

Long Intergenic Noncoding RNAs: New Links in Cancer Progression

Miao-Chih Tsai, Robert C. Spitale, and Howard Y. Chang

Abstract

The process of cancer metastasis involves a series of sequential and complex steps. Here we give a perspective on recent results regarding noncoding transcription in cancer progression, focusing on the emerging role of long intergenic noncoding RNAs (lincRNAs). LincRNAs target chromatin modification complexes or RNA-binding proteins to alter gene expression programs. Similarly to miRNAs, lincRNAs exhibit distinct gene expression patterns in primary tumors and metastases. We discuss how lincRNAs can be used for cancer diagnosis and prognosis and serve as potential therapeutic targets. *Cancer Res*; 71(1); 3–7. ©2011 AACR.

Introduction

Most deaths from cancer are due to metastasis (1), and the main barrier to the treatment of metastases is the biological heterogeneity of cancer cells in the primary neoplasm and in metastases. A full understanding of the gene regulation network during this transition is essential but still far beyond completion. Intensive investigations over the last few decades have focused on the role of protein-coding genes in the pathogenesis of cancer. The human genome contains only 20,000 protein-coding genes, representing <2% of the total genome, whereas a substantial fraction of the human genome can be transcribed, yielding many short or long noncoding RNAs (ncRNAs) with limited protein-coding capacity (2). Are ncRNAs the missing piece of the cancer jigsaw puzzle? One of the emerging themes in the study of noncoding transcripts is microRNAs (miRNAs), a class of small regulatory RNAs that mediate posttranscriptional silencing of specific target mRNAs (3). The identification of the miRNA *miR-34* as the direct target of the tumor suppressor gene *p53* (4) revealed for the first time that ncRNAs function in this crucial tumor suppressor pathway. Numerous miRNA expression profiling and functional studies have also associated miRNAs with cancer progression, diagnosis, prognosis, and treatment (5, 6). Therefore, the interplay between proteins and ncRNAs is an important topic in the field of cancer biology, and ncRNAs may be the missing links in well-known oncogenic and tumor suppressor networks. Long intergenic ncRNAs (lincRNAs), which range in size from several hundred to tens of thousands of bases, comprise another class of newly discovered ncRNAs.

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Here we provide an update and perspective on recent advances made in understanding lincRNA mechanisms in cancer progression.

LincRNA Expression and Cancer

Although >3,000 human lincRNAs have been identified, less than 1% of these have been characterized (7–9). Recent studies showed that lincRNAs are exquisitely regulated during development and in response to diverse signaling cues (8) and can be misexpressed in solid tumors and leukemias (10). Numerous *HOX* lincRNAs were found to be differentially expressed between primary breast carcinomas and distant metastases (11), and many *p53*-dependent lincRNAs were identified in response to DNA damage (12). The finding that several lincRNAs can control transcriptional alteration implies that the difference in lincRNA profiling between normal and cancer cells is not merely the secondary effect of cancer transformation, and that lincRNAs are strongly associated with cancer progression (13). Thus, the differential expression of lincRNAs may be profiled to aid in cancer diagnosis and prognosis and in the selection of potential therapeutics.

Although lincRNAs may have an impact on various human diseases (10, 13, 14), the basis of their molecular mechanisms is still largely unknown. Several lincRNAs can control gene expression by directly recruiting histone-modifying enzymes to chromatin (9). Chromatin modification and DNA methylation are key epigenetic events that are fundamentally disturbed during the development of cancer. Epigenetic alterations are potentially stable and heritable and can occur at a much greater rate than DNA mutations in somatic cells (15). Dysregulated lincRNAs may affect epigenetic information and provide a cellular growth advantage, in that their selection may result in the progressive and uncontrolled growth of a tumor. One such lincRNA, HOTAIR, is highly expressed in breast cancer metastases and in primary tumors predisposed to future metastases. Of importance, enforced expression of HOTAIR can target polycomb repressive complex 2 (PRC2; comprised of histone H3 lysine 27 methylase EZH2, SUZ12,

