Role of NPHS Gene in Mammalian Myogenesis and Rhabdomyosarcoma

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Introduction

Rhabdomyosarcoma (RMS) is an aggressive childhood cancer composed of undifferentiated myoblasts. Although it is the most common pediatric soft tissue malignancy, much remains to be unveiled about the exact mechanisms of the disease. Incomplete understanding of pathological mechanisms precludes novel therapies such as molecularly targeted treatment and differentiation therapies. We are exploring the underlying mechanisms of muscle development and RMS and the prospective therapies that understanding these mechanisms would make possible (1).

Background

Through use of drosophila, the lab has previously revealed that dysfunction in myoblast cell-cell fusion signaling acts as a driver of RMS. Furthermore, the lab obtained preliminary data that pointed towards Immunoglobulin Superfamily transmembrane Receptors (Ig-S-R) family members NPHS and KIRREL as regulators of mammalian myoblast fusion. It has recently been shown that these cells can be reprogrammed to differentiate and fuse, thus, redirecting them out of a proliferative state (2, 3).

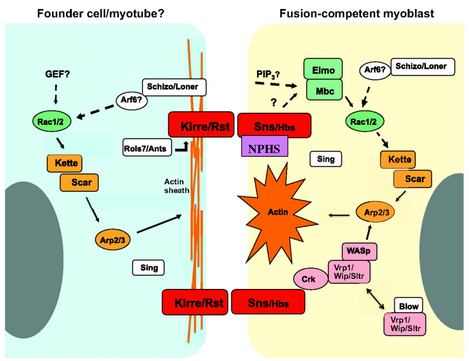

Figure 1: Ig-S-R family members Kirre and SNS interact to regulate myoblast cell adhesion and fusion during drosophila myogenesis. The mammalian orthologs of these genes are KIRREL and NPHS, respectively. It has been established that drosophila myoblast fusion involves founder cells that determine muscle pattern and fusion-competent cells that fuse with founder cells to form the muscle. Evidence shows that the Kirre receptor on the founder cell attracts fusion-competent cells through SNS (4). We are studying whether the mammalian orthologs function in a similar manner.

Purpose

The purpose of this research is to elucidate the function of NPHS in the mammalian myogenesis process and the pathogenesis of RMS, and to translate the results into newer and safer RMS treatments.

Results and Methods:

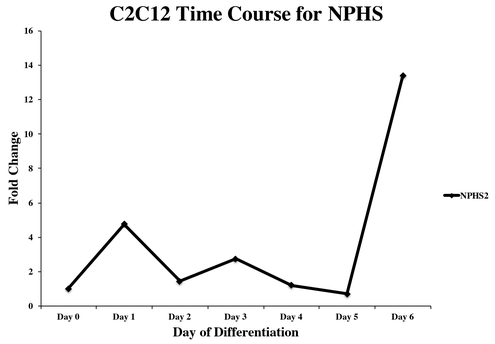

Figure 2: Regulated expression of NPHS during the seven day myogenesis process in C2C12 murine myoblasts. Upregulation is noted during key differentiation and fusion points.

The time course was done using a C2C12 murine myoblast cell line. RNA was extracted daily from the myoblasts during the seven day differentiation and fusion process. Subsequently, cDNA was made from this RNA. Finally, a qPCR was run to look at the expression levels of NPHS for days 0-6 of muscle development. We used 18S primers as our control.

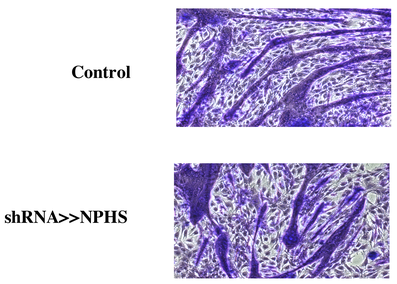

Figure 3: Knockdown of NPHS using shRNA in C2C12 murine myoblasts. Decreased fusion is seen with knockdown. The control is shRNA>>GFP.

Knockdown of NPHS was accomplished by transfection of a vector containing sequences necessary to express shRNA that is directed against NPHS. The control was a transfection with a vector containing an off target shRNA (shRNA>>GFP).

