TARGETING STRESS GRANULE DISSOLUTION WITH RALOXIFENE ALTERS TRANSLATION OF KNOWN GBM ONCOGENES AND PROMOTES CELL DEATH

Kathleen Attwood, A Robichaud, A Balgi, M Roberge, C McCormick, Adrienne Weeks

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BACKGROUND: Glioblastoma (GBM) is the most common primary malignant brain tumour, carrying a uniformly poor prognosis. Hypoxia is a feature of the GBM microenvironment, and previous work has shown that cells residing in hypoxic regions are treatment resistant. Hypoxia can trigger formation of stress granules (SGs), sites of mRNA triage that promote multiple cell survival mechanisms. We hypothesize that SGs play a role in hypoxia-induced therapy resistance.

METHODS/RESULTS: A screen of 1280 FDA-approved drugs identified 99 candidates that inhibited hypoxia-induced SG formation and 129 candidates that inhibited SG dissolution. Our screen identified raloxifene, a selective estrogen receptor modulator (SERM), which delays SG dissolution in a dose-dependent manner. SG dissolution typically occurs within an hour post-hypoxia, however pre-treatment of U251 glioma cells with raloxifene inhibited SG dissolution for up to 2-4 hours. Overall protein translation was compared in raloxifene pre-treated and untreated cells at various times post-hypoxia using a puromycin pulse assay. Raloxifene pre-treatment caused a translational decrease for up to 2 hours post-hypoxia, correlating to the persistence of SGs. This translational decrease was also found to correspond to increased phosphorylation of the SG-inducing protein eIF2α and decreased phosphorylation of S6 ribosomal protein- both hallmarks of a hypoxic state. The combination of raloxifene and hypoxia also resulted in synergistic killing of U251 cells. These effects are estrogen independent and interestingly varies depending on cancer cell type. The reason for this cancer specificity is currently under investigation.

CONCLUSIONS: We have demonstrated that targeting SG dissolution in GBM results in deleterious effects on translational control of known GBM oncogenes and promotes cell death.