and EED) genome-wide to alter H3K27 methylation and gene expression patterns, resulting in increased cancer invasiveness and metastasis *in vivo*. Loss of HOTAIR or PRC2 components inhibits cancer invasiveness, indicating potentially direct roles for lincRNAs in modulating cancer progression (11). HOTAIR was the first lincRNA to be identified as functioning in *trans* (16); however, other antisense lincRNAs are known to silence genes in *cis*. For example, ANRIL is antisense transcribed from the tumor suppressor genes *CDKN2B* and *CDKN2A*, where ANRIL interacts with CBX7 (a subunit of PRC1), resulting in heterochromatin formation and gene silencing (17–19).

LincRNAs may carry out many of their functions by acting as modular scaffolds for protein–chromatin interactions (20, 21). Tsai and colleagues (21) recently discovered that HOTAIR bridges together the PRC2 complex and the LSD1 H3K4 demethylase complex and recruits both complexes to target genes to coordinately alter several histone modifications and enforce gene silencing. As another example, TLS protein can be allosterically modified by interacting with ncRNA_{CCND1}, transcribing from the 5' regulatory regions of *CCND1* in response to DNA damage signals. The conformation change of TLS induces gene-specific TLS–CBP/p300 interactions, which in turn inhibit *CCND1* transcription (22). Another lincRNA, MALAT-1, which is highly expressed in many different tumors, can interact with SR splicing factors and modulates their distribution to nuclear speckles. MALAT1 then regulates alternative splicing by controlling the functional levels of SR splicing factors (23). LincRNAs can also affect global gene changes in response to signals to classic transcription factors. For example, *p53* induces lincRNA-p21, which in turn represses numerous genes globally by recruiting the repressor protein hnRNP-K (12).

Dozens of lincRNAs are differentially expressed in cancers with different clinical courses. In multivariate analyses with clinical and pathologic risk factors, HOTAIR RNA can serve as a significant and independent predictor of eventual metastasis and death (11). Because risk stratification by mRNA or miRNA profiling typically requires dozens if not hundreds of RNA species, the ability to use a single species of lincRNA (HOTAIR) represents a promising approach. These examples suggest that HOTAIR and other lincRNAs may be used as a novel prognostic tool in cancer management.

Mutations and Alternative RNA Structural Variation

The misexpression of lincRNAs in cancer naturally raises the question as to how structural variations in lincRNA genes, either germline or somatic, may contribute to cancer predisposition. To date, analyses of DNA copy number variations and rearrangements have focused on protein coding genes, although many noncoding sequences are included in recurrent structural aberrations of cancer genomes. Similarly, genome-wide association studies of cancer susceptibility have mainly identified single nucleotide polymorphisms in non-coding portions of the genome, some of which may be

transcribed (24). For instance, a region upstream of the 9p21 locus, encoding the cyclin-dependent kinase inhibitors *CDKN2B* and *CDKN2A* and *p53* activator *ARF*, is associated with cancer as well as cardiovascular disease and diabetes, and this region is transcribed to produce ANRIL, a long noncoding transcript that is implicated in controlling chromatin modification of the locus (17–19). Compared with mutations in protein coding genes, where certain single nucleotide mutations (such as a premature stop codon or frame shift) can completely abrogate protein function, structural variations in lincRNAs may have more subtle effects, which makes it more difficult to verify them experimentally (25). Better annotation of lincRNA expression patterns and primary structures should improve the detection and interpretation of genome aberrations that affect lincRNAs. Moreover, because known oncogenes and tumor suppressor genes are often dysregulated by multiple mechanisms, lincRNAs that are misexpressed in cancers should be the starting point for possible structural alterations in lincRNA genes.

