Analysis of the Promoter of Treacher Collins syndrome

Cecilia Blair
VCU Advisors: Jim Lister & Rita Shang

Introduction

Treacher Collins is an autosomal dominant disorder that affects one out of 50,000 live births every year. Treacher Collins has a very specific phenotype that affects craniofacial development. The essential features of Treacher Collins are hypoplasia of the cheek and mandibular bones, cleft palate, drooping of the lower eyelid, down slanting palpebral fissures, and middle ear ossicle malformation sometimes resulting in deafness. All these features cause the affected person to have a "fishlike" facial appearance.[1] Treacher Collins has variable expressivity, as seen in figure 1, and for this reason the syndrome is often passed unknowingly to affected offspring. Although inheritance is an obvious method of passing on the syndrome, 60% of all affected persons have Treacher Collins from de novo mutations.

Figure 1: All one family, and all positive for Treacher Collins syndrome.

Treacher Collins syndrome is due to mutations in the Tcof1 gene, which has been found to have linkage to chromosome 5q33.1. The Tcof1 gene is encoded by 28 exons ranging in size from 49 to 561 bp in length. The complete coding region has been identified using positional cloning. Tcof1 was found to encode the nucleolar phosphoprotien treacle, whose specific function still remains largely unknown.[2]

Mutations in the coding region of Tcof1 have been identified, but these do not account for all cases of Treacher Collins. There are reported accounts of Treacher Collin's syndrome that do not show any mutations within the coding region of Tcof1. This suggests that there might be mutations in the non-coding regions that affect expression such as the promoter region. Kathryn Shows, in Rita Shiang's lab, has been working with the promoter region of Tcof1 in a mouse germ line. She has found that by mutating specific binding sites upstream of the coding region

[1] Marszalek, 224.
[2] Dixon, 1473.

expressivity is effected. This indicates that mutations in the promoter region do cause changes in the expression of Tcof1 and possibly lead to the mutations indicative of Treacher Collins syndrome.

If we could observe the mutations that Shows found in the promoter sequence in a developing organism we could be able to see the effects of each specific mutation. Shows used mouse germ line cells to identify mutations within the promoter. The mouse is a close homolog to the human, but it has certain restrictions that make it expressive, lengthy, and difficult to use when trying to observe the effects of a mutation on development. The zebrafish on the other hand is a more efficient model for a number of reasons. During development zebrafish are transparent making it easy to observe development. One zebrafish can lay hundreds of embryos at a time making them easy to harvest and manipulate during the one-cell stage. Also because fish lay eggs there is no need to us dissection to observe fetus development as in mouse models.[3]

The question we ask ourselves now is if the work that we have done in the mouse promoter can be duplicated in the zebrafish. T. Michelle Holser in collaborative work with both Dr. Jim Lister and Dr. Rita Shiang has isolated the orthologue of the Tcof1 gene in zebrafish by using BLAST and searching for *Xenopus tcof1*. After tagging the Tcof1 gene in both the zebrafish and mouse models it was found that the two genes are expressed in homologous tissues suggesting that the two genes are also homologous. This suggests that we should be able to successfully use the mouse promoter region identified by Shows in the zebrafish. Fisher *et al* has been successful in showing that the RET gene is conserved in zebrafish as well has humans, indicating that it is possible to use the promoter region of a mammal on a fish.

Methods

We will use standard cloning procedures using the mouse promoter sequence, coupled with GFP, to observe the distribution of the Tcof1 promoter activity throughout the development of the zebrafish. This will be facilitated using a florescent microscope. If we are successful in

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[3] Lieschke.

using the mouse promoter region in the zebrafish we will then be able to systematically mutate segments of the promoter region and observe the effects on expression and development.

If we are unsuccessful in using the mouse promoter region in the zebrafish we have two other alternate strategies. **1)** We could go back to the mouse promoter and collect a bigger region. This would increase our chances of getting all the regions that are necessary for expression, such as enhancers that can be as far as 2k bp from the promoter it interacts with. **2)** Another approach we will probably take is using the fugu genome. The fugu is a puffer fish with a highly condensed genome.[4] The idea is that because the genome is condensed we would have a greater chance of getting all the segments that control gene regulation.

Implications

If we are successful in using the mouse promoter region on the zebrafish we will be able to test the effects of mutations of candidate regulatory elements. This would give us a better understanding of how mutations in the promoter region affect the development of an organism and the development of Treacher Collins syndrome in humans.

References

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[4] Rothenberg, 6540.