Head and Neck Cancer in Fanconi Anemia

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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.

Introduction

- Fanconi Anemia is genetic disorder affecting DNA crosslink repair genes resulting in bone marrow failure, developmental delays and increased risk of cancer[1]
- Spectrum of disease presentation and genetic aberrations is wide
- Risk of AML development is ~700 fold higher than general population[2]
- Current lifespan is 20-30
- Risk of solid tumors increases with age[2]
- Risk of HNSCC development is ~550 fold higher than general population[2]
- Traditional cytotoxic chemotherapies and radiation have dose-limiting toxicities, reducing survival rate (5 yr 39% in FA, 66% general)[3]
- Targeted therapies are needed to improve morbidity and mortality[4,5]
- Lack of DNA repair genes may lead to increased genomic instability
- PARP inhibitors, such as olaparib, have shown efficacy in tumors with DNA damage repair defects[6]
- EGFR inhibitors have shown efficacy in HNSCC with FANCD1 mutation[7]

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.

Results

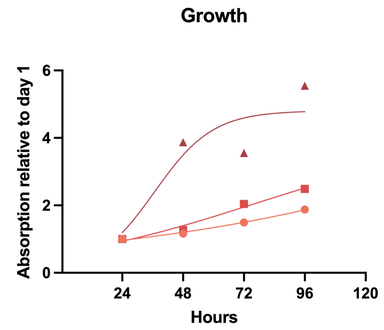
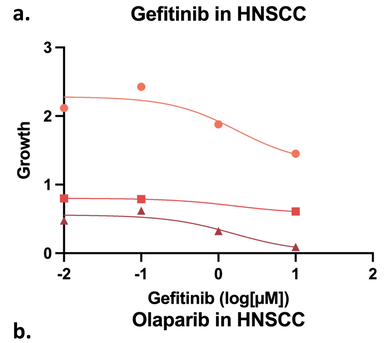


Fig. 1 - Growth of WT, FANCA KO, and FANCD2 KO JHU011 cells measured with MTT on day 1-4. Absorption was normalized to day 1 to observe doubling time.



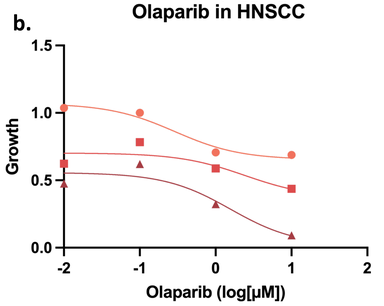


Fig. 2 - JHU011 cells were treated with (a) gefitinib and (b) olaparib and cell viability was measured to create growth inhibition curves

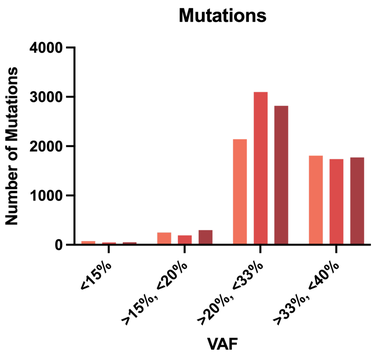


Fig. 3 - WES was performed using Illumina NextSeq and DRAGEN enrichment app analyzed the raw data to give mutation VAFs. Mutations within certain VAF ranges were plotted to give an estimate of tumor mutation burden in these samples.

Methods and Materials

DRUG TREATMENT. JHU011 cells were seeded as above and gefitinib and olaparib were added to media after 24 hours at stated concentrations and a 1:1000 DMSO vehicle control was used. Each day, cell viability was assessed as above WES. DNA was extracted from JHU011 cells using Qiagen DNeasy kit. Samples were run with Illumina NextSeq 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[5]
- Gefitiinib 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 (Fig. 2b)
- Olaparib showed an increased effect in FANCD2, which does not appear to be due to higher mutational load – WES may be informative
- Olaparib may be able to be combined with other targeted agents, such as gefitinib or PD-1 inhibitors
- WES of the JHU011 cells show an increase in mutations that fall into a VAF range consistent with somatic mutations, indicating a possibly higher TMB (Fig 3)
- Higher numbers of somatic mutations may increase the sensitivity to PD-1 immunotherapy, particularly with PARPi[8]

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

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