Generating a Bcl2L12 Melanoma Zebrafish Model

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Pingry Research

Introduction

Melanoma is the most lethal form of skin cancer, originating in melanocytes, skin pigment cells. In 2016, ~75,000 people in the US have melanoma and ~10,000 died from the disease [(1)]. Melanoma accounts for less than 1% of skin cancer cases, but accounts for the vast majority of skin cancer deaths.

In previous genomic studies of melanoma tumors, Bcl2L12 of the Bcl2 apoptotic protein family was found to be upregulated, implying its role as an anti-apoptotic oncogenic protein [(2)]. The goal of this project is to determine Bcl2L12's ability to accelerate melanoma.

To test this, we are expressing Bcl2L12 using a miniCoopR system and an existing zebrafish model from Dr. Leonard Zon's Lab at Harvard University that contains all the components for melanoma genesis. We are engineering a Tol2 transposon-based "miniCoopR" vector that will express Bcl2L12 in the zebrafish model.

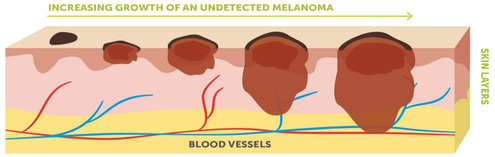

Figure 1 | Diagram of Melanoma growth over time [(3)]

Melanoma Genetics

The model zebrafish we are using as discussed in the next section is based upon two major genes: BRAF and MITFA, genes that control cell growth and transcription of proteins in melanocytes respectively.

BRAF^{V600E}

BRAF is an oncogenic protein that is involved growth signaling. BRAF is the most commonly mutated gene in melanoma. A specific mutation, V600E, where the 600th amino acid, Valine (V), is switched out for Glutamic Acid (E), was the most common mutation. This puts the protein in a state where it consistently promotes cell growth.

MITFA

MITFA is the "master" melanocyte transcription factor. It is involved in the specification and development of melanocytes. Without MITFA, melanocytes will not develop.

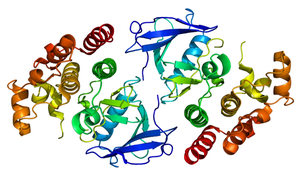

Figure 2 | BRAF Protein Model [(4)]

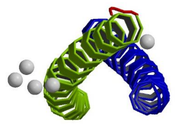

Figure 3 | Coiled Region of MITFA [(5)]

Zebrafish Model

Previous research by the Zon Lab found that transgenic BRAF^{V600E} in zebrafish caused nevi to develop [(6)] (**Fig. 5**). However, when tp53, a tumor suppressor gene, was knocked out, those nevi could develop into malignant melanoma [(6)] **(Fig. 6)**.

The model zebrafish strain we are using has all of the necessary components for melanoma genesis, except for MITFA: *Tg(mitfa:BRAFV600E);p53(-/-);mitfa(-/-)*

Figure 4 | Wild Type Zebrafish
There are no nevi developing on the zebrafish's skin [(6)]

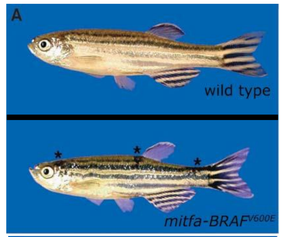

Figure 5 | BRAF^{V600E} Zebrafish
*Indicates nevi development, but not invasive [(6)]

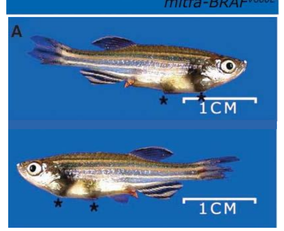

Figure 6 | BRAF^{V600E};p53$^{-/-}$ Zebrafish
*Indicates malignant nevi development, with tissue invasion [(6)]

MiniCoopR System

For this project, we are using the miniCoopR system. The power behind this system is the control we have over melanoma genesis. The model zebrafish we are using has all the necessary ingredients to develop melanoma, but since MTIFA is knocked out, melanocytes cannot develop. However, a MITFA minigene will be added through the miniCoopR vector that will allow for melanocytes to develop again, making melanoma development possible. In the process, a candidate oncogene can be added to the miniCoopR vector, which will be expressed in melanocytes if driven by a MITFA promoter.

We will express Bcl2L12 in the zebrafish using a "miniCoopR" vector plasmid. This plasmid, which we are currently making, will contain 3 major components: Tol2 miniCoopR Backbone, MITFA minigene, and target oncogene, which will be Bcl2L12 (**Fig. 7**).

We will then microinject the vector into *Tg(mitfa:BRAFV600E);p53(-/-);mitfa(-/-)* embryos. The candidate oncogene and MITFA minigene will then be incorporated into the zebrafish DNA through the Tol2 transposon system.

Figure 7 | miniCoopR Vector Structure [(6)]

Tol2 miniCoopR Backbone (MiniCoopR Backbone Vector)
This is the backbone of the miniCoopR plasmid vector. It contains the Tol2 transposons that allows for the vector to be cut, with the half containing the candidate oncogene and MITFA minigene to be incorporated into the zebrafish DNA.

MITFA Minigene (5' Entry Vector w/ MITFA Promoter)
This is the critical component that allows for melanoma to develop in the zebrafish melanocytes. The addition of a MITFA minigene, allows melanocytes to grow once again, and the transgenic model will thus have the potential to develop melanoma.

Candidate Oncogene (3' Entry Vector w/ PolyA Sequence)
For the candidate oncogene we selected the apoptotic protein Bcl2L12. It will be expressed by a MITFA promoter, which means it will only be expressed in melanocytes.

Vector Construction

We received the plasmid components listed above from Dr. Julien Ablain in the Zon Lab, each delivered as its own plasmid.

We are first transforming these plasmid components into bacterial cells for production and long term storage. We used NEB5α Competent *E. coli* cells to transform the 5' Entry Vector w/ MITFA Promoter and 3' Entry Vector w/ PolyA Sequence **(Fig. 8)**. The miniCoopR Backbone Vector requires a different type of cell: ccdB Survival Bacteria. From there, we can simply miniprep bacterial colonies to isolate the plasmid components for future use.

We also received two other plasmids: pENTR223 plasmid w/ Bcl2L12 ORF from the Harvard Plasmid Repository and a pCS2 Tol2 plasmid also from Dr. Ablain. The pCS2 Tol2 plasmid is used for the microinjection process of integrating the miniCoopR vector into the zebrafish DNA. However, the pENTR223 plasmid w/ Bcl2L12 ORF is used in a process called Gateway Cloning to construct the final miniCoopR vector.

Gateway Cloning

To construct the plasmid, we are using Gateway Cloning, which allows the transfer of components between different cloning vectors. The system makes use of recombination sequences named "att L 1" and "att L 2".

We have three entry vector components as listed previously. The Tol2 miniCoopR Backbone Vector serves as the destination vector. To insert the vector components into the destination vector, we will perform a LR Recombination reaction **(Fig. 8)**.

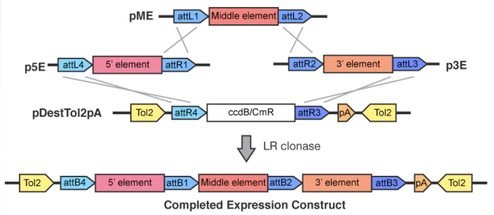

Figure 8 | LR Recombination Reaction [(7)]

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