Small non-coding RNAs and human disease

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Abstract:

Non-coding RNAs are functional RNAs which mainly function in regulatory processes. This review small nuclear RNA (snRNA), and small nucleolar RNA (snoRNA). The focus is on latest knowledge and understanding of these RNAs as well as their significance and role in cancer, cardiovascular disease, and diabetes.

Keywords:

Noncoding RNAs, snoRNA, snRNA, snoRNA C/D box, snoRNA H/ACA box cancer, diabetes, cardiovascular disease, obesity

Introduction

Regions of the genome that did not code for proteins were assumed to be non-functional and considered of no value [1]. These regions make more than 98% of the genome and contain regulatory sequences and sequences that do not code for proteins[2]. This was the case until the idea that ribonucleic acids (RNAs) could have an active role in protein synthesis was proposed[3]. This idea was reinforced with the discovery of an active RNA that functioned in the transferring amino acids to the messenger RNA (mRNA) –rRNA was yet to be discovered-. These RNAs were denoted as tRNA (transfer ribonucleic acid) [4]. Since then the study of non-coding RNA became popular and new non-coding RNAs are frequently being discovered[2]. Of these non-coding RNAs snRNA and snoRNA will be discussed. These RNAs belong to a group of non-coding RNAs known as small noncoding RNAs. snRNA and snoRNA rang in size between 70 to 200 nucleotides. SnRNA and snoRNAs are both confined to the nucleus[5]. SnRNA mainly assemble with proteins to make the subunits

of the spliceosome[6]. SnoRNAs mainly regulate the post transcriptional processing of RNAs[7].

SnRNA

Small nuclear RNA (snRNA) are stretches of non-protein-coding RNA. snRNAs were first documented as low molecular weight RNA fractions in a study aimed to analyze the different fractions of RNA using agarose-gel electrophoresis. These low molecular-weight RNA molecules were recovered after repeating the experiment multiple times, indicating that they were not degradation products of higher molecular weight RNAs[8]. These small RNA molecules were analyzed and found to be methylated and are localized to the nucleus of the cells[9]. Moreover, further analysis of these molecules revealed their high uridine content (hence, the letter U was used to denote the different classes of these RNA molecules) [10]. The next discovery that greatly affected the way scientists thought about small nuclear RNAs was the discovery of DNA. A couple of years after DNA was discovered, scientists discovered the presence of long RNA molecules (known as pre-mRNA today) within the nucleus. However, when compared to cytoplasmic mRNA, the pre-mRNA was much longer[11]. Sequences of mRNA and their corresponding DNA templates were analyzed in1977 and it was found that some sequences, known as introns, in the cytoplasmic mRNA were missing, or spliced out[12]. The question that arose next was how are introns spliced out? The answer to this question was found in snRNAs' contribution to the formation of spliceosomes. Beside their function in splicing, more recent studies have shown a regulatory role of snRNAs in gene expression.

snRNAs are divided into two classes based on common characteristics between members of each class. The first class is the Sm class, which comprise snRNAs U1, U2, U4, U5, U7,

U11, U12, and U4atac. These snRNAs are synthesized by RNA polymerase II. Whereas U6 and U6atac belong to the class Lsm and are synthesized by RNA polymerase III [13, 14]. Sm class RNAs are transcribed in the nucleus, exported to the cytoplasm where they are modified and returned to the nucleus where they assemble with proteins to form small nuclear ribonucleicproteins (snRNP). On the other hand, Lsm class RNAs are modified within the nucleus and do not require to be transported outside the nucleus.[14]. Moreover, two types of spliceosomes exist, major (contributing greater percentage of RNA splicing) and minor spliceosomes (contributing to a small percentage of RNA splicing The major spliceosome consists of U1, U2, U4, U5, and U6, whereas the minor spliceosome consists of U5, U11, U12, U4atac, and U6atac[6].

