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Review

Non-Coding RNA and Functions

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ABSTRACT

Only 2% of the eukaryotic genome is translated into proteins. The remaining %98 of the genome consists of non-protein-coding RNA, which is basically called non-coding RNA (ncRNA). Even though these noncoding transcripts are considered as transcriptional noise at the first sight, they have important roles in epigenetic mechanisms such as gene silencing, DNA methylation, and genome reorganization. Non-coding RNA and its functions are important for gene regulation, which is an explanation for the complexity of higher eukaryotes. Also, dysregulated transcription of noncoding RNAs are correlated with lots of diseases, such as cancer.

Keywords: Long non-coding RNA, non-coding RNA, small non-coding RNA.

Non-coding RNA (ncRNA) is defined as the RNA which generally lacks an open reading frame (ORF) and is, therefore, unable to encode proteins. Reciprocally, there are also noncoding transcripts that contain potential ORFs, but they are somehow not translated into proteins or translated into such proteins that are degraded quickly, which are therefore non-functional. As the definition is uncertain, Ulitsky and Bartel suggest defining it as the transcript that is unlikely to encode a functional protein.[1] Besides the regulatory ncRNA, the definition also corresponds to housekeeping ncRNA such as rRNA and tRNA, which are vital for cells. It is known that 98% of the transcription in the eukaryotic genome consists of transcripts of the non-protein-coding RNA. Another fact is that complex organisms have a higher percentage of non-protein-coding RNA in their genome, which leads us to think that ncRNA is an explanation for the complexity of higher organisms.[2,3] Even though most of their functions are still a mystery, they are widely known in higher eukaryotes for their regulatory functions, which were first projected in 1961 by Jacob and Monod.[4] General functions of ncRNA can be listed as epigenetic modifications like transcriptional and posttranscriptional gene silencing, chromatin remodeling, DNA methylation, and dosage compensation.[5] This review includes the details about functions of ncRNA.

LONG NON-CODING RNA (LNCRNA)

Long noncoding RNA (lncRNA) is practically defined as the ncRNA that is longer than 200 nt which distinguishes it from small ncRNA like PIWI-interacting RNAs (piRNAs). Despite the fact that lncRNAs are classified based on function, location in the DNA, or other features, usually they are collectively mentioned as lncRNA. A subclassification focuses on the functions of lncRNA in the context of oncogenesis and/or tumor suppression. Steroid receptor RNA activator (SRA) is an example of oncogenic lncRNA which raises adipogenesis and inhibits the transcription of inflammatory genes that are related to adipocytes in respect of insulin. Long intergenic noncoding RNA (lincRNA) is another subclass of the lncRNA and it can be distinguished by its location which is not shared with coding loci in the DNA.[3,6] In contrast to lincRNA, intronic lncRNA's are transcribed from the intron regions of the DNA. Sense and antisense lncRNAs are transcribed from

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sense and antisense strands of the DNA and their characteristics are that they may contain exons from protein-coding genes.[7]

Reports suggest that regulatory functions of lncRNA are mostly related to the DNA and transcription elements in the locus of lncRNA but not the lncRNA sequence itself. This characteristic of the lncRNA is the rational suggestion for the fact that lncRNA sequences are low-conserved. If the lncRNA somehow acts on the locus where it is transcribed, it is called cis-acting lncRNA and if not, it is then defined as trans-acting lncRNA.[8] Even though the classifications are not certain in this subject, it helps us to understand this new-discovered RNA world. This part of the review contains some most prominent lncRNAs and their functions.

Xist is Recruited For Dosage Compensation in Mammals

One of the earliest discoveries of lncRNA was made in the 1990s, and X-inactivation specific transcript (Xist) was one of them. Functions and biological relevance of Xist are still being studied. Xist has a major role in dosage compensation in mammals as it inactivates the second X chromosome in females (XX) and this inactive chromosome is named as Barr Body.[9] Studies with mouses show that Xist is crucial for dosage compensation in mammals, as the mutant mouses with Xist deletion have growth deficiency, and they prematurely died in embryogenesis.[10] Xist acts on the chromosome that is being transcribed, in other words, it acts in cis and inactivates more than 1000 genes on the X chromosome by spreading all the Xist RNA, which has the length of 17-kb on the X chromosome to be inactivated. Experiments show that Xist and polycomb complexes such as polycomb repressive complexes 1 and 2 (PCR1 and PCR2) are dependent on each other for cis-acting of Xist on the chromosome and besides that, there are more elements that take place. However, this process is not resulting in a completely inactive chromosome but some X-linked genes keep being expressed just like in the active X chromosome (Xa). These genes which escape from X-chromosome inactivation (XCI) are important as they relate to some diseases and susceptibility. Interestingly, females (XX) have more susceptibility for autoimmune diseases when compared to men (XY). It is related to the X-linked genes and the fact that supernumerary XXX chromosome mammals are more susceptible to autoimmune disease supports this idea. Consequently, Xist has an important role in this epigenetic mechanism of gene silencing.[9,11]

