Investigating the Mechanisms of Breast Cancer Metastatic Reactivation by Generating a New DNA Construct That Targets a Mediator Complex Subunit

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Introduction

- Metastasis is a serious threat to cancer patients, as it is one of the major causes of all cancer deaths.
- Genetic screening in mice and subsequent testing has identified Med4, a subunit of the Mediator complex, as a dormancy enforcer, or repressor of metastatic reactivation. The Mediator complex is a multiprotein coactivator that regulates RNA polymerase II-related transcription.
- Interestingly, cancer patient dataset analyses indicate that lower levels of Med4 correlate with poorer outcomes. Med4 also has significantly reduced expression in breast cancer cells.
- However, the specific role that Med4 plays in enforcing dormancy and the mechanisms of metastasis upon Med4 deregulation remain to be elucidated.

Objective

I silenced Med4 in dormant breast cancer cells by cloning microRNA-E-based (miR-E) short hairpin *Med4* (sh*Med4*) into lentiviral vectors and transducing the cells.
This approach allows for the investigation of the mechanisms of metastatic reactivation and how Med4 enforces dormancy.

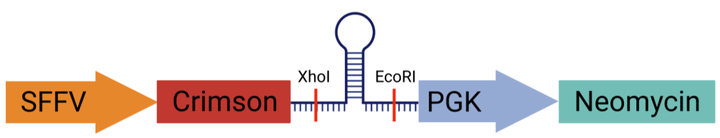

Fig 1: DNA Construct for Lentiviral Vector. SFFV and PGK are Pol-II promoters. Crimson is a reporter protein for miR-E-based sh*Med4,* which has XhoI and EcoRI restriction sites, and Neomycin is a marker gene for antibiotic resistance.

Methods

- Short hairpin RNA (shRNA) is an engineered RNA molecule that silences a specific gene via RNA interference.
- microRNA-adapted shRNA have a microRNA (miRNA) backbone incorporated into the shRNA molecule, allowing it to be expressed by Pol-II promoters and processed by the endogenous miRNA pathways. The processed shRNA then binds to the target mRNA and induces mRNA degradation or suppresses translation of that gene, silencing it.
- Utilizing this miRNA pathway leads to less toxicity, more precise processing of shRNA, and the ability to transcribe several shRNA's/reporter genes under one promoter, as compared to simple stem-loop shRNA.

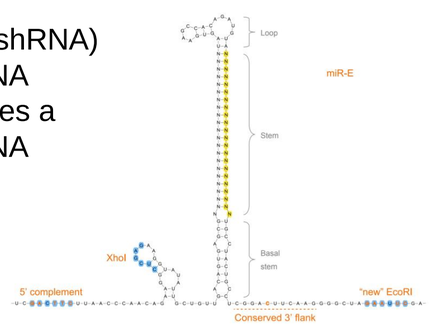

Fig 2: miR-E shRNA. The guide strand that binds to the target mRNA is highlighted in yellow. *Adapted from Fellmann et al.*

Methods

- Lentiviruses are useful carriers of vectors containing the shRNA sequence and allow for efficient knockdown of genes in mammalian cells. Lentiviruses can integrate their genome into the host cell genomes, allowing for stable, long-lasting expression of shRNA.

