Techniques for validating the genomic drivers of nanoparticle interactions in cancer cells

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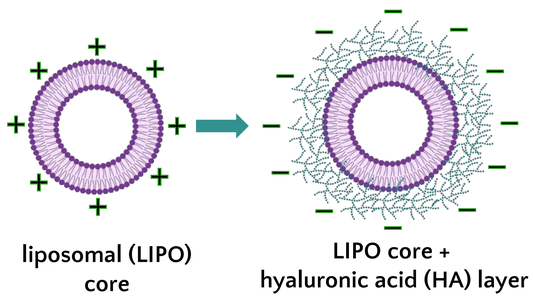
Background

Current challenges of cancer therapies
Off-target effects, lack of specificity, and difficulty penetrating cancerous tissue[1]

Nanomedicine is an exciting way address these issues.
Nanomedicine = using nanotechnology for medical and biological applications
- Reduce early degradation & clearance
- Improve tissue specificity
- Lower toxicity[2]

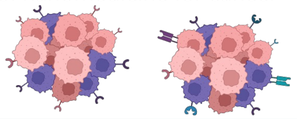
Layer-by-Layer nanoparticle (NP) assembly
- Tunable NP assembly platform allowing multiple therapeutic modalities and tissue targeting



liposomal (LIPO) core

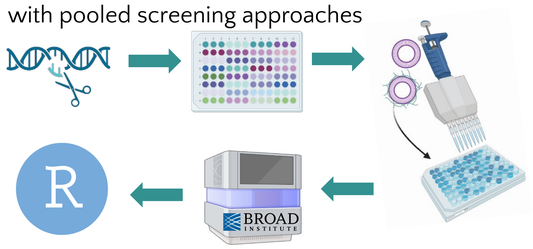
LIPO core + hyaluronic acid (HA) layer

Limitations of studying nano-bio interactions
- Tissue and cellular heterogeneity make it difficult to determine which chemical properties result in successful NP delivery in specific tissues[3]
- We hypothesize that an improved understanding of the biologic drivers of NP delivery will improve the utilization of nanomedicine for cancer

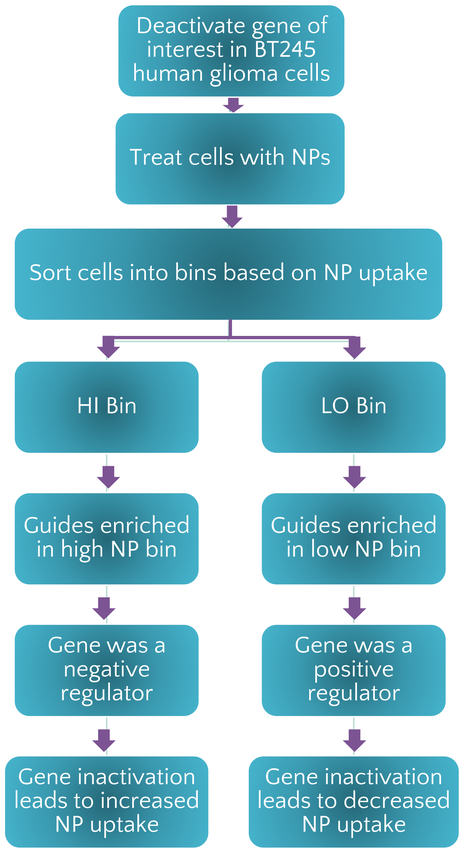


High-throughput techniques

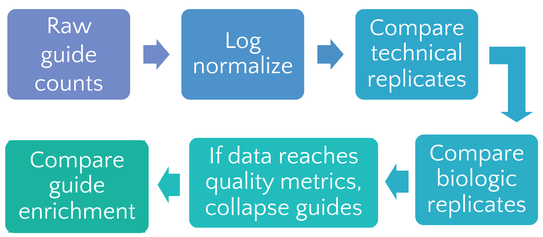
High throughput screening to validate the genomic drivers of NP-cell interactions
- We previously identified potential genes that regulate these interactions using previous methods[4]
- We seek to validate multiple genes simultaneously with pooled screening approaches