The main function of snRNAs is to form the spliceosome, a ribonucleoprotein (made of protein subunits and RNA molecules), which is the maestro of RNA splicing. The premature RNA has conserved intron sequences at the 5'(AG/GU) and 3' end known as consensus sequences. These sequences are recognized by the the snRNA component of the spliceosome. Once they have been recognized, a double transesterification at both ends occurs resulting in the release of the intron as a lariat[15]. The U1 first recognizes the 5' split site on the premature mRNA and initiate the assembly of the spliceosome[16]. The U2 then recognizes the branch site [17]. U4, U5 and U6 assemble together and from a tri-snRNA which assembles with the U1 and the U2 forming the complete spliceosome structure[15]. The spliceosome then catalyzes the double transesterification reaction, where the 2'OH on the branch site attacks the 5' splicing site and forming a lariat. The free 5'OH of the exon then attacks the 3' split sites, leading to the ligation of two exons together[15].

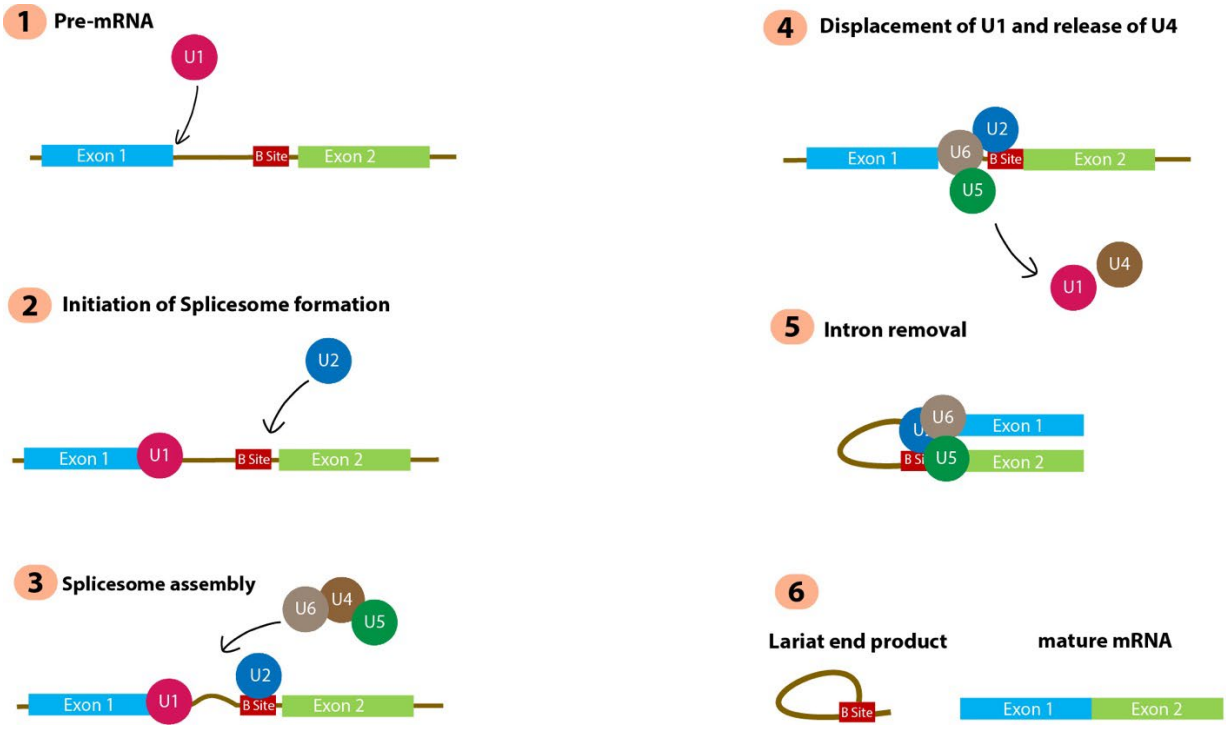

Figure 5: The process of splicing a pre-mature mRNA containing exons and introns into a mature mRNA containing only exons. **1)** U1 snRNP recognized and attaches to the 5' splice site of exon 1 through complementary base pairing. **2)** U2 snRNP recognizes and attaches to the branch site (B site) through complementary base pairing. **3)** U4, U5, and U6 tri-snRNP complex assembles and binds to the pre-mature mRNA creating the splicesome. **4)** U6 displaces U1 and U4 is released. **5)** The premature mRNA bends to bring the 5' splice close to the branch site. The spliceosome catalyzes the breakage of the 5' split site and the ligation of this site to the branch site. **6)** The splicing process is completed and the end products released are a lariat structure and a mature mRNA.

