Dissecting the Functions of lncRNA Genes Transcribed From tRNA Gene Loci

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Abstract

The human genome is pervasively transcribed, yet only 2% of its three billion base pairs are associated with protein-coding exons. As a relatively new class of RNA, the functions of the ~20,000 annotated long non-coding (lncRNA) genes remain poorly understood. Here, I dissect the functions of lncRNA genes transcribed from tRNA gene loci. This investigation began when I identified the lncRNA *LINC00324*, which spans a cluster of four tRNA genes, during the *in vitro* differentiation of primary human mesenchymal stem cells (MSCs) into chondrocytes. When I deleted the entire *LINC00324* genomic locus in chondrocytes, collagens were downregulated at the RNA and protein level. One of the tRNAs at the *LINC00324* locus is tRNA-Gly-TCC-3-1, and the corresponding Gly codon, GGA, is enriched in the downregulated mRNAs and proteins. Collagens, which are composed of glycine repeats, are especially affected. Since these results implicated *LINC00324* as a functional gene in chondrogenesis, in Chapter 3 I went on to search for *LINC00324*-like genes in the genome, and identified 70 tRNA-overlapping lncRNA genes, which I define as ‘tRNA-Overlapping LncRNAs’ (tROLs). Deletions of four tROLs result in changes in the expression of codon-biased genes, where downregulated genes are enriched in codons corresponding to tRNAs

overlapping disrupted tROLs. However, tROL lncRNA expression is controlled independently of the overlapping tRNA loci. Remarkably, tROL loci are located in gene-dense regions and interact extensively *in trans* between chromosomes. The tROL deletions result in the upregulation of significantly overlapping subsets of genes in the vicinity of tROL loci. Taken together, the results suggest that tROL loci coalesce and are dependent on each other's transcription to repress surrounding genes *in trans*. To further understand the mechanism of tROL function, in Chapter 4, I attempted to disentangle the function of the *LINC00324* tRNAs with the lncRNA's transcription and transcript in the observed phenotype using several CRISPR-based approaches in HEK-293 cells. PolyA transcriptional termination at different genomic positions and Cas7-11 knockdown of the *LINC00324* lncRNA transcript show moderate effects on translation. My investigation thus sheds light on a unique role for tROLs as a regulatory bridge between the non-coding and coding genomes.