IMPLICATIONS OF UNUSUAL NON-CODING RNAS IN HUMAN DISEASES

By
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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Bachelor of Science in Biotechnology

Department of Mathematics and Natural Sciences
BRAC University
Fall 2020

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled “Implications of unusual non-coding RNAs in human diseases” submitted by Tazkia Ahmed bearing the ID: 17136040 of Fall 2020 has been accepted in partial fulfillment of the requirement for the degree of Bachelor of Science in Biotechnology.

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Abstract

Over the last few decades, the occurrences of genetic diseases and the rationale behind them are still under the wheels of research. Researchers have unveiled various causes of genetic disorders and shed light on the underlying complex connections. Although, there has been extensive work on genetic diseases that have mainly focused on the mutation among the coding gene as they carry the blueprint for protein synthesis. However, the unexplored non-coding RNAs also play a unique and critical role in gene transcription regulation and protein generation. This review mainly focuses on those specific non-coding RNAs that are thought of as unconventional in terms of their roles in gene expression regulation and are thus are termed as unusual non-coding RNA. In particular, we summarize our current understanding of the relationship of unusual non-coding RNA with human diseases. We also explore the regions in non-coding RNA, responsible for stimulating and promoting human diseases and look for the possible avenues for developing diagnostic biomarkers and therapeutics.

Keywords: ncRNA, unusual, disease, diagnostic biomarker, therapeutics

Dedicated to my beloved parents.

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List of Acronyms

RNA	Ribonucleic Acid
nc RNA	Non-coding RNA
nt	nucleotide
piRNA	PIWI- Interacting RNA
circRNA	Circular RNA
snoRNA	Small nucleolar RNA
snoRNP	Small nucleolar ribonucleoprotein
scaRNA	Small Cajal body associated RNA
AGO	Argonaute proteins
PWS	Prader Willi Syndrome
lncRNA	Long non-coding RNA
LPS	Lipopolysaccharide
MM	Multiple Myeloma
CMC	Closed mitral commissurotomy

Chapter 1: Introduction

One of the most famous notions of biology is Central dogma stated by Francis Crick. According to the central dogma, the genetic information is passed down from the DNA to RNA for protein synthesis [1]. Extensive studies have shown that 70% of the genome is transcribed and amongst them, only 2% serve as the blueprint for protein [2]. This 70% are those intermediates that are non-functional and do not encode for the proteins and are thus known as the non-coding RNA (ncRNA). They were termed as "junk RNA". The ENCODE project ("The Encyclopedia of DNA Elements") focused on annotating and identifying all the functional units in the human genome sequence [3]. Previously, the world of the non-coding RNA was thought to be governed by transfer RNA (t-RNA), however, studies and research gives an insight into the huge diversity of other long and short ncRNAs and have slowly turned into a highly popular topic for research. These ncRNAs play role in different areas of cellular biology [4]. The world of non-coding RNA began to unveil itself recently for which the diverse functions of non-coding RNA are yet not fully known. However, commonly the non-coding RNA is known for the gene regulation in eukaryotes [5], control of chromosome dynamics, splicing [6], RNA editing, translational inhibition [6], transcriptional regulation [7], and epigenetic control [8]. These non-coding RNAs are classified on the basis of the length of nucleotide (Figure 1). The long non-coding RNAs are considered to be greater than 200 nucleotides and the short non-coding RNAs are usually co smaller than 200 nucleotides. The long ncRNA comprises pRNA, eRNA, gsRNA, lincRNA, and NAT (Figure 1). On the other hand, the short ncRNA includes siRNA, miRNA, piRNA, snoRNA, and sdRNA (Figure 1).

Few of the most intensively studied ncRNA are miRNA, siRNA, long non-coding RNA (lncRNA) whose biogenesis and its implications on cancer and other diseases had been greatly reported. For example, studies regarding miRNA and its role in cancer had been extensively researched. However, the other ncRNAs such as snoRNA, circRNA which are less researched but yet play a regulatory role in the molecular mechanism of ncRNA and are considered to be the unusual non-coding RNA for this review paper. For example one of the unusual non-coding RNA is the snoRNA. It has been reported that snoRNAs actively participate in the development of breast cancer [9]. These ncRNAs are least highlighted for which a compilation of the functional roles of this unusual nc RNA is yet to be compiled. This review paper thus sheds light on the biology of unusual non-coding RNAs and their functional roles in different human diseases.

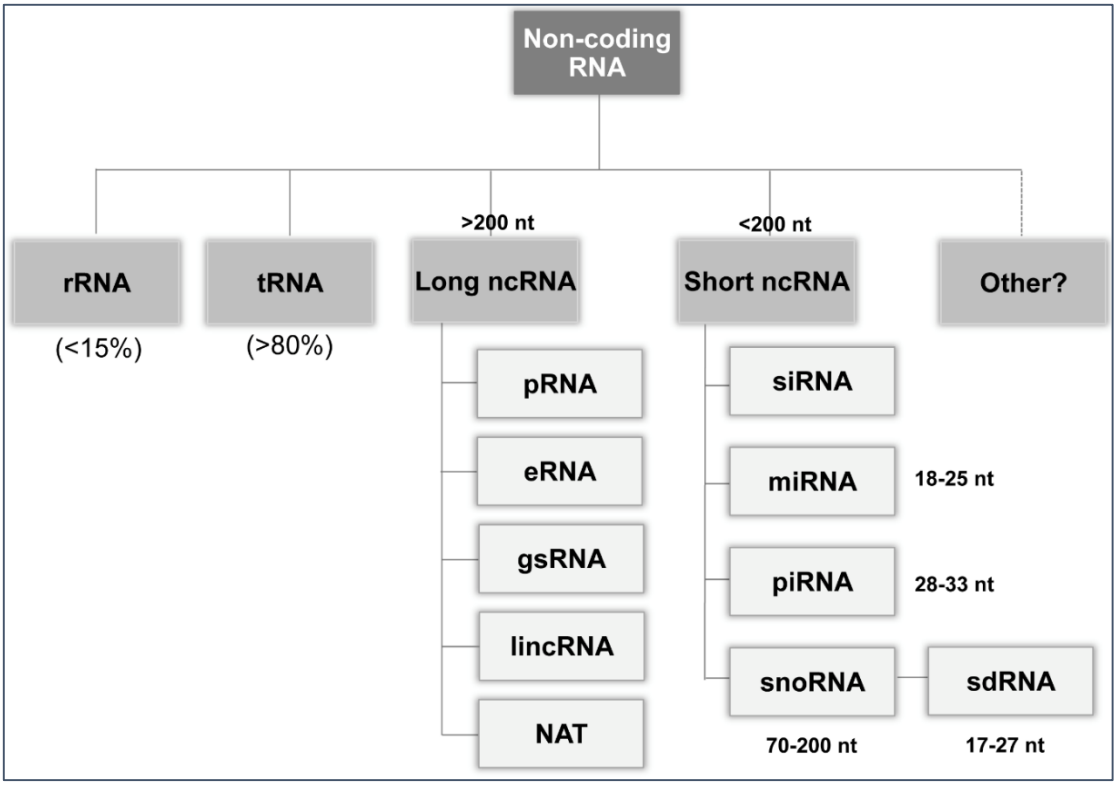

***Figure 1**: Classifications of non-coding RNAs (adapted from [10]).*

Chapter 2: Unusual non-coding RNAs and their biology

Non-coding RNAs are classified into a different subclass (Figure 1) and each subclass has its mechanism of action and follows a discrete pathway for various actions. The non-coding RNAs are linked with genes and functions as the key regulators which in turn can influence in the development of diseases [11]. Therefore, the mechanism of the non-coding RNA, their biogenesis, their transcriptional and post-transcriptional mechanism needs to be fully understood to properly comprehend the association of non-coding RNAs with diseases.

2.1 PIWI-Interacting RNAs (piRNAs)

PIWI-interacting RNAs (piRNAs) are the novel class of small non-coding RNAs of 24-31 nucleotides (nt) and are found in germline and somatic cells [12]. These piRNAs have a unique feature of 5'-terminal uridine on tenth position adenosine bias and also lack in the secondary structure motif [13]. The discovery of the piRNA took place in 2001 where it was recounted as small RNAs derived from the Su (Ste) tandem repeats of the Drosophila testes which were responsible for suppressing the Stellate transcripts to sustain the male fertility [14]. Following the biosynthesis pathway, it leads to the end product i.e. a mature piRNA from a piRNA cluster [15]. The piRNA/piwi complex is formed as a result of coherence between the piRNAs to the piwi proteins leading to the transposon silencing, genome rearrangement, epigenetic regulation, spermatogenesis, protein regulation, and germ stem cell maintenance [16]. The functionality of piRNA is not much clear but however, it is seen in an action widely in the case of epigenetic regulation, transposon silencing, protein regulation, and many others [16].

Biogenesis of piRNA

To generate mature piRNAs it mainly follows two pathways: the primary synthesis pathway and the 'ping pong' amplification pathway [17]. At first in the primary synthesis pathway, the riboendonuclease Zucchini first cleaves the primary transcript/piRNA and forms a 5' to 3' fragment which is later incorporated into the PIWI protein (Figure 2). The excess fragment is trimmed by a 3' to 5' exonuclease. The enzyme Hen1 acts on the 3'end for the methylation of the 2' hydroxyl group while the 5' end shows a strong inclination for uridine residues (Figure 2). After being processed into the ultimate final length, the piRNAs then bind with PIWI proteins respectively to form a piRNA/PIWI complex and then transports it back to the nucleus (Figure 2). In the secondary pathway, piRNA/AGO or piRNA/AUB complex is formed from

the piRNAs (Figure 2). These complexes contain the complementary sequences required to provide a substrate [16].

