The Emerging Roles of Long Noncoding RNA ROR (lincRNA-ROR) and its Possible Mechanisms in Human Cancers

Yan Pan  Chen Li  Jing Chen  Kai Zhang  Xiaoyuan Chu  Rui Wang  Longbang Chen

Department of Medical Oncology, Jinling Hospital, School of Medicine, Nanjing University, Nanjing, China

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Abstract
To date, there is only up to 2% of protein-coding genes that are stably transcribed, whereas the vast majority are non-coding RNAs (ncRNAs). These ncRNAs, also known as non-messenger RNAs (nmRNAs) or functional RNAs (fRNAs), include transfer RNAs, ribosomal RNAs, microRNAs and long non-coding RNAs (lncRNAs). With the advance of high-resolution microarrays and massively parallel sequencing technology, lncRNAs have gained extended attentions nowadays and are found to play important roles in tumorigenesis and progression of human cancers. Long intergenic non-protein coding RNA, regulator of reprogramming (linc-ROR), was first discovered in induced pluripotent stem cells (iPSCs), where it was controlled by the key pluripotency factors Oct4, Sox2 and Nanog. Linc-ROR has been shown to be dysregulated in many types of cancers, including breast cancer (BC), pancreatic cancer (PC), hepatocellular cancer (HCC), endometrial cancer (EC), and nasopharyngeal carcinoma (NPC). Also, linc-ROR functions as regulatory molecule in a large amount of biological processes. However, the underlying mechanisms of its contribution to carcinogenesis remain to be elucidated. In this review, we will emphasize on the characteristics of linc-ROR and their roles in different types of human cancers.

Introduction

It is well known that RNA plays a crucial role in the organization and regulation of genome by the activity of a large amount of protein-coding genes and non-protein coding RNAs (ncRNAs). According to the ENCODE project, the transcripts cover 62-75% of our genome, among which are mostly noncoding RNAs [1]. They appear to comprise a hidden layer of internal signals that control various levels of gene expression in physiology and
development, including chromatin architecture/epigenetic memory, transcription, RNA splicing, editing, translation and turnover [2]. It’s a long period before the different types of ncRNAs have been identified, which can be briefly grouped into two main classes: the small non-coding RNAs (miRNAs), whose functions have been better described and the lncRNAs [3].

miRNAs are short, 18-25 nucleotide, non-coding RNAs, which bind to target messenger RNAs (mRNAs), usually in their 3'-untranslated regions (UTR), and functions in RNA silencing and post-transcriptional regulation of gene expression [4, 5]. Evidences have shown that altered expressions of miRNAs are associated with carcinogenesis and development of various cancers [6-8]. By regulating hundreds to thousands of potential target genes, miRNAs form a novel layer of the complicated regulatory network in cells. LncRNAs are non-protein coding transcripts longer than 200 nucleotides and are mostly transcribed by RNA polymerase II from different regions across the genome [9]. LncRNAs can be usually divided into five categories, sense, antisense, bidirectional, intronic and intergenic lncRNAs [10, 11]. They are considered to be the frequent targets of positive selection and have poorly-conserved stretches of sequence that maintain functional domains and structures as well [12, 13]. Like proteins, the function of lncRNAs depends on their subcellular localization. Many lncRNAs are recognized as important modulators for nuclear functions [14, 15]. Generally, lncRNAs can influence almost every cellular behavior of the central dogma, from transcription to translation by diverse mechanisms including epigenetic alterations, lncRNA-miRNA/DNA interactions, protein-lncRNA interactions and genetic variations [16]. In fact, many of them can modulate gene expression by recruiting the chromatin-modifying proteins to specific sites in the genome [17].

