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IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Project Completion Report

Report

This project aimed to investigate the functional roles of housekeeping cis-regulatory elements in the human genome, focusing on the core promoter regions of four genes, ZNF544, ZNF551, ZNF530, and ZSCAN5A, on chromosome 19. These genes were identified as possible oncogenes through prior computational analysis, based on their expression patterns and potential links to tumor suppressor elements.

To test their function, we designed sgRNAs to target 1 kb regions around each promoter and cloned them using standard CRISPR/Cas9 protocols. Cloning was verified by Sanger sequencing, and lentiviral transduction into PC3 prostate cancer cells was followed by qPCR to confirm successful targeting and transcript downregulation (Figure 1).

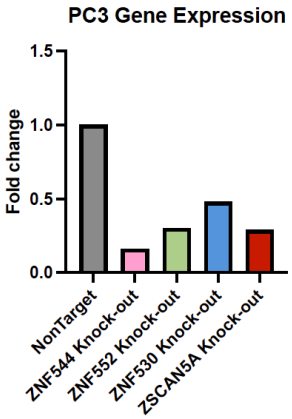

Figure 1. qPCR result upon CRISPR-Cas9 targeting of each region with specific sgRNAs.

We then assessed the phenotypic effects using colony formation and 3D spheroid assays. Targeting ZNF544 and ZNF551 reduced both colony growth and spheroid size, suggesting a functional role in supporting proliferation under oncogenic conditions (Figures 2 and 3). These effects were consistent across three replicates. ZNF530 and ZSCAN5A did not show similar results.

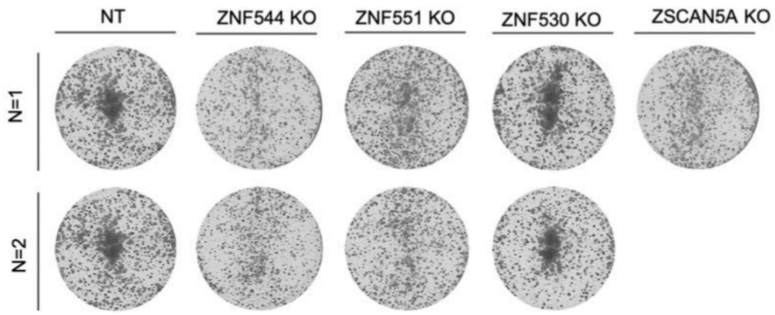

Figure 2. Colony formation assay.

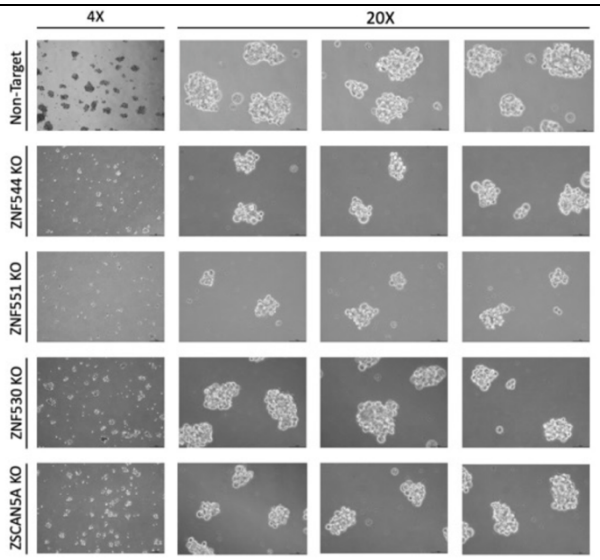

Figure 3. 3D cancer spheroid formation was optimized and analyzed for each oncogene.

While the proposal also included extending the work to other cancer cell lines and conducting RNA-seq for pathway analysis, this was not completed. Reasons included limited access to additional cell lines, and staffing constraints in the lab. We also had fewer updates from the experimental side during the second half of the project, which made it difficult to align next steps.