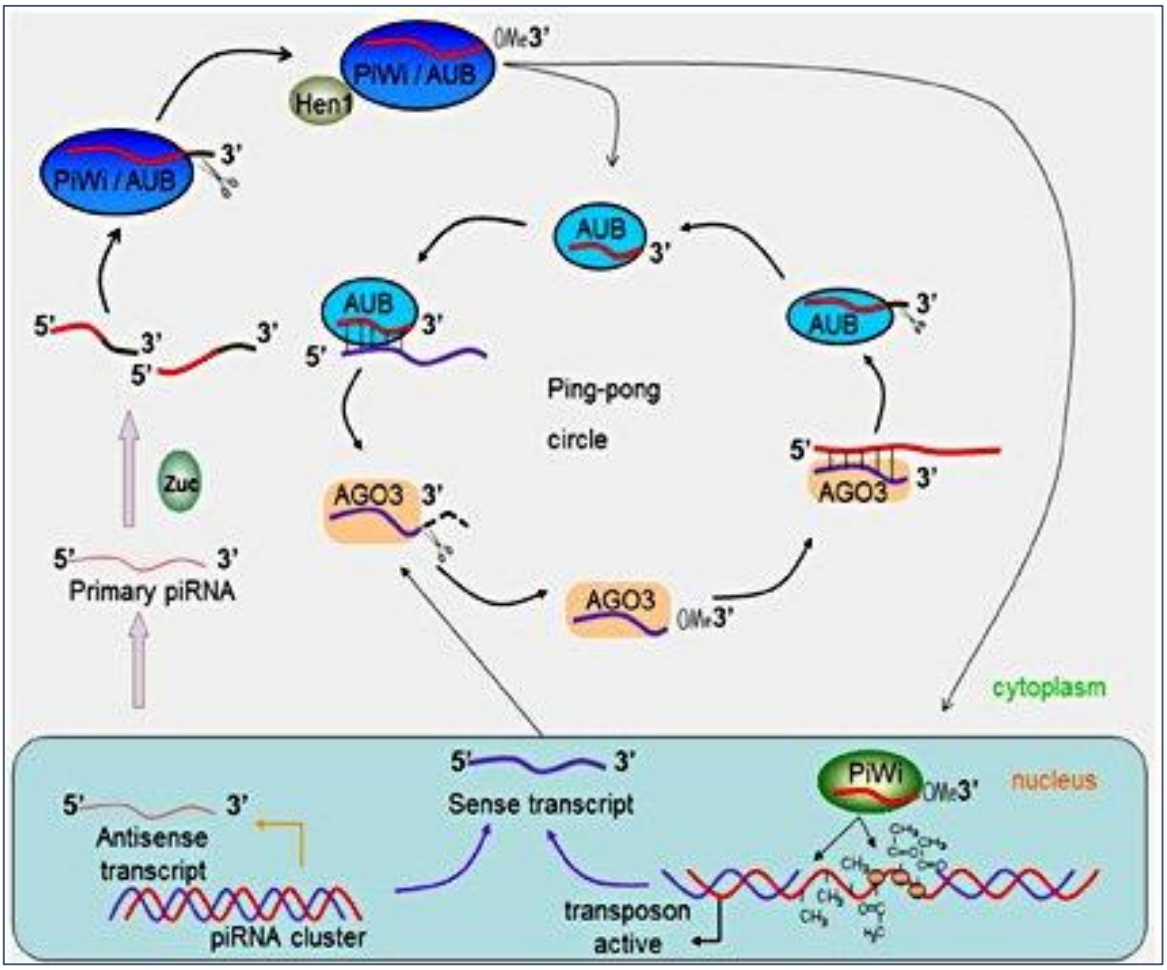

Figure 2*: Biogenesis of piRNA. The two pathways (primary synthesis pathway and ping pong amplification method) are followed to generate a mature piRNA (adapted from [16]).*

In the second stage i.e. after the primary piRNA generation, the piRNA enters the cytoplasm whereby it will be amplified by the "ping-pong" mechanism [18]. Here, piRNAs bind with AGO3 or AUB proteins and form piRNA/Ago or piRNA/Aub complexes which have the complementary sequences incorporated. PiRNA/Ago then forms a sequence of RNA that acts as a substrate for the formation of a new piRNA. This new piRNA has the capacity to load an Aub protein. Similarly, the newly formed piRNA/Aub protein complex will form more RNA substrates which in turn form a new piRNA/Ago3 complex (Figure 2). To summarize the ping pong method, the resulting product of the piRNA in the cytoplasm gives the food for another functional piRNA molecule to run [19].

2.2 Circular RNA (circRNA)

Circular RNAs (circRNAs) are considered to be a special group of non-coding single-stranded RNAs that have a high extraordinary stability [20]. The circRNA are covalently bonded to form a single-stranded continuous loop structure [21]. The circRNA is present in all kingdoms of life and are profound in eukaryotes. CircRNAs are formed from the splicing events and circularization of exons or introns [22]. The circRNAs were first observed in an electron microscope study of RNA virus [23] and were seen to exist in humans, mice, rats, fungi, and other organisms [24].

Biogenesis of circRNA

The circularization of circRNA takes place in several pathways. They are-

1. RBP associated pathway
2. Intron pairing pathway
3. Lariat-driven pathway

1. RBP associated pathway of circRNA biogenesis: The Muscleblind (MBL) and Quaking (QKI) binds the sequence motifs of flanking introns and joins the flanking introns together. This, in turn, causes the RBP to regulate the adjacent splice sites and thereby form circRNA formation. The inhibition of circRNA occurs due to the ADAR1, whereby the ADAR1 binds to the double-stranded RNA and melts the stem structure to form an ecircRNA and ElciRNA.

2. Intron pairing pathway: The circular RNA is produced by the direct base pairing of introns flanking complementary sequences or inverted repeats. The removal of the introns leads to the formation of ecircRNA or ElciRNA.

3. lariat-driven pathway: An exon skipping event leads to the formation of a lariat containing an exon. Following that the flanking intronic sequence is removed by the internal splicing of lariat. Thereby generating ecircRNA and ElciRNAs [25].

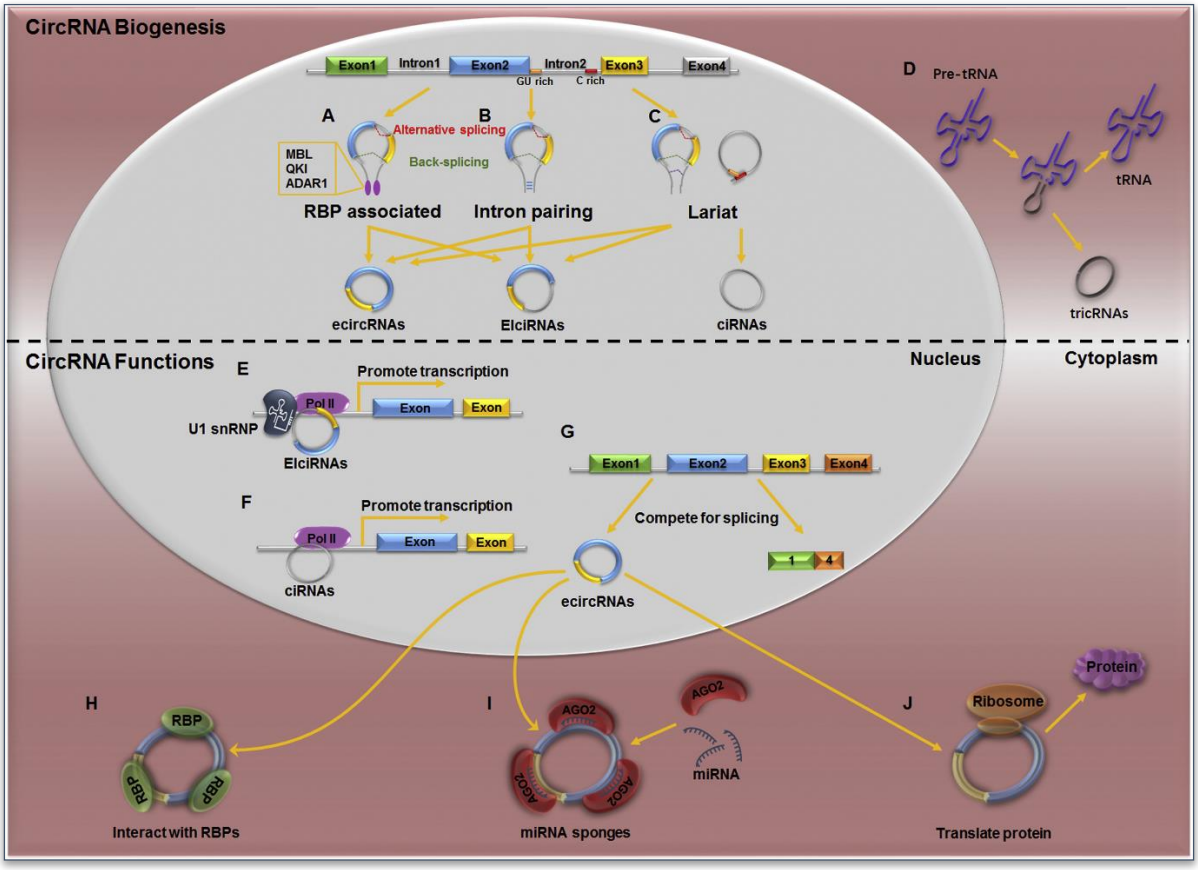

***Figure 3**: Biogenesis of Circular RNA. The upper half of the figure shows the biogenesis of circRNA along with the different routes used. The second half of the figure shows the different functions of circRNA involved (adapted from [26]).*

2.3 Small nucleolar RNA (SnoRNA)

The small nucleolar RNAs (snoRNAs) are commonly known as regulatory RNAs which play a role in the development of ribosomal RNAs (rRNAs). These are divided into two subtypes on the basis of their structure and conserved sequence motifs [10] i.e.: box C/D snoRNAs and box H/ACA snoRNAs [10, 27, 28]. The box C/D RNAs play a role in the 2'O—methylation while the box H/ACA is responsible for the pseudouridylation of nucleotides [29, 30]. Typically, the length of the eukaryotic box is 70-129 nucleotides and contains two conserved elements: boxes C (PuUGAUGA) and D (CUGA) which are present in the 5' and 3' terminal of the RNA molecule respectively. This forms a stem-bulge-stem structure that acts as a scaffold for the binding of small nucleolar ribonucleoprotein (snoRNP) and other proteins such as Nop1p (fibrillarin), Nop56p, etc. The key component of the snoRNP is the methyltransferase fibrillarin whose role is to catalyze the transfer of a methyl group from S-adenosylmethionine (SAM) to the 2'O position of the target nucleotide [30-34].