Recent recognition that lncRNAs function in various aspects of cell biology has focused increasing attention on their potential to contribute towards disease etiology [18-20]. A handful of studies have implicated lncRNAs in a variety of disease states and support an involvement and co-operation in neurological disease and oncogenesis [21, 22]. Expression analyses that compare tumor cells and normal cells have revealed changes in the expression of lncRNAs in several forms of cancer [23]. It has already been revealed that some lncRNAs, such as HOTAIR, are potential biomarkers in cancer diagnosis and prognosis [24]. MALAT1 (also known as NEAT2) was originally identified as an abundantly expressed ncRNA that is upregulated during metastasis of early-stage non-small cell lung cancer and its overexpression is an early prognostic marker for poor patient survival rates [25]. Besides, H19, a gene for a long noncoding RNA, is found to have a role in the negative regulation (or limiting) of body weight and cell proliferation [26]. Increasing expression of H19 is found in many kinds of cancers including adrenocortical neoplasms, choriocarcinomas and hepatocellular carcinomas [27, 28]. Among them, linc-ROR was identified in 2010, in which study, linc-ROR was reported to promote reprogramming of differentiated cells to iPSCs and maintenance of embryonic stem cells (ESCs) and iPSCs [29]. Linc-ROR is composed of four exons and is located at 18q21.31, in most cases, it plays oncogenic roles in cancers [30]. In this review, we will summarize the oncogenic roles of linc-ROR in human cancers, especially its identification, characterization and molecular mechanisms.

**Characteristics and mechanisms of linc-ROR**

**Identification and characterization of linc-ROR**

Linc-ROR was firstly described as a 2.6 kb (for exons) lncRNA by Loewer et al. in 2010, of which study, it was reported to act as a promoter of the derivation of pluripotent stem cells’ reprogramming [29]. Since linc-ROR was discovered, studies in this area have been profoundly extended during the past six years. Most of the sequence is composed of LINE, SINE and LTR elements and is located at 18q21.31 [30]. The location of linc-ROR is a binding site for pluripotency transcription factors (TFs) Oct4, Sox2 and Nanog, thus it was thought to function as a ceRNA to regulate the expression of those core TFs. As a result, linc-ROR was
proved to have huge impact on the self-renewal and differentiation in human embryonic stem cells (hESCs) by functioning as a sponge to trap miR-145 at the post-transcriptional level [31]. Dysregulation of linc-ROR has been found in many types of cancers include breast cancer, hepatocellular cancer, endometrial cancer and so on [32-38]. The expression level of linc-ROR is restricted to less differentiated cell populations in cancers where it might be involved in the stem-like properties, such as resistance to adverse environmental conditions or chemotherapy [39].

**Functional mechanisms of linc-ROR**

Linc-ROR is a typical lncRNA that plays important regulatory roles in interacting with miRNAs and maintaining stem cell pluripotency, triggering the epithelial-mesenchymal transition (EMT) as well. Linc-ROR is also involved in various key roles under hypoxia and in tumorigenesis promotion (Fig. 1). Mostly, linc-ROR promotes the tumorigenesis, however, it can also be an inhibitor in the proliferation of cancer cells and self-renewal of glioma stem cells (GSCs), partly by blocking KLF4 expression [40].

**Linc-ROR functions as a ceRNA to regulate the cell progresses**

Competing endogenous RNAs (ceRNAs) plays important parts in the regulatory network across the transcriptome by interacting with the microRNA respond elements (MREs) [41]. MicroRNAs negatively regulate gene expression at the post-transcriptional level by direct base pairing to target sites within untranslated regions of messenger RNAs, and it is estimated that over 60% of human protein-coding genes have been under selective pressure of miRNAs [5, 42, 43]. Recent studies have shown that lncRNA may function as competing endogenous RNA in modulating the concentration and biological functions of miRNAs [44-46]. In previous reports, experimental evidences for such a ceRNA crosstalk have been initially presented between the tumor suppressor gene PTEN and the pseudogene PTENP1 [47]. Linc-MD1 has also been identified as a ceRNA, which sponges miR-133 and miR-135.
to retain MyoD transcripts [44]. These findings help us to understand the importance of lncRNAs in functioning as ceRNAs.

Similarly, linc-ROR can function as a ceRNA and overexpression of it may reverse the negative regulation between miRNAs and their target genes. Related miRNAs consist of miR-145, miR-205, miR-133 and miR-34 [31, 32, 34, 36, 38, 48, 49]. Among which, the miR-145 is the one who gains most attention in the field of linc-ROR. Wang et al. postulated a regulatory feedback loop model between the linc-ROR and related miRNAs. Also, they found that linc-ROR functions as a ceRNA to regulate the expression of TFs including OCT4, SOX2, and NANOG by sponging the differentiation-related miRNAs in hESCs especially miR-145 [29, 31].