HOX Transcript Antisense RNA (HOTAIR)

HOTAIR genes are placed on chromosome 12, the intergenic locus on the HOXC clusters in humans. This lncRNA is transcribed in antisense to neighboring genes, which are HOXC1 and HOXC2. When HOTAIR is transcribed, it binds to the PCR2 -which contains a crucial transcriptional-regulatory enzyme that represses gene expression by tri-methylation of histone H3 Lys27 (H3K27me3)- at 5′ end, and binds to a complex -which contains Lysine Specific Demethylase 1 (LSD1)- at 3′ end. Hence, HOTAIR acts as a scaffold, and it represses the transcription on the targeted promoters. Therefore, HOTAIR represses HOX gene cluster D (HOXD) and it is also known that experiments with HOTAIR-deletion-mutant mouses exhibit de-repression of HOXD genes.

The high amount of HOTAIR is related to plenty of diseases such as cancer. Besides that, protein-coding HOX genes are related to hematological diseases, but somehow it is not evident that dysfunctional transcript of HOTAIR plays a role in it.[12]

Some experiments show that HOTAIR supplies the tumor microenvironment. For example, HOTAIR is significantly up-regulated in nasopharyngeal carcinoma (NPC) hence it also supplies angiogenesis. These studies revealed that HOTAIR-deletion mutants exhibit anti-angiogenesis factor in NCP and HOTAIR released from tumor cells contribute to angiogenesis. Other examples focus on the function of HOTAIR on the aspect of its relationship with cancer-associated fibroblasts (CAF), cancer stem cells (CSC), or immune cells.[13]

Appealingly, for the observed lung cancer patients (both in small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), it is reported that HOTAIR expression levels are high in tumor cells when compared to neighboring healthy cells.[12]

MALAT1

Another well-known lncRNA is the metastasis-associated with lung adenocarcinoma transcript 1 (MALAT1) which is also called nuclear-enriched abundant transcript 2 (NEAT2). MALAT1 is first discovered in NSCLC survival patients and moreover, MALAT1 is most-expressed in lung and pancreas cells in humans. MALAT1 has transcriptional and post-transcriptional functions. One of the molecular functions of MALAT1 is to localize the factors that are involved in alternative splicing (AS). Interestingly, it is not a critical regulator, but takes

a role in the presence of specific conditions and stress. According to expanding data about MALAT1, it possibly takes place on development of the central nervous system, myogenesis, up-regulation of vascular growth. Abnormal expression levels or dysregulated expression of MALAT1 findings in several neurological disorders such as Alzheimer's disease, retinal neurodegeneration, and Parkinson's disease make it a potential biomarker.[14]

After MALAT1 was first discovered in lung cancer patients, it was also found in more than 20 different tumor models. High expression level of MALAT1 is found relatable with tumor progression, metastasis, weak prognosis of solid cancers and hematopoietic cancers. Some other studies confirm that high expression level of MALAT1 is associated with metastasis in liver, breast and lung cancers.[15] Despite the fact that MALAT1 is mostly known to be a promoter for metastasis, research on breast cancer shows that MALAT1 possibly has a suppressor role.[16]

Maternally expressed gene 3 (MEG3)

Maternally expressed gene 3 is one of the studied lncRNA. Functions of MEG3 are not totally understood, but there are significant experiments that are made with MEG3. An experiment is performed with asthma patients, and the results confirmed the interaction of MEG3 and miRNA in gene regulation processes. While functioning, this lncRNA acts as an endogenous competing RNA.[17] Competition refers to that MEG3 or other such ncRNAs are interactively competing to bind miRNA which they share.[18] MEG3 transcript is known as a gene repressor. Another experiment with MEG3 results that MEG3 functions by interacting with chromatin structure.[19] When the MEG3 gene is inactivated, it results in an increase of the growth factor for vascular endothelial tissue and therefore cortical microvessels, according to a study about the correlation of lncRNA and angiogenesis. Angiogenesis is considered as a complex process that is involved in cancer pathogenesis. Therefore, if MEG3 is proved to be part of this process, it has the potential to be a therapeutic target for the treatment.[20] Earlier studies were suggesting MEG3 as a tumor suppressor. Nevertheless, later it is given that MEG3 is possibly an inhibitor for gastric cancer. It is shown that MEG3 increases the metastasis and proliferation of cancer cells by affecting the p53 signal pathway.[21] There are other studies that support the effect of MEG3 on the p53 signal pathway, which is important as a stress response.[22]

SHORT NON-CODING RNA (SNCRNA)

Classification based on length of the ncRNA contains this second subclass: small or short noncoding RNA (sncRNA). sncRNA are typically larger than 18 nt and smaller than 200 nt. Widely known properties of sncRNA are their regulatory roles in epigenetics.[23] Nevertheless, functions of sncRNA vary in an expanded repertoire. In addition to gene regulation or gene expression regulation, viral defense, and transposon control functions of small interfering RNA (siRNA) is an example.[24] Micro RNA (miRNA), piwi-interacting RNA (piRNA) or small nucleolar RNA (snoRNA) are the most prominent members of this class.[23,25] Besides its primary functions, it is shown that sncRNA modifications are also important in some physiological mechanisms such as stress response, epigenetic regulation, immunity, and metabolism.[26] Following part of the review is about the most well-known small noncoding RNAs and their functions.

