ARID1A Deletion Results in Enhanced Osteosarcomagenesis and Altered Chromosome Structure

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Background And Hypothesis

Osteosarcoma is a quintessential cancer of genomic instability. In the United States, 400 - 1,000 new cases are diagnosed per year. Roughly 75% of Osteosarcoma cases occur in individuals of 15-25 years of age. Patients with metastatic or recurrent diseases have a <20% chance of long-term survival despite aggressive therapies suggesting genetic complexity and chemoresistance characteristics.

Using a random mutagenesis screen in mice, we identified ARID1A as one of the most frequent mutations that decreased survival in our mice with osteosarcomas (Figure 1).

We hypothesized that, loss of ARID1A results in genomic instability and contributes to cancer progression and chemoresistance in osteosarcoma.

Materials And Methods

For in-vitro analysis two Osteosarcoma cell lines putatively expressing ARID1A (SUSA-1 and U2-OS) were used. Through RT-PCR, Western Blotting (rabbit anti-ARID1A antibodies), and Immunofluorescence assay, we verified the existence and location of ARID1A within the cell lines (Figure 3).

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We then proceeded to knockout ARID1A using CRISPR/Cas9 gene editing. Cas9 was introduced to cells via liposomal transfections of bacteria plasmid (pSpCas9(BB)-2A-Puro). The pCas9 included a puromycin resistance, which allowed for selection of successfully transfected pspCas9. Following Cas9 transfection, small guide RNAs highly specific for ARID1A were transfected into the cells. Based on our earlier experiments and results (Figure 2), we hypothesized that knockout of ARID1A would result in decreased survival (Figure 3).

Results And Conclusion

Upon deletion of ARID1A in Osteosarcoma cell lines (Figure 4), cells were tested for proliferation and migration. We found that deletion of ARID1A resulted in an increase in proliferation and migration. We have established our mice cohorts with and without ARID1A to see its effect in vivo. ARID1A wildtype cohorts have on an average 7 months of survival advantage over the mice with homozygous and heterozygous deletion of ARID1A (Figure 5).

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For our in-vivo study we have used a spatially, temporally and inducible controlled gene knockout using cre-recombinase system to control deletion of Arid1a only specific to osteosarcoma. We generated our genetic mice of interest by breeding mice with conditional alleles (TRAP58/RIR1/R1/ROR/CoxSCE72/Arid1a B6) and used Tamoxifen to induce Arid1a knockout in the mice. With 3D micro CT scanning and necropsy we are analyzing the primary and secondary tumor development (Figure 6).

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It is evident from our study that loss of ARID1A enhances a more aggressive phenotype in the cell lines and results in higher morbidity in mouse lines. We conclude that ARID1A is a potent tumor suppressor in Osteosarcoma and loss of it results in genomic instability and a more chemoresistant phenotype.

Future plan is to investigate the genomic instability in osteosarcoma with and without ARID1A to understand the molecular mechanism of chemoresistance. By doing so we will be able to identify therapeutic targets to develop more effective targeted therapies for chemoresistant cancers like aggressive osteosarcoma.

References And Acknowledgment

- Institutional Developmental Award (IDA) from the National Institute of General Medical Sciences of the NIH under Grant #2G35GM103408.