Review Article

Non-coding RNAs Could Be New Tools for Cancer Treatment

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Abstract

For 50 years, the term gene is synonymous with regions of the genome gene that coding by mRNAs and translate to protein. nonetheless, Genome wide Recent studies have revealed that regulating gene expression through degradation or translational inhibition of their point mRNAs and thus attend in a wide variety of physiological and pathological cellular processes including: development, cell proliferation, differentiation, and apoptosis pathways by thousands of regulatory non coding RNA such as lncRNAs and microRNAs. According to a recent survey, it is known this RNAs have vital role in regulation cellular pathways at transcriptional, posttranscriptional and epigenetic levels. These noncoding genes are often aberrantly expressed in a variety of human cancers. However, the biological functions of most ncRNAs remain largely in doubt. In this review, we proved that a remarkable part of the genetic etiology of cancer is imposed by noncoding regulatory sequences. The purpose of this review is aimed to give an outlook of using of noncoding RNA as diagnostic markers and therapeutic targets. These observations emphasized that the recognition of coding genes and Research continued evolution and function of non-coding RNAs for a comprehensive understanding human complex diseases like cancer are essential.

***Keywords*:** NcRNA, Expression, Transcription, Cell proliferation, Apoptosis, Cancer

Introduction

Cumulative evidence supports the importance of changes in the steps following the transcription of gene expression associated with cancer symptoms. The steps following transcription (i.e. pre-mRNA splicing and polyadenylation, with 'pre-mRNA' connoting the immature mRNA), stability and translation of mRNA (changes in mRNAs) and post transcriptional regulators (RNA-binding proteins and noncoding RNAs such as microRNAs and long non-coding RNAs) are very diverse and constantly growing. Understanding this diversity is therefore challenging for cancer treatment.

Noncoding RNAs are a diverse family of regulatory transcripts that are effective in all the steps of gene expression, from transcription and mRNA stability to mRNA translation. Recent evidence has revealed the critical role of noncoding RNAs in the pathogenesis of cancer.

Large-scale cDNA sequencing projects along with technological advances such as tiling arrays and new generation RNA sequencing have provided a new perspective on the complexity of transcriptome, i.e. a set of mRNA molecules or transcripts expressed in a cell (Consortium, 2012). While a number of protein-coding genes (20,000-25,000) have retained their broad consensus, recent human transcriptome studies have uncovered a significant number of non-coding RNAs (ncRNA). These transcribed elements lack the capacity to encode a protein and are confusingly abundant in all the organisms studied to date, from yeast to humans (Birney et al., 2007; Kapranov et al., 2007) .These non-coding portions of the genome produce a wide variety of mostly regulatory RNAs that often differ in terms of biogenesis, properties and function and are divided into short RNAs, such as microRNAs (Lin and Gregory, 2015), and long RNAs (>200 nt), depending on their size (Carninci et al., 2005; Guttman et al., 2009).

Extensive studies have been conducted to examine the role of ncRNAs in cell biology and cumulative evidence shows that these RNA molecules play significant roles in cellular functions and their restructuring leads to various severe pathological conditions, including cancer (Calin and Croce, 2006; Tsai et al., 2011).

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Preliminary evidence suggests that ncRNAs, especially long ncRNAs (lncRNAs), have a key role in tumor development (Huarte and Rinn, 2010), and that lncRNA-mediated biology has a major role in cancer progression(Prensner et al., 2011).LncRNAs are classified into several broad categories by their mechanisms of the regulation of mRNA transcription and translation (Fatemi et al., 2014)or LncRNAs can regulate apoptosis and cell cycle(Kino et al., 2010), or, as positive regulators of gene expression, increase the expression of neighboring genes (Andersson et al., 2014), LncRNAs play a significant role in epigenetic regulation, and acting as a modular scaffold, they assemble protein complexes to position epigenetic enzymes to the specific sequences (Guttman et al., 2011; Khalil et al., 2009). Some of important LncRNAs involved in cancer listed in table-1.

Approaches to Cancer Treatment

Targeted cancer therapies are medications or other substances that prevent the growth and spread of cancer by interfering with specific molecules (molecular targets) that are involved in the growth, development and spread of cancer.

