Long Non-coding RNA and Cancer
Intro To Biology - Scientific Report

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Abstract

Non coding RNAs do not take part in protein translation, and for a long time were considered non-functional. Recent studies on ncRNA has shown varied types and functions of ncRNA. They greatly affect the transcription and translation indirectly and are very important for us to study. This report showcases the involvement of long ncRNA (a type of ncRNA) in human cancer.

1 Introduction

In the recent times, it has been found that 70-90% of the human genome is transcribed by a mere 2-3% of the genome is acting as the blueprints and is actively involved.[1, 2]. The RNAs which are capable of protein transcription are called coding-RNAs and the part which lacks this capability are called non-coding RNAs. They are of two broad types ncRNA based on their length, small ncRNA, which are less than 200 nucleotides long and long ncRNA, which are longer than 200 nt to a few kilobases long.

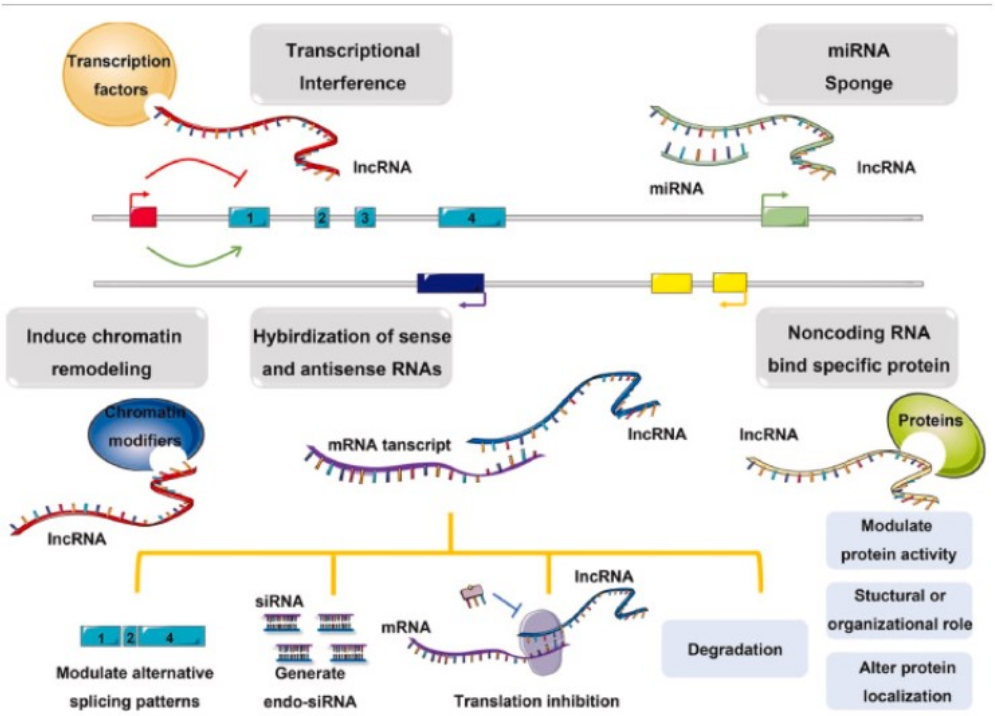
Recent studies of sncRNA and its sub parts, like microRNAs are shown to play a major role in cancer. But the human genome encodes lncRNAs in huge numbers, they lack open reading frames(ORF allow the translation of protein). Thus they are very varied and this allows them to take part in a myriad of cellular and molecular functions.[1]. For example, epigenetics(heritable changes in DNA without mutations), nuclear import, alternative splicing, as structural components, possibly as regulators of mRNA decay and precursors to small RNAs [2], also functioning as signals, decoys, scaffold etc[3].

2 Long non-coding RNA and Cancer

As these lncRNAs are involved in many varied pathways, any change in their working or in them can lead to difficult conditions, which are now studied. And one of the repercussions can be its involvement in cancer. Such as any disruption in genomic imprinting and transcriptional regulation[2]. Many lncRNA are structurally similar to mRNAs, as some of them have 5' capping, alternative splicing and also polyA+ tails[3].

There are many lncRNAs which are antisense of coding genes and are intronic. Antisense means that they are form complementary strand of other mRNA/gene and thus can affect the activity in transcription/translation.

