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**The development of the zebrafish as a model for Treacher Collins syndrome.**

Treacher Collins syndrome (TCS) or mandibulofacial dysostosis (MFD) is an autosomal dominant disorder that occurs at a frequency of 1 in every 50,000 live births. The prevailing phenotype of TCS is down slanting palpebral fissures, coloboma of the lower eyelid, midfacial hypoplasia, mandibular hypoplasia, cleft palate, external ear malformations, and middle ear ossicle malformation sometimes resulting in deafness (Marszalek *et al.*, 2002). In 1996 the gene that underlies TCS was mapped to human chromosome 5q31.3-32 and named Treacher Collins-Franceschetti Syndrome 1 (*TCOF1*). *TCOF1* is a low complexity protein encoded by 26 exons. In 1997 the protein product of *TCOF1* was uncovered and named treacle (Dixon *et al.*). Within the coding region of *TCOF1* 51 mutations have been found that result in a truncated treacle protein (Splendore *et al.*, 2000). Forty percent of the cases of TCS are due to clear inheritance of a mutation in the coding region, but the remaining 60% arise from *de novo* mutations (Marszalek *et al.*, 2002). Currently the only known treatment, depending on the severity of the phenotype, is reconstructive surgeries starting at a very young age.

Treacle is most highly expressed in neural folds, which are comprised of neural crest cells (Dixon *et al.*, 2000). Neural crest cells generate a diverse number of cell types including bones, tendons, neurons, glia, and melanocytes. The phenotype of TCS is the result of a mutation in *TCOF1,* which could lead to the formation of a truncated protein (Edwards *et al.*, 1997; Gladwin *et al.*, 1996) though the major mechanism that underlies TCS is the haploinsufficiency of the treacle protein (Shows *et al*., 2005). The lack of a sufficient amount of functioning treacle, causes a subset of neural crest cells to enter into the apoptotic pathway. The cells enter into the apoptotic pathway during embryogenesis when the branchial arches are forming leading to the craniofacial abnormalities of TCS (Dixon *et al*., 2000). This suggests that treacle is required for proper neural crest cell formation and proliferation (Dixon *et al.*, 2006). It has been proposed that based on the

subcellular location and similarity of treacle to rat nucleolar phosphoprotien 140, that treacle is involved in ribosome biogenesis (Dixon *et al*., 1997; Isaac *et al.*, 2000). Ribosome biogenesis is needed for protein synthesis. Therefore, when there is a non-functional treacle protein it is hypothesized that there is not enough ribosomal assembled to keep up with the high rate of protein synthesis in the rapidly proliferating neural crest cells (Dixon *et al.*, 2000).

This summer my project is focusing on establishing the zebrafish as a model organism for TCS. The zebrafish is an excellent model because it can quickly reproduce, produces hundreds of embryos in one spawning, is easily manipulated, and has a transparent embryo. At the one cell stage of the embryo's development it is easy to perform microinjection of a construct to be expressed or oligonucleotides to knockdown expression of any gene (Lieschke and Currie, 2007).

Through BLAST analysis a gene in the zebrafish genome was found to have a protein product that had 20.8% homology to human treacle, and named *zTcof1*. Although this may not seem like a high correlation between humans and zebrafish we have found that expression patterns are similar, as seen in figure 1. To further support the use of *zTcof1* for studies into TCS we are testing *zTcof1* to make sure that it has a similar phenotype to that of TCS.

One way that we are exploring *zTcof1* is by using a fish line that is heterozygous for an insertion mutation in *zTcof1*. We are using this model to establish similarities in the TCS phenotype and zebrafish that are homozygous for the *zTcof1* mutation. Although TCS is a dominant disorder we hypothesis that in the zebrafish there is a maternal transcript that overrides the mutant *zTcof1* transcript in heterozygous fish, causing the phenotype to only show up in fish that are homozygous for the trait. In order to correlate the similarities between TCS and a mutation in *zTcof1* we are examining apoptosis, proliferation, and cartilage development in the branchial arches. Thus far we have found similarities in phenotype in both the formation of the branchial arches and an increase in apoptosis of the neural crest cells.

Another approach that we are taking to examine the *ztcof1* gene is using morpholino oligonucleotide (MO). MOs are chemically modified oligonucleotides that

have similar base stacking capabilities as natural genetic material, but they have a morpholino moiety instead of a ribose (Ekker, 2000). Phosphorodiamidate linkage is used which causes the backbone of the structure to be neutral. With the two aforementioned changes to the natural genetic material a highly soluble polymer that is able to hybridize to a single-stranded nucleic acid sequence with high specificity is created (Ekker, 2000). The MO binds to its target mRNA sequence and blocks either translation or proper splicing depending on which part of the gene it is designed for.

We are injecting *zTcof1* MOs into the zebrafish embryos at the one cell stage. In addition to the *zTcof1* MOs we are also co-injecting *p53* MOs to determine if knockdown of p53 can rescue the *zTcof1* MO phenotype. *p53* is a gene that is known to initiate apoptosis. In addition, Jones *et al*. (2008) showed that with the inhibition of *p53* function, in a TCS mouse model, there was rescue of neural crest cells from apoptosis. In the absence of sufficient amounts of treacle, *p53* initiates apoptosis in the neural plate (Jones *et al.*, 2008). We also saw rescue of the TCS phenotype with the co-injection of *zTcof1* and p53 MOs.

In addition to looking for a rescue of the phenotype we are also looking at the expression of other genes that are know to be regulated with *Tcof1*. To explore this we look at both *zTcof1* and *p53* MO co-injected embryos and *zTcof1* MO injected embryos. On these embryos we will perform *in situ* hybridizations to look for the expression patterns of *CNBP*, *Tbx1*, and *Col2a1*. The reason for looking at *CNBP* and *Tbx2* is based on a microarray analysis that was performed. The microarray showed that the expression of both genes increases when *tcof1* is knocked down (Mogass et al., 2004). We therefore want to see if this happens in a developmental model, and also if the expression is rescued by the *p53* MO. *Col2a1* is a collagen gene that is involved in chondrogenesis. Previous studies have shown that this collagen gene's expression decreases when z*Tcof1* is mutated. This suggests that z*Tcof1* is involved in chondrogenesis. We want to confirm this information, and see how the expression of *Col2a1* is effected by the co-injections of the *zTcof1* and *p53* MO.

Through these experiments described above we hope to establish zebrafish and *zTcof1* as a model organism for TCS. This will also enable scientists to more rapidly

explore the function of treacle.

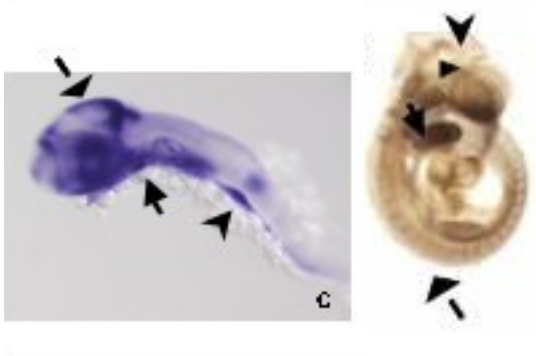

**Figure 1**: Shows the similarity in *tcof1* expression in a zebrafish (Left) and mouse (Right). Arrows indicate where *tcof1* mRNA is being expressed in homologous tissues.

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