THE OHIO STATE UNIVERSITY
BIOMEDICAL SCIENCES
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THE OHIO STATE UNIVERSITY
COLLEGE OF MEDICINE

Megan Duggan
PhD Candidate

“The Role of Novel NRAS Isoforms in Melanoma Disease Progression and BRAF Inhibitor Resistance”

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BRT 105
9:00 AM
VITA

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ABSTRACT

Melanoma accounts for the vast majority of skin cancer-associated deaths and the incidence is increasing faster than any other cancer in the US. It is estimated that 87,000 new cases of melanoma will be diagnosed in the US in 2017. NRAS is the second most commonly mutated oncogene in melanoma, altered in 15-20% of cases, and efforts to therapeutically target NRAS and other RAS oncogenes have proven difficult. Recently, our group discovered that the NRAS gene transcript is differentially spliced to give rise to 5 distinct NRAS isoforms of varying size, expression patterns and downstream effects. Thus, we characterized the expression patterns and phenotypic functions of these isoforms in melanoma.

When melanoma tumor tissues were assessed for their expression of the NRAS isoforms, all five isoform transcripts were found to be expressed, with canonical NRAS (isoform 1) consistently expressed to the highest degree. NRAS isoform 1 mRNA expression was also significantly increased in metastases compared to primary melanoma lesions. Isoform 5 mRNA expression was found to be significantly correlated with survival, as high levels of isoform 5 in metastases were associated with enhanced survival in these patients with stage IV disease. Forced over-expression of each the isoforms led to enhanced proliferation, but invasiveness was only increased with over-expression of isoforms 1 or 2. Over-expression of isoform 4 led to significantly decreased ability to engage in anchorage-independent cell growth. Downstream signaling analysis indicated that the isoforms varied in their ability to mediate signaling through the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways. In vivo growth of A375 cells over-expressing each of the isoforms showed that cells over-expressing isoform 5 had significantly decreased tumor growth. Finally, A375 cells over-expressing isoforms 2 or 5 showed marked resistance to vemurafenib treatment in vitro. The results from this study indicate that the five different isoforms of NRAS play varying
roles in melanoma phenotype and progression and that they can potentially serve as biomarkers for therapeutic response and disease prognosis.

Activating mutations in BRAF are found in 50% of melanomas and although treatment with BRAF inhibitors is effective, resistance often develops. Therefore, we decided to further investigate the role of these NRAS isoforms in BRAF inhibitor resistance. NRAS isoform 2 was discovered to be up-regulated in the setting of BRAF inhibitor resistance in melanoma, in both cell lines and patient tumor tissues. When isoform 2 was over-expressed in BRAF mutant melanoma cell lines, melanoma cell proliferation and \textit{in vivo} tumor growth were significantly increased in the presence of BRAF inhibitor treatment. shRNA-mediated knockdown of isoform 2 in BRAF inhibitor resistant cells restored sensitivity to BRAF inhibitors compared to controls. Signaling analysis indicated decreased MAPK pathway signaling and increased PI3K pathway signaling in isoform 2 overexpressing cells compared to isoform 1 overexpressing cells. Immunoprecipitation of isoform 2 validated a binding affinity of this isoform to both PI3K and BRAF/RAF1. The addition of an AKT inhibitor to BRAF inhibitor treatment resulted in a partial restoration of BRAF inhibitor sensitivity in cells expressing high levels of isoform 2. Consequently, it was concluded that NRAS isoform 2 may contribute to resistance to BRAF inhibitors by facilitating PI3K pathway activation.

Taken together, these studies attempt the first thorough characterization of novel splice isoforms of NRAS in the setting of melanoma skin cancer. The role these isoforms play in the development and progression of the disease, and the potential for these isoforms to serve as prognostic and therapeutic biomarkers in melanoma is demonstrated, and importantly NRAS isoform 2 is found to play a role in the development of BRAF inhibitor resistance in melanoma. Additional studies to understand the regulation of the splicing of these isoforms, their role in the interaction of melanoma with the immune system, and their functions in the context of NRAS mutations will serve to enhance our knowledge of NRAS biology and inform future treatment strategies for patients with melanoma.

Duggan M, Stiff A, Olaverria Salavaggione G, Bainazar M, Latchana N, de la Chapelle A, Eisfeld A, Carson WE. Identification of NRAS isoform 2 overexpression as a novel mechanism facilitating BRAF inhibitor resistance in malignant melanoma. Oral Presentation: OSU Biomedical Sciences Graduate Program Retreat; 2016 Dec 8; Columbus, OH.


**Duggan MC, Regan K, Stiff A, et al.** NRAS mRNA is differentially spliced to give five distinct isoforms: implications for melanoma therapy. *In preparation.*
AWARDS AND HONORS

2014  OSU Graduate School University Fellowship
2015  OSU Council of Graduate Students Career Development Grant
2015  NIH Systems and Integrated Biology T32 Fellowship
2016  AACR Women in Cancer Research Scholar Award
2017  Hayes Research Forum, 3rd Place Oral Presentation

FUTURE PLANS

Following graduation, I plan to continue my research in the Carson laboratory for an additional year as a Postdoctoral Fellow. After that time, I will pursue a position in cancer immunology research in either academia or industry. Ultimately, I hope to become a translational researcher working on the development of novel cancer therapeutics.