Figure 1: Regulation mechanism of lncRNA[4]
(1) Inducing chromatin remodeling and histone modification; (2) It forms complementary double chains with the transcription of the protein encoding gene, inhibits the protein translation, and leads to the mRNA degradation; (3) iamge Binding to specific proteins, lncRNA transcripts can regulate the activity of the corresponding proteins



2.1 Characteristics of lncRNA[3]

- **Chromatin remodeling**
  They take part in transcriptional regulation by chromatin remodelling. One case is of, *KCNQ1OT1* which acts as signal lncRNA which mediate gene silencing associated marks, like dimethylation of of some sites on histone proteins. The *cis* and *trans* configuration corresponds for close targets and distant targets repectively. This lncRNA is upregulated in colon cancer. Other cases seen include *HOX* transcript antisense RNA (*HOTAIR*), which forms "multiple double stem-loop structures that bind to lysine-specific demethylase 1 (LSD1) and PRC2 histone-modification complexes"[3, 5]. And this modification to PRC2 can affect cancer development.

- **Chromatin looping in cancers**
  Another way of regulation is done by enhancer lncRNA called as eRNA related to chromatin looping. Fluorescence *in situ* hybridization (FISH) is used to study and identify looping. Recently, it has been seen enhancer regions can bind with transcription factor p53, which produce RNA which interact with neighbouring genes and stabilize it. Integrators complexes possess endonuclease activity and are required for 3' end processing of non-polyA nuclear RNA genes which maintain the levels of eRNA. These eRNA and p53

combination is important for tumor suppression, but the decrease in Integrators can in turn decrease this combination and weaken the suppresion.

- **As ceRNAs in cancers**
  Transctiption is also modulated by the specialized lncRNAs called as ceRNAs(Competing-endogenous RNA) which sequester regulatory factors like miRNAs, catalytic proteins and transcription factors etc. One example studied is lncRNA *HULC* which is seen to be highly upregulated in HCC (hepatocellular carcinoma, a type of liver cancer), it suppresses the activity of miRNA-372 which forms a positive feedback loop for and HULC upregulates.[6].

- **As NATs (natural antisense transcripts)**
  The mammalian genome has over 20% pairs of sense-antisense transcripts. NATs can be both coding and noncoding(as lncRNA). NATs can activate or suppress their counterpart strands of gene and thus regulate, by attaching to the other strand and making it inactive or vice versa. An example of NAT in cancer is Wrap53 which is antisense to p53, an important tumor suppressor. Thus it is an oncogenic lncRNA which inhibits TP53(which produces p53).

2.2 Hallmarks of Cancer[1]

Lets look at the basic 6 hallmarks, of cancer in human, and how lncRNAs act and affect in these steps:

1. **Sustaining proliferative signaling**
   Cancer cells have deregulated signaling that allows them to maintain proliferative signaling which is required for non-stop growth and division without any external simulation. Which the normal cells are not capable to do. A recent report showed, E3 ubiquitin ligase Mdm2 regulates p53 protein negatively and promotes cancer. However when Mdm2 bounds to p53 mRNA, it does the opposite, it promotes the activity of p53. Thus Mdm2 plays an essential role in p53's capacity to counter DNA damage. There are examples too where lncRNA promotes cancer, like steroid receptor RNA activator (SRA) which acts as an ncRNA and promotes tumorigenesis in mammalian breasts.

2. **Evading growth suppressors**
   Cancer cells also have the ability to evade growth suppressors, which complements the proliferative signalling. Some tumor suppresive genes include Tp53, PTEN, or Retinoblastoma gene that code protein. Some pathway adopted to suppress these genes is that the lncRNA compete with them to attach to the DNA, so the suppressors cant perform their actions, for examples lncRNA blocking the cancer suppressor PSF protein. Other pathway, which is taken by lncRNA *ANRIL* (antisense non-coding RNA in the INK4 locus) is that it interacts with a subunit of PRC2 (polycomb repression complex 2) and use this to suppress the p15, a tumor suppressor gene. Decrease in ANRIL will increase p15, which can suppress tumor growth.

3. **Enabling replicative immortality**
   Cancer cells unlike normal cells can replicate infinitely. This property of a cell to divide a specific number of times is decided by the condition of telomere in the DNA. Normally this shortens after every division and finally the cell cant divide anymore. But in cancer cells, a special enzyme telomerase regulates the length of the telomere. Interestingly it depends on lncRNA, TERT (Telomerase Reverse Transriptase) and a primer TERC (Telomerase

RNA Component) together maintain the length of telomere, making TERC essential for immortalization. In cancer cells, it has been observed that there is high concentration of TERC, and this fact is also used for therapeutic purposes, which attack this TERC. Recently a new lncRNA was discovered termed as TERRA(telomeric repeat-containing RNA) which is thought of having negative regulation on telomerase. Thus ncRNA can not only promote but suppress cancer too.