These TFs have been proved to be expressed in various tumors and are associated with an undifferentiated phenotype [50-53]. The key role of the core TFs was highlighted by the fact that the exogenous introduction of these TFs into murine or human adult cells induced pluripotency by reprogramming these cells into iPSCs, which are functionally and phenotypically similar to ESCs [54, 55]. However, under the circumstance of strong differentiation conditions, this effect of linc-ROR could be consumed when there were abundant miRNAs. Furthermore, linc-ROR plays a similar role, besides, it has effect not only on pluripotent cells, but also on cancer stem cells (CSCs) [38]. The inhibition of linc-ROR could be utilized to decrease the chance of CSCs being the origin of cancer, which could facilitate related studies and cell therapies. Based on these two studies, Gao et al. indicated that linc-ROR can serve as a prognostic factor in pancreatic cancer, which was supported by the findings that silence of linc-ROR can suppress the proliferation, invasion and tumourigenicity of pancreatic cancer stem cells (PCSCs), thus reducing the malignant characteristics of PCSCs [48]. Recently, Zou et al. indicated that linc-ROR acts as a molecular ‘sponge’ to competitively regulate the expression of Sox2 with miR-145, thereby achieving pluripotency maintenance in human amniotic epithelial cells (HuAECs) and improving the efficiency of their differentiation into β islet-like cells, which have been proven by transplanting into diabetic patients or animal models, they can help to restore islet function and therefore can be a potential treatment [56-58]. Notably, Duru et al. suggested that the aggressive clones derived from CD49f+/CD44+/CD24- single cells activate the OCT4/ SOX2/linc-RoR signaling axis to maintain self-renewal of cancer stem cells and regulate differentiation in breast cancer [35]. What’s more, the enhanced expression of K14, ARF6, and miR-10b might relate to migration and invasion of these specific clones. In addition of TFs, the linc-ROR/ miR-145 axis also has influence on hypoxia process, Takahashi et al. reported that linc-ROR is a hypoxia-responsive lncRNA which sponges miR-145 and modulates the expression of hypoxia inducible factors (HIF-1α) and its target genes, such as VEGF, TGF-β and PDK1 [36]. HIF-1α is broadly expressed in human cancers and is served as a therapeutic target in HCC [59, 60]. In triple-negative breast cancer, recent study has proved that linc-ROR functions as a ceRNA which sponges miR-145 and therefore upregulate the expression of ARF6 [32]. The ARF6 regulates adhesion and invasion properties of breast tumor cells through E cadherin modulating [61]. Moreover, linc-ROR was identified as a prognostic biomarker and exerts its impact by associating with miR-145 in colon cancer [62].

In spite of functioning together with miR-145, linc-ROR is also reported to regulate EMT by acting as a ceRNA for miR-205 in breast cancer cells [34]. The study provides evidences that linc-ROR prevented the degradation of miR-205 target genes, such as ZEB2, which is a EMT inducer. In addition, there are evidences that increased linc-ROR promotes the re-expression of fetal gene (ANP and BNP) and cardiomyocyte hypertrophy related genes via behaving as a ceRNA to inhibit the expression and function of miR-133 [49, 63]. By suppressing miRNAs, linc-ROR may contribute to the regulation of genetic networks during development and tissue regeneration and may lead to new therapies for many diseases.

**Linc-ROR triggers the EMT progress**

The EMT is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal stem cells,
these are multipotent stromal cells that can differentiate into a variety of cell types. EMT is featured by down-regulation of epithelial makers and intercellular molecules (E-cadherin and occludins), as well as up-regulation of mesenchymal markers (N-cadherin and cadherin-11) [64]. Many transcription factors that can repress E-cadherin directly or indirectly can be considered as EMT-TF (EMT inducing TFs), such as ZEB1 and ZEB2 [65, 66]. The role of linc-ROR in the tumorigenesis of epithelial cancers involves EMT triggering and stemness acquisition, and suppression of linc-ROR may reverse the EMT process [34]. The linc-ROR prevents the degradation of miR-205 target genes, including the EMT inducer ZEB2, thus inducing EMT and promoting breast cancer progression and metastasis. Further, Chen et al. demonstrated that linc-ROR is an important marker for multidrug resistance of breast cancer by inducing the EMT [33], which was relevant to paclitaxel and 5-FU resistances in human malignancies [67-69]. Moreover, linc-ROR may mediate migration and metastasis in pancreatic cancer cells partly by activation of ZEB1 through inhibition of p53 expression [70]. ZEB1 can repress the members of miR-200 family, resulting in activation of EMT and maintenance of stemness in pancreatic cancer [71]. Certainly, further is needed to support it.

