AMERICAN ACADEMY OF OTOLARYNGOLOGY-HEAD AND NECK SURGERY FOUNDATION

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<u>COVER SHEET FOR FINAL PROGRESS REPORT</u>

Type of Grant:
_____AAFPRS Leslie Bernstein Grant
_____AAFPRS Leslie Bernstein Resident Research Grant
_____AAFPRS Leslie Bernstein Investigator Development Grant
_____AAOA Foundation/AAO-HNSF Combined Research Grant
_____AAO-HNSF Resident Research Award
_____AAO-HNSF Maureen Hannley Research Training Award
_____AAO-HNSF Percy Memorial Research Award
_____AAO-HNSF Health Services Research Grant
_____AAO-HNSF Rande H. Lazar Health Services Research Grant
_____AHNS Pilot Grant
_____AHNS Alando J. Ballantyne Resident Research Pilot Grant
__X___AHNS/AAO-HNSF Young Investigator Combined Award
_____AHNS/AAO-HNSF Surgeon Scientist Combined Award
_____AHRF Wiley H. Harrison Memorial Research Award
_____ALA/ALVRE Award
_____ANS/AAO-HNSF Herbert Silverstein Otology and Neurotology Research Award
_____ARS New Investigator Award
_____ARS Resident Research Grants
_____ASPO Research Grant
_____ASPO Daiichi Innovative Technology Grant
_____PSEF/AAO-HNSF Combined Grant
_____The Triological Career Development Award
_____XORAN Resident Research Grant

Start date: 7/1/2010   **Stop date:** 6/30/2012

Principal Investigator: Thomas J. Ow, MD

Institution: University of Texas MD Anderson Cancer Center

Title of Project: TP53 gain of function mutation in head and neck squamous cell carcinoma

Abstract:
Rationale: P53 mutation is very common in head and neck squamous cell carcinoma (HNSCC), and has been correlated with decreased survival. Loss of tumor supressor function in p53 has been shown to be important in the multi-step process of tumor progression. However, specific mutations in p53 can also cause it to gain oncogenic properties. These mutations have been termed p53 'gain of function' (GOF) mutations, and many of these have been shown to promote malignant and metastatic behavior in models of human cancer.

Hypothesis:
We hypothesize that p53 'gain of function' mutations can promote progression and metastasis of HNSCC.

Specific Aims: To test this hypothesis: 1) We will demonstrate that p53 GOF mutations increase the metastatic potential of HNSCC in an orthotopic nude mouse model of oral tongue cancer. 2) We will evaluate the effects of the most common p53 mutations on the malignant and metastatic behavior of HNSCC and evaluate them for GOF activity. 3) We will explore key gene targets and signaling pathways of mutant p53 that affect malignant and metastatic potential.

Study Design: The HN30 and HN31 cell lines are an isogenic, tumor/lymph node pair. HN31 carries two mutations in p53 and is very aggressive in the nude mouse oral cancer model. HN30 expresses wild-type p53 and forms very indolent tumors. We will reduce p53 with shRNA in both of these cell lines, as well as introduce the HN31 p53 mutations into HN30. We will examine malignant and metastatic behavior of each of these stable cell lines in the nude mouse model. We will be able to determine p53 gain of function, loss of function, and dominant negative effects of the HN31 p53 mutations after studying these cell lines. Common p53 mutations will then be examined in a similar fashion using this system. In addition to *in vivo* studies, *in vitro* experiments, including growth in soft agar and cell migration assays will be correlated with *in vivo* metastatic behavior. Gene expression arrays, western blot, and coimmunoprecipitation studies will be used to explore gene targets and cell signaling molecules that interact with mutant p53 to promote the metastatic phenotype.

Conclusions: The HN30/HN31 cell lines present a unique opportunity to examine the role of p53 in metastasis. Identification and characterization of specific p53 mutations and associated pathways that increase metastatic potential will be important in the prognosis and treatment of HNSCC

Briefly describe progress in completing the project:
We have shown that stable knockdown of p53 with shRNA in HN30 leads to aggressive tumor growth and invasion the orthotopic mouse model of oral tongue cancer, with little effect on metastatic potential. Knockdown of p53 in HN31 reduced aggressive behavior slightly, but HN31 remains highly metastatic to cervical lymph nodes. Expression arrays yielded genes associated with p53 knockdown, and additionally a separate subset that are associated with metastatic potential. Exploration of TP53 mutation in patient tumor and lymph node samples suggest that TP53 gene status can indeed differ between tumor and lymph node specimens.

What work was completed?
Stable TP53 knockdown cell lines and controls were created for HN30 and HN31. The behavior in an orthotopic mouse xenograft model was demonstrated among these lines in both preliminary and repeat experiments, as described above.   *In vitro* characteristics, including cell migration, invasion, and proliferation were explored in these cell lines, with good correlation with *in vivo* tumor behavior. Gene expression was examined from RNA

extracted from harvested tumor samples to explore genes that were linked to loss of p53 and genes that were linked to lymph node metastasis. As a translational offshoot from this grant, primary and lymph node tumor samples are being examined to determine the frequency with which TP53 status differs between primary site and metastatic disease.

What work was not completed?
HN30 TP53 3"-UTR-knockdown cell lines transfected with the HN31 mutations were not created because the gain-of-function activity of the HN31 mutations did not appear to strongly effect the aggressive phenotype. Instead, we are focusing on genes in the expression data that are most effected in this cell line that are associated with such a profound change in behavior after p53 loss.   We are also exploring the p53-unrelated genes that are associated with lymph node metastasis. We are continuing to mine the expression data to identify and validate these key genes.

Were all of the funds spent?   YES

Have the results been presented? Poster? Oral? What meeting? What publication?

Ow, TJ; Sano, D; Sandulache, VC; Pickering, CR; Skinner, HD; Zhao M; Xie, TX; Zhou, G; Myers, JN. Missense mutation in TP53 increases aggressive tumor characteristics in metastatic head and neck squamous carcinoma (Oral Presentation, Accepted to the Combined Otolaryngology Section Meeting, AHNS section, April 2011)

A manuscript entitled "Heterogeneity in TP53 mutation status between primary tumor and lymph node metastasis in head and neck squamous cell cancer may affect tumor behavior" is currently being drafted and should be submitted to *Head and Neck* in the near future.

Clinical Applications, Either Immediate or Potential, of This Research:
TP53 mutation is common, but it is not always associated with aggressive tumor phenotype and poor outcome. Our work explores a cell line in which tumor behavior is strongly linked to TP53 status. We hope that further study of this cell line will reveal genetic alterations or cellular characteristics that are linked to aggressive tumor behavior in the setting of TP53 mutation. We also have shown that TP53 status can be different between primary disease and lymph node metastatic disease. This finding has major implications, as genetic heterogeneity, even in major driver genes such as TP53, will have to be considered as we improve the personalized approach to cancer therapeutics.

Other Pertinent Information:

None