

The Adenoid Cystic Carcinoma Research Foundation Fellowship
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Research Summary

Our previous work on adenoid cystic carcinoma (ACC) suggested that the Sox4 protein may play an important role in how normal cells in secretory glands turn in malignant cancer cells. The Sox4 gene encodes for a protein that regulates the activity of genes, and it is likely that it turns on and off other genes in a specific way to cause this effect. The overall goal of this project is to create a mouse model in which the Sox4 protein is over-expressed in salivary gland epithelium, the predominate tissue affected by ACC. The mouse model will allow us to study the effects of Sox4 in greater detail, and may allow us to partially recreate the ACC disease state in a system that can be used to test new therapies.

Our progress so far is that we have obtained the mouse-version of the Sox4 gene and have used recombinant DNA technology to clone it into a DNA plasmid vector that we can use to introduce it into the mouse genome. To confirm the increased levels of Sox4 in the mice, we need an antibody that recognizes the mouse Sox4 protein. We tested several existing antibodies to see if the mouse protein can be detected in mouse tissue culture cells. None of the antibodies worked, so we first decided to try a strategy in which we add some extra material, called a "FLAG tag" to the Sox4 protein to which good antibody reagents are available. Since we added additional sequences to the Sox4 protein, we needed to confirm that the FLAG tag would not affect Sox4 function. We devised a system in which we could introduce the standard (untagged) Sox4 gene and the FLAG-Sox4 gene into mouse tissue culture cells and test the ability of the Sox4 proteins to regulate a reporter gene. Our results suggested that the FLAG-Sox4 protein did not function the same as the untagged Sox protein, so we won't be able to use this strategy either. We have decided to make our own antibodies in rabbits against mouse Sox4. We made recombinant DNA of the mouse Sox4 gene that will let us make large amounts of protein in bacteria. We are purifying this protein and are preparing to inject it into rabbits for production of the antibody.

Since we have decided to use the untagged Sox4 gene in the recombinant mouse, the next step is to test that the system that we devised to get the protein to be expressed in salivary gland epithelium works. The trick that we will use is to insert DNA sequences in front of the Sox4 gene so that it normally can't be made into protein. This interfering DNA is flanked by two special small DNA sequences called Lox-P sites. When another protein called Cre is present, the DNA in between lox sites is cut out. The plan is to make our recombinant mice with the Lox-P Sox4 gene and mate them with mice that express Cre in salivary gland epithelium (these transgenic mice are currently available). Before we go on to do these complicated animal experiments, we are currently testing our Lox-P Sox4 gene to make sure that our system works first in a test tube, and then in cells in culture (plastic dishes).

Immediate next steps

Before we go on to create the transgenic animals and perform the subsequent time consuming breeding experiments, we are currently testing our Lox-P Sox4 gene to make sure that our system works. We are currently setting up a system to test the recombination in an in vitro assay using purified recombinant cre. Additionally, we plan to test this system in our tissue culture cells, co-transfecting our Lox-P Sox4 construct with a cre-expressing plasmid and using the luciferase assay described above to assay for functional Sox4 protein. We would anticipate no Sox4 activity with transfection of the Lox-P Sox4 construct alone, and to only see Sox4 activity with the cre co-transfection.

Upon successful testing of the system, we will then commence with creation of the transgenic animals.