Many different targeted therapies have been approved for use in the treatment of cancer, including hormone therapy, signal transduction inhibitors, regulators of gene expression, apoptosis inducers (programmed cell death), angiogenesis inhibitors, immunotherapy (to boost the immune system to attack tumor genetic mutations) and toxin delivery molecules (delivery of toxin into cancer cells).

Hormone therapy slows or stops the growth of hormone-sensitive tumors that depend on certain hormones for their growth. Hormone therapy helps treat cancer by blocking the production of the hormones in the body or by interfering with their action. Hormone therapy is useful in the treatment of breast and prostate cancer (Khalil et al., 2009; Sweeney et al., 2015).

Signal transduction inhibitors block the activities of the molecules involved in signal transmission –a process by which cells respond to the signals received from the environment. During this process, when the cell receives a certain signal, the signal is relayed within the cell through a series of biochemical reactions and ultimately leads to appropriate response(s). In some cancers, malignant cells are stimulated, but not by external growth factors and are constantly divided. Signal transduction inhibitors disrupt this improper signaling (Steeg, 2003). Regulators of gene expression modify the function of proteins involved in controlling gene expression.

Apoptosis inducers subject cancer cells to a process of controlled cell death called apoptosis. Apoptosis is a method of cleansing the body of unneeded or abnormal cells, but cancer cells use strategies to evade apoptosis, i.e. cellular processes that cause genetic and physiological changes in them. Apoptosis inducers cause the death of cancer cells by circumventing these strategies (Hassan et al., 2014).

Angiogenesis inhibitors block the growth of new blood vessels into the tumor (a process called tumor angiogenesis). Tumors need a blood supply to grow beyond a certain limit, as blood provides the oxygen and nutrients needed for the continued growth of tumors. Treatments that prevent angiogenesis may thus stop tumor growth as well. Some targeted therapies that inhibit angiogenesis interfere with the function of vascular endothelial growth factor (VEGF), which is a substance that stimulates the formation of new blood vessels. Other angiogenesis inhibitors target other molecules that stimulate the growth of new blood vessels (El-Kenawi and El-Remessy, 2013).

Immunotherapy is a method of treatment that destroys cancer cells by triggering the immune system. Some types of immunotherapy consist of monoclonal antibodies that identify specific molecules on the surface of cancer cells. Monoclonal antibody binding to the target molecule leads to the immune destruction of cells that express the target molecule. Other monoclonal antibodies bind to certain immune cells to help them kill more cancer cells. (Kyi and Postow, 2014). Monoclonal antibodies that deliver toxic molecules can cause the death of cancer cells in a certain way. When the antibody binds to its target cell, the toxic molecule that is bound to the antibody (for instance, a radioactive substance or toxic chemicals) is absorbed into the target cell and eventually causes cell death. The toxin will not affect cells that lack a target for the antibody; that is, it does not affect the healthy cells and seeks only the target cells (for instance, it does not affect the vast majority of the cells in the body).

Cancer vaccines and gene therapy are sometimes considered targeted therapies, as they have a special role in the growth of cancer cells. More information about these therapies can be obtained through NCI fact sheets on cancer vaccines and biological therapies for cancer (Imai and Takaoka, 2006).

Traditional Treatments

Including surgery, radiotherapy and chemotherapy, either alone or in combination with other methods, are the most commonly used

methods used for the treatment of cancer. The method of treatment used differs depending on the type of cancer, the extent of the disease, its rate of progression, the patient's conditions and the response to the treatment.

Surgery

Although the development of other therapeutic strategies has reduced the rate of surgical intervention in the treatment of certain cancers, surgery is still the oldest and principal form of cancer treatment. Despite the advances in surgical techniques, the capacity of surgery to control cancer is limited by the fact that, at the time of surgical intervention, two-thirds of cancer patients have tumors that have spread beyond the original site.

