Role of KIRREL in mammalian myogenesis and Rhabdomyosarcoma

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Introduction

Rhabdomyosarcoma (RMS) is the most common sarcoma, an aggressive tumor that grows on soft tissue and prefers childhood tissues. Despite notable advances, RMS models have suffered from various experimental limitations and treatments for high-risk RMS have not improved for three decades. New genetic tools are needed to promote RMS gene discovery and enable the development of new precision therapies.

Background

RMS is comprised of myoblasts that are blocked from terminally differentiating into muscle. Through previous studies in the Galindo Lab, it was discovered that TANC1, an adaptor molecule that regulates cell-cell fusion, is a key gene in RMS. Misexpression of TANC1 dysregulates signaling and promotes RMS, while correction the TANC1 misexpression alone can reprogram RMS myoblast to differentiate. While little is known about myoblast fusion genetics, data in flies and mammalian cells suggest that Immunoglobulin Superfamily Transmembrane Receptors (Ig-S-R) family members Kirrel and Nephrin are fusion regulators that partner with TANC1 to drive fusion and RMS.

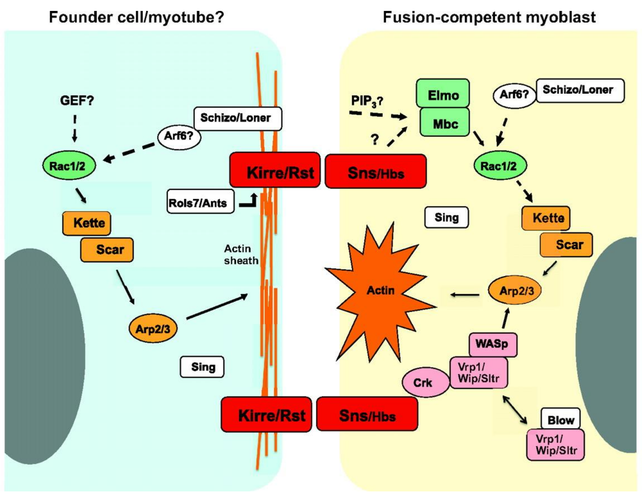
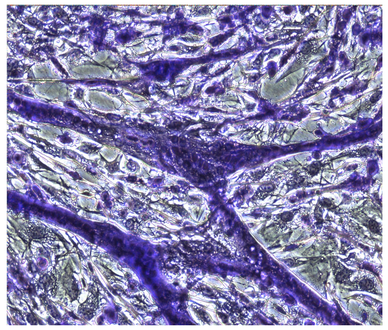


Figure 1: It's well established that *Drosophila* muscles arise from the fusion of one founder cell with several fusion competent myoblasts. The named genes and pathways have been shown experimentally to function in founder cells and fusion-competent myoblasts of *Drosophila* embryos. Ig-S-R family members Kirre and SNS appear to drive myoblast cell adhesion and fusion during drosophila myogenesis. The mammalian orthologs of these genes are KIRREL and NPHS, respectively.

Results

Knockdown of KIRREL3 in C2C12 Murine Myoblasts

Control



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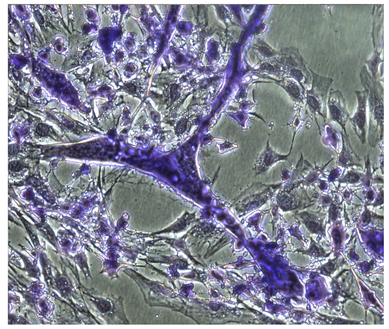

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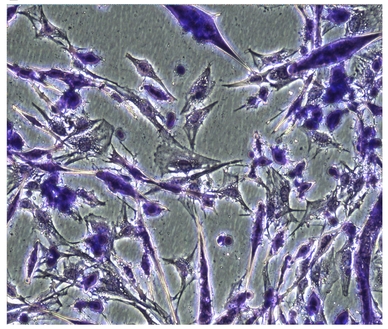

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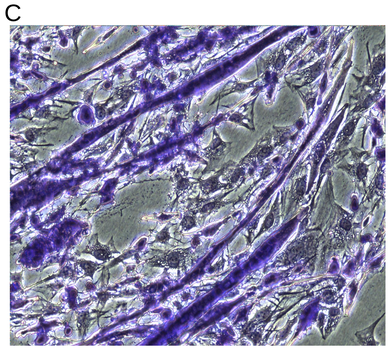

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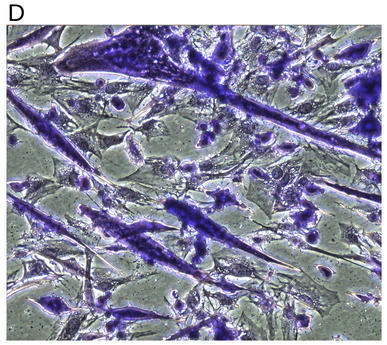

Figure 2: shRNA-mediated knockdown of KIRREL3 in C2C12 cells. Cells expressing shRNA against KIRREL3 (A-D) showed decreased and less organized myotube formation compared with the cells expressing shEGFP (control).

Methods

KIRREL1 Molecular Cloning

The Kirre1 insert first had to be cloned into the intermediate vector PSP73. The insert was then cloned into the desired vector, pEGFP-N3. At each step, sequencing was used to verify successful molecular cloning.

KIRREL3 Molecular Cloning

The KIRREL3 vector was cloned directly into PFLAG-CMV

shRNA-silencing

Silencing of KIRREL3 in C2C12 cells (a mouse myoblast cell line) was achieved using four different short hairpin (shRNA) sequences that target murine KIRREL3. The relative mRNA of Kirre-3 was therefore reduced compared with the control cells. After knocking down KIRREL3, the cells differentiated for 6 days. Crystal Violet staining allowed visualization of the differentiated myotubes in the C2C12 cells expressing shRNA against KIRREL and the cells expressing control shRNA

Conclusion and Future Work

Sequencing showed that the KIRREL1 insert was correctly cloned into the PSP73 intermediate vector. However, multiple attempts at then inserting KIRREL1 into pEGFP-N3 were not successful. It appeared as though one of the enzymes used did not cleave at the anticipated sites. KIRREL3 molecular cloning was also difficult, as one of the enzymes used had a cut site in the middle of the vector. I attempted to do a double digestion, but this yielded too little DNA product. Both KIRREL-1 &-3 molecular cloning techniques must be troubleshooted so that overexpression studies may be conducted in the future.

Since we believe KIRREL3 is essential for myotube formation, we hypothesized that when KIRREL3 is silenced, the C2C12 cells would be slower to form myotubes, and the tubes would be smaller. Using crystal violet to visualize the differentiated myotubes, it did appear that the KIRREL3 silenced cells formed fewer and smaller myotubes when they differentiated. However, the experiment was only conducted once and needs to be repeated a few more times to validate the results.

References

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2. Abmayr, S. M., and Pavlath, G. K. (2012) Myoblast fusion: lessons from flies and mice. *Development* 139,641-656
3. Galindo, R. L. R01 Research Plan.