SnoRNA

Similar to snRNA are small nucleolar RNAs (snoRNA), which are short strands of single stranded RNA (ssRNA) that are confined to the nucleus and range in size from about 70 to 150 nucleotides[5]. In contrast to snRNA which play a role in cellular splicing and regulation of transcription, snoRNAs play major role in RNA modification. SnoRNAs are classified into two families based on structural similarities. Both families are involved in post transcriptional modifications of rRNAs, and to a lesser extent tRNA and snRNA[5, 7]. The first family is designated C/D box, because it has two conserved regions the C (UGAUGA) region and the D (CUGA) region. Members of this family are mainly involved in the methylation of rRNA.

Methylation is achieved by the addition of a methyl group to nucleotide within the premature RNA[18]. The second family is the H/ACA box, which also have two conserved sequences, the H (ANANNA) region and the ACA (ACA) region. This family of snoRNA is involved in pseudouridylation. Pseudouridylation is the process in which the nucleotide uridine is converted to an isomer known as pseudouridine. In a pseudouridine molecules, the uracil is attached to the ribose through a carbon-carbon bond rather than the original nitrogen-carbon bond found in the uridine molecule[19]. Both families of snoRNAs carry their function by recognizing sequences that are near the nucleotide that is to be modified. The catalysis of actual modification is carried by the proteins attached to the snoRNAs[20]. Similar to snRNAs, each family of snoRNA has a specific set of four proteins attached to it. Proteins that attach to the C/D box include fibrillarin, Snu13p, Nop56p, Nop58p. The proteins associated with H/ACA box include Cbf5p, Nhp2p Nop10p, and Gar1p [21]. These proteins assemble with the snoRNA to form the complex responsible for executing the fine post-transcriptional nucleotide modification. This complex is known as small nucleolar ribonucleoprotein (snoRNP) –not to be confused with snRNP which is the functional unit of a spliceosome- [5]. SnoRNAs seem to be involved in alternative splicing[22]. Snord115 has been shown to play a role in the inclusion of an exon known as Vb into the serotonin receptor. Deletion of snord115 was associated with also Prader-Wili Syndrome [22, 23]. In addition, snoRNA seems to play a role in oncogenesis[24-26] and the cell's susceptibility to stress[27].

Small non-coding RNAs in selected diseases

Cancer

The mention of snRNAs and their relation to cancer has been minimal in the literature. However, family 7SK has been described as potential therapeutic agents against cancer.

Overexpression of 7SK induced cellular apoptosis in different cancerous cell lines by inhibiting the activity of positive elongation factor b (P-TEFb). Another interesting observation is that stem cells had higher concertation of 7SK than differentiated cells[28, 29]. These finding demonstrates the regulatory that 7SK in cellular proliferation.

SnoRNAs have been linked to the proliferative ability of cancerous cells in different types of cancers and to the metastatic ability of these cancerous cells. A class of snoRNA known as snora23 increased significantly in metastatic tumor cell lines, and the knock down of genes responsible for its synthesis reduced cellular proliferation and metastasis[26]. SnoRNA55 snoRNA was also shown to induce growth and metastasis in prostate cancer patients[30]. Moreover, classes of snoRNA such as U3 and U8 were shown to interfere with the function of p53 gene in metastatic cells, and their absence lead to normal cellular apoptosis, indicating their oncogenic effect[31]. In addition, snora74B also increases in patients with gallbladder cancer, and its silencing in vitro resulted promoted normal cellular apoptosis[25]. It is clear that snRNA can be used in therapy, whereas snoRNAs play an important role in the progression of cancer, and hence their control may present therapeutic benefits in cancer treatment.

