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14. ABSTRACT
We continue to make progress on the proposed work. For the first aim, we have rederived and crossed a floxed p63 mouse line with our Scgb1a1-CreER; lsl Sox2-IRES GFP mouse line. The resulting mouse line has no decrement in carcinogenesis. We are currently deleting p63 in human lung cancer cell lines to further validate the finding that p63 is dispensible for Sox2-induced tumorigenesis. In aim 2, we have attempted further transplants of proximal Sox2-expressing bronchial epithelial cells into the alveoli. However, we have not seen tumor formation even in the presence of supporting fibroblast cells. For aim 3, speed congenics continues to create pure strains of Scgb1a1-CreER; lsl Sox2-IRES GFP mice. Comparison of the mice will allow identification of modifier genes contributing to Sox2-induced lung cancer.

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Progress Report: The Mechanism of Sox2-Induced Lung Cancer

Introduction

The purpose of the proposed research is to clarify the mechanism of Sox2-induced non-small cell lung cancer. The first aim involves investigating p63 as a direct target of Sox2 in tumor cells. The second aim investigates the tumor-initiating ability of proximal and distal respiratory epithelial cells when Sox2 is overexpressed. The third aim tests the hypothesis that strain-specific modifier genes affect the phenotype of mice inducibly overexpressing Sox2 in Scgb1a1-expressing cells. We have continued work on all three aims.

Aim 1

We have made significant progress on this aim over the past year. The main purpose of this aim is to identify the importance of the p63 gene in Sox2-induced oncogenesis. Because p63 has been demonstrated to be important in the maintenance of squamous epithelia, because it leads to squamous metaplasia when overexpressed in the mouse, and because p63 is expressed in SOX2+ human squamous lung cancers and in SOX2+ basal cells, we hypothesized that p63 is a direct target of Sox2 and that it is essential in Sox2Õs ability to intiaite lung cancer. Because p63 is expressed in 2 different isoforms, we first developed primers for each isoform. Reverse transcription-polymerase chain reaction (RT-PCR) was carried out using these primers, and the results are depicted in Figure 1. As the figure demonstrates, the TA-p63 isoform is upregulated by Sox2 in our Scgb1a1-CreER-induced mouse model. The delta-N isoform is not detected.

The next goal was to perform chromatin immunoprecipitation (ChIP) using an antibody for Sox2. However, the small number of cells recovered per mouse (using digestion of the intraparenchymal lung epithelium followed by trypsinization and sorting for GFP) made performance of ChIP from these cells impractical. We thus changed course to use the SOX2-overexpressing human squamous cell lung carcinoma cell line H520. Expansion of these cells gave us enough material for efficient ChIP. After the cells were fixed and sonicated to shear the DNA, immunoprecipitation was performed using the Seven Hills Biochemical Sox2 antibody. Crosslinking was then reversed, and massively parallel sequencing was carried out using the Duke Sequencing core. Analysis of these sequence reads surprisingly demonstrated that p63 is not a direct target of SOX2. Thus, it may be that p63 is being upregulated by another direct target of SOX2.

The next item in the Statement of Work involves breeding an inducible p63-knockout allele into the Scgb1a1-CreER; Rosa26-Sox2-IRES GFP mouse line to assess the necessity of p63 for Sox2 induced tumorigenesis. Our close collaborator Barry Stripp provided re-derived inducible p63-knockout (floxed p63) mouse line from frozen embryos. We set up breeding cages to produce Scgb1a1-CreER; Rosa26-Sox2-IRES GFP; floxed p63 mice. Again surprisingly, deletion of p63 has no discernible effect on Sox2-induced oncogenesis in this model (Figure). We will continue to examine larger numbers of these mice as well as to test the effect of lentiviral knockdown of p63 in human SOX2-expressing lung cancer cell lines.

Aim 2

The main goal of Aim 2 is to explore whether there is a microenvironmental contribution to Sox2-induced oncogenesis in the mouse. We planned to isolate proximal Scgb1a1-CreER; lsl Sox2 IRES GFP cells via flow sorting for transplantation into the retroorbital veins of immunodeficient Rag1-/- mice. While our preliminary data demonstrated that this transplantation led to tumors when the transplant was performed using K-RasG12D-expressing cells, we have not obtained tumors using the Sox2-overexpressing proximal cells. Possible explanations for this lack of tumor formation include lack of ability of the proximal cells to form tumors no matter the environment as well as a possible need for cotransplantation with stromal cells. However, transplants of distal epithelial cells have also not grown robustly. We have reasoned that the transplanted cells may require fibroblasts for support. We thus initiated transplants using mouse fibroblasts 1:1 with Sox2-overexpressing proximal and distal Clara cells. Again, no robust tumor formation was identified in the recipient mouse lungs. We are repeating these experiments using direct injection of

