

Long non-coding RNAs as key regulators of cancer metastasis

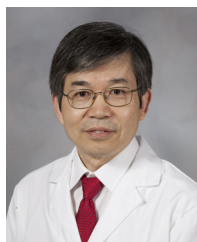
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ABSTRACT

The recent advances in functional genomics have discovered that a large number of long non-coding RNAs (lncRNAs) are pervasively transcribed from the human genome. Increasing evidence further indicates that lncRNAs are important for gene expression during cell differentiation and development through various mechanisms such as nuclear organization, post-transcription regulation, alternative splicing, and epigenetic regulation. Thus, aberrant expression of lncRNAs can cause abnormality in those cellular functions and lead to various pathological conditions. One of such fatal consequences is cancer metastasis which is responsible for more than 90% of cancer-related deaths. A good understanding of how lncRNAs regulate different genetic and epigenetic changes during different stages of cancer metastasis is important not only for general cancer biology but also for identification of novel biomarkers and therapeutic targets for treatment of metastatic cancer. A significant progress has been made regarding the role of lncRNAs in cancer for past several years. In this study, we first discuss general functions of lncRNAs and then highlight recent findings of how lncRNAs impact cancer metastasis, and finally we provide our perspectives on clinical implications of lncRNAs.

Key words: Cancer metastasis; epigenetics; gene regulation; long non-coding RNA

INTRODUCTION

It is well-known now that protein-coding genes account only about 2% of the human genome,^[1] whereas the vast majority of the transcripts do not code for protein.^[2] Although these non-coding RNAs were considered “transcriptional noise”, their functions are increasingly valued for defining the cellular complexity of organisms. For instance, the number of protein-coding genes in humans is only a 2-fold more than that in worms such as *Caenorhabditis elegans* do,^[1] implying that the protein alone is not sufficient to determine the complexity of organisms. Instead, this complexity may be achieved by efficient programming, which helps in handy

expression and functioning of protein in a different context. The versatility and plasticity of non-coding RNAs help in such programming of protein function by regulating their expression and assembly in contextual cues.^[3]

Non-coding RNAs include a broad category of RNA molecules. Some of them are constitutively expressed in the cells, and they may play a housekeeping role such as ribosomal RNA, transfer RNA, small nuclear RNA, and small nucleolar RNA (snoRNA). In contrast, other non-coding RNAs may be spatiotemporally expressed, and they often play a regulatory role.

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LncRNA-ATB

This lncRNA is activated by cytokine TGF- β (lncRNA-ATB) that is well-known for its role in tumor metastasis. TGF- β modulates different signaling pathways involved in EMT, migration, invasion, and metastasis.^[66-68] A long time treatment of cells with TGF- β induces EMT (decreased E-cadherin and increased N-cadherin, vimentin, slug, twist1, ZEB-1 and ZEB-2). Similar treatment of hepatocellular carcinoma (HCC) cells with TGF- β activates the lncRNA-ATB in a time- and dose-dependent manner. Clinically, lncRNA-ATB level is high in HCC tumors as compared to adjacent normal tissue. Similarly, a high level of lncRNA-ATB is positively correlated with microvascular invasion and portal vein tumor thrombosis. Consistent with these observations, injection of HCC tumor cells overexpressing lncRNA-ATB into orthotopic mice promotes metastasis to different organs.^[69] One of the possible mechanisms is through enhancement of EMT by interfering the action of miR-200 which can inhibit EMT by suppressing ZEB-1 and ZEB-2.^[70] This 2.5 kb long lncRNA carries 6 binding sites for miR-200. Therefore, lncRNA-ATB traps miR-200 and prevents degradation of ZEB-1 and ZEB-2 by miR-200. The high level of ZEB-1 and ZEB-2 ultimately promotes EMT and invasiveness of different cells *in vitro* and *in vivo*. In addition, lncRNA-ATB enhances colonization of migrating cells by enhancing the function of IL-11-STAT3 signaling pathway. In this case, lncRNA-ATB binds to IL-11 mRNA and stabilizes it. The increased stability of IL-11 facilitates its secretion. As a ligand, IL-11 promotes phosphorylation of STAT3. This autocrine mitogenic signal helps in robust cell survival and effective colonization in distant organs.^[69]

LncRNA-low expression in tumor

Low expression in tumor (LET) was originally identified in HCC cells.^[71] Along with HCC, a reduced level of LET is also

found in lung squamous carcinoma and colorectal cancer as compared to adjacent normal tissue. Overexpression of lncRNA-LET suppresses metastasis of HCC and colon cancer cells *in vivo*.^[72] lncRNA-LET could limit HCC metastasis in both hypoxic and normoxic condition by different mechanisms. In hypoxic condition, lncRNA-LET interferes with the function of hypoxia-inducible factor-1 α (HIF-1 α), a transcription factor that regulates a number of genes under tumor hypoxia, and promotes angiogenesis and metastasis.^[73] The high expression of lncRNA-LET suppresses HIF-1 α through inhibiting NF90 which is required for accumulation of HIF-1 α mRNA. However, hypoxia keeps the level of lncRNA-LET low by deacetylating its promoter. As a result, HIF-1 α is increased promoting metastasis. In normoxic condition, lncRNA-LET inhibits expression of CDC42 (which is required for trans-endothelial migration) of circulating tumor cells. The low level of lncRNA-LET in HCC keeps CDC42 high and this results in profound metastasis of HCC.^[72]

