EXPLORING THE FUNCTION OF LNCRNA GENES MAPPED TO THE DFNA58 LOCUS

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INTRODUCTION

Hearing loss is among the most genetically heterogeneous disorders known. Long non-coding RNAs (lncRNAs) are increasingly recognized as important regulators of gene expression, including modulation of neighboring genes.

At the DFNA58 locus, a genomic duplication has been identified in a large Brazilian family with postlingual, progressive hearing loss. This duplication encompasses three lncRNA genes of unclear function that are not evolutionarily conserved. Notably, two of these lncRNAs were found to be overexpressed in the peripheral blood of 10 affected individuals carrying the duplication.

To investigate their potential functional role, this study employed in vitro transcription and transfection experiments in murine tumor cells, focusing on one of the duplicated lncRNAs (Fig,1). Specifically, the study examined its impact on the expression of protein-coding genes located within the same duplicated region of human chromosome 2p14, which lacks orthologous lncRNA genes in the syntenic murine chromosomal region.

AIMS

1. To determine the sequence and generate in vitro–transcribed RNA of lncRNA genes from the DFNA58 locus.
2. To transfect murine cells lacking these lncRNAs (e.g., NIH3T3) with the synthesized transcripts.
3. To evaluate whether DFNA58 lncRNA transcripts alter the expression of neighboring protein-coding genes, thereby clarifying their potential role in hearing loss.

METHODOLOGY

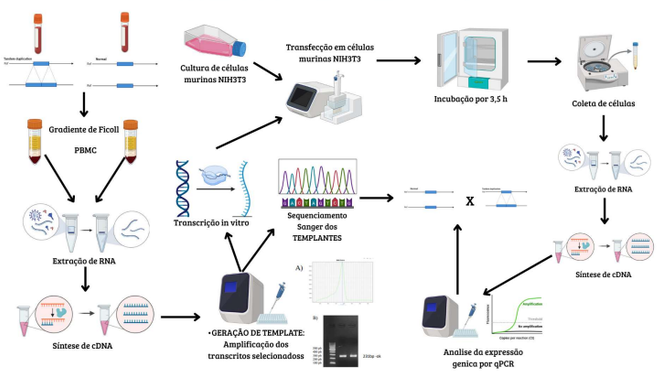


FIG. 1: Schematic representation of the methodological workflow, including lncRNA transcript characterization, primer design, amplification and sequencing, in vitro transcription, cell transfection, and subsequent gene expression analysis.

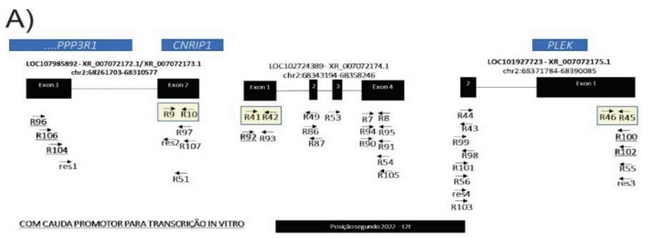
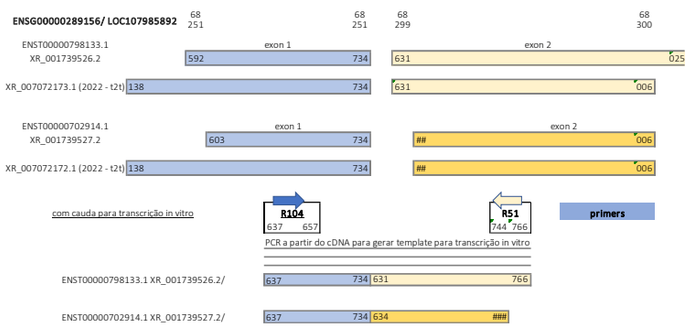


FIG.2: A) Exon structure, primer locations

RESULTS



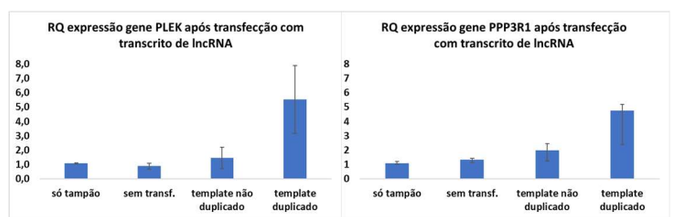


FIG.2: B) Diagram of exons and available primers for gene ENSG00000289156/LOC107985892 C) gene expression modulation D) Transcript sequencing for lncRNA genes with duplication and nonduplicated

CONCLUSION

Analysis of lncRNA exon structures across three databases and genome assemblies revealed inconsistencies, suggesting multiple alternative transcripts and justifying this study.

Attempts to amplify full-length DFNA58 lncRNA from blood cDNA were unsuccessful, but exon mapping and primer design (Figure 1B) enabled the detection of at least two distinct transcripts. Sequencing confirmed two isoforms in both duplicated and non-duplicated samples. While isoform levels were similar in non-duplicated samples, duplicated ones showed predominance of ENST00000798133.1.

After transfection of in vitro–transcribed RNA into NIH3T3 cells, RT-qPCR showed increased expression of neighboring genes only with transcripts from the duplicated template. Overexpression of Plek and Ppp3r1 occurred specifically when ENST00000798133.1 was more abundant, suggesting duplication alters transcript ratios and modulates adjacent gene expression. Therefore, these experiments showed that partial lncRNA fragments also induced gene expression in murine cells, supporting their potential role as precursors of smaller regulatory RNAs.

For Cnrip1, however, low basal expression limited conclusions. Overall, lncRNA duplication appears to fine-tune transcript balance, influence neighboring gene expression, and contribute to the formation of smaller functional RNAs.

This study highlights the potential of targeting lncRNA function as a basis for future genetic therapies.

REFERENCES

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AGRADECIMENTOS:
AOS PACIENTES.