; Li *et al.,* 2012). Such fragments are present in many organisms, including mammals (Bortolin-Cavaille & Cavaille, 2012; Kishore *et al.*, 2010; Ender *et al.,* 2008; Bai *et al.,* 2014; Brameier *et al.,* 2011; Burroughs *et al.,* 2011), a primitive protozoan *Giardia lamblia* (Saraiya & Wang, 2008), budding yeast *Saccharomyces cerevisiae* (Żywicki *et al.,* 2012; Walkowiak *et al.,* 2016) and the Epstein-Barr virus (Hutzinger *et al.,* 2009). Interestingly, these fragments associate with proteins different than the full-length snoRNAs do, and therefore possibly fulfill distinct cellular functions. Moreover, such snoRNA cleavage in yeast cells is most prominent under non-optimal growth conditions, which include UV irradiation, anaerobic growth, growth in a high or low pH

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Abbreviations: 5-HT2CR, 5-hydroxytryptamine receptor 2; Ago, Argonaute protein; CRHR1, corticotropin-releasing hormone receptor 1; EBV, Epstein-Barr virus; DPM2, dolichol phosphate-mannose biosynthesis regulatory protein; HEK293, human embryonic kidney 293 cells; hnRNP, heterogeneous nuclear ribonucleoprotein; HNSCC, head and neck squamous cell carcinomas; MAPK/ERK, mitogen-activated protein kinases; originally called ERK, extracellular signal-regulated kinases; miRNA, microRNA; mRNA, messenger RNA; ncRNA, non-protein coding RNA; NGS, next generation sequencing, NSCLC, non-small-cell lung carcinoma; PBRM1, protein polybromo-1; PCR, polymerase chain reaction; PWS, Prader–Willi syndrome; qRT-PCR, quantitative real-time reverse transcriptase PCR; RALGPS1, Ral GEF with PH domain and SH3 binding motif 1 protein; RNP, ribonucleoprotein complex; rRNA, ribosomal RNA; sdRNA, small RNA derived from snoRNA; SL-RT-PCR, stem-loop reverse transcription combined with polymerase chain reaction; snoRNA, small nucleolar RNA; TAF1, transcription initiation factor TFIID subunit 1; TGF-β, transforming growth factor β

medium or growth in a medium with no amino acids or sugars (Żywicki *et al.,* 2012; Tyczewska *et al.,* 2016). Such response to changing environment may therefore indicate that snoRNA processing may play a crucial role in the stress-dependent metabolism regulation, similar as in case of tRNA processing (Mleczko *et al*., 2014). In this article, we highlight the current state of the art concerning non-canonical roles of snoRNAs and their derivatives, sdRNAs, with emphasis on their role in human disorders.

PROCESSING OF snoRNAs TO sdRNAs – DISCOVERING NOVEL FUNCTIONS OF KNOWN RNAs

In 2008, an unbiased sequencing study (aimed at capturing all potential human small RNAs in a range of 19–40 nt) led to the observation of specific processing and accumulation of small RNAs originating from well-characterized non-coding RNAs, including snoRNAs (Kawai *et al*., 2008). In the next year, by systematic analyses of deep-sequencing libraries from diverse eukaryotic organisms, Taft and coworkers (2009) revealed that small RNAs with evolutionarily conserved size and position, are derived from the vast majority of snoRNA loci in animals (human, mouse, chicken, fruit fly), thale cress and fission yeast. Later on, a comparison of human small RNA deep sequencing data sets revealed that box C/D sdRNA accumulation patterns are conserved across multiple cell types (Scott *et al*., 2012). Recently, in 2014, Laiho and coworkers performed deep sequencing of small RNomes in subcellular compartments of the HeLa cells (Bai *et al.,* 2014). The nucleolar small RNAs were predominantly represented by 19–20 nt and 25 nt reads and 93% of them were mapping to the box C/D snoRNAs. The most abundantly represented locus among the sdRNA reads in the nucleolar fraction was SNORD44 (RNU44) – 71.4% of all nucleolar sdRNAs. Few of the sdRNAs were detected in the cytoplasmic fraction, which could suggest their functional potential in processes different than those in which the mature precursors act (since full-length snoRNAs are localized and perform their function in the nucleolus). The list of discovered snoRNA-derived small RNAs is presented in Table 1.