Radiotherapy

In this method, cells get destroyed by radiation for two reasons: Either because they are no longer able to proliferate as a result of excessive genetic damage or because radiation induces apoptosis or programmed cell death. Cancer cells are more sensitive to radiation compared to healthy cells, since they are constantly proliferating; this greater rate of proliferation makes cancer cells weaker than healthy cells, which are not always proliferating, and as a result, cancer cells are less able to recover from radiation damage. Radiation therapy is the most effective method for eradicating an undetectable disease at the periphery of the tumor and the least effective method for killing cells at the center of a large tumor. In general, 'chemotherapy' refers to the use of chemical compounds or medications for eliminating diseases; nevertheless, the term is often exclusively used for cancer and interchangeably with anticancer agents. Chemical compounds developed for chemotherapy destroy cancer cells by preventing their proliferation. Unlike surgery or radiotherapy, which often fail to treat widespread metastasis, medications can spread throughout the body through the bloodstream and attack the tumor cells growing anywhere, except for a few places in the body that are known as sanctuary sites, i.e. areas in which medications cannot access the cancer cells (Tannock, 1998).Research into lncRNAs in cancer and the identification of a number of lncRNAs (long non-coding ribonucleic acids) have led to the generation of new hypotheses about the biology of cancer cells. The present study reviews the current perceptions of ncRNAs in cancer with a special emphasis on lncRNAs as new triggers of angiogenesis. The present review focuses on the general features of lncRNA, their mechanisms of action and their role in the development of cancer.

Non- coding RNAs Gene Therapy

LncRNAs have an advantage over protein coding genes as potential biomarkers and therapeutic targets, as their gene expression is more tissue specific, which makes them attractive as a biomarker and therapeutic target. LncRNAs are remarkably stable in body fluids and tissues; they are also valuable biomarkers in liquid biopsies and facilitate the inhibition of invasive procedures (Qi and Du, 2013; Tong and Lo, 2006). LncRNAs can be used with therapeutic targets in a variety of methods, including RNAi mediated gene silencing, antisense oligonucleotides, targeted plasmid, small molecule inhibitors and gene therapy, as discussed below (Sánchez and Huarte, 2013; Takahashi and Carninci, 2014). Evidently, lncRNAs are crucial to the epigenetic control of gene expression and comprise potential therapeutic targets for conventional antisense technologies. In particular, in cases where a lncRNA is directly linked to the pathogenesis of the disease, conventional RNAi or antisense oligonucleotides can be used for regulating gene expression.

The Hallmarks of Cancer

Hanahan and Weinberg (2000) described six properties required for cell transformation, coined as the hallmarks of cancer. These properties include self-sufficiency in growth signals, insensitivity to antigrowth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis (HANAHAN AND WEINBERG, 2000). LncRNAs are regulatory molecules that are involved in most of these functions and key patterns thus emerge (Gutscgner and Diederichs, 2012).

Self-sufficiency In Growth Signals

LncRNAs often increase self-sufficiency in growth signals by activating the signal receptors in the first step of signal transduction. Multiple lncRNAs specifically bind nuclear receptors either alone or in a ribonucleoprotein complex (CATHCART ET AL., 2015). LncRNAs often induce self-sufficiency in growth signals by activating the signal receptors in the first step of the signal transduction. Some lncRNAs, such as PVT1, affect cell proliferation by regulating receptor abundance, as previously shown for PVT1 and thyroid-stimulating hormone (Zhou et al., 2016).

Insensitivity to Antigrowth Signals

Inhibiting or evading growth can also be regulated by lncRNAs –a process that is often carried out by the effect of RNA on tumor suppressors that regulate

cell cycles such as cyclins, CDKs, CDK inhibitors and p53(KITAGAWA ET AL., 2013). PANDA suppresses protein CDKN1A through PRC1 while ANRIL (a type of RNA) suppresses target tumor suppressor protein p15 (CDKN2B) through PRC2 (Kotake et al., 2011; Puvvula et al., 2014). Some lncRNAs regulate the expression of tumor suppressors by affecting different parts of transcription and translation. Transcription initiation can be affected by the scaffolding of transcription factor complexes, as in the case of LincRNA-p21 and p21 (CDK2 inhibitor) (DIMITROVA ET AL., 2014).