**Linc-ROR plays an important role for the cell to better respond to various stresses**

It is well known that under stress, such as DNA damage, the expression of the tumor suppressor p53 is remarkably elevated, which is regulated by variety of ways including translational and posttranslational control [72, 73]. It's reported that under the circumstance of DNA damage, linc-ROR can significantly suppress p53 by interrupting the interaction of heterogeneous nuclear ribonucleoprotein I (hnRNP I) with p53 mRNA through a translation repression mechanism [74]. hnRNP I is an RNA binding protein that carries several RNA binding domains and is well known for its role in mRNA splicing [75]. Studies also indicated that linc-ROR is under control of p53, which means the linc-ROR level is increased when p53 is induced. What's more, in nasopharyngeal carcinoma, linc-ROR played an important role in the progression of NPC and the mechanism by which NPC resists chemotherapy might be that linc-ROR suppress p53 signal pathway [76]. Similar to this pathway, the linc-ROR also plays an oncogenic role in regulating the c-Myc (enhancing its mRNA stability) by interacting with hnRNP I and AU-rich element RNA-binding protein 1 (AUF1) [77, 78]. Moreover, the nuclear factor-erythroid 2-related factor (NRF2), which is activated under high cellular stress and is well established as a master regulator in cellular defending against chemical carcinogenesis [79, 80], directly binding linc-ROR promoter and represses its expression [81]. The study also proved that the exposure to estrogen metabolites can downregulate NRF2 expression and result in upregulation of linc-ROR, which might contribute to the estrogen-mideated breast tumorigenesis.

It is also revealed that at low to mild stress conditions, low levels of p53 upregulate the NRF2-dependent antioxidant response through p21 to reduce the neutralize reactive oxygen species (ROS) and promote cell survival, on the other hand, when cells have been damaged under high stress conditions, elevated p53 levels suppress the NRF2-mediated cell survival pathway and presumably initiate the apoptotic cell death processes [82]. So, p53, NRF2, linc-ROR and c-Myc may form a regulatory network upon DNA damage, the high expression of p53 inhibits the activation of NRF2, which leading to the upregulation of linc-ROR, as a result, the high expression of linc-ROR can suppress the p53 level, at the same time inhibit the expression of c-Myc by interacting with hnRNP I and AUF1 [74, 77, 81]. This forms a feedback loop which confirms the importance of linc-ROR in playing an oncogenic role.

Apart from DNA damage, cellular toxicity and stress occurring during exposure to therapeutic agents such can elicit survival responses that eventually result in resistance to these agents. Takahashi et al. indentified a role of linc-ROR as a mediator of cell-to-cell communication through extracellular vesicles, which is selectively upregulated by TGF-β under the treatment of sorafenib in the hepatocellular cancer [37]. These implicated IncRNAs in extracellular vesicles as mediators of the chemotherapeutic response, and support that linc-ROR targeted therapy can enhance chemosensitivity in HCC.
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Linc-ROR decoys gene-specific histone methylation to promote tumorigenesis

Histones undergo posttranslational modifications that alter their interaction with DNA and nuclear proteins. Modifications of the tail include methylation, acetylation, phosphorylation, ubiquitination, SUMOylation, dditurnylation, and ADP-ribosylation. Histone modifications act in diverse biological processes such as gene regulation, DNA repair, chromosome condensation (mitosis) and spermatogenesis (meiosis). Fan et al. firstly reported that linc-ROR acts as a decoy oncoRNA to block the recruitment of chromatin regulatory factors (G9A methyltransferase), spreads a tumorigenic signal to the downstream target TESC [83]. TESC is a gene which is considered as a novel oncogene to abolish histone H3K9 modification of its promoter and consequently induce abnormal tumor growth and metastasis [84, 85]. However, further studies are required to validate the role of linc-ROR in tumorigenesis.

Linc-ROR-related cancers

Recently, increasing evidences indicate that linc-ROR plays a role in tumorigenesis and tumor progression. In tumorigenesis, linc-ROR acts as an oncogene, except for one in glioma [40]. In general, linc-ROR is associated with diverse biological processes including proliferation, differentiation, apoptosis, invasion and metastasis in various human cancers (Table 1). However, the molecules that mediate linc-ROR’s effects must be understood before linc-ROR can be applied in cancer treatment. A series of studies has established the importance of linc-ROR as a marker of cancers. Marked upregulation of linc-ROR is observed in various cancers, including breast cancer [32-34], pancreatic cancer [48, 70], hepatocellular cancer [36, 37], endometrial cancer [38], and nasopharyngeal carcinoma [76], while linc-ROR acts as a tumor suppressor in glioma [40]. Although linc-ROR is overexpressed in various types of cancer, the target genes are rather variable depending on the host cell type. Because the studies on the linc-ROR is still limited, further research into the linc-ROR pathway and molecules is needed.

