Abstract #11 (0346_0513_000017)

EVALUATION OF TEMOZOLOMIDE AND IRINOTECAN COMBINATION THERAPY IN HUMAN RHABDOMYOSARCOMA CELLS.

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Background:
Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma, accounting for 5% of all childhood cancers. Temozolomide (TMZ) and Irinotecan (IR) are commonly used to treat reoccurring cases of RMS, but their mechanisms of action remain unclear.

Objective:
Previously, we have shown that TMZ induces autophagy and apoptosis in RMS cells, thus we hypothesized that treatment of RMS cells with IR together with TMZ would induce cell death via an autophagy-associated mechanism.

Methods:
We determined the effects of TMZ and IR on cell viability in RH30 and A204 cells (two RMS cell lines) using MTT assays and determined the type of cell death with flow cytometry. Western blot analysis, live cell imaging, immunofluorescence, and TEM were used to assess levels of autophagy and mitophagy.

Results:
Treatment of RH30 and A204 cells with TMZ+IR showed significantly more death than treatment with either drug alone ($p<0.001$). Moreover, the cellular death was found to be apoptotic. TMZ and IR co-treatment induced both autophagic and mitophagic flux in both cell lines. Interestingly, levels of expression of several BCL2 family proteins—including BCL2L13, NIX, and BNIP3—change significantly after drug treatment. p53 was shown to play an important role in regulating the expression of these proteins and inducing mitophagy.

Conclusion:
Our findings show that both autophagy and apoptosis are occurring after treatment with IR and TMZ, and we hope to further examine the connection between autophagy, mitophagy, and apoptosis in these cells by investigating p53 and its role as a master regulator of these processes. We hope that by elucidating the cell death mechanism in RMS cells, we can work towards proposing a novel chemotherapy treatment for RMS.