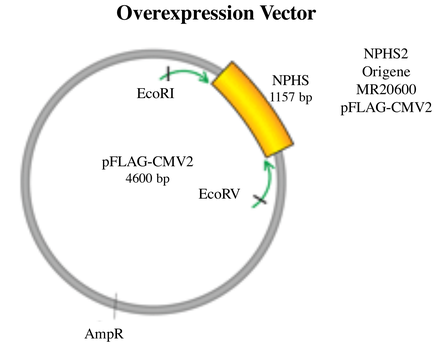

Figure 4: pFLAG-CMV2 vector with NPHS insert. This vector was used for overexpression studies.

A vector was designed with the NPHS gene incorporated. The vector used was pFLAG-CMV2, and restriction enzymes HF-EcoRI and HF-EcoRV were used to cut the vector and NPHS cDNA. The vector and gene of interest were then ligated together.

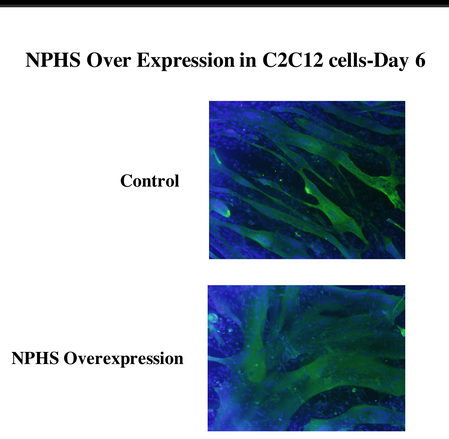

Figure 5: Overexpression of NPHS in C2C12 murine myoblasts. A dramatic increase in fusion is seen with overexpression. The control is pFLAG-CMV2.

In order to achieve overexpression, the vector described above was transfected into C2C12 murine myoblasts, and fusion was observed over the seven day myogenesis process. A pFLAG-CMV2 vector without the NPHS gene was used as a control.

Summary

The transmembrane receptor NPHS appears to be a crucial regulator of differentiation and fusion during the mammalian myogenesis process. Preliminary results show that the expression of NPHS throughout the myogenesis process is tightly regulated, with clear upregulation of the gene during important differentiation and fusion points. Knocking down NPHS leads to a decrease in fusion, while overexpression of NPHS leads to a clear increase in fusion, exemplifying the fact that NPHS is playing a vital role in myogenesis, and hence, is a factor in the pathogenesis of RMS.

Future Research

We will continue to look at the function of NPHS both in mammalian myogenesis and in pathogenesis of RMS. Next steps include repeating and expanding studies in expression of NPHS in myogenesis, effects of knockdown of NPHS, and effects of overexpression of NPHS. Furthermore, we will also look at what proteins interact with NPHS and what expression of NPHS looks like in RMS cells. Similarly, studies will be done to illuminate the function of KIRREL in the myogenesis process. These will include studies equivalent to those done for NPHS, looking at expression, effects of knockdown, effects of overexpression, interactions of KIRREL, and expression of KIRREL in RMS cells. To further understand the similarities between drosophila and mammalian myogenesis, it will be important to specifically study the interaction of NPHS and KIRREL with each other. Ultimately, the results of this work will help reveal the pathogenesis of RMS and will lead to much needed newer and safer treatments for patients.

References

1. Kashi, V. P., Hatley, M. E., and Galindo, R. L. (2015) Probing for a deeper understanding of rhabdomyosarcoma: insights from complementary model systems. *Nat Rev Cancer* 15, 426-439
2. Galindo RL, Allport JA, and Olson EN. A Drosophila model of the rhabdomyosarcoma initiator PAX7-FKHR. Proc Natl Acad Sci USA. 2006; 103: 13439-13444.
3. Avirneni-Vadlamudi, U., Galindo, K. A., Endicott, T. R., Paulson, V., Cameron, S., and Galindo, R. L. (2012) Drosophila and mammalian models uncover a role for the myoblast fusion gene TANC1 in rhabdomyosarcoma. *J Clin Invest* 122, 403-407
4. Abmayr, S. M., and Pavlath, G. K. (2012) Myoblast fusion: lessons from flies and mice. *Development* 139, 641-656

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