Concerning the function of sdRNAs in the cells, the first and breakthrough functional study came from the Meister group. It demonstrated the miRNA-like activity of an sdRNA originating from ACA45 snoRNA (Ender *et al.,* 2008). This particular sdRNA was identified in the Ago-associated RNA library. Importantly, the sequencing reads were found to be conserved in mammals, suggesting that they are, indeed, specific processing products. In terms of both, processing and function, ACA45 sdRNA was similar to miRNAs. Firstly, this sdRNA was produced in the HEK293 cells in a Dicer-dependent manner. Secondly, an endogenous target RNA has been identified (CDC2L6 – a component of the mediator complex) and experimentally confirmed. ACA45 sdRNA was able to inhibit the activity of a CDC2L6 target in a miRNA-like pattern. Notably, the same authors identified seven additional sdRNAs with miRNA-like processing features in the follow-up experiments. Parallel NGS studies of the small transcriptome in mice revealed the presence of snoRNA-originating miRNA-like molecules in embryonic stem cells and demonstrated that sdRNAs exhibit tissue specific expression (Babiarz *et al.,* 2008; Babiarz *et al.,* 2011).

At the same time, two functional snoRNA-originating miRNA-like RNAs were described in the protozoan *Giardia lamblia*, a unicellular parasite whose genome does not encode Drosha: miRNA2 (Saraiya & Wang, 2008) and miRNA5 (Li *et al.,* 2011). These miRNA-like sdRNAs associated with the Ago proteins and were pro-

Table 1. The list of discovered snoRNA-derived RNAs.

Organism	Processed snoRNA	References
Epstein-Barr virus	v-snoRNA1	Hutzinger *et al.,* 2009
Giardia lambia	GlsR17, GlsR2	Saraiya & Wang, 2008; Li *et al.,* 2011
Saccharomyces cerevisiae	snoRNA: 78, 77, 128, 51, 76, 17a, 66, 67, 73, 18, 54, 83, 30, 8	Żywicki *et al.,* 2012 Walkowiak *et al.,* 2016
Mus musculus	MBII-52	Kishore *et al.,* 2010; Bortolin-Cavaille & Cavaille, 2012
Homo sapiens	ACA45, ACA36B, ACA56, ACA3, ACA17, ACA50, ACA47, ACA25, RNU44, SNORD48, SNORD21, SNORA48, SNORA64,SNORA73, SNORA8, SCARNA15, SCARNA15, snR39b, H/MBII-52, HBI-100, HBII-336, HBII-429, HBII-142, U27, U83a, U74, U15a. U92, U3, U78, U17a, U17b, sno-miRNA-28, hsa-miRNA-1291	Bortolin-Cavaille & Cavaille, 2012; Kishore *et al.,* 2010; Ender *et al.,* 2008; Bai *et al.,* 2014; Brameier *et al.,* 2011; Burroughs *et al.,* 2011; Yu *et al*., 2015, Pan *et al.,* 2013
Canis familiaris *Rattus norvegicus* *Mus musculus* *Canis familiaris*	ACA45	Ender *et al.*, 2008
embryonic chicken cell line	GSE10686, GSM270187, GSM270188, GSM270189	Taft *et al.,* 2009
mouse embryonic stem (ES) cells	GSE12521, GSM314552, GSM314553, GSM314552	Taft *et al.,* 2009
Drosophila melanogaster	GSE7448, GSM180328, GSM180329, GSM180330, GSM180331, GSM180332, GSM180333, GSM180334, GSM180335, GSM180336, GSM180337, GSE11086, GSM280082, GSM280083, GSM280084	Taft *et al.,* 2009
Arabidopsis thaliana	GSE12037, GSM304282, GSM304283, GSM304284, GSM304285	Taft *et al.,* 2009
Schizosaccharomyces pombe	GSE12416, GSM311595, GSM311596	Taft et *al.,* 2009