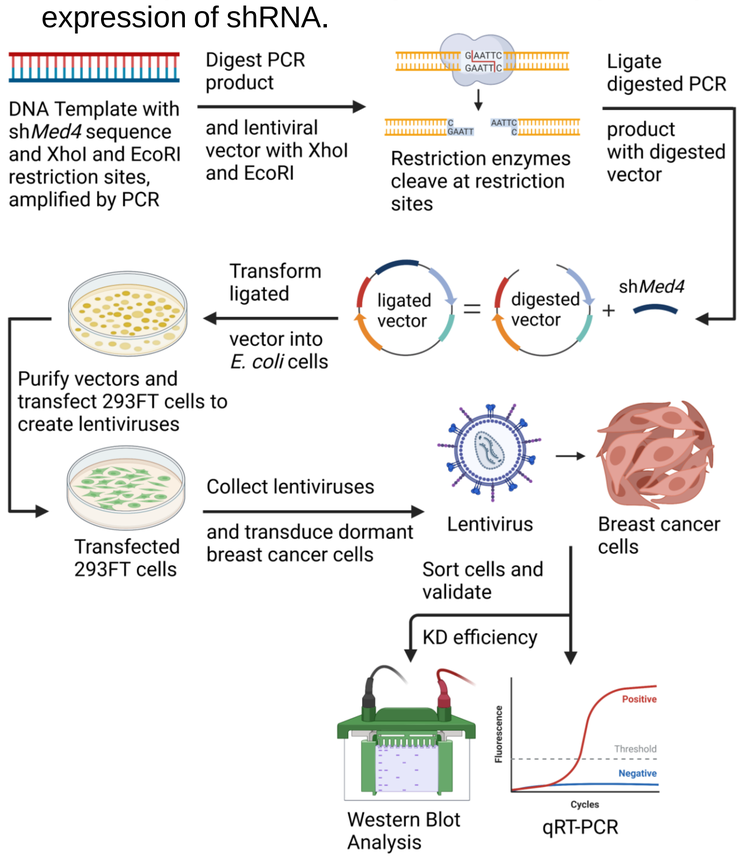

Fig 3: Overview of Methods. DNA template containing shMed4 sequence was cloned into the lentiviral vector, stable dormant breast cancer cell lines were generated with the lentiviral vector incorporated into the genome, and knockdown efficiency was validated.

Results

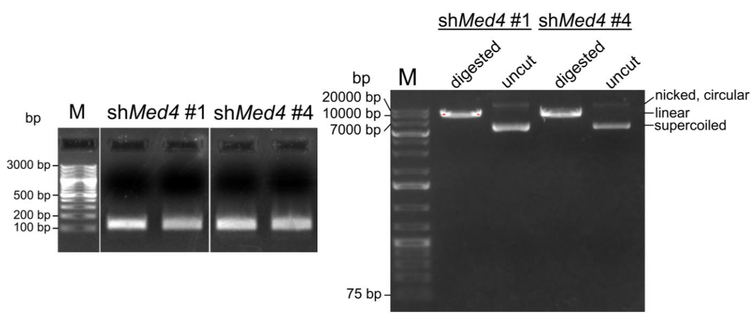

Fig 4: Agarose gels to confirm successful PCR of DNA template and vector purification. Left: Digested PCR product of DNA template on 2% agarose gel. The digested product is at the expected size for all samples, a little over 100 bp. Right: Digested and uncut vector containing DNA template on 1% agarose gel after maxiprep and subsequent isolation in preparation for transformation into *E. coli* cells. The digested vectors for both sh*Med4* #1 and sh*Med4* #4 are linear and around the expected size, between 10 kb and 20 kb. As expected, the uncut vectors for sh*Med4* #1 and sh*Med4* #4 contain a nicked, circular vector and supercoiled vector, without any single-stranded, circular vector present.

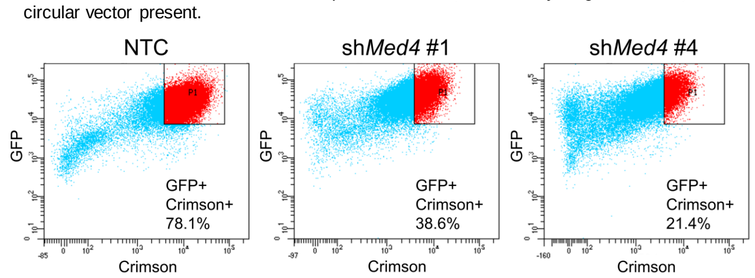

Fig 5: Fluorescence-activated cell sorting based on Crimson and Green Fluorescent Protein (GFP) expression. Crimson signals for sh*Med4* expression and GFP is linked to luciferase for in vivo imaging of metastasis. Cells with the highest Crimson and GFP signals were sorted and tested for validation of sh*Med4* knockdown efficiency.