Available assays for linc-ROR measurement

With the rapid development of molecular biology and laboratory technology, new markers and detecting approaches are constantly emerging. In order to explore the features of linc-ROR, some methods are introduced to characterize the functions of it, including qPCR, in situ hybridization, immunoprecipitation(IP), lncRNA pull-down, lncRNA northern blot analysis, lncRNA knockdown and so on [86-88].

As one major mechanism for IncRNA to exert its function is to serve as a scaffold via RNA–protein interaction, it is important to investigate which IncRNAs are binding to a protein of interest. RNA-IP has been developed to identify IncRNA species that bind to a protein of interest [89]. On the other hand, if the research focus is to identify the proteins that are bound to a given IncRNA, IncRNA pull-down will help to identify the protein molecules

Table 1. The roles of linc-ROR in human cancers and its relevant target genes

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Biological process</th>
<th>Relative genes</th>
<th>Prognostic role</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>Metastasis, invasion(promotion)</td>
<td>ARF6/ZEB2</td>
<td>Poor</td>
<td>[32-34]</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>Proliferation, metastasis, invasion(promotion)</td>
<td>OCT4/SOX2</td>
<td>Poor</td>
<td>[35]</td>
</tr>
<tr>
<td>Hepatocellular cancer</td>
<td>Proliferation, apoptosis, metastasis, invasion (promotion)</td>
<td>Nanog</td>
<td>Poor</td>
<td>[48]</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>Proliferation, apoptosis(promotion)</td>
<td>ZEB1</td>
<td>Poor</td>
<td>[70]</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>Invasion, migration(promotion)</td>
<td>HIF-1a</td>
<td>Poor</td>
<td>[36]</td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>Proliferation, migration (promotion)</td>
<td>TGF-β</td>
<td>Poor</td>
<td>[37]</td>
</tr>
<tr>
<td>Glioma</td>
<td>Proliferation(inhibition)</td>
<td>c-Myc/KLF4</td>
<td>Poor</td>
<td>[40]</td>
</tr>
</tbody>
</table>
that interact with a specific lncRNA [90]. Moreover, as a novel class of RNA transcripts, it is important to characterize the expression of lncRNAs in various systems. RT-PCR can gives the exact number of lncRNA by comparison with DNA standards using a calibration curve [91]. While the northern blot can be used to determine lncRNA abundance and to identify different splicing variants of a given lncRNA, in situ hybridization can also provide information regarding the expression level of a given lncRNA [92, 93]. More importantly, it can reveal the cellular or tissue localization of the lncRNA of interest. Knocking down the expression of a target gene has been a gold standard assay to elucidate its endogenous function [94].

Conclusion and Future Directions

Non-coding RNAs are long known to mediate gene expression regulation by a variety of mechanisms [2]. Studies in small non-coding RNAs has been prosperous since past, such as microRNAs, however, long non-coding RNAs are arising as important regulators of gene expression. During the past 25 years, the idea of RNA targeted therapies has grown from a concept into a clinical reality [95]. The lnc-ROR was previously described as a 2.6 kb lncRNA by Loewer et al. in 2010 [29], and different studies provide evidences for the crucial roles of linc-ROR in the initiation and progression of various cancers. Many evidences indicate that linc-ROR represent a potent tumor promoter, and aberrant linc-ROR expression is found common in different cancers. Its tumor-promoting function can be attributed to its regulation of target genes involved in multiple pathways, including proliferation, invasion, angiogenesis and cancer stem cells. Linc-ROR is closely associated with many signaling pathways, therefore, the discovery of novel targets is still required to obtain a comprehensive understanding of the biological roles of linc-ROR. Our knowledge of linc-ROR as a potential biomarker of various cancers and a potential target for cancer therapy has considerably increased. However, further investigation is necessary to discover about linc-ROR.

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Disclosure Statement

The authors declare that they have no conflicts of interest related to this work.

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