Biogenesis of snoRNA

The biogenesis of snoRNA can take place in different ways (Figure 4) [28]. For example, the synthesis of snoRNA occurs due to its transcription from independent genes (commonly seen in yeast and plants) [35]. Similarly, some snoRNAs usually follow the pathway where the snoRNAs are extracted from introns or polycistronic RNA [35, 36]. However, the synthesis of snoRNA in vertebrates is commonly seen to follow the process whereby the snoRNA is formed from the excised and linearized introns [28].

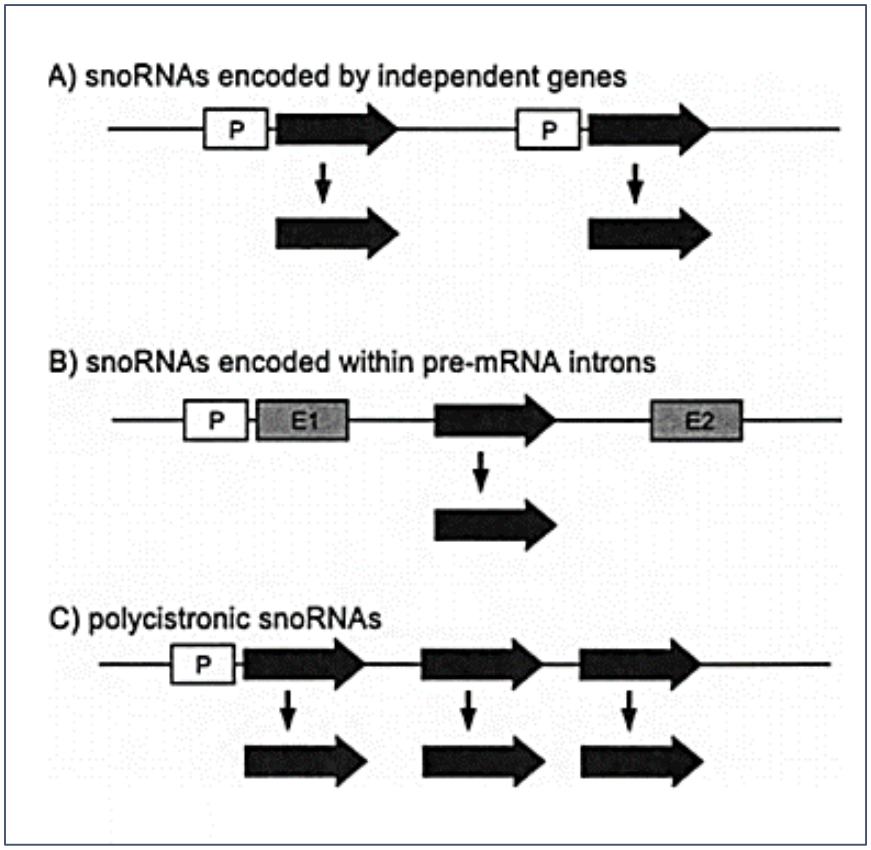

***Figure 4**: Biosynthesis of snoRNA. Biosynthesis of snoRNA can follow in either of the three different processes. The promoter region is marked as P and the grey box which is exon is marked as E and lastly, the snoRNAs are indicated by the black arrow (adapted from [37]).*

However, the established mechanism of snoRNA includes a 5-step method (Figure 4).

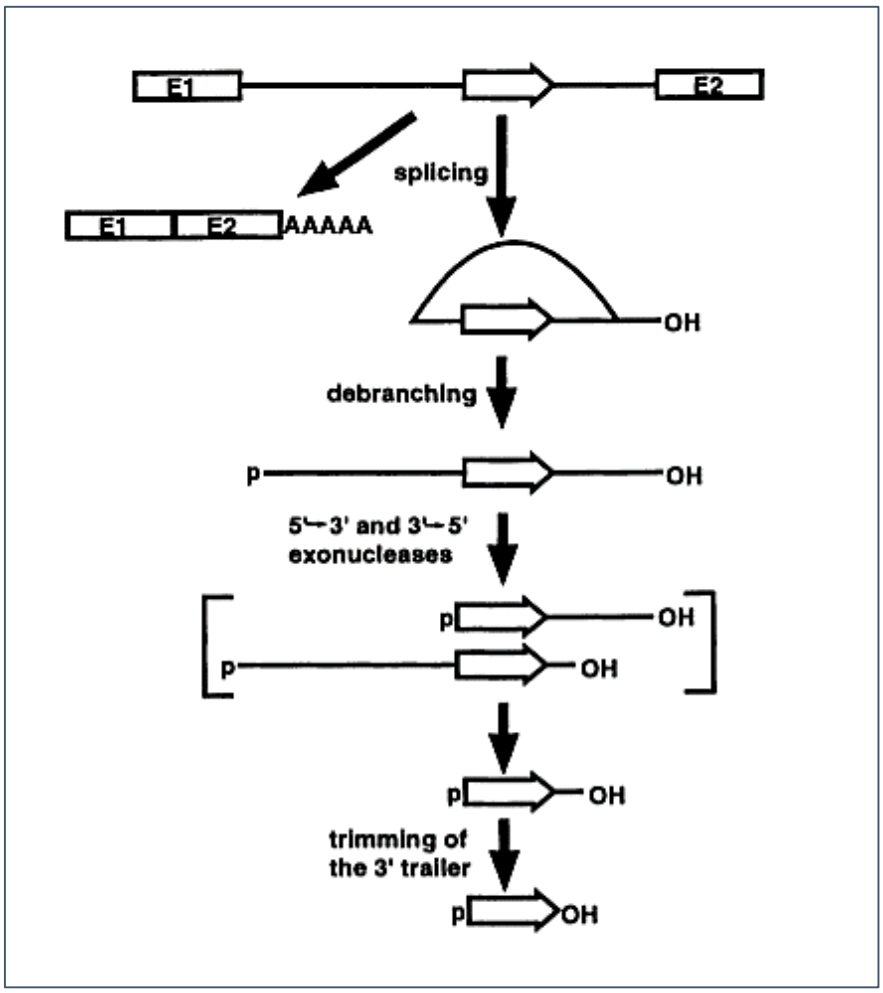

Figure 5*: An elaborated pathway of the synthesis of snoRNA (adapted from [37]).*

At first, the exons marked as E1 and E2 (Figure 5) are spliced followed by debranching. The exonuclease 5' to 3' and 3' to 5' is responsible for excising from the two ends and is ceased at the nick of the perimeter of snoRNA [38]. This occurs due to the tightly-packed RNP in the snoRNA region [38]. This leads to the formation of snoRNA. However, the open ends of the snoRNA might lead to the further processing and maturation of snoRNA therefore the last step includes the capping of RNA or the circularization of snoRNA [38].

2.4 Enhancer RNA

Enhancer RNAs or eRNAs are a special class of RNA that is involved in the regulation of gene transcription. The concept of eRNA is linked with that of the enhancers. Transcription of cells is manifested by the enhancers [39]. These enhancers are defined as the short regulatory elements of DNA accessibility which are edged by transcription factors, co-regulators, and RNA polymerase II (RNAP II) [39]. While looking into enhancer intently, it was found out

that active transcription was taking place in the enhancer region and formed a product titled "enhancer RNA" or "eRNA" [40, 41]. Although the role of eRNA is fully not clear but researchers suggest that the transcription of the enhancer is similar to the derivative of the transcription and amongst the various function of eRNA, it particularly has a special role in cell regulation [39].

Biogenesis of eRNA

The biogenesis of eRNA takes place in three steps- bidirectional initiation, elongation, and termination. The first step of initiation starts off with the recruitment of Pol II at the enhancer region [40-42]. The enhancer region is enriched with general and specific transcription factors (TF) and histone H3Kme1 and H3K27ac [40] (Figure 6). Typically, the histone filled enhancer regions do not generate eRNAs. However, the histone modification activity along with eRNA is used to predict whether the enhancers are active or not [40]. The eRNA production begins with bi-directional initiation whereby transcription occurs in both directions in the same region. The phosphorylation of Ser^5 and Ser^2 in the C-terminal domain (CTD) of Pol II occurs specifically in the initiation and elongation phase and also acts as a mark or indicator of elongation at the protein-coding gene bodies [43]. During the elongation period, the Pol II along with the TFs are mainly phosphorylated at Tyr^1 and Ser^5 [43-45](marked with brown circles in Figure 6). Furthermore, the transcription factors play a vital role in eRNA biogenesis. They are mostly involved in the regulation of cell growth and proliferation. However, in this case, the transcription factors (Spt5 and P-TEFb) are involved in the modulation and regulation of transcriptional pausing and elongation of eRNAs [45]. Subsequently, a large complex with 12 subunits known as Integrator comes into action in the transcriptional termination enhancer process [46]. The Integrator corresponds with the Pol II domain and results in a release from Pol II [46]. The WD Repeat Domain 82(WDR82) along with the protein phosphatase 1(PP1) is also responsible for the termination of eRNAs and thus releases eRNA [47].