Targeting Small ncRNAs Associated with Cancer

A major goal in the development of cancer therapeutics is to identify molecular targets that are specific for cancer cells (26). The differential expression and biological importance of specific ncRNAs in cancer suggest that ncRNAs may be useful targets (27). Here we briefly discuss efforts to target miRNAs and then speculate on the potential of targeting long ncRNAs for cancer treatment (Fig. 1).

miRNAs have been effectively targeted in preclinical models by means of small molecules and exogenously introduced complementary RNA sequences (28). High-throughput screens, both *in vitro* and *in vivo*, have identified small molecules that affect several steps in miRNA biogenesis, including pre-miRNA processing, or interact directly with the miRNA, thus blocking miRNA hybridization with its endogenous 3'-UTR sequences (28). For example, a small-molecule inhibitor of *miR-122* can selectively induce apoptosis in liver cancer cells (29). Screens involving small molecules often are initiated with little prior chemical knowledge of a successful binder. Because every RNA sequence is different, it is difficult to generalize the chemical functionality of anti-miRNA molecules. Further, although studies such as those mentioned above are leading to promising compounds, the modest binding affinity of these compounds [often in the micromolar range (30)] suggests a need for improvement. A more targeted approach that exploits the miRNA sequence directly may be more advantageous.

Antagomirs are synthetic RNA molecules that are designed to directly hybridize with miRNAs, thus potentially limiting the availability of the miRNA for Argonaute loading and 3'-UTR hybridization. Antagomirs are chemically modified to prevent premature degradation by RNases, thereby increasing their half-life *in vivo* (31). Krützfeldt and colleagues (32) were the first to show that antagomirs can inhibit specific miRNA function in living mice. Subsequently, Ma and colleagues (33)

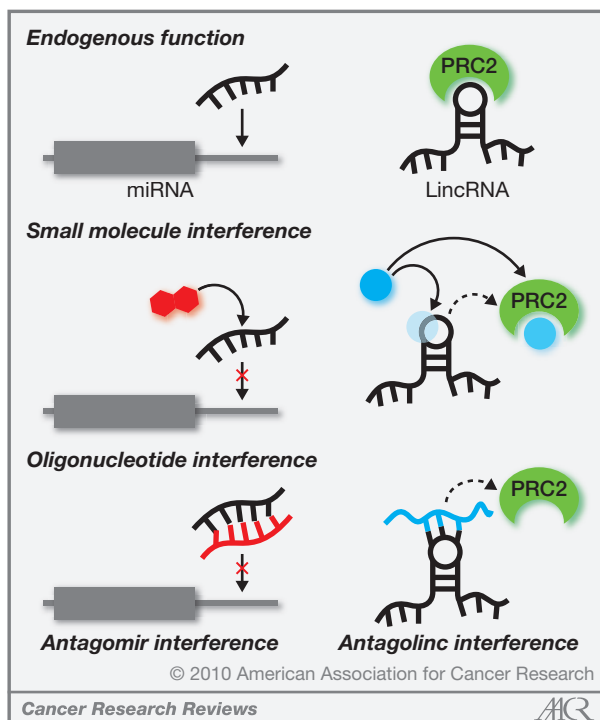


Figure 1. Targeting cancer-specific ncRNAs. Small molecules and complementary antagomir sequences have been identified that bind to miRNAs, disrupting base pairing. We speculate that targeting the lincRNA structure could inhibit binding to chromatin-modifying complexes without disrupting the binding of different RNAs.

showed the utility of antagomirs in preventing the onset of metastasis. The authors reported that systematic treatment of tumor-bearing mice with antagomirs targeting *miR-10b* increased the expression of its target, *HOXD10*, a tumor suppressor gene that is downregulated in metastasis. In another study (34), intravenous administration of the antagomir did not reduce primary mammary tumor growth, but it did strikingly suppress the formation of lung metastases in a sequence-specific manner. These studies preliminarily suggest that antagomirs may be powerful molecules that can disrupt the miRNA pathway and may be potential chemotherapeutics. Although we are just beginning to understand the potential of lincRNAs as therapeutic targets, it is conceivable that the avenues that have been explored for other ncRNAs can be used to target lincRNA-protein interactions. In the next section, we highlight some of the potential avenues that can be tailored to lincRNAs and their protein partners.