Diabetes

Although no direct relation has been established between snRNA nor snoRNA and diabetes, oxidative stress was linked to a some classed of snoRNAs. Oxidative stress is induced in diabetic patients and it is one of the main contributors to diabetic consequences such as nephropathy and retinopathy[32]. Hence, we a link between diabetes and snoRNAs can be established. U33a, U33, and U35a have been linked to lipotoxicity in diabetes. These

snoRNAs made the cells susceptible to apoptosis as a result of lipotoxicity and oxidative stress. Down regulation of U32a U33 and U55a lead the cells to become capable of withstanding lipotoxic stress and undergo normal life cycle and apoptosis[27].

Cardiovascular Disease

Similar to diabetes, neither snRNAs or snoRNAs were directly associated with cardiovascular diseases, however, the small of snRNAs as therapeutic agents to treat hypertrophic myocardiopathy was described in mice studies. U7 snRNA inserted into a vector can be used to transport antisense oligonucleotides (AON) to cardiac cells to promote exon skipping and correct this genetic disorder[33].

Obesity and Nutritional deficiency

There was no direct link between snRNAs or snoRNA and obesity or nutritional deficiencies. However, studies have shown that snord116, a class of snoRNAs, plays a role in the hyperphagia seen in those suffering from Prader Wili Syndrome (PWS). Loss of snord116 gene cluster was observed in a PWS patient and the associated hyperphagia and hypogonadism were imputed to the function of snord116 in the hypothalamus [34]. Moreover, animal models have shown that the loss of the snord116 gene increased food intake in mice, however it did not induce obesity in mice. This discrepancy between humans and mice suffering from the same deletion can be attributed to differences in mice and human metabolism[35].

Small non-coding RNAs in health and disease

Small nuclear RNAs are involved in post transcriptional processing of the pre-mRNA in healthy individuals. However, the relation of snRNAs to disease have not been widely

studied. SnRNPs and their associated proteins (LSm proteins) are the main structures studied, and research have been focused on the proteins moiety of snRNP rather than RNA moiety[36]. This can be attributed to the fact that snRNAs are known to function in site recognition rather than in the catalytic activity on the spliceosome during splicing.

It is worth to mention that snRNA could plays an active role in diseases related to exon skipping and viral infections. However, snRNAs could provide new therapeutic routes and delivery techniques[28]. It has been shown that utilizing U1 snRNA gene may provide a safe therapeutic possibility to patients suffering from splicing diseases such as hemophilia, cystic fibrosis and spinal muscular atrophy with mutated 5' exonic splice sites mutations. Mutating the 5' prime end of U1 snRNA would improve the splicing site recognition by the U1 snRNP within the spliceosome [37]. It has also been shown that inserting a functional beta-globin gene to a U7 snRNA gene through a vector to beta-thalassemia HeLa cells has significantly increased the production of normal beta-globin, providing an easier therapeutic method than gene replacement [38]. Intriguingly, snRNA induction has been associated with viral infections, and controlling the levels of snRNA in host cells provides means to tackle these infections [39].

On the other hand, many species of snoRNA have been associated with disease and they have been studied in greater depth and associations between many snoRNAs and disease have established. Example of snoRNA species associated with disease include snora23 (in cancer), U33 (in lipotoxicity), snord116 (in hyperphagia) which were discussed earlier. New research on snoRNAs (specifically SNORA7A) provide evidence that these RNAs play a role in multipotent stem cells proliferation and self-renewal. Hence, these RNAs could provide tools to tackle the problem of short survival of stem cells by improving their self-renewal capabilities[40]. In addition, it snoRNAs have also been implicated in joint disease, and the of these RNAs to track joint ageing and related diseases such as osteoarthritis was suggested.

