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Cell death mechanism of irinotecan in combination with temozolomide in human rhabdomyosarcoma cells

Philip Kawalec, University of Manitoba

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 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 (alveolar and embryonal RMS cells, respectively) cells using MTT assays, and determined the type of cell death via the Nicoletti method. Western blot analysis was used to assess markers of apoptosis and autophagy. To confirm TMZ and IR-induced autophagy, cells were transfected with LC3-GFP; after transfection and drug treatment, cells were stained with LysoTracker or MitoTracker dyes to study changes in autophagic flux and mitophagy, respectively. Statistical analyses were completed using One-Way ANOVA tests.

Results:

Treatment of RH30 and A204 cells with TMZ and IR in combination showed significantly more cell death than treatment with either drug alone ($p<0.001$). Moreover, flow cytometry showed the cellular death to be apoptotic. TMZ and IR co-treatment induced autophagic flux in both cell lines, which was confirmed with LC3-GFP live cell imaging. Interestingly, levels of expression of several BCL2 family proteins vary significantly between the two cancer types after drug treatment.

Conclusion:

Our findings show that both autophagic and apoptotic processes are occurring after treatment with IR and TMZ, and that there exist large differences in how the two RMS cancers respond to chemotherapy. We hope to further investigate the connection between autophagy and apoptosis in these cells, with the goal of proposing a novel treatment for RMS based on modulating autophagic flux to induce apoptotic death.