Potential New Cancer Therapy: lincRNAs

Although efforts to target cancer-specific miRNAs have proven successful, designing molecules to inhibit long ncRNAs presents different opportunities and challenges. The fact that lincRNAs can be depleted by siRNAs provides a straightforward approach to inhibit the function of lincRNAs implicated in cancer. Gupta and colleagues (11) showed that depletion of HOTAIR inhibited the matrix invasiveness of a breast cancer

cell line. In practice, we and others (21) have found that to achieve a successful knockdown of lincRNAs, one must screen more siRNAs compared with mRNAs, possibly because of the extensive secondary structures in lincRNAs.

Structural insights into riboswitches, the ribosome, RNase P, and catalytic RNAs are beginning to be exploited for the design of specific small-molecule inhibitors that can target RNA and ribonucleoprotein (RNP) complexes (35–37). For example, investigators have used the atomic structure of the bacterial ribosome, the most complex RNP structurally characterized to date, to identify functional sites within rRNA for therapeutic targeting by antibiotics (38). These results underscore the importance of structural studies to aid in the design of therapeutic agents against complex RNAs. Detailed structural studies of recently discovered lincRNAs associated with cancer are still forthcoming.

LincRNAs such as HOTAIR serve as a structural scaffold for protein complexes and possess complex RNA structural motifs (21). Because the chromatin complexes that interact with lincRNAs are ubiquitous, targeting the proteins themselves may lead to deleterious phenotypes. However, targeting the RNA-protein interface and focusing on the cancer-specific transcript (e.g., HOTAIR) may provide an inroad to cancer-specific therapeutics. Targeting transcripts the size of lincRNAs may seem like a daunting task; nevertheless, there is a precedent for fragmenting large RNP complexes into more manageable sizes. This strategy has been applied to the design of ligands that can bind expanded rCUG and rCAG repeat RNAs that are expressed in myotonic dystrophy type 1 (DM1) and interact with Muscleblind-like 1 (MBNL1) protein. Ligands identified *in vitro* have also been shown to inhibit the formation of RNA-protein complexes in mouse myoblasts (39). These results, along with other findings, provide strong evidence that examining defined, functionally relevant RNA fragments *in vitro* can lead to the discovery of small-molecule binders, and the information gained can be used in the cellular context to disrupt RNA-protein interactions.

Although one can investigate lincRNAs by applying methods and principles similar to those used to target miRNAs with small molecules, one must use a different strategy for antagomirs. Because the structural content of lincRNAs is expected to be vast, designing antagomirs based on the primary sequence may yield unsuccessful results. However, using a more unbiased approach may yield oligonucleotide sequences that bind to lincRNAs. Methods such as systematic evolution of ligands by exponential enrichment (SELEX) provide an unbiased way to identify high-affinity binders of a selected macromolecule (40). Just recently, SELEX was used to identify RNA sequences that bind to pri-miRNAs (41). pri-miRNAs are larger than their mature products and contain substantial predicted secondary structure. Therefore, a similar approach may yield RNA sequences that can bind to lincRNAs at key lincRNA-protein interfaces, thereby disrupting the interaction. We suggest the term "antagolincs" for this yet to be established class of molecules. Once an RNA sequence is identified through SELEX, the same RNA sequence can be transformed into the more chemically stable construct, in similarity to an

antagomir. The administration of an antagomir against HOTAIR would then lead to competitive inhibition of a chromatin remodeling complex, such as PRC2, by binding to the lincRNA. This RNA–RNA interaction may then normalize the chromatin state to inhibit cancer cell growth and metastasis.

Conclusions

Just as dysregulation of miRNAs is now recognized as a universal feature of many types of cancer, many lincRNAs that affect cancer initiation, progression, and treatment are surely waiting to be discovered. Cancer-associated lincRNAs may provide new approaches to the diagnosis and treatment of cancer. Systematic identification of lincRNA expression patterns and characterization of lincRNAs and their associated

proteins should pave the way for designer therapeutics that can target lincRNAs for cancer and other disease states.

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No potential conflicts of interest were disclosed.

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