Targeting the Dark Matter of the Genome: Functional Insights into lncRNAs RP11-796E10.1 and RP11-595B24.2 in Colorectal Cancer

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1. Abstract

This study focused on two uncharacterized lncRNAs, **RP11-595B24.2** and **RP11-796E10.1**, which showed tumor-specific expression. Their expression was analyzed in the colorectal cancer cell lines HT-29 and SW480 and compared in the control non-cancerous fibroblast line MRC-5. In HT-29 cells, antisense oligonucleotide (ASO)-mediated knockdown successfully reduced their expression in an isoform-dependent manner and the most efficient knockdowns led to a time-dependent reduction in cell viability. These findings suggest a prooncogenic role of the investigated lncRNAs and highlight their potential as therapeutic targets in CRC.

2. Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related deaths. It arises from genetic and epigenetic alterations affecting proto-oncogenes and tumor suppressor genes. Despite advances in screening and therapy, incidence is expected to increase by 60% by 2030, highlighting the need for new molecular targets [1].

LncRNAs are RNA molecules longer than 200 nucleotides that do not encode proteins but regulate gene expression at multiple levels. Many display high tumor specificity, making them attractive therapeutic candidates. The two lncRNAs RP11-595B24.2 and RP11-796E10.1 were identified as epithelial tumor-specific but remain uncharacterized [2].

ASOs are short synthetic nucleic acids that bind complementary RNA sequences and induce their degradation via RNase H. They offer a powerful strategy to silence RNAs with high specificity [3].

3. Aims and leading questions

Aim 2: Test the efficiency of antisense oligonucleotides (ASOs) in reducing lncRNAs RP11-796E10.1 and RP11-595B24.2 in CRC cell lines (SW480, HT-29). *Leading question:* Can specific ASOs effectively downregulate these lncRNAs?

Aim 3: Assess the impact of ASO-mediated knockdown on cell viability and proliferation. *Leading question:* How does silencing of RP11-796E10.1 and RP11-595B24.2 affect CRC cell growth?

4. Materials and Methods

HT-29 cells were used for ASO knockdown and viability assays (SW480 insufficient growth). Two ASOs per lncRNA were transfected with Lipofectamine®2000. Expression was measured by RT-qPCR, normalized to YWHAZ, and compared to water and control ASOs.

Effect on viability (Aim 3):
HT-29 cells were seeded after transfection and analyzed with CellTiter-Glo® at 0, 24, 48 and 72 h. Each condition was tested in triplicate with technical quadruplicates.

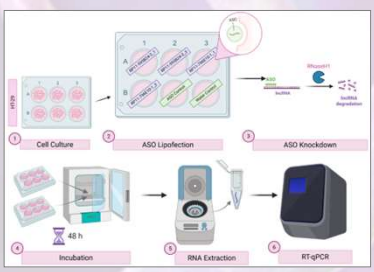


Fig. 1: Experimental workflow for Aim 2.

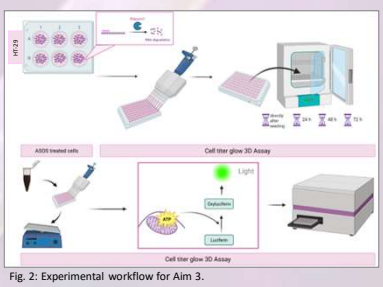


Fig. 2: Experimental workflow for Aim 3.

5. Results

ASO-mediated knockdown (Aim 2):

qPCR in HT-29 cells showed reduced expression of both lncRNAs.

- RP11-595B24.2: strongest effect with ASO 595B24-2_3
- RP11-796E10.1: most efficient knockdown with ASO 796E10-1_1

Effect on viability (Aim 3):

CellTiter-Glo® assays showed time-dependent loss of viability after knockdown. ASO 595B24-2_3 caused a clear reduction already at 24 h, whereas ASO 796E10-1_1 led to a progressive decline up to 72 h. Control ASOs showed no effect, confirming target specificity.

As only three replicates were available, no statistical analysis was performed. Figures 3 and 4 show representative expression and viability data; full results are presented in the diploma thesis.

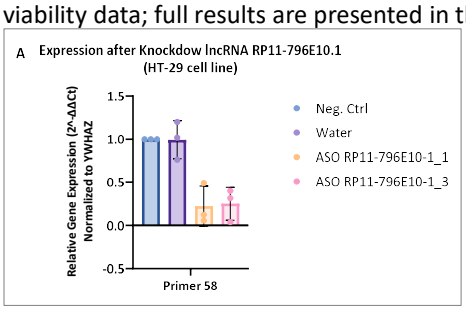


Fig. 3: Relative expression of RP11-796E10.1 in HT-29 cells after treatment with two ASOs, measured with primer 58. Normalized to YWHAZ and calculated using the 2^-ΔΔCt method. Mean ± SD of three biological replicates. Created with GraphPad Prism 10 (Pizzardi, L., 2025).

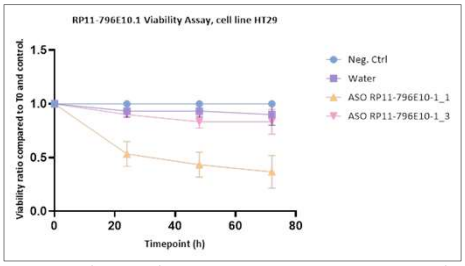


Fig. 4: Viability of HT-29 cells after ASO treatment targeting RP11-796E10.1, measured at four time points over 72 h. Values are shown relative to T0 and control. Mean ± SD of three biological replicates. Created with GraphPad Prism 10 (Pizzardi, L., 2025).

6. Discussion and Conclusion

The knockdown experiments demonstrated that ASOs were able to reduce the expression of both target lncRNAs, RP11-595B24.2 and RP11-796E10.1, in HT-29 colorectal cancer cells. The observed knockdown efficiency varied depending on the primer pairs used, as these primers target different isoforms of the respective lncRNAs. Importantly, the most efficient ASOs (RP11-595B24-2_3 and RP11-796E10-1_1) not only produced the strongest reduction in transcript levels but also led to a clear, time-dependent decrease in cell viability. Reduced viability correlated with knockdown, indicating a direct effect of lncRNA suppression rather than unspecific ASO toxicity. Control ASOs did not affect viability, further confirming target specificity.

These results point towards a pro-oncogenic function of RP11-595B24.2 and RP11-796E10.1 in HT-29 cells, highlighting them as promising candidates for therapeutic targeting in colorectal cancer.

Referenzen

[1] Tsukanov, V. V., Vasyutin, A. V., & Tonkikh, J. L. (2025). Risk factors, prevention and screening of colo-rectal cancer: A rising problem. World Journal of Gastroenterology, 31(5), 98629. https://doi.org/10.3748/wjg.v31.i5.98629

[2] Tsagakis, I., Douka, K., Birds, I., & Aspden, J. L. (2020). Long non-coding RNAs in development and dis-ease: Conservation to mechanisms. The Journal of Pathology, 250(5), 480–495. https://doi.org/10.1002/path.5405

[3] Lauffer, M. C., van Roon-Mom, W., & Aartsma-Rus, A. (2024). Possibilities and limitations of antisense oligonucleotide therapies for the treatment of monogenic disorders. Communications Medi-cine, 4(1), 6. https://doi.org/10.1038/s43856-023-00419-1

Figures

Fig. 1 / Fig. 2 Own illustration – created with BioRender (Pizzardi, 2025)
Fig. 3 / Fig. 4 Own illustration – created with GraphPad Prism 10 (Pizzardi, 2025)