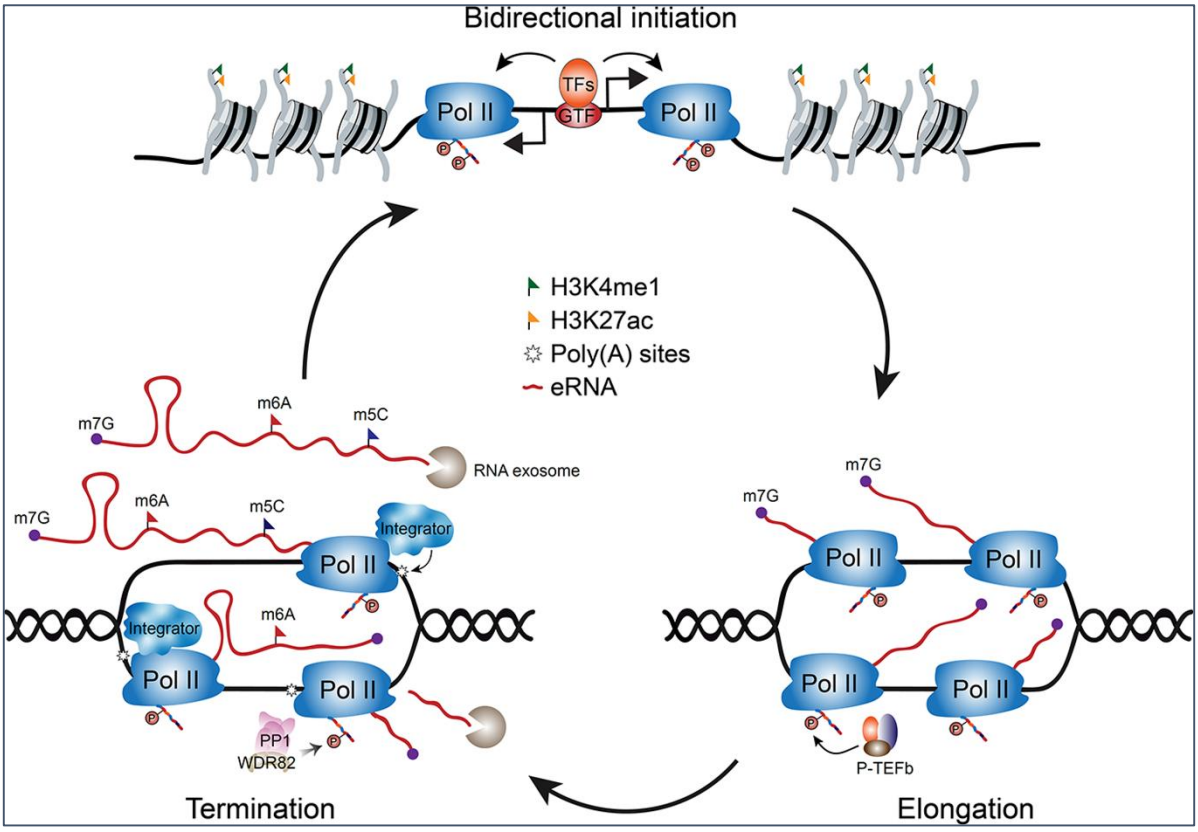

Figure 6*: The Biogenesis of enhancer RNA. The biogenesis of enhancer RNA occurs in three steps- bidirectional initiation, elongation, and termination. The key players involved are PolII, TF, and histone markers (H3K4me1 and H3K27ac) (adapted from [48]).*

However, after the formation of eRNA from chromatin, it is easily degradable by nuclear RNA exosomes, and therefore to protect it from degradation it undergoes modifications such as m5C and m6A which therefore makes them stable [49].

2.5 Small Cajal-body Association (ScaRNA)

The small Cajal body associated RNAs (ScaRNA) are the new and unique type of non-coding RNA which are part or subclass of the snoRNA family. The scaRNA specifically localizes to the Cajal body, which is a nuclear organelle. The Cajal body is a site whereby the synthesis of small nuclear riboproteins(snRNP) is formed. Overall, the scaRNA plays a major role in the alterations of different RNAs including rRNAs, snRNAs, and mRNAs. Alongside that, the scaRNA has many other responsibilities too for example pseudouridylation, etc.

Biosynthesis of scaRNA

The majority of the scaRNA has a primary transcript attached to them as they are considered as the intronic sequence [50]. These primary transcripts are emancipated from the sequence either by endonucleases or by splicing following the mRNA process [51, 52]. However, not all

the scaRNA has intronic sequences and the exceptions include SNORD3, SNORD13, SNORD118, SCARNA2, and SCARNA17 [52, 53]. These scaRNAs instead have intrinsic promoters that work to their advantage [52].

The synthesis of SCARNA2 and SCARNA17 begins at the conserved motifs upstream of the intragenic loci where they are found [54]. The exonuclease starts removing and degrading from the two ends of the intronic sequence [55]. This degradation is stimulated due to the binding of the protein near the sno/scaRNA terminals [51, 55]. The process of degradation is an ongoing process until a bound protein hinders the process which results in the release of sno/scaRNA [55]. This whole process takes place in nucleoplasm where they are synthesized, processed, and transported to the Cajal body [53].

2.6 Antisense RNA (asRNA)

Antisense RNA is considered to be the embodiment of non-coding RNA required for gene regulation in cells such as DNA, RNA and chromosome structures, transcription and translation, and RNA and protein stabilities [56]. The antisense RNA molecule is basically a distinctive type of DNA transcript which is complementary to mRNA [57].

Formation and regulatory mechanism

Antisense RNA is said to be a DNA transcript for which it does not have a biosynthesis process. However, the regulatory mechanism is known and discussed below. The mechanism of the antisense RNA is said to be similar to that of *lac repressor* [58]. In the lac operon system, there were few components similar to antisense RNA and here one of the main components is the operator. The operator is responsible for controlling the expression of a gene. The operator binds to either the genes (Figure7 Model 1) or with the cytoplasmic messengers flanking genes (Figure 7 model 2) [57]. When the repressor (for example a small RNA or antisense RNA in this case) binds to the operator region it is activated and thus disables the function of the gene which thereby causes the protein synthesis to be inhibited (Figure 7 Model 2). The antisense RNA follows the same mechanism and is also involved in disease association.

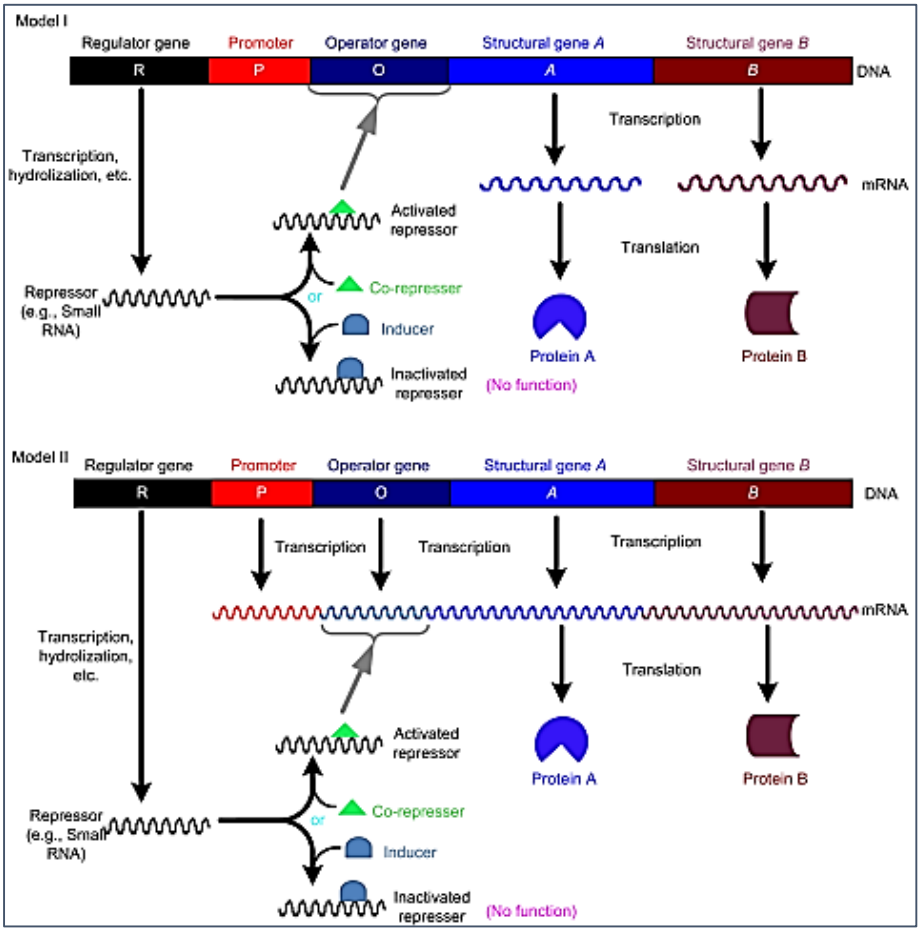

Figure 7*: Regulatory mechanism of antisense RNA. The mechanism of antisense RNA can be illustrated in two models. Model I and model 2 both represent the regulatory mechanism followed by antisense RNA (adapted from [57]).*

The Antisense RNA based regulation is much more preferred over other regulation techniques mainly because of two reasons. Firstly, in a situation where the protein requires a specific set of conditions to be expressed or repressed the antisense RNA can easily yield the condition accordingly [58]. Secondly, because of less complex pathways involved and therefore takes less time [58].

