A Potential Novel Treatment for Neurofibromatosis Type 1 via RAS Inhibition

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Methods

Introduction

Neurofibromatosis type 1 is a genetic disease that results from either heritable or spontaneous autosomal dominant mutations in the NF1 gene. Neurofibromatosis type 1 individuals frequently suffer benign tumors known as Plexiform Neurofibromas which develop from cranial and peripheral nerve sheaths. Plexiform Neurofibromas have the potential to develop into a highly deadly malignant peripheral nerve sheath tumor (MPNST). As low as thirty-four percent of individuals survive MPNST for more than 5 years and there is no effective treatment or cure.

The NF1 gene encodes the protein Neurofibromin. Neurofibromin is a negative regulator of the transform protein Ras. Thus, inactivation of NF1 leads to a constitutive increase in the active form of RAS. This is a transforming event that drives the disease.

In an attempt to combat the problem of a lack of a therapeutic treatment for Neurofibromatosis Type 1, and indeed, RAS driven cancer in general, the Clark and Trent laboratories have performed in silico screening of two million compounds followed by bioassay to identify a small molecule, referred to as F3, that binds and inhibits active RAS. The compound is effective against models of mutant RAS driven tumor formation in vitro and in vivo.

Here, for the first time, we test the compound against a disease driven by hyper-activation of the wild type RAS protein, rather than the mutant form. Therefore, in this project we hypothesize that the use of F3 will limit RAS activated signaling of its mitogenic pathways in Neurofibromatosis Type 1 causing a suppression of cell growth and tumorigenicity. We aim to determine a mechanism for which this occurs as well as evaluate the functionality of F3 and its derivative in both two-dimensional and more physiologically relevant three-dimensional tissue culture assays of Neurofibromatosis Type 1 cell systems.

Cell Lines: IPN and S462.TY cells were cultured in DMEM with 10% fetal bovine serum (FBS) and 1% penicillin streptomycin in an incubator at 37°C in 5% CO2.

Results

Neurofibromatosis is caused by mutations in NF1 leading to aberrant RAS activation

Identification of a novel small molecule designated F3 that binds RAS

F3 slightly decreases growth of malignant peripheral nerve sheath tumor cells in 2-D

F3 suppresses downstream RAS activated Signaling pathways

F3 inhibits tumorigenicity in 3-D growth

F3 has little effect on the growth of immortalized normal Schwann cells

F3 exhibits more potency at increased concentrations

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