Understanding the Role of Wnt Signaling in Schwann Cell Tumors

Amanda L. Halfond, Adrienne L. Watson, Dr. Eric P. Rahrmann, Leah K. Anderson, Andrew D. Greeley, and Dr. David A. Largaespada

Biology, Society and the Environment, Health and Natural Sciences Department, College of Liberal Arts, University of Minnesota

Background

Malignant Peripheral Nerve Sheath Tumors (MPNSTs) originate in the Schwann cell and often occur in patients with Neurofibromatosis Type 1 (NF1), but can also form spontaneously (3). NF1 is a genetic disease that occurs in 1 in 3,000 live births, and predisposes patients to benign neurofibromas. 10% of NF1 patients will have one of their benign neurofibromas undergo malignant transformation into an MPNST, the leading cause of death in NF1 patients (3). The average survival rate with patients with MPNSTs is 21 months and due to the lack of complete understanding of the genetic basis for MPNST development, the common treatments for these MPNSTs are surgery and non-specific chemotherapy (3). A current goal in the field is to understand which signaling pathways drive MPNST development and progression, with the hopes of discovering novel targeted therapies to improve the treatment these patients.

There are many known pathways that drive cellular growth and proliferation in cancer, and we are interested in identifying which of these pathways play a role in MPNSTs. Specifically, we are focusing on the potential role of Wnt signaling in the development and progression of Schwann cell tumors, as this pathway has been implicated in a mouse *Sleeping Beauty* forward genetic screen that has been successfully completed in the lab. The genes identified in our *Sleeping Beauty* screen include *Gsk3β, Axin1, Tnks,* and *β –catenin,* which all have well characterized roles in the Wnt signaling pathway (1). In addition, data from human gene expression microarrays and tissue

microarrays have also implicated this pathway in human Schwann cell tumors (data unpublished). The Wnt pathway controls cellular proliferation, and increased Wnt signaling has been shown to cause tumorigenesis in many cell types (1). My hypothesis is that Wnt signaling also plays a role in MPNST development.

Gsk3β and *Axin* are part of the β -catenin destruction complex, and when mutated or downregulated, result in increased signaling through the Wnt pathway (1). *β –catenin* and *Tnks* are both positive regulators of Wnt signaling and function by increasing the amount of signaling through the Wnt pathway leading to cellular proliferation (1). The goal of this project is modulate Wnt signaling in both normal and malignant Schwann cells and monitor the effects on cell proliferation and colony formation.

Methods

Aim1: Validation of Gene Knockdowns

Gene knockdowns will be done using lentiviral transduction of short hairpin RNAs (shRNAs). To activate Wnt signaling in normal human Schwann cells, *Axin1* and *Gsk3B* will be knocked down. To decrease Wnt signaling in MPNST cell lines, *β –catenin* and *Tnks* will be knocked down. The normal human Schwann cell lines that will be used, HSC 2λ and HSC 1λ, are both derived from normal patient sciatic nerve and immortalized to allow *in vitro* studies. The MPNST cell lines that will be used, S462-TY and STS-26T, are both well characterized and derived from MPNST patients, one developed in the context of NF1 and one who exhibited a spontaneous MPNST (4). To validate gene expression changes, we will use quantitative PCR to look at changes at the mRNA level, and western blotting techniques to look at changes that occur at the protein

level. We will assess changes in the specific gene we have knocked, as well as changes in the overall output of Wnt signaling by looking at gene that are transcriptionally activated by Wnt signaling such as *C-myc* and *CyclinD1*. Our controls will be parental cell lines and non-silencing shRNA transduced cell lines.

Aim2: Functional Assays to Assess Changes in Proliferation and Colony Formation

In vitro functional assays will be conducted to assess changes in proliferation and soft agar colony formation that these cells undergo when we modulate Wnt signaling. The proliferation will be assessed through the use of the MTS assay, in which a tetrazolium reagent is introduced into the cells and is reduced as cells proliferate to give a formazine by-product which absorbs light at 490nm. The cells will be plated in a 96 well plate format, and the absorbance at 490nm will be measured over 6 days using an automated plate reader. My hypothesis is when we increase Wnt signaling in normal Schwann cells there will be an increase in proliferation and when we decrease Wnt signaling in MPNST cell lines there will be a decrease in proliferation. Soft agar assays will be utilized to determine changes in colony forming ability of our cells when we modulate Wnt signaling. Normal cells do not form soft agar colonies, while cancer cell lines are capable of forming colonies. If Wnt signaling does play a role in MPNST development, then I would predict that colony formation will decrease if Wnt signaling is down-regulated in MPNST cell lines. If Wnt signaling is increased in normal human Schwann cells, then they may gain the ability to form soft agar colonies.

Impacts

With the results of these experiments, we hope to identify whether activation of Wnt signaling is a mechanism that normal Schwann cells or benign neurofibromas use to

undergo malignant transformation. This may eventually lead to new targeted therapies to control tumor growth and prolong the life span of patients with MPNSTs.

References:

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