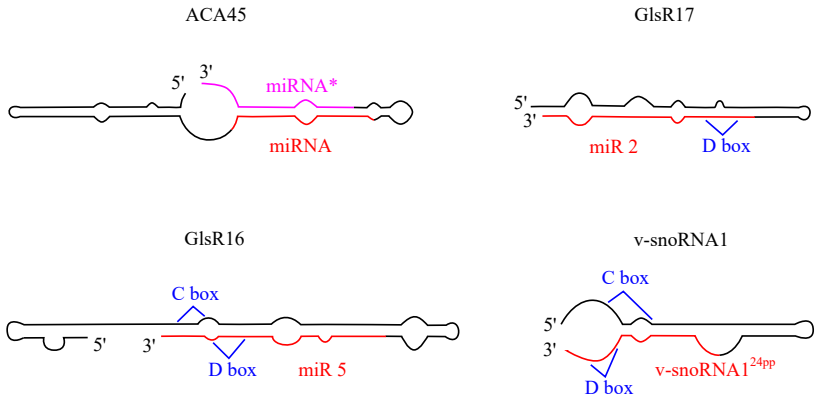

Figure 1. Examples of sdRNAs with microRNA functions.
Predicted secondary structures of the following snoRNAs are presented: human ACA45, *G. lamblia* GlsR17 and GlsR16, EBV v-snoRNA1. microRNA positions are marked in red. For C/D box snoRNAs, C boxes and D boxes are marked in blue.

duced in a Dicer-dependent but Drosha-independent manner. 26-nucleotide long miRNA2 is a processing product of GlsR17 snoRNA and is localized in the cytoplasm. miRNA5 of the same length is derived from a C/D box snoRNA, GlsR2. In this case the authors also identified a putative miRNA target sites in the 3'-UTRs of mRNAs and verified the activity of miRNA2 *in vivo*. The expression of Renilla luciferase (RL) reporter mRNA containing six identical miRNA2 target sites in the 3'-UTR was reduced by 40% when co-transfected with miRNA2 (Saraiya & Wang, 2008). Moreover, Li *et al.* took the same approach and discovered that the presence of endogenous miRNA5 in *Giardia* reduces the RL expression by 18%, whereas the presence of additional synthetic miRNA5 further decreases the expression by a total of 39% (Li *et al.,* 2011). The same pathway was described one year later in cells infected with the Epstein-Bar virus (EBV). In this system, EBV expresses a miRNA-like precursor endogenously encoded by a viral v-snoRNA1 to suppress the viral DNA polymerase upon induction of the lytic cycle (Hutzinger *et al.,* 2009). Numerous miRNAs-like molecules derived from C/D box snoRNAs which exhibit mRNA silencing features were additionally identified in human cell lines: HeLa, Jurkat (T cells) and RPMI8866 (B cell) (Brameier *et al.,* 2011). 11 RNAs derived from SNORD2 (snR39b), SNORD3@ (U3), SNORD78 (U78), SNORD93 (HBII-336), SNORD100 (HBII-429), SNORD66 (HBII-142), SNORD27 (U27), SNORD83a (U83a), SNORD74 (U74) and SNORD15a (U15a) were capable of translation regulation of reporter-gene mRNAs. Three other miRNA-like RNAs turn out to be cleaved from H/ACA box snoRNAs and they also regulated expression of the reporter gene. What is of special importance, the silencing activity differed among all investigated cell types. Representatives of the experimentally validated functional miRNA-like sdRNAs and their position within the secondary structures of snoRNA counterparts are presented in Fig. 1.

Recent research aiming at deep sequencing of small non coding RNAs from patients with prostate cancer and normal prostate at different disease stages, shed a new light on the importance of snoRNA fragments in cancer tissues (Martens-Uzunova *et al.,* 2015). Surprisingly, it turned out that at least 78 of the detected sdRNAs, including SNORD44, SNORD78, SNORD74 and SNORD81, demonstrate a strong differential expression in prostate cancer patients, which was even stronger than in the case of miRNAs. Most of sdRNAs originated from equivalent locations of their precursors and often one predominant sdRNA was observed per precursor snoRNA. High levels of SNORD78 in a patient who developed metastatic disease suggested that this sdRNA could be a novel prognostic biomarker for the prostate cancer patients with a high risk of developing metastasis. What is worth to mention, Martens-Uzunova and coworkers also discovered that the tRNA fragments could be present in high amounts in metastatic samples (Martens-Uzunova *et al.,* 2012).

The p53 protein is an tumor suppressor gene and it plays a crucial role in the prevention of oncogenic transformation. In 2015, Nielsen and his group published novel and unobvious results demonstrating a role for p53 in repression of a family of polycistronic C/D box snoRNAs, of which at least one is processed into an operative miRNA-like RNA (Yu *et al.,* 2015). This snoRNA fragment represses TAF9B-mediated stabilization of p53 and promotes cell proliferation, which in turn leads to breast cancer.