Evading Apoptosis

Apoptosis or controlled cell death is one of the key pathways for the control of carcinogenesis (ROSSI AND ANTONANGELI, 2014). Some lncRNAs act in the regulation of transcription of key apoptotic genes. For example, lncRNA INXS is expressed from the intron of Bcl-X and regulates its splicing into a pro-apoptotic isoform inhibitor of apoptosis (DEOCESANO-PEREIRA ET AL., 2014).

Sustained Angiogenesis

Multiple lncRNAs are mainly involved in the regulation of nutrient supply to the tumor by regulating the VEGF, which is essential for the formation of blood vesicles. According to recent reports, the transcription of VEGF is regulated by lncRNAs HOTAIR (Fu et al., 2016).

Tissue Invasion and Metastasis

Multiple lncRNAs increase the invasiveness of cancer cells and facilitate metastasis. Examples include the RNAs h19 and MALAT1 in colorectal and nasopharyngeal carcinoma (Raveh et al., 2015; Yang et al., 2015) .

LncRNAs in Cancer

This section discusses a number of important deregulated lncRNAs in cancer and their mechanisms of action and potential clinical applications.

KCNQ1OT1 (KCNQ1 Overlapping Transcript 1) is another imprinted, paternally expressed 91.5 kb transcript produced from the KCNQ1 locus, a few hundred kilobases away from H19 (Mohammad et al., 2008), that regulates gene expression epigenetically by interacting with chromatin remodeling complexes like PRC1, PRC2 and G9a proteins for silencing KCNQ1 (Nakano et al., 2006; Pandey et al., 2008). It is a CRISPR RNA and the chromosomal aberrations (any general changes in the chromosome structure is called aberration) associated with it include Beckwith-Wiedemann syndrome, which is a congenital overgrowth syndrome (Higashimoto et al., 2006; Weksberg et al., 2002), colorectal cancer (Nakano et al., 2006) hepatocellular carcinoma (Wan et al., 2013) and pediatric adrenocortical tumors (Wijnen et al., 2012).

NEAT1 (Nuclear Enriched Abundant Transcript 1) is a gene that produces two transcripts: the 37 kb NEAT-1-1 short isoform and the 32 kb NEAT-1-2 long isoform. Although the expression of the long isoform is much lower compared to the short isoform, NEAT1 is widely expressed across several tissues. NEAT1 is found exclusively in the paraspeckles (dynamic nuclear structures) in the nucleus (Naganuma and Hirose, 2013; Sunwoo et al., 2009) and plays an important role in the regulation of gene expression in transcription and after transcription, and its reduced expression leads to the disintegration of paraspeckles (Clemson et al., 2009). In fact, NEAT1 and NEAT2 (MALAT1) transcription shows that their model of binding to the human genome depends on hundreds of active genes. NEAT1 is strongly induced in breast cancer cells and is also involved in the transformation of myeloid cells into acute promyelocytic leukemia or APL (Zeng et al., 2014). In addition, its positive over-regulation in ATRA (All Trans Retinoic Acid) induces the differentiation of NB4 (APL) cells that could be inhibited by specific siRNA for NEAT1 (Zeng et al., 2014). Silenced NEAT1 in Burkitt's lymphoma cells leads to a reduced viability, increased apoptosis and therefore an abnormal cell morphology, thereby suggesting their oncogenic nature (Halford, 2013).

GAS5 (Growth Arrest Specific 5) at 1q25.1 locus produces two splice variant lncRNAs and its intron also leads to the formation of several snoRNAs (Mourtada-Maarabouni et al., 2008). GAS5 acts as a tumor suppressor and facilitates normal growth inhibition and apoptosis through the repression of GR (glucocorticoid receptor) mediated transcription (Pickard and Williams, 2014). GAS5 interacts specifically with the DNA binding domain of GR and inhibits the binding of GR to its target genes, including cIAP2 (cellular Inhibitor of Apoptosis 2), bringing about apoptosis, independent of other triggers in cancer cells. GAS5 also represses progesterone receptor and androgen receptor in a ligand-dependent method (Mourtada-Maarabouni et al., 2008). It also induces the inhibition of mTOR (mammalian Target of Rapamycin), which regulates protein synthesis and cell growth and proliferation. Observations have proved the fact that the

antiproliferative effect induced by Rapamycin can be repressed by silencing GAS5 in primary T cells as well as in the leukemic cells (Mourtada-Maarabouni et al., 2010).In turn, GAS5 is regulated by a negative feedback loop with miR-21(Zhang et al., 2013) .The down-regulation of GAS5 and/or its snoRNAs along with genetic aberrations at the locus (chromosomal locus) are associated with mild carcinogenesis in several cancers, including breast cancer (Mourtada-Maarabouni et al., 2009).