Experimental Overview



Data analysis pipeline

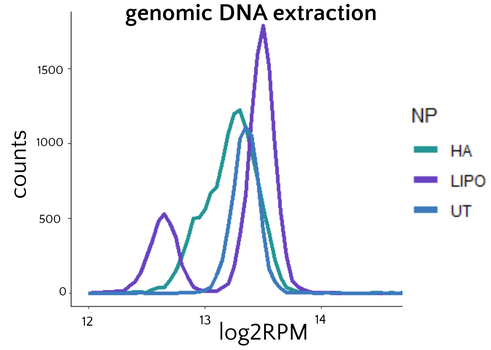


$$\text{Log}_2 \text{ normalized Reads (RPM)} = \text{Log}_2\left(\frac{\text{Reads for one guide in one sample}}{\text{Total reads for all guides in one sample}} \times 1e6 + 1\right)$$

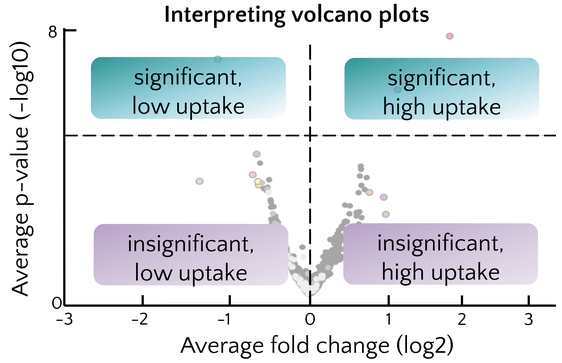
$$\text{Log}_2 \text{ fold change} = \text{Log}_2 \text{ normalized reads of HI} - \text{Log}_2 \text{ normalized reads of LO}$$

Results

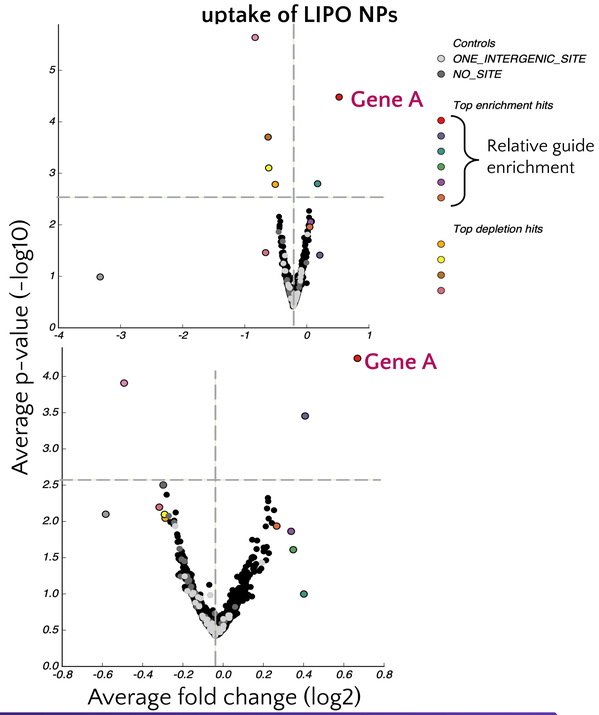
Log_2RPM > 12 indicates adequate sample input and genomic DNA extraction



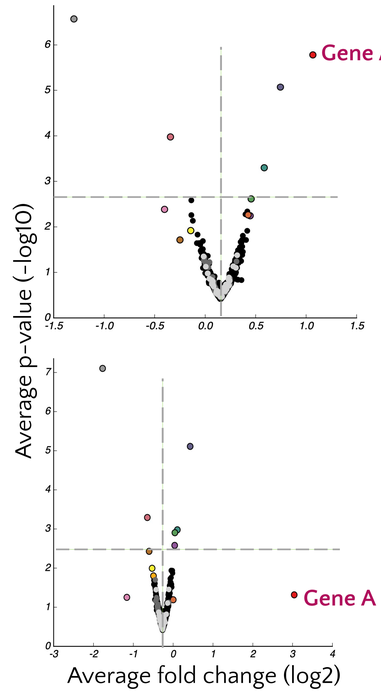
Interpreting volcano plots



Volcano plot shows genes significantly involved in uptake of LIPO NPs



Similar results for HA NPs



Conclusion

- Developed a strategy to analyze results of CRISPR pooled screening with two different NP formulations
- Validated genes identified using computational methods
- Identified a previously less-explored gene that contributed to decreased uptake of LIPO and HA NPs

Next Steps
- Use the techniques developed here to study a larger library of NPs
- Perform screening on other cancer cell lines

References

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[4] Boehnke N, Straehla JP, Safford HC, Kocak M, Rees MG, Ronan M, et al. Massively parallel pooled screening reveals genomic determinants of nanoparticle delivery. Science 2022;377:eabm5551. https://doi.org/10.1126/science.abm5551.
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