Moreover, snoRNA-derived miRNAs can also modulate cellular drug disposition (Pan *et al.,* 2013). The hsa-miRNA-1291 is a small non-coding RNA derived from H/ACA SNORA34 which modulates cellular drug disposition and chemosensitivity through binding to the 3'UTR of *ABCC1* mRNA and negative regulation of its expression. ABCC1 is a membrane transporter that is expressed ubiquitously in human tissues and contributes to cellular disposition of numerous xenobiotics. Such regulation of ABCC1 expression is responsible for a significantly increased level of intracellular drug accumulation and chemosensitivity. Furthermore, it has been found that hsa-miRNA-1291 is significantly downregulated in human pancreatic ductal adenocarcinoma, when compared to normal pancreas. Better understanding of regulation of ABC transporters might therefore help to develop a rational drug therapy for cancer patients.

Interestingly, using an alternative computational approach for non-coding RNA detection based on the properties of promoter regions of well-characterized ncRNAs, in 2015 Qu et al. were able to detect two dicistronic genes encoding precursors that are processed into mature snoRNA and miRNA molecules in *Arabidopsis thaliana* (Qu *et al.,* 2015). Both, the snoRNAs and the miRNA transcribed from the two identified dicistronic snoRNA-miRNA775 and sno-miRNA779 genes have a common precursor and they use two distinct maturation pathways that preserve the integrity of both ncRNAs.

Although the components of the machinery necessary for miRNA action are conserved in diverse eukaryotic species, including budding yeast *Saccharomyces castellii* and *Candida albicans*, they have been lost in the budding yeast *Saccharomyces cerevisiae* (Houseley *et al.,* 2008). However, the presence of snoRNA processing products was demonstrated by high throughput sequencing studies and further validated with small RNA detection techniques in *S. cerevisiae*. Although yeast sdRNAs were observed in very low abundance in small-RNA sequencing studies (Zywicki *et al.,* 2012), they could be easily detected with

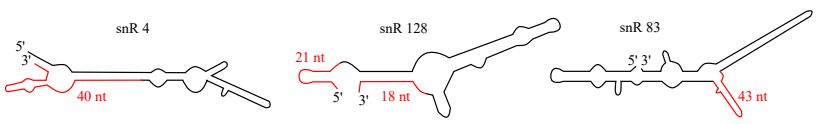

Figure 2. Atypical sdRNAs in *Saccharomyces cerevisiae*.
Predicted secondary structures of the following snoRNAs are presented: snR 4, snR 128 and snR 83. sdRNA positions as well as their length are marked in red. Different types of sdRNA locations within the secondary structure of snoRNAs: 3'-part-derived, 5'-part-derived or middle-part-derived.

stem-loop RT-PCR (SL-RT-PCR) method with as little as 50 ng of low molecular weight RNA (Walkowiak *et al.,* 2016). These results confirmed the presence of shortened version of both types of snoRNAs (C/D and H/ACA box) in baking yeast. The processing events were most prominent under non-optimal yeast growth conditions. What is interesting, yeast snoRNA fragments have different length than typical microRNA-like sdRNAs, ranging from 18 nt to 43 nt and the processing sites may be located within the 3' part of snoRNA, 5' part, both of them or snoRNA middle part (Fig. 2). What is of special importance, these sdRNAs have been co-purified with the yeast ribosomes (Zywicki *et al.,* 2012). Therefore, the sdRNA/ribosome association might implicate a novel, yet undiscovered regulatory role of sdRNAs in ribosome biosynthesis and/or function in *S. cerevisiae*, independent from the microRNA-like pathway. Considering the fact that 30% of known genes involved in human diseases have yeast orthologs, and that the oxidative stress has been linked to diseases such as cancer, or to aging processes, sdRNA interactions with ribosomes in yeast might improve our understanding of defense mechanisms ranging from microorganisms to humans. Possible functions of snoRNA-derived fragments are summarized in Table 2.