Chapter 3: Roles of unusual non-coding RNA in human diseases

The non-coding RNA is responsible for critical physiological and cellular processes. Emerging studies have shown that a change in the signaling pathway or upregulation or downregulation of these non-coding RNA could lead to human diseases. These unusual non-coding RNAs can thus cause various diseases by following different pathways and mechanisms which will be broadly described below.

3.1 piRNA

3.1.1 piRNAs in cancer

piRNAs are responsible for the dysregulation in tumor tissues, they can either act as a regulator or an inhibitor in the tumor cells [59]. Recent studies have shown the role of piRNAs in different cancer types. A few of them are elaborated below.

Role of piRNA in Breast Cancer

Amongst the different types of cancer, Breast cancer accounts for 25% of all the cancer type and is commonly diagnosed [60]. One of the piRNAs responsible for breast cancer is piR-36712/PIWIL complex. This piR-36712/PIWIL1 is responsible for the inhibition of cell proliferation, invasion, and migration through the p53/p21/SEPW1P RNA. The endogenous RNA (ceRNA) i.e SEPWEP1 competes with SEPW1 RNA for miR-7 and miR-324. The upregulation of piR-36712 causes the SEPW2 expression to decrease or down-regulate thereby causing the inhibition of ubiquitin-medicated degradation and activates the p53 and p21 thus resulting in G1 cell cycle arrest [59]. Another notable piRNA responsible for breast cancer is piR-932. The piR-932 forms a complex with PIWIL2 to influence latexin promoter CpG island methylation in Breast Cancer stem cells. This complex suppresses the latexin expression thus promoting epithelial-mesenchymal transition (EMT) in breast cancer [61]. This latexin is a tumor suppressor and causes the decrease in old stem cell transformation into Cancer Stem Cell (CSCs), reduces cell replication, and increases apoptosis [62, 63]. Thus, the suppression of latexin leads to the up-regulation of tumor expression.

Table 1: Illustrative list of piRNAs involved in different types of cancer (Adapted from [64]).

piRNA	*Cancer Type*	*Function*	*Expression in Tumors*
piR-36712	Breast Cancer	Suppressed cell proliferation, invasion, and migration by combining with SEPW1P RNA	Down
piR-021285		Inhibited cell proliferation and invasion by ARHGAP11A methylation	Down
piR-932		Caused EMT through promoting promoter region CpG island methylation of Latexin	Up
piR-DQ598677		Form pi-RISC to degrade targeted genes like miRNA	Down
piR-34871	Lung Cancer	Correlated with RASSF1C expression, promoted cell proliferation, and colony formation by reducing AMPK phosphorylation of ATM-AMPK-p53-p21cip pathway	Up
piR-52200			
piR-35127			Down
piR-46545			
piR-651		Promoted cells and tumor proliferation and inhibited apoptosis, induced cyclin D1, and CDK4 expression	Up
piR-55490		Inhibited LC cells and tumor proliferation by binding 3’ UTR of mTOR mRNA	Down
piR-823	Gastric Cancer	Inhibited proliferation of cancer cells, and caused cells aberrant “stem-like” state by weakening tumor supporter genes methylation	Down
piR-651		Promote cell proliferation and associated with TNM stages	Up
piR-FR222326		Positively associated with overall survival	Up
piR-FR290353		Associated with recurrence-free survival	Up

piRNA	*Cancer Type*	*Function*	*Expression in Tumors*
piR-FR064000 piR-FR387750			
piR-1245	Colorectal Cancer	Accelerated cell growth, promoted migration and invasion as well as anti-apoptosis by binding to its downstream targeted mRNA in nuclear exosomes, associated with poor differentiation, TNM state, and poor overall survival	Up
piR-54265		Promoted proliferation and metastasis, inhibited apoptosis, correlated with shorter progression-free survival time and overall survival time, caused therapy resistance to anti-tumor agents by regulating STAT3 phosphorylation	Up
piR-823		Enhanced cells proliferation and suppressed apoptosis by promoting HSF1 phosphorylation at ser326 and inducing Stat3 phosphorylation	Up
piR-015551		Influenced the colorectal cancer development by causing gene mutation.	Up
piR-Hep1	Hepatocellular Carcinoma	Promoted cells proliferation and invasion via upregulating phosphorylated AKT of PI3K/AKT signaling pathway	Up
piR-LLi-24894		Associated with low-grade lesions of hepatocellular carcinoma	Up

piRNA	*Cancer Type*	*Function*	*Expression in Tumors*
Hsa-piR-013306		Involved in the hepatic carcinogenic process	Up
piR-32051 ***piR-39894*** ***piR-43607***	Kidney Cancer	Linked with renal cell carcinoma of high tumor stage and metastasis and cancer-specific survival	Up
piR- 57125		Inhibited cancer metastatic	Down
piR- 30924 ***piR-38756***		Associated with cancer metastatic	Up/Down
piR-823	Hematological Malignancy	Promoted proliferation, inhibited apoptosis, and modulated cell cycle progression of multiple myeloma cells by regulating DNA methylation and angiogenesis	Up
piR-651		Associated with shorter disease-free survival and shorter overall survival in classical Hodgkin lymphoma patients	Up
piR-30188	Glioblastoma	Suppressed tumor cell proliferation, invasion, and migration and promoted the delivery of therapeutics into the glioma micro-environment via binded to MEG3	Down
piR-8041		Promoted cells proliferation and inhibited death by interacting with mRNA MAPK	Down
piR-DQ593109		Increased the permeability of the blood-tumor barrier and promoted the delivery of therapeutics into glioma micro-environment via binded to MEG3	Down
piR-DQ590027		Increased the permeability of glioma-conditioned normal blood-brain barrier and promoted the transport of	Down

piRNA	*Cancer Type*	*Function*	*Expression in Tumors*
		macromolecular chemotherapeutics into glioma tissues by binding to MIR17HG	
piR-39980	Fibrosarcoma	Inhibition of cell proliferation o=via interacting with RRM2	Down
piR-52207	Ovarian Cancer	Promoted cell proliferation migration and tumorigenesis by binding to targeted mRNA (NUDT4, MTR, EIF253, MPHOSPH8)	Up
piR-33733		Inhibited cells apoptosis by binding to targeted mRNA (ACTR10, PLEKHA5)	Up
piR-017061	Pancreatic Cancer	Not clear	Down

3.1.2 Role of piRNA in Neurodevelopmental Disorder

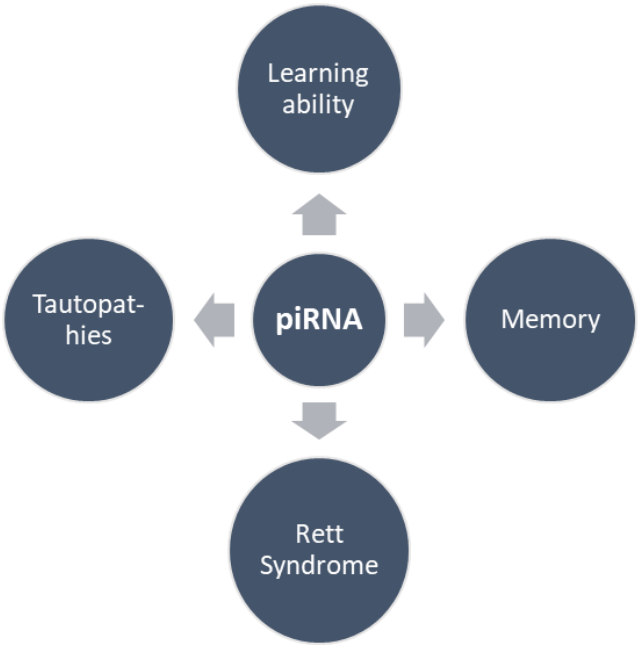

Figure 8*: Neurodevelopmental problems caused due to piRNA.*

Recent studies have shown that the piRNA pathway functions by controlling the activity of transposable elements in the germline. These transposable elements are those that can replicate and incorporate themselves in the new genomic locations however mutation in the germline genome can lead to sterility [65]. The PIWI-piRNA pathway has a significant role in brain

development and is found to be the key factor in many neurological diseases. Active transposition occurs during neurogenesis whereby the neurons are entitled to genetic diversity and occurs commonly in humans [66, 67]. Similar to the mammalian hippocampus, the fly transposition occurs in memory-relevant neurons inside the mushroom body (which is crucial for the learning and memory region) [65]. It suggests that there might be a mechanism whereby PIWI/piRNA mediated suppression results in the transposition rate in a brain region involved in learning and memory. Hence the collective disruptive transposition may lead to the neural problem and other neurological diseases [66].

It is also assumed that piRNA dysregulation leads to Rett Syndrome (RTT), a neurodevelopmental disorder. Rett Syndrome is commonly said to be caused due to the methylation of methyl-CpG binding protein 2 (MeCP2). Studies show that the piRNA levels are significantly more where there is MeCP2 knockout [68]. The activity of the transposable element LINE-1 is increased with the reduction of MeCP2 activity and can thus be concluded that piRNA dysregulation can lead to Rett syndrome [69].

