Investigating the role of SOCS3 as a tumor suppressor in Zebrafish

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Abstract

JAK/STAT is a fundamental signaling cascade that when inappropriately activated, can lead to the development of tumors. This is due to JAK/STAT involvement in cell proliferation, differentiation, cell migration, and apoptosis. The SOCS3 gene is a cytokine suppressor in the pathway. Zebrafish have two copies of SOCS3: SOCS3a and SOCS3b. To determine the role of SOCS3 in the development of cancers and tumors, we used the CRISPR/Cas9 system to target these genes. After mutagenesis, some zebrafish showed mutation. We will further use the CRISPR/Cas9 method to make stably mutant lines for SOCS3a and SOCS3b.

Hypothesis

When SOCS3a and SOCS3b genes are mutagenized in the fish, their function as a tumor suppressor in the JAK/STAT pathway will be interrupted.

JAK/STAT Pathway

The JAK/STAT pathway is a key signaling cascade for many cytokines and growth factors. When activated, JAK (Janus Kinase) stimulates cell proliferation, differentiation, cell migration, and apoptosis.

Methods

1) CRISPR/Cas9 System

This system, based on a bacterial CRISPR (Clustered Regularly Interspaced Palindromic Repeats)-associated nuclease (Cas9) from Streptococcus pyogenes, is used to target genes in organisms and make double stranded cuts in DNA, ultimately resulting in mutagenesis. Guide RNAs target Cas9 to a specific site in the genome.

Figure 3. DNA cleaved by Cas9 can be repaired by non-homologous end joining (NHEJ) or homology directed repair (HDR). NHEJ introduces insertions or deletions leading to mutations at the cleavage site.

Gene Targeting of Zebrafish SOCS3

Figure 4. Targeting SOCS3a and b on the genomic level.

2) Injection to produce mutagenesis

Figure 5. To cause mutagenesis, the Cas9 mRNA and the SOCS3 gRNA are injected into the zebrafish embryos in the one-cell stage. Embryos are raised to adulthood and the adult fish are crossed to wild type. F1 generation fish are screened for inheritance of SOCS3 mutant alleles.

Results

Table 1. Phenotypes of injected and uninjected zebrafish embryos.

<table>
<thead>
<tr>
<th>Plate</th>
<th>Injected</th>
<th>Not Injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 severe/monster</td>
<td>1 mild</td>
</tr>
<tr>
<td>2</td>
<td>0 severe/monster</td>
<td>3 normal</td>
</tr>
<tr>
<td>3</td>
<td>0 mild</td>
<td>5 normal</td>
</tr>
</tbody>
</table>

Future Plans

- Fix the SOCS3a digest by changing the experimental conditions
- Establish stable lines with mutant alleles of SOCS3a and b
- Inject into Sox2-2a-GFP transgenic to test whether disruption of SOCS3 signaling alters progenitor cell proliferation in the nervous system

Sources


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