snoRNAs IN MALIGNANCY DEVELOPMENT

Cancer is one of the major causes of human death, accounting for 8.2 million deaths in 2012 worldwide (World Health Organization statistics). It is therefore of crucial importance to identify and characterize the genetic alterations causing distinct types of cancer. One of the processes that could lead to the transformation of normal cells into the tumor cells are defects in ribosome maturation and function. Ribosomal RNA biogenesis is known to be more robust in cancer cells than in normal cells (Belin *et al.,* 2009). Since snoRNAs are involved in the regulation of ribosomal RNA modification, it is therefore logical to suspect their possible roles in cancer development. It can be assumed that increase in the snoRNA levels is necessary for the acceleration of rRNA maturation, ribosome assembling and protein synthesis during tumorigenesis. Indeed, in 2002 Chang and coworkers showed for the first time that snoRNAs are involved in the development of cancer (Chang *et al.,* 2002). They found that h5sn2 snoRNA which belongs to H/ACA box family was highly expressed in normal brain but its expression was reduced in meningioma, pointing that loss of snoRNA h5sn2 is involved in brain tumorigenesis.

snoRNAs have been also found to be involved in development of the non-small-cell lung cancer (NSCLC), which is the leading cause of cancer deaths in the world (1.59 million deaths in 2012, according to the World Health Organization). Unfortunately, for most patients the available treatments are not sufficient, which clearly provides the reason for improving the NSCLC early detection. In 2010 Liao and coworkers presented the first study which globally analyzed the snoRNA expression patterns in human tumor tissues (Liao *et al.,* 2010). Interestingly, the set of snoRNAs was overexpressed in the lung tumor tissue and compared with their normal counterparts. In this study, Liao and coworkers proposed a potential diagnostic test for NSCLC by measuring plasma snoRNA expressions by real-time quantitative reverse transcriptase PCR (qRT-PCR). Later on, the function of one of the overexpressed H/ACA box snoRNAs, SNORA42 (U42) was studied in greater detail (Mei *et al.,* 2012). siRNA knockdown of SNORA42 resulted in a reduced cancer cell growth and compromised tumorigenicity in animal models by inducing p53-dependent apoptosis. On the contrary, overexpression of SNORA42 activated cancer cell growth. Moreover, the expression level of SNORA42 in lung tumor tissue specimen was found to be inversely correlated with the survival of NSCLC patients. In 2015, a significant upregulation of SNORD78 (U78) levels in NSCLC tissues was observed by Zheng and coworkers (2015). In this case, silencing of SNORD78 expression inhibited cell proliferation by inducing the phase G0/G1 arrest and cell apoptosis.

snoRNA expression profiling may be useful in the diagnosis of some subtypes of Peripheral T-cell lymphoma and the prognostication of cancer patients treated with chemotherapy. In 2012, it has been revealed that multiple snoRNAs are globally down-regulated in the T-cell lymphoma cells when compared with normal tissues; the same was also observed for miRNA (Valleron *et al.,* 2012). Particularly interesting was the prognostic value of SNORD71 (HBII-239) snoRNA, which was significantly overexpressed in the case of angio-immunoblastic T-cell lymphoma and Peripheral T-cell lymphoma that were not otherwise specified. Such overexpression predicts a good prognosis for patients. What is also worth to mention, the SNORD71 fragment (which was called miRNA-768) is also overexpressed in tissues of patients with good prognosis and can be a powerful prognostic tool.

Another example of snoRNA downregulation in tumor cells comes from the research on hepatocellular carcinoma (Xu *et al.,* 2014). Lowered expression of SNORD113-1 was correlated with cancer progression, cancer development and patient survival. Functional analyses revealed that overexpression of this snoRNA inhibited viability, tumorigenicity and cell growth of tumor cells, probably by the MAPK/ERK and TGF-β pathway-dependent mechanisms. Hence, SNORD113-1 was proposed to be not only a potential diagnostic tool for hepatocellular cancer but also a potential therapeutic target.

One of the most common mutations causing many different types of human cancers, including breast and prostate cancer, is a deletion of chromosome 6 and its q14-q22 fragment (Dong, 2001). In 2008, Dong and his group revealed that SNORD50a (U50) gene can act as a 6q tumor suppressor (Dong *et al.,* 2008). They have discovered that a 2-bp germline homozygous deletion of SNORD50a was associated with clinically significant prostate cancer. What is more, one year later the same group revealed that deletion of SNORD50a leads to de-

Table 2. Examples of diverse snoRNA and sdRNA functions with emphasis on human diseases.