4. **Activating invasion and metastasis**
   It is the ability of cancer cells to promote distant metastases, and involves a very complex system. Two lncRNAs have been identified that have a huge impact in promoting invasiveness and metastasis:

   - MALAT1(Metastasis-Associated Lung Adenocarcinoma Transcript 1, MALAT-1). It is extremely abundant in human cells and retained in the nucleus and helps in pre-mRNA alternative splicing. It also acts as a regulator for post-transcriptional RNA modification. It is upregulated in cancer cells, and thus acts as a marker for prognosis. It promotes cell motility of lung cancer cells by regulation of transcription of genes related to motility, and thus is an metastasis agent supporting cancer invasion and travel.
   - HOTAIR(HOX Antisense Intergenic RNA). It is antisense to HOXC gene cluster and 2.2kb in length. It is important in epigenetic regulation, and thus is its deregulation is cancer promoting. In epithelial cancer, in excess of HOTAIR it alters a H3K27(a histone) methylation which alters expression of target genes, this in turn increases cancer invasiveness and metastasis. HOTAIR also has similar effects on breast cancer.

5. **Inducing angiogenesis**
   Angiogenesis is the formation of new blood vessels from existing vessels. As cancer cells grow and increase in size, the intake of Oxygen, nutrients, water and transportation of waste etc becomes difficult through diffusion, thus cancer initiates angiogenesis. Some lncRNAs have been discovered that have a say in its regulation. One such example is αHIF, which is a NAT (decribed before), anitsense to 3' untranslated region of HIF1α, an important regulator of angiogenesis. αHIF suppresses the activity of HIF1α. Excess presence of αHIF triggers HIF1α mRNA decay, and this starts a negative feedback loop. αHIF is detactable in humans and is masrked for poor prognosis in breast cancer.

6. **Resisting cell death**
   A cell can die in a controlled way owing to 3 pathways:

   (a) Apoptosis. Which is induced by various internal or external stimuli. Many cancer cells suppress apoptosis agents and become immune to treatment.

   (b) Autophagy. Which is like a suicide of cell to breakdown the cell organelles and use them as fuel for other purposes, in harsh conditions. It can help cancer to survive or eliminate them.

   (c) Necrosis. This also like autophagy has dual effect on cancer. The necrotic cells can attract proinflammatory cells, which promotes cancer cells to invade, proliferate and activate angiogenesis.

   Recent studies show that some lncRNA have control on apoptosis through indirect or direct pathways. For example, CUDR (cancer upregulated drug resistant), is an lncRNA which

inhibits apoptosis in squammous cancer cells. Other example is of PCGEM1(Prostate-specific transcript 1) which is an lncRNA involved in prostate cancer cells and also has a role in inhibiting apoptosis.
The role of lncRNA in autophagy and necrosis is yet to be discovered.

3 Recent advancements

As more and more studies are going to know more about ncRNA and lncRNA, due to the advancements in the recent times in sequencing and identification, we have come to know more about their interaction with cancer. A very few are mentioned below:

Identification and Annotation of lncRNA[3]

The sequencing techniques have greatly improved from the last two decades, and the cost of the process also dropped, which allows for much efficient sequencing. RNA-seq is one of the widely used techniques which is based on NextGen sequencing. "It involves converting RNA to cDNA, which is followed by fragmentation and ultra-high-throughput sequencing by methods such as Illumina HiSeq."[3] RNA-FISH is used for annotating lncRNA in different organisms. It involves using fluorescent probes and hybridization in cells followed by imaging. Recently, in 2020 Nobel prize in Chemistry was awarded to Emmanuelle Charpentier and Jennifer Doudna, for the development of CRISPR-Cas9 genome editing. CRISPR can also be used to study lncRNA in more detail.

Melanoma[7] - a recent research

A recent research studied the role of a long non coding RNA in melanoma, which is the most serious type of skin cancer. A nuclear lncRNA termed as Disrupted In Renal Carcinoma 3 (DIRC3) is found to be responsible for regulated tumor suppression indirectly. It activates another tumor suppressor *IGFBP5* by changing the chromatin strucure and suppressing the transcription factor SOX10, which is responsible for regulating the genes that promotes melanoma proliferation along with another factor MITF. Its depletion in human cells, has resulted in increased malignant transformation. This research can be developed to be used therapeutically to fight melanoma.

4 Conclusion

Long non-coding RNAs are the least studied RNAs and constitute the major part of genome. They have a huge variety of functions some of which are very important for the transcription/translation process. Although they dont have the potential to code protein but they affect their functioning to a great extent.
Much more has to be studied about them and their functioning. With the current advancements we are becoming more capable to study them in detail. Their linking with cancer and diseases in general makes them crucial for study. They can be used for diagnosis and prognosis and if possible for treatement in near future. With the help of this we can know much more about cancer, and identify and implement clinical therapeutic measures to fight it.

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