Results

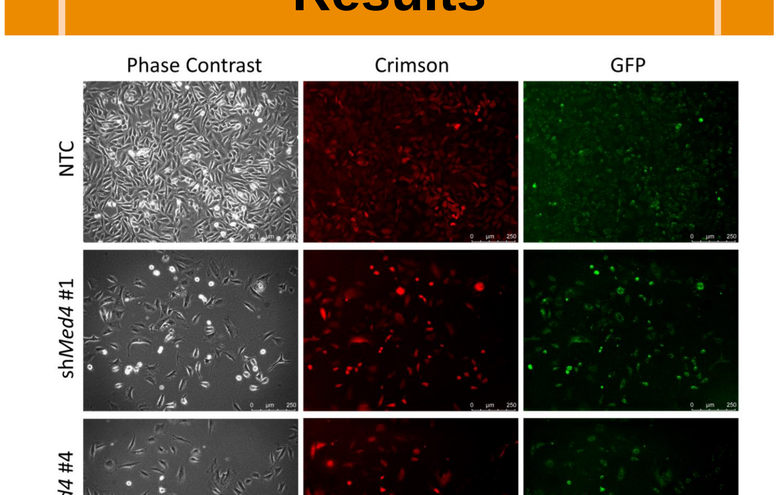

Fig 6: Fluorescence microscopy images. Crimson and GFP fluorescence was detected within the cells.

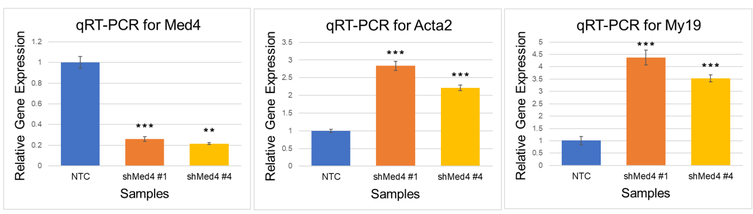

Fig 7: Validating knockdown efficiency with Real-Time Quantitative Reverse Transcription PCR (qRT-PCR). Decreased *Med4* mRNA levels were seen for sh*Med4*#1 and sh*Med4* #4 compared to NTC. sh*Med4* #1 and #4 had increased *Acta2* and *My19* (actin) mRNA levels. *Gapdh* mRNA levels were similar for all samples. * = p-value < 0.05, ** = p-value < 0.01, *** = p-value < 0.001.

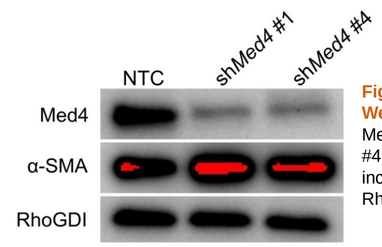

Fig 8: Validating knockdown efficiency with Western Blot Analysis. Decreased amounts of Med4 protein were detected for sh*Med4* #1 and #4 compared to NTC. sh*Med4* #1 and #4 had increased protein levels for α-SMA (actin). RhoGDI levels were similar for all samples.

Conclusion

- Silencing of Med4 using this DNA construct was successful based on the qRT-PCR and Western Blot results.
- This knockdown method can allow for additional studies that explore the interactions and signaling pathways related to Med4 and metastatic reactivation.
- Further investigation can include RNA-sequencing, immunofluorescence staining, drug treatment, and co-knockdown of genes.
- Understanding the mechanisms through which depletion of Med4 promotes metastatic relapse may lead to the identification of novel biomarkers and therapies that may benefit a substantial fraction of breast cancer patients.

References

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Figure 3 created with BioRender.com.

Acknowledgements

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