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REVIEW

Characteristics of Antisense Non-coding RNA in the INK4 Locus and Its Roles in Disease

Jin-hua He and Yu-guang Li*

Department of Laboratory, Panyu Central Hospital, Guangzhou 511400, China

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Abstract With the development of genome-wide sequencing technology, 195 types of functional long non-coding RNAs (lncRNAs) have so far been found, and their cellular roles are gradually being revealed. Now lncRNAs have become a hotspot in the life science. These small molecules exist in almost all higher eukaryotes, and have very important regulatory roles in these organisms. This review briefly summarizes recent progress in researches on antisense non-coding RNA in the INK4 locus.

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LONG non-coding RNAs (lncRNAs), which are different from other smaller non-coding RNAs, have been found to affect species evolution, embryonic development, metabolism, and tumorigenesis. Research on lncRNAs will make revolutionary changes in the understanding of cell structure and regulatory networks, as well as provide scientific evidence for diagnosis and treatment of several diseases, especially in human tumors.^{1,2} The lncRNAs include antisense non-coding RNA, intronic transcript, large intergenic non-coding RNA, promoter-associated lncRNA, and 3' untranslated region-associated lncRNA. Mammalian genomes encode numerous natural antisense transcripts, but the function of these transcripts is not well understood. Functional validation studies indicate that antisense transcripts are not a uniform regulatory RNA group, but belong to multiple categories with some common features. Recent evidence indicates that the antisense transcripts are frequently functional and have a wide range of biological roles *via* diverse transcriptional and post-transcriptional gene

regulatory mechanisms.³

EXPRESSION AND FUNCTION OF ANTISENSE NON-CODING RNA IN THE INK4 LOCUS

The antisense non-coding RNA in the INK4 locus (ANRIL) exists in multiple splicing isoforms, such as CDK2BAS. It is also detected as an unspliced transcript of 34.8 kb (specified as p15AS), which, however, seems to correspond to an isoform with the first intron of CDK2BAS retained. Antisense to the tumor suppressor gene p15/CDKN2A, it is located in hg19 chr9:21,994,790-22,121,093.^{4,5}

ANRIL is expressed in tissues and cell types affected by atherosclerosis, including peripheral blood mononuclear cells, whole blood, and atherosclerotic plaque tissue. Increased ANRIL level is found in patients carrying the atherosclerosis risk haplotype expression, and the level is directly correlated with the severity of atherosclerosis.⁵

In vitro over-expression of p15as/ANRIL RNA was originally demonstrated to induce epigenetic silencing of p15 gene expression.⁵ Recently, ANRIL RNA has been found to interact and regulate chromobox 7 (CBX7), a component of

the Polycomb Repressive Complex 1 (PRC1), which methylates histone H3 lysine 27 to promote epigenetic silencing, and is also up-regulated in prostate cancer. The interaction of ANRIL RNA with a conserved chromodomain of CBX7 is essential for the ability of PRC1 to repress the INK4b/ARF/INK4a locus and control cell senescence.^{6,7}

ROLES OF ANRIL IN DISEASES

Coronary heart disease and periodontitis are genetically related by at least one susceptibility locus, which is possibly involved in ANRIL activity and independent of diabetes-associated risk variants within this region, promising new insight into the underlying shared pathogenic mechanisms of these complex common diseases.⁸ ANRIL collocates with the high-risk haplotype. It is expressed in tissues and cell types that are affected by atherosclerosis and a prime candidate gene for the chromosome 9p CAD locus.⁹ ANRIL is a large antisense non-coding RNA within the 403-kb germ-line deletion, with a first exon located in the promoter of the *p14/ARF* gene and overlapping the two exons of *p15/CDKN2B*. Expression of ANRIL mainly co-clusters with *p14/ARF* in both physiological (various normal human tissues) and pathological conditions. The existence of ANRIL within the *p15/CDKN2B*-*p16/CDKN2A*-*p14/ARF* locus is putatively involved in melanoma-neural system tumor pedigrees and in melanoma-prone families with no identified *p16/CDKN2A* mutations as well as in somatic tumors.¹⁰ *CDKN2A-CDKN2B* is low-penetrance susceptibility allele contributing to the risk of developing glioma and providing insight into the etiology of this primary brain tumor.¹¹ The antisense non-coding RNA in the INK4b/ARF/INK4a locus is also important for the expression of the protein-coding genes in cis, but the mechanism has remained elusive. CBX7 within the PRC 1 binds to ANRIL, and both CBX7 and ANRIL are found at elevated levels in prostate cancer tissues.⁶

In conclusion, ANRIL is expressed at a high level in peripheral blood mononuclear cell, and atherosclerotic plaque tissue. It may serve as a diagnostic marker for atherosclerosis. Some studies indicate that ANRIL contributes to the development of some tumors, such as somatic tumors, prostate cancer, etc. Natural antisense transcripts are involved in different gene regulatory pathways, but it is still not clear which intrinsic properties of natural antisense RNA molecules, or extrinsic features (such as protein interactions, cellular and developmental context), are

decisive for any given pathway.³ Some fundamental and powerful regulatory mechanisms related to ANRIL remain to be elucidated.

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