Fruit Fly Models of Noonan Syndrome to Screen for Therapeutic Drugs

Caitlin Brown

***Abstract* — Noonan Syndrome (NS), the common RASopathy, can be modeled in *Drosophila melanogaster*, the fruit fly. This genetic disease causes hematological abnormalities, developmental delays, and cardiac problems. Most patients die before reaching adulthood due to heart defects. There is no cure for this disease, but fruit fly genomes can be manipulated to model NS and help find therapeutic drugs that will cure NS in humans. Six NS mutations were inserted into fly genomes, which caused them to develop defective wings with ectopic veins. Four drugs were given to the flies to see which was most successful in rescuing the ectopic vein phenotype.**

I. INTRODUCTION

RASopathies are a group of genetic disorders caused by hyper-activating mutations within the RAS pathway, a pathway that cells use to communicate to each other for cell proliferation and death. Noonan Syndrome (NS) occurs in approximately one in 1,000 children. Most NS patients die before reaching adulthood due to hypertrophic cardiomyopathy (HCM) or pulmonary valve stenosis. Although there is currently no cure for the disease, several treatment regimens are available, shown to partly reduce side effects of NS.

The research goal was to use transgenic fruit fly models of NS to identify therapeutic drugs which would work best in reducing lethality and organ malformation associated with the disease. Transgenic fly models were made by adding NS mutations to the fly genome, which caused them to develop defective wings with ectopic veins. NS mutations cause over-activation of the RAS pathway; thus, the ideal NS drug would be one that decreases such activity [1]. The objective was to test potential drugs on the flies and look for wing rescue, which would indicate that the drug was successful. It was hypothesized that the 7447 drug at 1 μ M would be the most effective therapeutic compound in rescuing the ectopic vein phenotype due to its success in past experiments.

II. METHODS

To create a fruit fly model, NS mutations were inserted in the fly genome using a GAL4/UAS system, a tool used to create offspring with desired mutations. In this mating cross, female flies had the GAL4 driver, while the male flies had the UAS gene of interest. When the flies mated, the GAL4 protein activated transcription of the target UAS gene, and the offspring had a specific NS mutation [2]. Female flies were crossed with six male fly lines: UAS RasV12, phl, UAS hRAF S259A, UAS mek E203K, UAS mek Y130C, and UAS mek F53S. Each male fly line was crossed with three sets of female flies to allow for three trials per fly line. Once the offspring of the crosses developed into adults, their wings were dissected and observed under a microscope. When it was confirmed that all offspring developed ectopic wings, the next phase of drug testing began. Four drugs (331, 535, 3717, 7447) at optimal concentrations (1 μ M, 10 μ M, or 100 μ M) were added to fly food and administered to offspring flies that had ectopic wings. Varying concentrations were used to determine if 100 μ M would be too powerful and kill the fly, or if 1 μ M would be too weak and have no effect on the fly. Once the offspring developed into adults, their wings were observed to see if the drugs rescued the ectopic wings.

III. RESULTS AND DISCUSSION

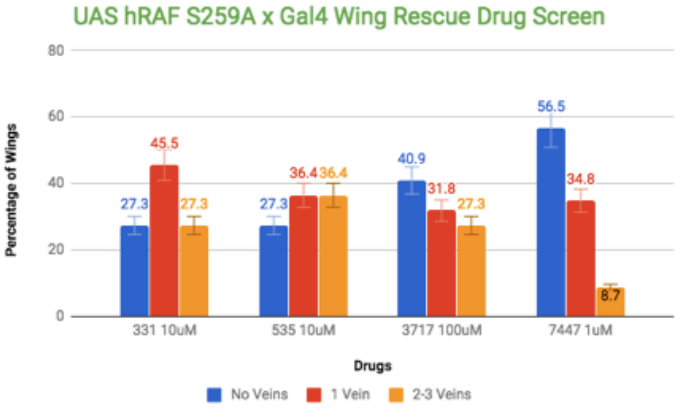

Figure 1: *Percentage of wings rescued by the top four drugs at optimal concentrations on UAS hRAF S259A fly line. 7447 at 1 μM was the most successful drug, rescuing 56.5% of wings.*

The drug screen experiment indicated that the 7447 drug at 1 μ M was the most successful drug in rescuing the ectopic wing phenotype caused by NS mutations, particularly in the UAS hRAF S259A fly line. As shown in Fig.1, 56.5% of fly wings were fully rescued by the 7447 drug, and they had no ectopic veins present. Flies with no veins indicated that the drug was able to regulate RAS pathway activity, while flies with two to three veins were the most severe, meaning the drug had little to no effect on the pathway. This data shows that the 7447 drug is likely to regulate RAS pathway activity and would be the best drug for humans with NS.

This research will guide chemists in developing improved versions of NS drugs for fly models, and eventually humans, that will have the same chemical structure as the 7447 drug and are aimed at preventing fatal side effects of NS, such as HCM. All four drugs will be tested on induced pluripotent stem cells and mammalian models of NS to see if the results correspond with this experiment. The ultimate goal is to find a cure for NS in humans.

IV. ACKNOWLEDGMENTS

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V. REFERENCES

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