When genes collide: PAX3::FOXO1 in childhood cancer

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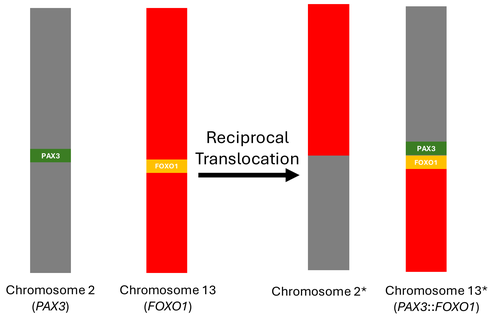
Overarching Goal – Can we develop non-invasive treatment for treating fusion-positive rhabdomyosarcoma?

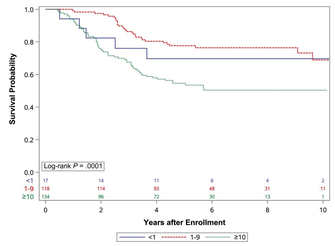
1. Why fusion proteins cause health problems



- Soft tissue sarcoma account for ~7% of cancers in children and about half of them with soft tissue sarcoma have rhabdomyosarcoma (RMS)[1]
- The PAX3::FOXO1 fusion variant (also known as fusion-positive RMS) of alveolar rhabdomyosarcoma (ARMS) is the most aggressive subtype and primarily occurs in the limbs and trunk of the body
- Surgery has been the preferred treatment, but non-invasive treatments would be easier for kids to adapt.

Nine-month-old with orbital rhabdomyosarcoma[2]

Fusion-positive Rhabdomyosarcoma Overall Survival (OS) by Age at Diagnosis



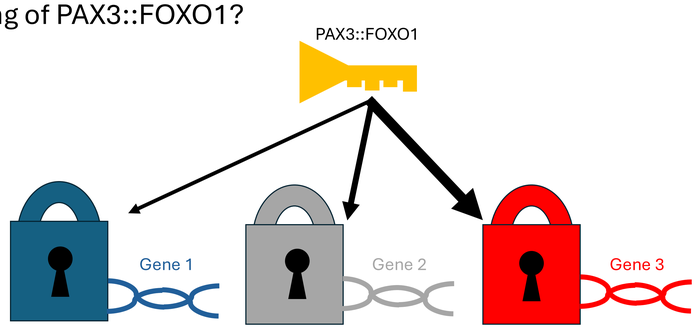
Some statistics from the study[3]:

- 71% of the 257 patients with newly diagnosed, fusion-positive ARMS, had PAX3::FOXO1
- Patients were all on a form of trial treatment (ARST0531, D9803, and D9602)
- Among children, biological males are 1.5x more likely to get the disease then biological females

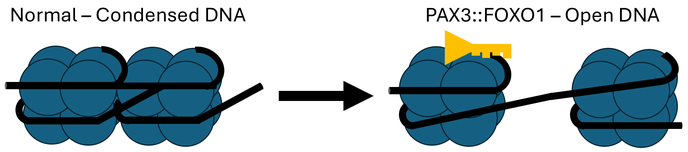
2. How fusion proteins activate genes

A. Is there preferential binding of PAX3::FOXO1?

- PAX3::FOXO1 acts as a transcription factor and as a pioneer factor
- It can bind at **thousands** of sites across the human genome to activate cancer promoting genes



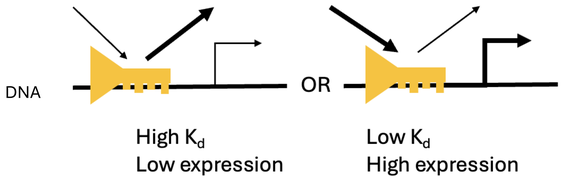
B. How does PAX3:FOXO1 alter the genome?



- Through its pioneering ability and activation of enhancers, PAX3::FOXO1 rearranges the essential architecture of the genome to drive cancer

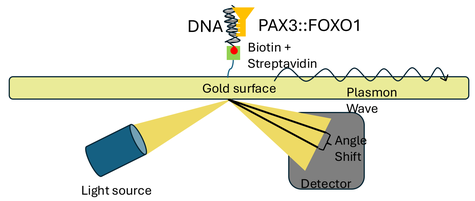
3a. Measuring preferred genes activated by PAX3::FOXO1

Does PAX3::FOXO1 bind at a specific DNA region better than other regions and what are those regions?



Surface Plasmon Resonance

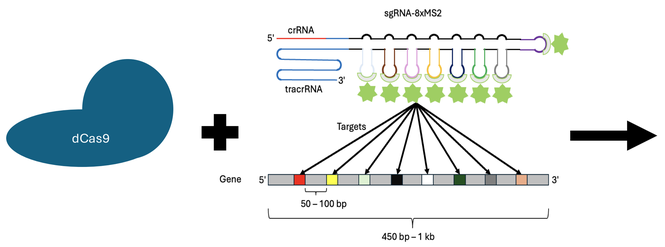
- Provides kinetic data (k_{on} and k_{off})

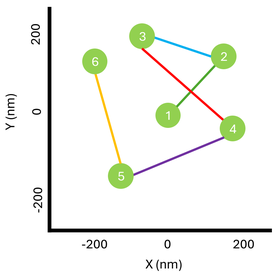


Low dissociation constants (K_D) suggest high binding affinities.

3b. Using CRISPR-Sirius to visualize genes affected by PAX3:FOXO1

How does PAX3::FOXO1 impact the dynamics of target genes?





In this variant of the CRISPR system, catalytically inactive Cas9 (dCas9) stabilizes single guide RNAs (sgRNAs) to gene of interest and can bind to fluorescent proteins[4]

Under a microscope, we can visualize and track target genes (*MYCN*, *FGFR4*, and *MYOD1*) in living cells!

Conclusion

These studies aim to further understand the role of PAX3::FOXO1 in FP-RMS to discover potential direct targets for therapeutics

- Addressing how PAX3::FOXO1 binds to specific regions of DNA will provide insights into the preferences of binding and if there are specific regions of DNA that can be targeted
- Monitoring PAX3::FOXO1 target genes in real-time will reveal changes in the genome architecture and transcriptome which are important for driving ARMS

References

[1]Skapek, S. X., Ferrari, A., Gupta, A. A., Lupo, P. J., Butler, E., Shipley, J., Barr, F. G., & Hawkins, D. S. (2019). Rhabdomyosarcoma. *Nature reviews. Disease primers*, *5*(1), 1. https://doi.org/10.1038/s41572-018-0051-2

[2]Heske, C. M., Chi, Y., Venkatramani, R., Li, M., Arnold, M. A., Dasgupta, R., Hiniker, S. M., Hawkins, D. S., & Mascarenhas, L. (2020). Survival outcomes of patients with localized *foxo1* fusion-positive rhabdomyosarcoma treated on recent clinical trials: A report from the soft tissue sarcoma committee of the children's Oncology Group. *Cancer*, *127*(6), 946–956. https://doi.org/10.1002/cncr.33334

[3]Friedmann, A. M., Tarbell, N. J., Schaefer, P. W., & Hoch, B. L. (2004). Case 4-2004. *New England Journal of Medicine*, *350*(5), 494–502. https://doi.org/10.1056/nejmcpc030036

[4]Ma, H., Tu, L. C., Naseri, A., Chung, Y. C., Grunwald, D., Zhang, S., & Pederson, T. (2018). CRISPR-Sirius: RNA scaffolds for signal amplification in genome imaging. *Nature methods*, *15*(11): 928–931. https://doi.org/10.1038/s41592-018-0174-0

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