Neuronal cell death in neurogenerative tauopathies occurs as a result of Tau aggregation i.e induced decondensation of heterochromatin and leads to the reduction of PIWI and piRNA activity thus causing transposable element dysregulation [70]. In an Alzheimer's patient, it has been observed that the piRNA was found in abundance and have thus suggested that the up-regulation of piRNA might be the cause of the neurodegenerative disease [71, 72].

3.2 circRNA

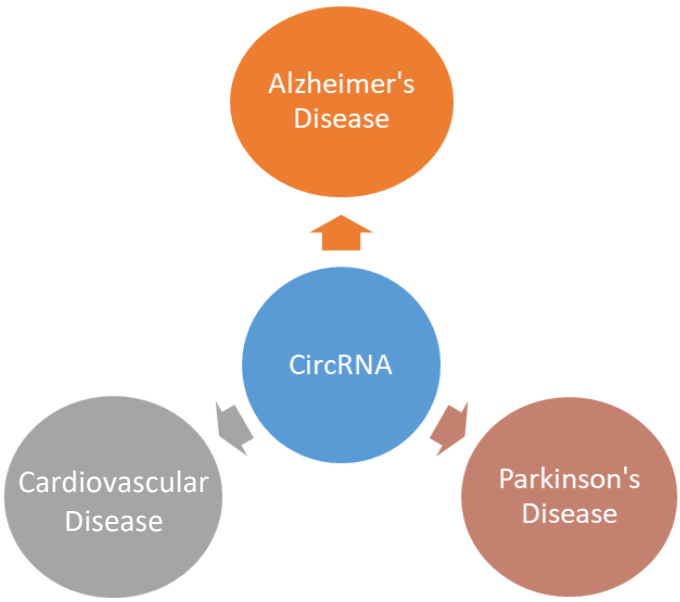

***Figure 9**: Diseases caused by circular RNAs.*

Researchers have found out the association between circRNA with autophagy [73], apoptosis [74], cell cycle [75], and proliferation which points out that circRNA might have a role in different diseases.

3.2.1 circRNA and Alzheimer's Disease (AD)

The role of circRNA in Alzheimer's disease is still not elucidated to indicate that circRNA is the potential reason for AD. However, studies show that the microRNA-7 which is profoundly present in the human brain is also associated with circRNA and is known as ciRS-7 [20]. The ciRS-7 functions as an endogenous, anti-complementary miRNA "sponge" which takes in the role of miRNA-7 [20], and this miR-7 circRNA system is observed to be down-regulated as seen in AD patients and is tested and confirmed by using the Northern blot hybridization technique and the circularity-sensitive circRNA probe RNase R. This was reinforced by a study which explains that the ciRS-7 also impedes the NF-KB pathway translation and causes localization to the cytoplasm and this, in turn, suppresses UCHL1 which is responsible for up-regulating APP and BACE1 degradation [76]. APP and BACE1 are responsible for the production of amyloid β in AD. Thus this validates and concludes that ciRS-7 could be an effective target for the treatment of AD [77].

3.2.2 circRNA and Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder that mainly influences the dopamine-producing neurons in the specific parts of the brain. The microRNA-7 which is profoundly found in the brain is linked with the circRNA to form ciRS-7 and is also known as CDR1as. The CDR1as is responsible for the suppression of miR-7 which then inhibits the expression of α synuclein, an indicator of the PD brain [78]. This benefits the cells against oxidative stress and adds a layer of protection against neuron death.

Most studies of circRNA are usually carried out in vitro until very recently Piwecka et al. made a knockout murine model for Cdr1as which shows that the brain of these transgenic mice had higher levels of miR7 genes such as c-Fos and concluded that it had defects in synaptic transmission and information processing [79]. The study hence suggests that circRNA plays an important role in neurological diseases [80].

3.2.3 circRNA in Cardiovascular Disease

CircRNAs have portrayed themselves as a safeguard and protector of our hearts. The circRNA involved in this action is HRCR which is thought to provide protection from cardiac hypertrophy and heart failure by binding to the inflammatory onco-moRmiR-223. When treated with angiotensin II, the level of circRNA_000203 and circRNA_010567 increases in cardiomyocytes. These circRNA are responsible for the downregulation of miR-26b-5p, miR-141, and miR-141 which in turn up-regulates the TGFB1 gene. The influence of this gene causes the suppression of fibrosis-associated protein and encourages the production of fibrosis in the myocardium [81, 82].

3.3 snoRNA

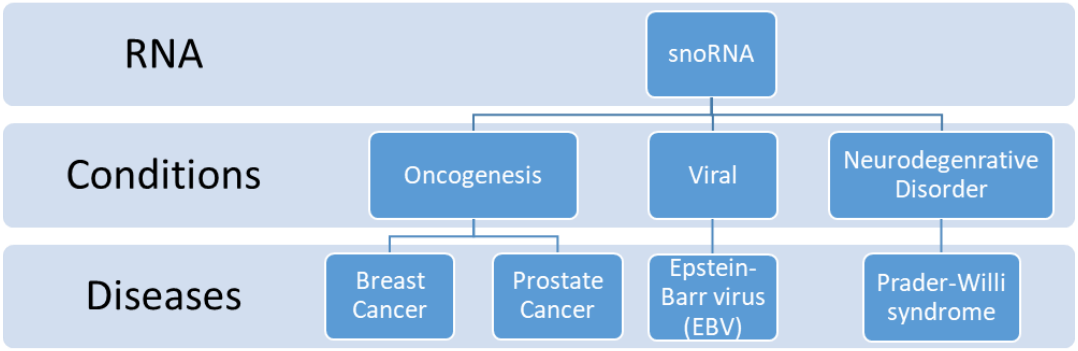

***Figure 10**: Association of snoRNA and human diseases.*

Studies suggest that snoRNAs are involved in the regulation of posttranscriptional modification of ribosomal RNAs for which the change in snoRNA expression level can affect the physiological condition and cause disruption of the vital processes and lead to various diseases [83].

3.3.1 snoRNA and oncogenesis

In a recent study of breast cancer cells, it was seen that the expression level of snoRNA U22, U3, U8, U94, and U97 box C/D snoRNAs had higher expression levels compared to the normal human cell [83]. The correlation between the expression of snoRNA, snoRNP (the core component of fibrillarin), and Myc oncogene was observed. Changes in the level of snoRNA cause the change in the expression of snoRNP which had a connection with the oncogene. The increased amount of fibrillarin was observed in breast cancer and prostate cancer. Similarly, when the expression level of fibrillarin was decreased along with other proteins then there had

been a significant decrease in the box C/D snoRNA level which was seen to have suppressed cancer cell growth and reduced the oncogenicity.

In the case of non-small cell lung carcinoma, the small nucleolar box H/ACA RNA SNORA42 was seen to be overexpressed [84]. The decrease in the specific SNORA42 cancer cell can cause apoptosis in p53 dependent manner. Contrarily, the increased expression of SNORA42 was found to trigger cancer cell growth [84].

The levels of snoRNA in cancer cells are regulated by different mechanisms one of which could be the methylation of CpG islands which are found close to the snoRNA gene. In HCT-116 colorectal cancer cell, 49 snoRNA genes which were within 2kb from 5' termini of CpG island were analyzed [85]. On giving a closer look, it was seen that hypermethylation of CpG islands near the host genes for SNORD123, SNORA70C, and SNPRA59B was established to be specific for cancer cells. Further analysis shows that the expression of box C/D and H/ACA snoRNAs in HCT-116 and SW48 cells were repressed or reduced in comparison to the cells near SNORD123, SNORA70C, and 59B snoRNA where CpG island methylation did not take place. This gives us an idea that the epigenetic control mechanism for the regulation of snoRNA expression could be the reason for various cancer cells [86].

3.3.2 snoRNA and Human Neurodegenerative Disorder

The relationship between snoRNA and human neurodegenerative disorder is linked by the genes involved in snoRNA. Prader-Willi syndrome (PWS) is a human neurodegenerative disorder and is associated with snoRNA. The common reason behind the Prader Willi syndrome (PWS) is due to the loss of paternal gene expression from a maternally imprinted region 15q11-q13 on chromosome 15. Two copies of two box of C/D RNAs-SNORD115 (HBII-52) and SNPRD116 (HBII-85) are present in the locus 15q11-q13. The deletion of SNORD116 snoRNAs is considered to be one of the significant causes of PWS. In a mouse model experiment, it was proved that the change in DNA methylation pattern caused the expression level of snoRNAs to change specifically the SNORD115 and SNORD116 [87]. Intensive studies using the mice model concluded that the change in SNORD115 could lead to abnormal brain development and may also be the cause of autism [88].

3.3.3 snoRNA and Viral Diseases

In a study on herpes viruses, it has been seen that the snoRNAs are encoded in viral genomes and a box C/D RNA which is known as v-snoRNA1 (small nucleolar RNA 1) was found to be

infected with Epstein-Barr virus (EBV) in B lymphocytes [89]. Although the viral box C/D RNA has C/D RNA which has C/D and C'/D' pairs of boxes complementary to the human 18S and 28s rRNA yet it does not prove the 2'-O methylation of the potential rRNA target nucleotides in infected cells. Fragment of the viral box C/D RNA, v-snoRNA^{24pp} was also found infected with EBV. Therefore, it can be concluded that the presence of C/D RNA in the viral genome proves that viruses are able to employ the snoRNA- dependent mechanisms for the regulation of their life cycle.