sno/ sdRNA	Name	Type	Disease/function	Organism	References
full-lenght snoRNAs	SNORD116 (HBII-85)	C/D	PWS	Clinical specimens	Duker *et al.,* 2010
	SNORD115 (HBII – 52)	C/D	PWS/alternative splicing	Clinical specimens	Kishore & Stamm, 2006
	SNORD32a (U32a), SNORD32a (U33), SNORD34 (U34), SNORD35a (U35a)	C/D	lipotoxic and oxidative cellular stress response	Chinese hamster ovary cells	Michel *et al.,* 2011
	SNORD50a (U50)	C/D	prostate and breast cancer	Human prostate / breast cancer cell lines and clinical specimens	Dong *et al*., 2008; Dong *et al.,* 2009
	SNORD42 (U42)	C/D	NSCLC	Human lung cancer cell lines and clinical specimens	Mei *et al.,* 2012
	SNORD78 (U78)	C/D	NSCLC	Clinical specimens	Zheng *et al.,* 2015
	SNORD113-1	C/D	hepatocellular carcinoma	Human hepatocellular carcinoma cell lines and clinical specimens	Xu *et al.,* 2014
	SNORD35B (U35B)	C/D	HNSCC	Clinical specimens	Zou *et al.,* 2015
	SNORD71 (HBII-239)	C/D	peripheral T-cell lymphoma	Clinical specimens	Valleron *et al.*, 2012
	h5sn2	H/ACA	brain tumors	Clinical specimens	Chang *et al.,* 2002
snoRNA-derived fragments	SNORD44, SNORD78, SNORD74, SNORD81	C/D	prostate cancer	Clinical specimens	Martens-Uzunova *et al.,* 2015
	MBII-52	C/D	PWS/alternative splicing	TgPWS mouse model	Bortolin-Cavaille & Cavaille, 2012, Kischore *et al.*, 2010
	GlsR17, GlsR2	C/D	miRNA-like functions	Giardia lambia	Saraiya & Wang, 2008 Li *et al*., 2011
	SNORD2 (snR39b), SNORD78 (U78), SNORD93 (HBII-336), SNORD100 (HBII-429),SNORD66 (HBII-142), SNORD74 (U74), SNORD15a (U15a)	C/D	miRNA-like functions	HeLa, Jurkat (T cells) and RPMI8866 (B cell)	Brameier *et al*., 2011
	SNORD3@ (U3)	C/D	miRNA-like functions	HeLa and RPMI8866	Brameier *et al.,* 2011
	SNORD83a (U83a)	C/D	miRNA-like functions	Jurkat and RPMI8866	Brameier *et al.,* 2011
	SNORD27 (U27)	C/D	miRNA-like functions	Jurkat	Brameier *et al*., 2011
	Sno-miRNA-28	C/D	miRNA-like functions	Breast cancer cell lines (MDA-MB-231 MCF10A)	Yu *et al.,* 2015
	SNORA34 (ACA34), SNORA81 (HBI-61)	H/ACA	miRNA-like functions	HeLa, Jurkat, RPMI8866	Brameier *et al.,* 2011
	SNORA36b (ACA36b)	H/ACA	miRNA-like functions	HeLa, Jurkat	Brameier *et al.,* 2011
	SCARNA15 (ACA45)	H/ACA	miRNA-like functions	HEK293	Ender *et al.,* 2009
	hsa-miRNA-1291	H/ACA	miRNA-like functions	PANC-1	Pan *et al.,* 2013

velopment and/or progression of breast cancer (Dong *et al.,* 2009). Interestingly, in breast cancer, homozygosity of the deletion was rare in both, the cases and controls. This difference between prostate cancer and breast cancer could suggest that breast cells are more susceptible to SNORD50a mutation, when comparing to prostate cancer cells.

Head and neck squamous cell carcinoma (HNSCC) is one of the six most common cancers in the world (Ferlay *et al.* 2008). Recent research aiming at sequencing of a transcriptome of head and neck squamous cell carcinoma tissue revealed 33 significantly deregulated snoRNAs, ranging from about 4-fold to 3-fold for SNORD116-20 and SNORD60, respectively (Zou *et al.,* 2015). Moreover, lower expression of SNORD35B (U35B), a snoRNA downregulated in HNSCC, served as an adverse prognostic factor for patient survival.