Intriguingly, SNORD116 was found to be increased. Loss of this snoRNA result in PWS and its associated phenotypes, which include decreased mineral bone density (BMD). This finding shed the light on the contribution of this snoRNA in the pathogenesis of joint diseases, specifically osteoarthritis[41].

References

1. Ohno, S. *So much" junk" DNA in our genome*. in *Brookhaven symposia in biology*. 1972.
2. Alexander, R.P., et al., *Annotating non-coding regions of the genome.* Nat Rev Genet, 2010. **11**(8): p. 559-71.
3. Crick, F.H. *On protein synthesis*. in *Symp Soc Exp Biol*. 1958.
4. Holley, R.W., et al., *Structure of a ribonucleic acid.* Science, 1965. **147**(3664): p. 1462-1465.
5. Makarova, J.A., et al., *New functions of small nucleolar RNAs.* Biochemistry (Mosc), 2013. **78**(6): p. 638-50.
6. Hall, S.L. and R.A. Padgett, *Conserved sequences in a class of rare eukaryotic nuclear introns with non-consensus splice sites.* J Mol Biol, 1994. **239**(3): p. 357-65.
7. Carmen, L., et al., *Existence of snoRNA, microRNA, piRNA characteristics in a novel non-coding RNA: x-ncRNA and its biological implication in Homo sapiens.* Journal of Bioinformatics and Sequence Analysis, 2009. **1**(2): p. 031-040.
8. Tsanev, R., *Direct spectrophotometric analysis of ribonucleic acid fractionation by agar-gel electrophoresis.* Biochim Biophys Acta, 1965. **103**(3): p. 374-82.
9. Weinberg, R.A. and S. Penman, *Small molecular weight monodisperse nuclear RNA.* J Mol Biol, 1968. **38**(3): p. 289-304.
10. Hodnett, J.L. and H. Busch, *Isolation and characterization of uridylic acid-rich 7 S ribonucleic acid of rat liver nuclei.* J Biol Chem, 1968. **243**(24): p. 6334-42.
11. Sharp, P.A., *The discovery of split genes and RNA splicing.* Trends Biochem Sci, 2005. **30**(6): p. 279-81.
12. Berget, S.M., C. Moore, and P.A. Sharp, *Spliced segments at the 5' terminus of adenovirus 2 late mRNA.* Proc Natl Acad Sci U S A, 1977. **74**(8): p. 3171-5.
13. Reddy, R., et al., *The capped U6 small nuclear RNA is transcribed by RNA polymerase III.* J Biol Chem, 1987. **262**(1): p. 75-81.
14. Matera, A.G., R.M. Terns, and M.P. Terns, *Non-coding RNAs: lessons from the small nuclear and small nucleolar RNAs.* Nat Rev Mol Cell Biol, 2007. **8**(3): p. 209-20.
15. Will, C.L. and R. Luhrmann, *Spliceosome structure and function.* Cold Spring Harb Perspect Biol, 2011. **3**(7).
16. Moore, M.J., C.C. Query, and P.A. Sharp, *Splicing of precursors to mRNAs by the spliceosome.* COLD SPRING HARBOR MONOGRAPH SERIES, 1993. **24**: p. 303-303.
17. Shao, W., et al., *A U1-U2 snRNP interaction network during intron definition.* Mol Cell Biol, 2012. **32**(2): p. 470-8.
18. Tollervey, D. and T. Kiss, *Function and synthesis of small nucleolar RNAs.* Curr Opin Cell Biol, 1997. **9**(3): p. 337-42.
19. Peculis, B., *RNA processing: pocket guides to ribosomal RNA.* Curr Biol, 1997. **7**(8): p. R480-2.
20. Balakin, A.G., L. Smith, and M.J. Fournier, *The RNA world of the nucleolus: two major families of small RNAs defined by different box elements with related functions.* Cell, 1996. **86**(5): p. 823-34.
21. Ellis, J.C., D.D. Brown, and J.W. Brown, *The small nucleolar ribonucleoprotein (snoRNP) database.* RNA, 2010. **16**(4): p. 664-6.
22. Kishore, S. and S. Stamm, *The snoRNA HBII-52 regulates alternative splicing of the serotonin receptor 2C.* Science, 2006. **311**(5758): p. 230-2.