Colon cancer-associated transcript 1

Colon cancer-associated transcript 1 (CCAT1) was found up-regulated in colon cancer tissue, circulating blood cells of colon cancer patient and metastasis cases, indicating its role in colon cancer progression.^[74] Besides, high expression of CCAT1 is also associated with primary tumor tissue, lymph node metastasis, and metastatic cases of gastric carcinoma.^[75] The elevated level of CCAT1 reduces the survival of HCC patients. In both gastric cancer and HCC cell lines, overexpression of CCAT1 enhances the proliferation and migration of cells driven by c-Myc, an oncogenic transcription factor required for cell survival. On one hand, c-Myc binds to promoter of the CCAT1 and up-regulates its level in cancer cells.^[75] On the other hand, CCAT1 prevents degradation of c-Myc by interaction with let-7, a known miRNA that can target c-Myc through its 3'-UTR.^[76]

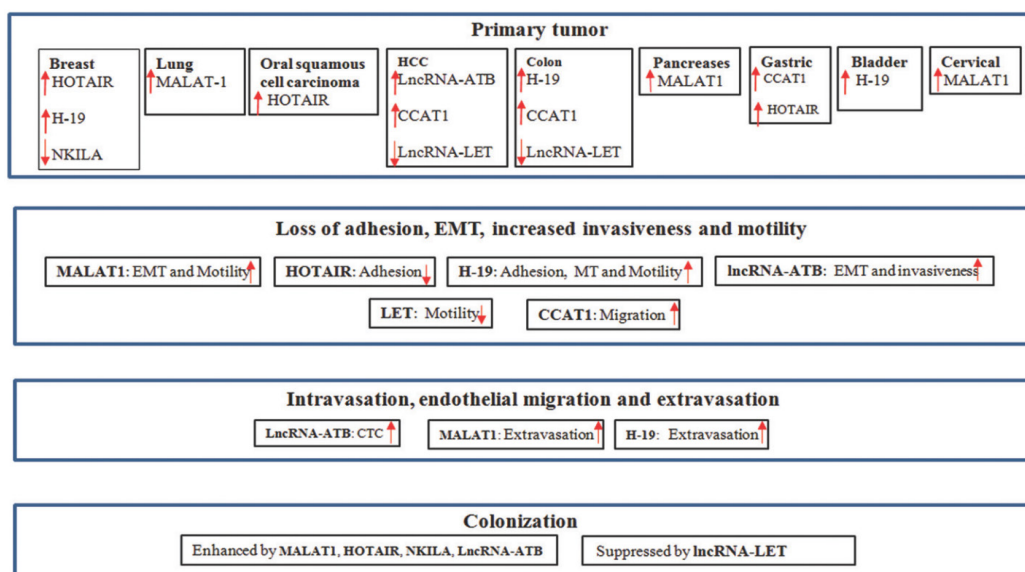


Figure 2: Long non-coding RNAs involved in different stages of cancer metastasis

DEREGULATION OF LNCRNA EXPRESSION IN CANCER

It is well-known that most of lncRNAs are transcribed through RNA polymerase II, just like protein-coding genes; they are also spliced products via canonical genomic splice site motifs, frequently ended with a poly A tail. Importantly, lncRNAs are often regulated by well-established transcription factors and are expressed in a tissue-specific manner.^[77] For example, we have shown that wild-type p53 can transcriptionally induce linc-RoR and loc285194, but mutant p53 cannot.^[17,24] On the other hand, c-Myc, as an oncogene, can regulate a group of lncRNAs.^[78] In cancer, c-Myc is often amplified or up-regulated, which may explain why some lncRNAs are often deregulated.

CONCLUSION AND PERSPECTIVE

A tremendous progress has been made in our understanding of the genes and events involved in metastasis in recent years. Moreover, emerging evidence indicates that lncRNAs have also joined this complex regulatory network and may serve as very important regulators at different stages of metastasis (e.g. EMT, invasion, migration, and colonization) often through their expression levels [Figure 2]. However, overall, lncRNA research in this field is still at the infancy stage. Given the complex interactions of lncRNAs with DNA, RNA, and protein, a systematic approach may be needed to better understand the molecular mechanism of lncRNA-mediated metastasis. With the development of advanced technology such as CRISPR/Cas9, it is now feasible to perform knockout or knockin experiments and these research tools will no doubt speed up new discovery. In this system, nuclease Cas9 assisted by a sequence-specific guide RNA (gRNA) which is functionally similar to RNAi, cuts targeted DNA sequence.^[79] Once the double strand break is made, the cell employs one of two major DNA repair mechanisms, non-homologous end joining (NHEJ), and homologous recombination (HR). Unlike HR, the NHEJ mechanism often leads to deletions or insertions, and thus it is an error-prone repair, a feature important for knockout. The HR mechanism would allow for introducing mutations or correcting a mutant sequence by knockin. Increasing evidence indicates that this technology has a potential to transform the field of cancer genetics such as the development of next-generation models of human cancer.^[80]

Given the nuclear localization nature for a number of lncRNAs, genetic manipulations at the DNA level provides a better alternative to RNAi approach which mainly works through RISC complex in the cytoplasm. Our recent study indicates that a dual gRNA/Cas9 system combined with donor vector for HR can greatly improve the efficiency of obtaining complete lncRNA knockouts in various cancer cell lines.^[81] As this field advances, we anticipate that more lncRNAs will be identified to be important players in cancer metastasis. More importantly, further

characterization of this regulatory system will reveal many of detailed mechanisms. As a result, these studies will help develop novel strategies for cancer treatment. Furthermore, lncRNAs may serve as biomarkers for diagnosis/prognosis as supported by profiling studies of clinical specimens. Finally, given their important role in metastasis, lncRNAs may also prove to be valuable targets for cancer therapy. In particular, ribonucleoprotein complexes through lncRNAs are critical to lncRNA-mediated metastasis, drugs that block or enhance such interactions may have a bright future.

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Conflicts of interest

There are no conflicts of interest.

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