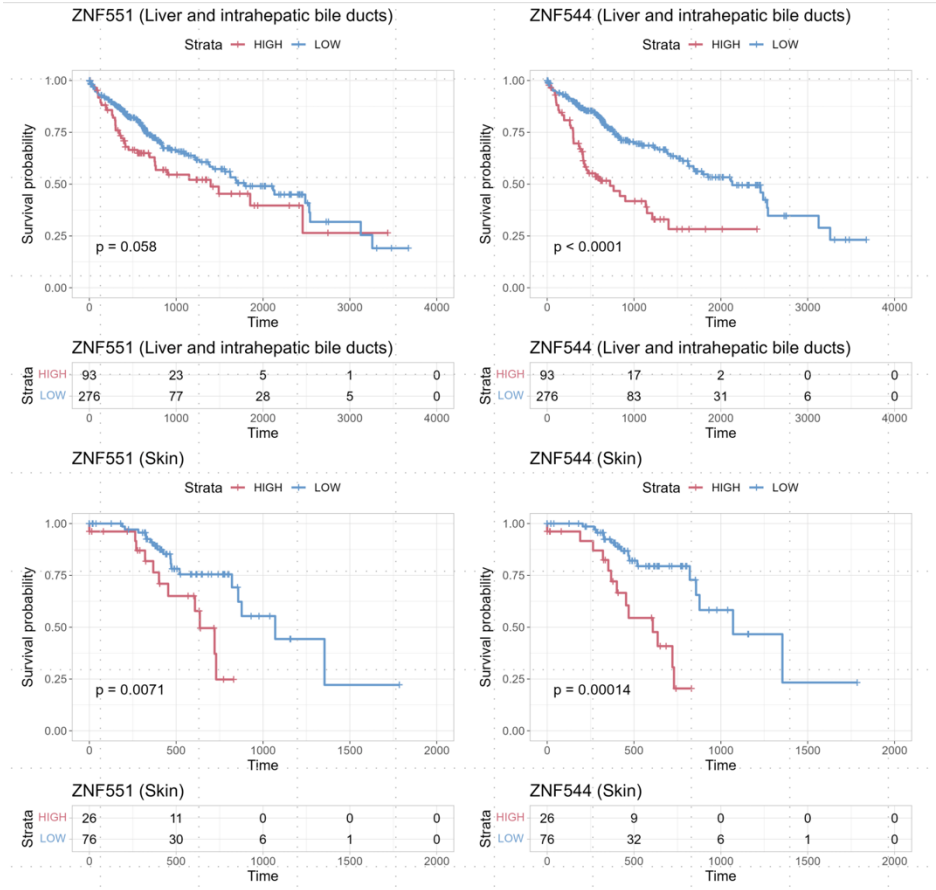

Figure 4. TCGA cancer subtypes with clear oncogene effects

Still, the results in PC3 cells were clear and reproducible. Public database analyses, including TCGA and ENCODE, showed increased expression of ZNF544 and ZNF551 in several cancer types, but survival correlations were inconsistent. This may reflect subtype-specific effects that require further investigation (Figure 4 and 5).

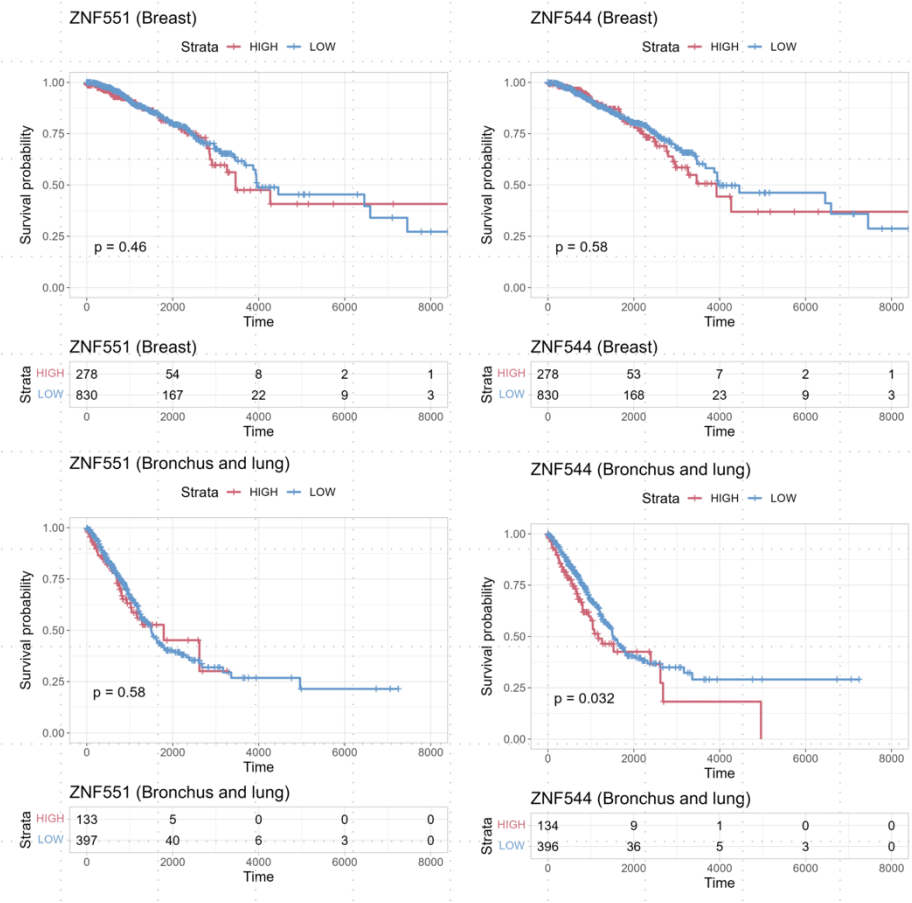

Figure 5. TCGA cancer subtypes with ambiguous oncogene effects

Several attempts were made to expand the analysis to other cancer types using data from DepMap. Lung and breast cancer models were considered, but experiments could not proceed due to limited resources. Nonetheless, discussions helped clarify potential directions, including the value of combining functional assays with public data analysis and chromatin conformation datasets.

Conclusion

This study provided functional evidence that the promoter regions of ZNF544 and ZNF551 affect cancer-related phenotypes in PC3 cells. While the work remained focused on a single cell line, it offers a useful framework for testing other candidate regulatory elements.

Future work may benefit from additional funding and expanded access to cell models. We hope the findings can contribute to broader efforts to understand the role of housekeeping regulatory elements in cancer.

We thank the IMSUT International Joint Usage/Research Center for supporting this project.