Micro RNA

Micro RNA (miRNA) is a sncRNA that has a length of 19 to 25 nt. A critical role of miRNA is to act on the targeted region of mRNA -3' untranslated region- hence providing post-transcriptional regulation. A miRNA duplex that is transiently formed then promotes gene silencing on mRNA by including in RNA-induced silencing complex. The immature second strand of miRNA is then degraded.[27] This mechanism is also called RNA interference (RNAi), in which mRNA is sequence-specifically silenced by a double-stranded RNA (dsRNA) which is miRNA duplex in this case.[28]

Micro RNA also represses gene expression to decrease but not completely silencing it.[27] It has a considerable role in tumor tissue development and this is confirmed, in lung tumor subjects, to be specific. Researchers discovered that miRNA expression is more than normal for the patients with lung tumor. Thus, miRNA is a reasonable biomarker to use in diagnosis of such cancers. The expression of miRNA is more than normal, it is related with other solid or hematological tumors as well. Other than its role as a biomarker, there is no study in which miRNA is used as a treatment. Anyway, with animal experiments, given that it is possible to manipulate miRNA to regulate gene expression mechanisms for therapeutic use. It should be reminded that these studies are in the earlier stages as now.[29]

PIWI-Interacting RNA (piRNA)

P-element induced wimpy testis (PIWI) interacting RNA (piRNA) is a sncRNA which corporates with PIWI class of proteins. PIWI refers to a subclass of Argonaute proteins which was first discovered in Drosophila, while Piwi refers to a specific protein. piRNA functions by corporating with PIWI proteins and this way PIWI-dependent transposon gene silencing, epigenetic regulation, genome reorganization, and germ stem cell development processes are performed.[30] Other functions of piRNA are concluded from plenty of research with different animals. Given that piRNA functions involved in processes such as embryonic patterning, metabolic homeostasis.[31] As a consequence of its crucial functions on gene regulation, piRNA also correlates with cancer. With a lot of research, it is shown that piRNA is dysregulated in tumor tissues as it promotes or suppresses tumor formation. piRNA is suggested as a biomarker for diseases such as gastric cancer, colorectal cancer, multiple myeloma, and classical Hodgkin lymphoma.[32]

Small Nucleolar RNA (snoRNA)

Small nucleolar RNA (snoRNA) is a member of sncRNA even though its length may change from 60 to 300 nucleotides. This is a reminder of the uncertainty of the classification. Even so, snoRNA refers to the sncRNA that is stored up in the nucleolus. snoRNA is either transcribed in separate pieces or in one unit by splicing from the intronic regions. It is indicated that snoRNA is positively related to gene silencing. snoRNA takes place predominantly in the development and stabilization of immature ribosomal RNA (rRNA) and post-transcriptional gene regulation. snoRNA is found to be functioning in stress-response pathways and other processes of homeostasis. An increase of snoRNA is observed due to palmitate and hydrogen peroxide treatment. This means that snoRNA suppresses this oxidative stress response. Additionally, it is observed that de novo synthesis of cholesterol is increased in the absence of snoRNA. Besides that, another important function of snoRNA is that it can function as miRNA, in other words, a high amount of miRNA in the cytosol are derived from the snoRNA that are in the nucleolus. Additionally, snoRNA takes a crucial role by involving in the ribonucleoprotein (RNP) complexes.[33,34] Small nucleolar host gene (SNHG) is the major agent to regulate the expression of snoRNA and there are studies focused on it rather than snoRNA itself. SNHG and snoRNA is found correlated with tumorigenesis. miRNAs which are the product of some snoRNA -thus called sno-miRNA sometimes- is also found related with tumorigenesis.[35] Tumor suppression of snoRNA is confirmed with other experiments as well. Its functions on tumor stem cells are indirectly observed as the knockout of snoRNA is an inhibiting factor for development of tumor stem cells. snoRNA is therefore another potential biomarker for cancer.[34]

Conclusion

The Discovery of ncRNA changed our perspective on gene expression. The scientist tried to classify them based on their functions, length, localization of the gene etc. Numerous studies revealed various functions of ncRNA. In the light of these studies, we gained a new vision on how genes are regulated and how it is related to biological mechanisms. ncRNA is found to be having important roles at molecular and physiological levels. Dysregulated expression of ncRNA is correlated with physiological diseases and cancer. ncRNA is considered as a potential biomarker in cancer. However, we still know very little about ncRNA and our knowledge is expanding day by day. Further, studies on ncRNA are going to be helpful in medical sciences and genetics, hence, gaps in the literature should be focused on.

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