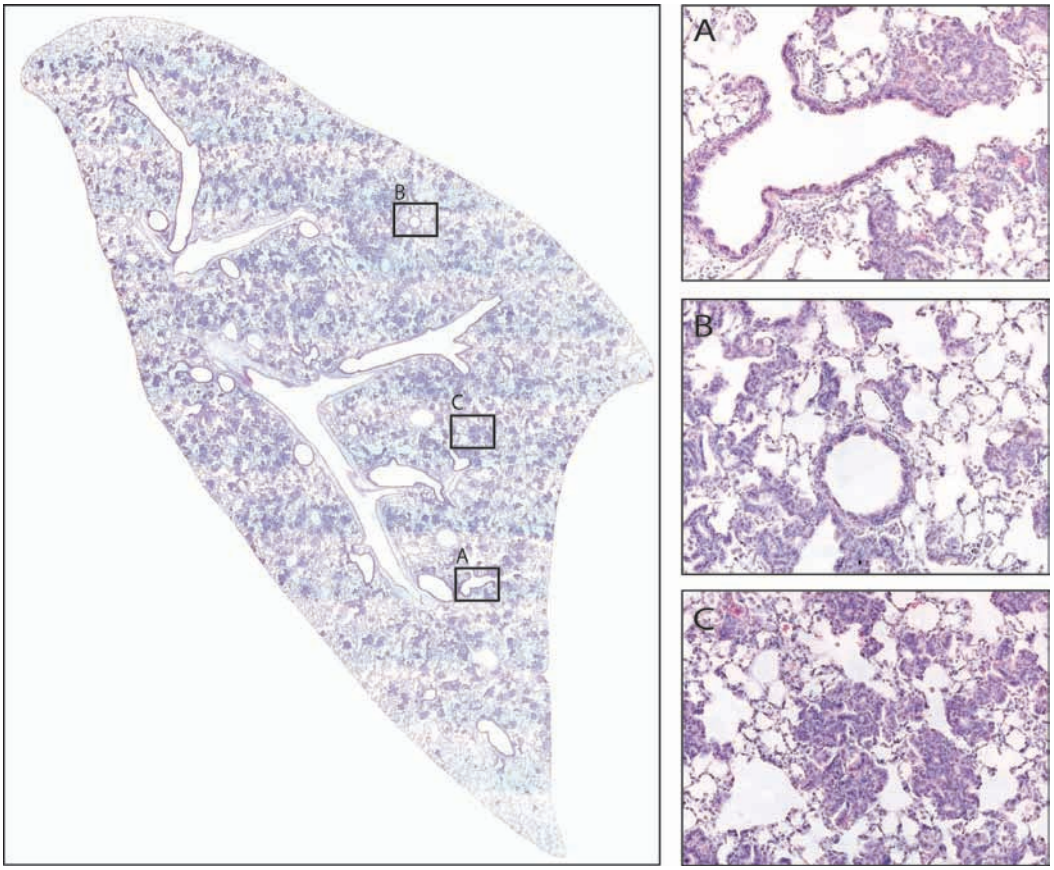

Figure 1: Representative image of a lung from Scgb1a1-CreER; lsl Sox2 IRES GFP; floxed p63 mouse. H&E stain.

the cells into the mouse lungs with the reasoning that tumor cells are being cleared in the bloodstream. Once the transplants are successful, we will analyze tumors that form as Statement of Work 2b and 2c state.

Aim 3

The final aim of the grant is to explore the role of modifier genes in Sox2-induced neoplasia. Approximately half of the Scgb1a1-CreER; Rosa26-Sox2-IRES GFP mice develop cancer on a mixed 129/C57/Bl6 background. We propose to breed our mixed mouse line to pure C57/Bl6, 129, and AJ backgrounds. The crosses have begun in our animal facility. Duke has initiated an in-house speed congenics program. The first progeny mice have been submitted for massively-parallel sequencing. This will direct further breeding. Once we have pure lines, the genomic analyses stated in SOW task 3 will be carried out.

KEY RESEARCH ACCOMPLISHMENTS

- Determination of the isoform of p63 induced by Sox2 over expression
- Identification of direct SOX2 transcriptional targets by ChIP-sequencing
- Elucidation that p63 is dispensable for SOX2-induced tumorigenesis in the mouse alveoli

REPORTABLE OUTCOMES

As yet, we have no reportable outcomes. We have begun to put together a manuscript regarding the dispensability of p63 in Sox2-induced lung cancer. We envision at least 2 other manuscripts arising from the ChiP-sequencing.

CONCLUSION

We believe that we are making steady progress toward understanding the mechanism of SOX2-induced tumoirgenesis. We need to examine more floxed p63 mice to definitively confirm our findings.