A recent analysis of lung transcriptome of mice infected with severe acute respiratory syndrome coronavirus (SARS-CoV) shows differential expression of 30 small RNAs were seen to be overlapping with annotated snoRNAs. In the GAS5 locus, two snoRNAs that were differentially regulated by respiratory virus infection were found [90]. In the chikungunya virus, the GAS5 intronic snoRNAs U44, U76, and U78 were found to have high levels of expressions in human cells [91]. It can also be assumed that the GAS5 intronic snoRNAs are correlated with the regulation of T cell growth [92].

Lastly, the hypothesis that viruses are capable of using other pathways to evade the host immune system is established. However further studies are required for the complete understanding of the role of snoRNA in viral infections [93].

3.4 eRNA

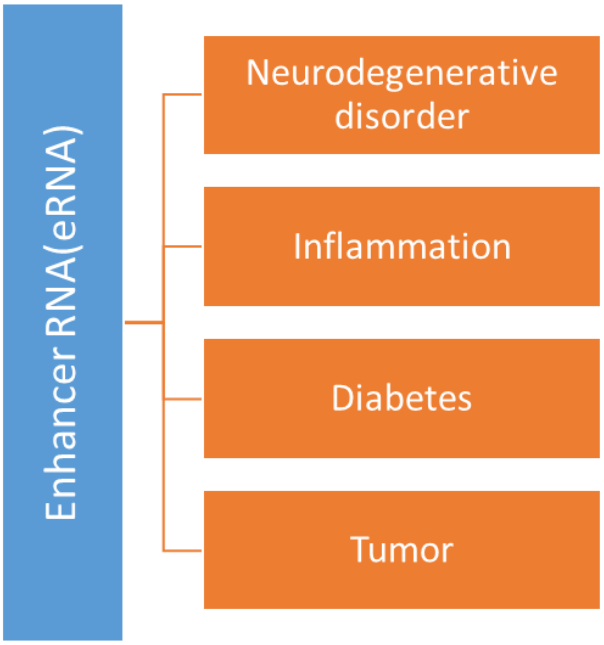

***Figure 11**: The involvement of Enhancer RNA and diseases.*

3.4.1 eRNA and neurodegenerative disorder

Enhancers have a notable function in the development of hereditary neurodevelopmental and neuropsychiatric disorder and as it is thought to carry mutations [94]. Le et al. reported that most of the enhancers in the striatum of the mouse brain were seen to be converted to enhancer RNA [95]. In addition to that, the number of enhancers in the brain might be a little over a hundred and were seen to have an effect on the mouse with Huntington's disease (HD) [95]. In comparison with a normal individual, it was observed that the eRNAs were down-regulated causing a subsequent effect on the target genes of HD [94]. Moreover, the association of the eRNAs with autism spectrum disorders were accredited due to the presence of the diseased genetic variants in enhancer RNAs [96].

3.4.2 eRNA and inflammation

The proinflammatory response is interdependent on the NF-KB signaling pathway which is governed by the IL1β, enhancer RNA [94]. On that account, it has been observed that the removal of IL1β-eRNA leads to a decrease of IL1β, the inflammatory media production, and thus has showcased the eRNA's potential immune system regulatory behavior [94]. Alongside that, the relationship between eRNA and inflammatory response is demonstrated and confirmed by the action of Bmal1 [97]. The Bmal1 is responsible for the control of macrophage inflammation [97]. Change in Bmal1 leads to the change in immune response and thus inflammation [97].

3.4.3 eRNAs and diabetes

Diabetes is a chronic disease of the pancreas which is characterized on the basis of the action of insulin and blood glucose level [98]. An anti-diabetic drug, Rosiglitazone is used for maintaining the glucose level in the blood and subsequently boosts insulin sensitivity [94]. In a study, it was seen that the efficacy of the drug was increased highly when the enhancer RNA levels at specific sites were increased which made the researchers draw a parallel line to conclude the connection between enhancer RNA and diabetes [94].

3.4.4 eRNA and tumor

By using AR ChIP- seq analysis, it was found out that hundreds of eRNAs were differentially expressed in prostate cancer [99]. It was highly affirmed due to its constant upregulation of PSA eRNA in the case of castration-resistant prostate cancer (CRPC) cells, patient-derived

xenografts (PDX), and patient tissues [100]. Moreover, in breast cancer cell MCF-7 it was seen that the estrogen-induced transcription of eRNA was upregulated [101]. However, it cannot be stated that only overexpression of eRNA causes cancer. This statement is endorsed by the study which illustrated that patients with throat cancer had a significantly lower number of enhancer RNAs [94].

3.5 scaRNA

As discussed earlier the role of scaRNA and its pathogenesis shows that the defects of scaRNA or its affiliated molecule are a probable reason for human diseases. These diseases occur in different forms and different organ system i.e. nervous system, musculoskeletal and cardiovascular system. An extensive study has revealed the diseases caused due to scaRNA and its association is discussed below [102].

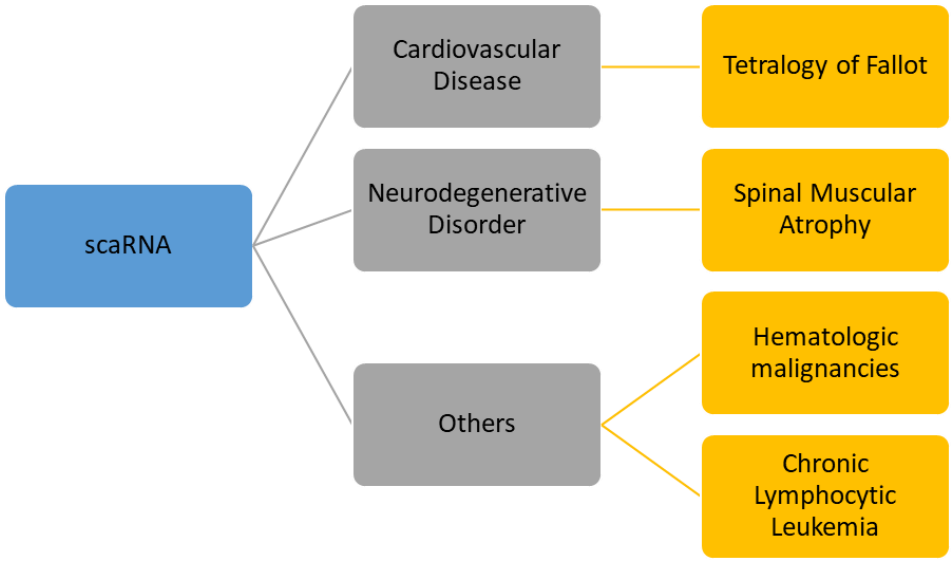

***Figure 12**: Associations of scaRNA with disease.*

3.5.1 Cardiovascular diseases and its affiliation with scaRNA

In a study, it had been found that cardiac diseases, alternative splicing, and heart development are closely associated with scaRNA in closed mitral commissurotomy (CMC) [103, 104].

Tetralogy of Fallot (TOF)

One of the most widely known congenital heart diseases is Tetralogy of Fallot. This has a prevalence rate of 0.5/1000 births and is caused due to the combination of four defects i.e. right ventricular septal defects, overriding aorta, and right ventricular hypertrophy [105, 106]. These congenital heart defects are coupled with that of the dysregulation of alternative splicing which

is linked with scaRNA [107, 108]. As discussed earlier, we know that scaRNAs are involved in splicing and are thus thought to have a confounding effect on congenital heart diseases [104].

Researchers concluded that alternative splicing isoforms are observed in genes that are responsible for heart development especially in infants with TOF [104]. Abnormal splicing is observed in places such as GATA4, muscleblind-like protein 1 (MBNL1), and genes of the Wnt pathway whereby the scaRNA is inhibited due to the lack of snRNA [104]. Infants with TOF have been characterized by the low expression of 12 scaRNA in the right ventricle [104]. These 12 scaRNA in turn influence the U2 and U6 snRNAs which affects the heart tissue [104]. Similarly, in the patients with TOF, the GATA4 and the Wnt pathway are also altered and modified [106, 109].

3.5.2 ScaRNA and Neurodegenerative disorder

Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) is one of the most common neuromuscular degenerative disorder with an occurrence rate of 1 in 11,000 births [110]. The Spinal muscular atrophy occurs as the motor neuron 1(*SMN1*) gene ceases to function and leads to weak muscle with poor reflexes [111, 112]. However, the gravity of the seriousness of the disorder is dictated by the gene *SMN2* [111]. According to the seriousness, it can be categorized into four types (I-IV) with ascending order of severity [111]. The involvement of scaRNA is related with spliceosome activity. For the smooth functioning of the spliceosome, the Gar1 is amalgamated into scaRNP and also requires the biogenesis of snRNA both of which require the SMN [102]. Therefore the disruption or deactivation of SMN would affect the spliceosome activity and in turn cause Spinal muscular atrophy [102].

3.5.3 ScaRNA in other diseases

Hematologic Malignancies

The scaRNA has a contributing role in the pathogenesis of Multiple myeloma (MM) [102]. Multiple Myeloma is a chronic terminal cancer that pertains in hypercalcemia, anemia, renal failure, and cortical bone degradation [113, 114]. Chu, L., et al., in his study asserts that the upregulation of specific translocation t (4; 14) is affiliated and linked with oncogenesis [113].

To elaborate, the overexpression of *SCARNA22* (also known as *ACA11*) also causes oxidative stress, evasion of chemotherapy and is also responsible for regulating the gene responsible for Wolf-Hirschhorn syndrome in the MM cells [113]. Moreover, through the global expression profile of sno/scaRNA in MM, it validates that specific translocation causes the SCARNA22 to be overexpressed in MM cells [115]. Another example of this notion is the secondary plasma cell leukemia (sPCL) [115].