snoRNAs IN THE PRADER–WILLI SYNDROME

The Prader–Willi syndrome (PWS) is a rare genetic disorder manifested by mental retardation, poor muscle tone, incomplete sexual development, cognitive and behavioral disabilities. The major cause of the PWS is the loss of paternal gene expression from a maternally imprinted region 15q11–q13 on chromosome 15. This locus contains numerous copies of snoRNAs and already in the year of 2000 it was reported that three human snoRNAs, expressed in brain tissue, map to the PWS region (de los Santos *et al.,* 2000). Recent discoveries have indeed pointed out that snoRNAs play a significant role in this disease. It has been observed that even a small microdeletion at 15q11.2 critical region that included the SNORD116 cluster (HBII-85), caused a Prader-Willi syndrome phenotype in an 11-year old patient (Duker *et al.,* 2010). Other studies had shown additional evidence that the same cluster of snoRNAs with unique, yet overlapping microdeletions lead to the loss of a paternal copy of the SNORD116 and PWS phenotype (Sahoo *et al.,* 2008; de Smith *et al.,* 2009).

The genetic loss of SNORD115 (HBII-52) likewise results in the PWS (Kishore & Stamm, 2006). It appeared that this missing snoRNAs is of special importance in PWS, since it changes the splice site selection. HBII-52 holds a sequence complementary to an alternatively spliced exon Vb of serotonin receptor 5-HT2CR and therefore regulates the alternative splicing of 5-HT2CR by binding to a silencing element in exon Vb. Lack of HBII-52 leads to defects in pre-mRNA processing and, as a consequence, patients with the Prader-Willi syndrome with loss of paternal HBII-52 gene have different messenger RNA isoforms. As a follow-up of these studies, the same group identified five additional pre-mRNAs (DPM2, TAF1, RALGPS1, PBRM1 and CRHR1) containing alternative exons that are regulated by MBII-52, a mouse homolog of HBII-52 (Kishore *et al.,* 2010). Notably, the analysis of a single member of the MBII-52 cluster had shown that the MBII-52 expressing unit generates shorter RNAs that originate from the full-length MBII-52 snoRNA, through an additional processing steps. These novel RNAs interact with hnRNPs and not with the proteins associated with canonical C/D box snoRNAs. These data indicated that not a traditional C/D box snoRNA MBII-52, but a processed version lacking the snoRNA stem is a predominant MBII-52 RNA missing in PWS. This processed snoRNA is a few nucleotides shorter at the 5' end than the full-length snoRNA and functions in alternative splice-site selection (Bortolin-Cavaille & Cavaille, 2012; Kischore *et al.,* 2010).

snoRNAs AS METABOLIC STRESS REGULATORS

SnoRNAs can be also regulators of metabolic stress response pathways, as observed in Chinese hamster ovary cells (Michel *et al.,* 2011). Four C/D box snoRNAs: SNORD32a (U32a), SNORD32a (U33), SNORD34 (U34) and SNORD35a (U35a) located in the ribosomal protein rpL13a locus, are highly conserved across mammalian species. In addition to their primary role in the ribosomal RNA modification, they also regulate a lipotoxic and oxidative cellular stress responses. Loss of three snoRNAs encoded in the rpL13a locus is sufficient to induce resistance to oxidative and lipotoxic stresses *in vitro* and prevents the spread of oxidative stress *in vivo*.

CONCLUSIONS

Small nucleolar RNAs are a large class of small noncoding RNAs which primarily guide chemical modifications of other RNAs. Rapid development of high-throughput and deep-sequencing technologies has significantly broadened our view of snoRNA characteristics and functionality. However, in higher eukaryotes, many orphan snoRNAs were discovered without known target and function, suggesting that they may play different roles in the cellular life. Moreover, a growing number of evidence clearly shows that many snoRNAs are processed into shorter functional forms, whose generation is still not completely understood. Small nucleolar RNAs may be processed into short miRNA-like RNAs as well as longer sdRNAs. miRNA-like molecules derived from snoRNAs can play a role in regulation of gene expression, whereas many other sdRNAs possess yet undiscovered functions. In the 1990s and at the beginning of the 21st century many investigations gave numerous information concerning the structure and function of canonical snoRNAs. Recently, many studies revealed new functions of snoRNAs and their fragments. The knowledge in the field of snoRNAs and sdRNAs is still developing and more information is being gained. Thus, in the next years we may discover a great number of novel snoRNAs and sdRNAs functions.

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