HULC (Highly Up-regulated in Liver Cancer), size 1.6 kb, is transcribed from the 6p23.3 locus (Panzitt et al., 2007) reached this finding with the help of Hepato Cellular Carcinoma (HCC) specific microarrays as the most highly up-regulated lncRNA in this cancer. Just as a typical mRNA, it has two exons and a poly A tail and is strongly localized in the cytoplasm and cooperates with ribosomes in the cleansing process but does not encode for any protein. It separates miRNAs and is involved in inhibiting the suppression of miRNAs that induce repression. Liu et al. (Liu et al., 2012) reported that the SNP, rs7763881, in HULC, is significantly associated with HCC susceptibility in HBV (Hepatitis B Virus) carriers. In addition, the reduced expression of CREB (cAMP response element-binding protein) and the use of a PKA (Protein kinase A) inhibitor reduces the regulation of HULC, showing that phospho CREB is required to activate HULC (Wang et al., 2010). HULC is oncogenic in nature and is highly up-regulated in both tumors and the plasma of HCC patients, but it has never been detected in any other tissues or cancers related to them (Panzitt et al., 2007). It, therefore, acts as a specific non-invasive biomarker for HCC (Xie et al., 2013). In addition, it is not expressed in primary colorectal cancers, but is detected in colorectal cancers metastasizing to the liver and associated with specific cancer symptoms for the hepatic tissue. Highly Up-regulated in Liver Cancer (HULC) is a definite symptom of hepatic cancer (Matouk et al., 2009). LncRNAs bind to miRNA-binding regions to separate the miRNAs and thus regulate the activity of miRNAs (Wang et al., 2010).

PCAT1 (Prostate Cancer Associated ncRNA Transcript 1) is a 7.8 kb lncRNA transcribed from the 8q24.13 locus.This RNA is up-regulated in metastatic cancers and high grade prostate tumors. Prensner et al. (Prensner et al., 2011) identified 121 prostate cancers associated with PCATs by RNA sequencing analysis from prostate cancer tissues in which PCAT1 is highly up-regulated. The reduced expression of PCAT1 in androgen dependent prostate cancer cell line leads to the alteration of hundreds of genes (Prensner et al., 2011). PCAT1 has also been reported to play an important role in double strand DNA break repair and to inhibit the homologous recombination of DNA (Prensner et al., 2014). It is a transcriptional repressor of DNA repair genes, just as BRCA2 tumor suppressor, and is instead regulated by PRC2. The overexpression of PCAT1 is associated with increased sensitivity to PARP inhibitors due to the reduction in RAD51 foci formation. PCAT1 is a negative prognostic marker for prostate cancer (Prensner et al., 2011). These prostate specific lncRNAs appear to be very useful in the process of treatment as diagnostic and prognostic markers in prostate cancer because traditional markers such as PSA have only a limited prognostic value. Several lncRNAs contribute to the regulation of p53 tumor suppressor signaling (Pickl et al., 2014). MEG3, a maternally expressed imprinted lncRNA on Chr14q32 activates p53 and facilitates p53 signaling, including the enhancement of p53 binding to target gene promoters (Zhou et al., 2007). MEG3 binds to p53 signals in meningioma and suppresses MEG3 overexpression, cell proliferation in meningioma and hepatocellular carcinoma cell lines (Braconi et al., 2011; Zhang et al., 2010). In human tumors,asignificant reduction in MEG3 expression is observed with the frequent hypermethylation of its promoters in pituitary tumors (Gibb et al., 2011) and leukemias(Benetatos et al., 2010). Overall, these findings suggest that MEG3 is a tumor suppressor. MEG3 is a modified lncRNA gene expressed in the maternal allele. The modification of this gene is induced through the binding of cytosine to methylation controlling binding proteins such as CTCF (Rosa et al., 2005). MEG3 is silent in many cancer cells due to DNA methylation (Benetatos et al., 2011; Zhao et al., 2005). MiR-29 and miR-148 can regulate DNA methyltransferase (DNMT) 1 and 3 by increasing the expression of MEG3 in hepatocellular cancer and gastric cancer, respectively (Braconi et al., 2011; Yan et al., 2014). MEG3 is a relatively poor prognosis in gastric cancer, pituitary adenomas, tongue squamous cell carcinoma and lung cancer (Lu et al., 2013; Sun et al., 2014). Yin et al. found that the low expression of MEG3 is associated significantly with low histological grade (proximity of the tumor to the main tissues) and deep tumor invasion in colorectal cancer (Yin et al., 2015). However, the metastasis mechanism of cancer cells MEG3 is not very clear. Examinations showed that MEG3 may suppress tumor proliferation through p53- dependent and/or p53-independent pathways (Lu et al., 2013; Zhou et al., 2007).