Chronic Lymphocytic Leukemia (CLL)

Chronic Lymphocytic Leukemia(CLL) is an irremediable cancer of mature B-cells which clusters together and is usually found in the blood, bone marrow, and lymphatic tissue [116]. Ronchetti, D., et al., throws light on the association of the development of cancer with scaRNA [117]. The expression of the non-coding RNAs specifically the unstable and high-risk group i.e. SNORA74A, SNORD116-18, SCARNA9, and SCARNA17 were taken into consideration for a study amongst the patients with CLL [117]. Through the expression profiling, it was found out that the SNORA74A, SNORD116-18, and SCARNA17 were unregulated while SCARNA9 was down-regulated which was then correlated to the modulation of chronic lymphocytic leukemia (CLL) [117].

Table 2: Illustrative list of pathologies caused by the dysfunction of scaRNA (Adapted from [102]).

Defect	***Normal Function***	***Pathology***	***Clinical Manifestations***	***Reference***
Decreased U2, U6	Components of spliceosome	Tetralogy of Fallot	i)Right ventricular outflow tract obstruction ii) Ventricular septal defects iii) Overriding aorta iv) Right ventricular hypertrophy Symptoms range from mild/asymptomatic to marked cyanosis	[107, 118, 119]

Defect	*Normal Function*	*Pathology*	*Clinical Manifestations*	*Reference*
Decreased SMN	Biosynthesis of snoRNPS, snRNPs, and telomerase RNP complex	Spinal Muscular Atrophies	Progressive muscle weakness with diminished or absent reflexes	[108, 120, 121]
Mutations in DKCI	Pseudouridine synthase forms RNP complex	Dyskeratosis Congenita	Classic triad of nail dystrophy, abnormal pigmentation, and leukoplakia. Highly correlated with increase susceptibility to bone marrow failure and cancer.	[111, 112, 121]
Up regulation of SCARNA 22	Suppression of oxidative stress and promote cell proliferation	Multiple Myeloma	Malignancy of plasma cells	[110, 122]
Various		Chronic Lymphocytic Leukemia	Malignancy of B-lymphocytes, aggregation of mature B-cells in the blood, bone marrow, and lymphatic tissue	[123, 124]

3.6 Anti-Sense RNA

Anti-Sense RNA has become a popular topic which is sought after by many researchers. The main reason behind this is because of its regulatory role in gene expression at multiple levels [125]. Therefore, it is also used as a treatment in anticancer, antivirus treatment, etc.

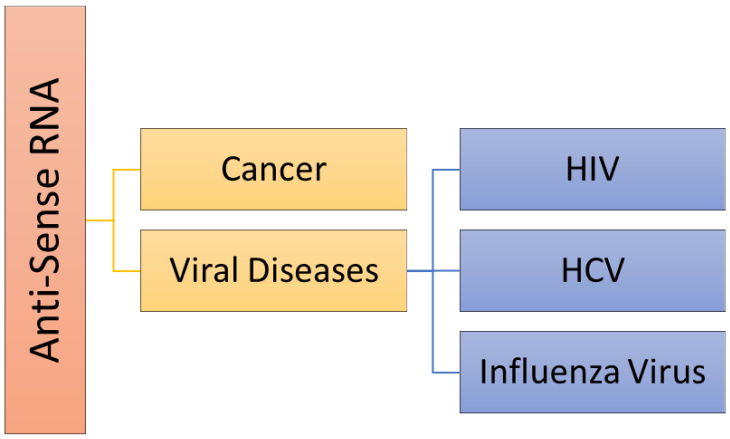

***Figure 13**: Diseases caused due to Anti-sense RNA.*

3.6.1 Antisense RNA and cancer

Studies show that in cancer cells, the expression of antisense RNA is relatively higher than that of the normal patients [125]. In a study conducted by Lorio et al (2015) shows the difference in the expression level of ncRNA in normal and cancerous tissue [126]. Another example lies with the use of antisense RNA as an anti-cancer treatment. In primary tumors and cancer cells, it has been discovered that Stat5 or survivin is overexpressed which indicates makes it the target of our treatment and is thus needed to be suppressed [125, 127]. Here antisense RNA comes into action and silences it [127].

3.6.2 Antisense RNA and viral diseases

Viral diseases such as Human Immunodeficiency Virus (HIV), Hepatitis C virus (HCV), influenza virus (IV) are some of the most commonly known and vindictive viruses. Amongst them, HIV is a lentivirus and affects the immune cells [128]. However, studies have shown that the lentivirus vector consists of 937 bp of antisense sequence and is seen to repress the HIV-1 envelope gene expression [129]. Based on this property, the HIV anti-virus treatment was made where the antisense gene was incorporated along with a recombinant antisense RNA to repress the replication of HIV-1 [130].

Another example includes Hepatitis C virus (HCV). Verstegen et al., (2015) in his review stated the influence of antisense RNAs on HCV replication [131]. Due to its regulatory role, it is also targeted as therapeutics in HCV treatment whereby the integration of antisense RNAs into the highly conserved 5’ region of the HCV genome results in the inhibition of translation of HCV RNA [125].

Chapter 4: Non-coding RNAs as biomarkers and therapeutics

Extensive study and research on non-coding RNA have led to the conclusion that the ncRNA can be used as genetic regulators. It has also led to the believe that it can also be thought of as an ideal diagnostic biomarker [132] and also as a therapeutics. For example, in the case of circRNA, 400 circRNAs were reported to be used as a potential biomarker for several diseases [133]. Expression profiling of hsa-circRNA-0061276 reveals that this circRNA is expressed higher in plasma cells of a patient with gastric cancer and is thus termed as a prognostic biomarker [134]. Another promising biomarker for cancer is the piRNAs [59]. In the case of colorectal cancer, the piR-54265 is unregulated and is expressed in blood [59]. These piRNAs are usually found in circulating tumor cells (CTC) and are considered to be a viable option as a tumor biomarker. This is mainly due to the fact that piRNAs are very stable [135, 136] and can oppose ribonuclease degradation [137]. Similarly, for Gastric cancer (GC), the expression profile of piRNAs (piR-651 and piR-823), in contrast to those of serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 levels, are able to identify and characterize more accurately [59]. Other unusual non-coding RNAs such as scaRNA, snoRNA, etc. also play an intermediate role as biomarkers too.

However, not all non-coding RNAs can be used as biomarkers. Non-coding RNAs such as enhancer RNAs are unstable in blood and are not expressed therefore are not considered to be potential biomarkers. However, it plays an important role as therapeutics in some diseases. For example, in castration-resistant prostate cancer (CRPC) cells, the enhancer RNA (eRNA) was up-regulated and expressed which were recorded using AR ChIP- seq analyses [94]. The eRNA was targeted and used as a therapeutics in CPRC [94]. Usually, therapeutic agents present themselves as an inhibitory function whereby it inhibits the pathway or the molecule thereby controlling its overexpression and thus its regulation. Another example of eRNA, serving as a therapeutic is in the case of Helicobacter pylori and its associated diseases which are gastritis and gastric cancer [138]. In a patient with H.pylori, the BRD4 is recruited and initiated transcription [139]. This BRD4 is also responsible for enhancer and eRNA activity [139]. However, to mitigate H.pylori associated diseases the JQ1 targets BRD4 and inhibits it [138]. This in turn results in the reduction of gene synthesis associated with H.pylori diseases [138].

Increasing interest in snoRNA has also lead to the usage of snoRNA as therapeutics. In HIV-infected patients, a drug namely tenofovir disproxil fumarate (TDF) is used and functions as an inhibitor of HIV reverse transcriptase [83]. This drug is used as a part of treatment i.e. the

antiretroviral therapy. Experiments revealed that TDF was used to inhibit the SNORND32a gene along with two other genes –GNAS, GOT2 gene [140]. Change in the snoRNA level by TDF could lead to cell death and thus act towards the prevention of HIV [140].

Reports show that non-coding RNA can be used effectively as both biomarkers and as a therapeutic agent in much other wide diversity of diseases.

Chapter 5: Conclusion

The world of non-coding RNA is expanding with the help of research and thus opens door to many diverse opportunities. The emergence of non-coding RNA begins with its widespread disruption of classical non-coding RNA such as miRNA in the context of human diseases [141]. However, a close look into the unusual non-coding RNA shows a new level of the complexity of nature. These unusual non-coding RNAs were thought to be the niche players but however, it was found out that these unusual non-coding RNAs play a major role in cellular biology and also functions in gene expression regulation. This was linked with the development of human diseases and was found to have both a direct and indirect hand in its cause. The non-coding RNAs which had an effect on the formation of the disease were then targeted to develop a powerful drug or therapeutics. Usually, the therapeutics would be an inhibitor molecular which would disrupt the unique pathway causing the disease. Moreover, these non-coding RNAs are also abundantly expressed in some diseases such as gastric cancer (GC) and can thus be used as a potential biomarker. In some cases, these non-coding RNAs were found to be a more effective prognostic biomarker due to its sensitivity and time required. Additionally, more

A dive into the mystery of the unusual non-coding RNA would give us exposure to the many other windows of opportunity. A further detailed study would lead to the development of drugs and personalized medicine to combat various diseases. Lastly, it is very important to continue to dive into more research in this sector using the updated information and latest techniques involved. In conclusion, non-coding RNA will expand its world through research and will bring about significant and fascinating updates to prevent and combat diseases.

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