Head and Neck Cancer in Fanconi Anemia

Casey O’Brien, BS1; Tingting Huang, MS2; Bonnie Lau, MD, PhD1,2
1Geisel School of Medicine, 2Dartmouth Hitchcock Medical Center

Abstract
Creating models to study FA mutated solid tumors, such as HNSCC, will give more insight into the disease and improve treatment options. We sought to understand the role of some FA mutations in HNSCC by creating a model in JHU011 cell lines by knocking out FANCA and FANCD2. After creating and validating these cell lines, we analyzed the effect of the mutations on growth rate, finding that it was increased in both edited lines compared to the WT. Further exploration will be done to understand the increased growth rate. After WES, we found that there was an increase in somatic mutations after introduction of the knockdown, which also may have contributed to increased growth. We used these models to test the efficacy of a PARPi, olaparib, and an EGFRi, gefitinib and found an increased effect in the edited HNSCC cells. With the WES data, we plan to measure the TMB as well as the EGFR pathway to understand the increased effectiveness of these targeted agents. Hopefully this can be used to further expand the study of targeted agents for FA HNSCC.

Methods and Materials

CRISPR. JHU011 cells were maintained in DMEM +10%FBS+5%P/S in a humidified incubator. This cell line is established from a squamous cell carcinoma of the larynx of a 45 y/o M. FANCA and FANCD2 were knocked out of JHU011 cells using Addgene’s protocol for LentiCRISPR v2. Cells were selected with puromycin 2μg/mL for 4 days. Knockouts were confirmed with qPCR with Actin as a control using SYBR Green following their protocol.

Growth. JHU011 cells were seeded at 2000 cells/well in a clear bottom 96-well plate and every 24 hours MTT cell viability assays were performed following CyQUANT MTT rapid protocol over a period of 96 hours.

WES. WES of the JHU011 cells show an increase in mutations that fall into a VAF enrichment app. NextSeq run with Illumina Mid Output 300 cycle kit and DRAGEN enrichment app.

Discussion
• The knockout of FANCA and FANCD2 appear to increase the growth rate of the HNSCC JHU011 cell lines - with more of an effect in FANCD2 (Fig. 1)
• FANCD2 is important for the localization of the FA core complex to sites of DNA damage
• Gefitinib is an EGFR inhibitor that shows greater efficacy in FA mutated HNSCC JHU011 cells, with FANCD2 KO cells showing a more robust response (Fig. 2a)
• WES may be used to understand possible effects of FANCD2 mutations on EGFR pathway
• There were issues with data acquisition for gefitinib
• Olaparib is a PARPi; PARP functions to repair DNA damage and may be more active in cells with higher mutational burden and decreased DNA repair defects
• EGFR inhibitors have shown efficacy in HNSCC with FANCD1 mutation

Conclusions and Future Directions
• Increase in growth rate could be due to FA mutations themselves or due to mutations that occur as a result of the FA mutations - assess with WES
• Olaparib and gefitinib have efficacy individually - combine the two agents to determine if there is a synergistic effect
• Use mouse models to examine toxicity
• Could combine PD-1 inhibitor with olaparib
• Appears to be an increase in somatic mutations after the introduction of FA mutations but measure with WES
• Use WES data to determine impact of FA mutations on other genes and pathways

References

Acknowledgements
This work could not have been completed without the help from everyone in the Lau Lab. Thank you to Laura Price, Jacob McCoy and Bernice Leung for the advice and support. Thank you to Geisel School of Medicine for the stipend allowing me to dedicate my time to this project. The NCCC Shared Resources were necessary to complete this work.