Sensitization of APC knock-out cells to Doxorubicin *in vitro* using KU55933, an ATM inhibitor.

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Background and Hypothesis:

Triple negative breast cancer (TNBC) has the lowest 5-year survival rate among breast cancer subtypes, with Adenomatous Polyposis Coli (APC) mutations/deletions found to be a leading cause of Doxorubicin (DOX) resistance. Additionally, increased expression of pATM/ATM in APC knock-out (APC^{KO}) cells was previously observed, providing a possible explanation to chemotherapy resistance. We hypothesized that treatment with the ATM inhibitor, KU55933, would sensitize APC^{KO} cells to DOX *in vitro* using γH2AX as a marker of DNA damage.

Materials and Methods:

The TNBC cell line, MDA-MB-231, was previously modified using CRISPR/Cas9 to knock out APC. The current study uses the non-targeting control (NTC) and 2 clonal knock-out cell lines (APC^{KO} or clones 1 and 2). Cells were cultured in DMEM with 10% FBS, 1% penicillin/streptomycin, and plasmocin, and treated with either H2O, DOX, KU, or DOX+KU for 24 hours. For western blot analysis of γH2AX, membranes were blocked at room temperature in 5% nonfat dry milk and incubated with primary anti-γH2AX overnight at 4°C using actin as an internal control. Immunofluorescence studies included drug treatment of cells with the four previously mentioned treatments and probing for γH2AX using anti-γH2AX antibody.

Results:

Immunofluorescence studies displayed increased γH2AX expression, indicating DNA damage in the combination (DOX+KU) treatment group in the APC^{KO} cell lines. Western Blot analysis showed a significant difference in the DOX treated 231 NTC and clone 2 cell lines vs H2O treated cells.

Conclusion and Potential Impact:

Data from this study will be used to pursue further research in the efficacy of KU in sensitizing APC^{KO} cells to DOX treatment and have significant implications for the future of chemotherapy research and dosing for TNBC patients. Future studies may include testing the ability of KU to overcome DOX resistance *in vivo* and assessment of APC^{KO} cell recovery after drug treatment in different growth media.