23. Kishore, S., et al., *The snoRNA MBII-52 (SNORD 115) is processed into smaller RNAs and regulates alternative splicing.* Hum Mol Genet, 2010. **19**(7): p. 1153-64.
24. Fang, X., et al., *SNORD126 promotes HCC and CRC cell growth by activating the PI3K-AKT pathway through FGFR2.* J Mol Cell Biol, 2016.
25. Qin, Y., et al., *SNORA74B gene silencing inhibits gallbladder cancer cells by inducing PHLPP and suppressing Akt/mTOR signaling.* Oncotarget, 2017. **8**(12): p. 19980-19996.
26. Cui, L., et al., *Small Nucleolar Noncoding RNA SNORA23, Upregulated in Human Pancreatic Ductal Adenocarcinoma, Regulates Expression of SYNE2 to Promote Growth and Metastasis of Xenograft Tumors in Mice.* Gastroenterology, 2017.
27. Michel, C.I., et al., *Small nucleolar RNAs U32a, U33, and U35a are critical mediators of metabolic stress.* Cell Metab, 2011. **14**(1): p. 33-44.
28. Abasi, M., et al., *7SK small nuclear RNA transcription level down-regulates in human tumors and stem cells.* Med Oncol, 2016. **33**(11): p. 128.
29. Keramati, F., et al., *7SK small nuclear RNA inhibits cancer cell proliferation through apoptosis induction.* Tumour Biol, 2015. **36**(4): p. 2809-14.
30. Crea, F., et al., *Integrated analysis of the prostate cancer small-nucleolar transcriptome reveals SNORA55 as a driver of prostate cancer progression.* Mol Oncol, 2016. **10**(5): p. 693-703.
31. Langhendries, J.L., et al., *The human box C/D snoRNAs U3 and U8 are required for pre-rRNA processing and tumorigenesis.* Oncotarget, 2016. **7**(37): p. 59519-59534.
32. Tangvarasittichai, S., *Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus.* World J Diabetes, 2015. **6**(3): p. 456-80.
33. Gedicke-Hornung, C., et al., *Rescue of cardiomyopathy through U7snRNA-mediated exon skipping in Mybpc3-targeted knock-in mice.* EMBO Mol Med, 2013. **5**(7): p. 1128-45.
34. 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, 2009. **18**(17): p. 3257-65.
35. Qi, Y., et al., *Snord116 is critical in the regulation of food intake and body weight.* Sci Rep, 2016. **6**: p. 18614.
36. Cooper, T.A., L. Wan, and G. Dreyfuss, *RNA and disease.* Cell, 2009. **136**(4): p. 777-93.
37. Fernandez Alanis, E., et al., *An exon-specific U1 small nuclear RNA (snRNA) strategy to correct splicing defects.* Hum Mol Genet, 2012. **21**(11): p. 2389-98.
38. Gorman, L., et al., *Stable alteration of pre-mRNA splicing patterns by modified U7 small nuclear RNAs.* Proc Natl Acad Sci U S A, 1998. **95**(9): p. 4929-34.
39. Meseda, C.A., et al., *Non-coding RNAs and heme oxygenase-1 in vaccinia virus infection.* Biochem Biophys Res Commun, 2014. **454**(1): p. 84-8.
40. Zhang, Y., et al., *H/ACA Box Small Nucleolar RNA 7A Promotes the Self-Renewal of Human Umbilical Cord Mesenchymal Stem Cells.* Stem Cells, 2017. **35**(1): p. 222-235.
41. Steinbusch, M.M., et al., *Serum snoRNAs as biomarkers for joint ageing and post traumatic osteoarthritis.* Sci Rep, 2017. **7**: p. 43558.