Table 1. LncRNA involved in cancer.

LncRNA	Genomic location	Official Full Name	Expression in patients or cancer cells	Function in tumorigenesis
KCNQ1OT1	11p15	KCNQ1 Opposite Strand/Antisense Transcript 1	Increased expression in colorectal cancer(Nakano et al., 2006)	NA
NEAT1	11q13.1	nuclear paraspeckle assembly transcript 1	Down-regulated in acute promyelocytic leukemia cells(Zeng et al., 2014)/ increased expression in breast cancer cell lines (Choudhry et al., 2015)	Oncogene
GAS5	1q25.1	Noncoding RNA growth-arrest-specific transcript 5	Down-regulated in breast cancer (Mourtada-Maarabouni et al., 2009)	Tumor suppressor
HULC	6p24.3	Highly up-regulated in liver cancer	Increased in HCC and colorectal cancer liver metastasis)Wang et al., 2010) / (Liu et al., 2012)	Oncogene
PCAT1	8q24	Prostate cancer associated transcript 1	Increased in a subset of prostate cancers(Prensner et al., 2011)	Oncogene
MEG3	14q32.2	Maternally expressed gene 3	Down-regulated in multiple cancers)Benetatos et al., 2011)	Tumor suppressor
ANRIL	9p21.3	Antisense NcRNA in the INK4 Locus (CDKN2B antisense RNA 1)	Inversely relates to p15 expression in cancer(Kotake et al., 2011)/ (Yap et al., 2010)	Oncogene

ANRIL (Antisense Noncoding RNA at INK4 Locus), also known as p15AS, is an antisense transcript of CDKN2B at 9p21.3 locus that has several alternatively spliced isoforms, including 3.9 kb and 34.8 kb transcripts(Kotake et al., 2011) (Yu et al., 2008). The mis expression of ANRIL is associated with a variety of diseases, including cancer.(Iacobucci et al., 2011; Popov and Gil, 2010). ANRIL creates changes in gene expression through epigenetic methods as it binds to PRC1 and PRC2 and induces gene silencing at the INK4b-ARF-INK4a locus (Kotake et al., 2011). It binds specifically to SUZ12 (Suppressor of Zeste 12 homolog), a subunit of PRC2, and induces the repression of p15, a tumor suppressor gene; as a result, the inhibition of ANRIL induces p15 and reduces cell proliferation (Kotake et al., 2011).
Nevertheless, these data are obtained from studies conducted on different cell types and it is not clear whether ANRIL binds to both complexes simultaneously or not. In addition, ANRIL has a highly complex splicing pattern with numerous variants, including circular RNA isoforms, and its expression has been detected in many tissues.

Conclusion

Any research involved in cancer treatment and prevention is very important due to worldwide cancer problems and among of new techniques non-coding RNAs are so important because the can affect very specific. Nowadays these small molecules presented as targeted tools for cancer therapy so knowing any mechanism about them are so important for researcher, in this review we mentioned some critical issues about them including a brief introduction and describe they role in cancer, treatment and prevention by reviewing some good related articles. We believe these small molecules will be work as a big and potent tools in cancer treatment and will play their clinical roles very soon.

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