Research Background

Diffuse Intrinsic Pontine Glioma (DIPG) is a glial tumor occurring primarily in children and so far, has seen very low survival rates. DIPG makes up about 10% of all childhood central nervous system tumors and occurs in the pons, a part of the brainstem. Due to its critical location, surgery is not an option as the brainstem controls many important bodily functions such as breathing and blood pressure. This paired with the fact that DIPG tumors tend to grow at an aggressive rate gives need to the development of novel therapeutics.

DIPG tumors possess a key mutation in Histone 3, H3K27M (a substitution mutation of lysine for methionine at position 27 of Histone H3) existent within 80% of DIPGs. This mutation inhibits the activity of the Polycomb repressive complex 2 (PRC2), which promotes the H3K27trimethyl (me3) mark on chromatin and an increase in the activating H3K27acetyl (ac) mark. As a result of increased K27 acetylation, there is an activation of oncogenic pathways and further tumor cell proliferation.

Interestingly, a chromatin remodeler, RE1 silencing transcription factor (REST) is highly associated G9a chromatin remodeler alone or in combination with a drug that blocks the activity of the histone H3K27 acetyl mark-BRD4 will decrease the growth of DIPG cells.

Hypothesis

We hypothesized that targeting the REST-associated G9a chromatin remodeler alone or in combination with a drug that blocks the activity of the histone H3K27 acetyl mark-BRD4 will decrease the growth of DIPG cells.

Methods

Cell Culture: DIPG cell lines used include (DIPG-IV, -VII, and -XIII) (expressing the H3.1K27M, wildtype (WT), and H3.3 K27M, respectively. Isogenic DIPG cells engineered to express higher-REST levels were also used in the assays. All DIPG cell lines were grown in DIPG cell culture media with added growth factors (Shaik et al., 2017).

ATP Assay: "All materials used in ATP Assay come from the PerkinElmer ATPlite assay system" Cells were plated in 96 wells plate. Following 120 hours of incubation with JQ1 or UNC0638, cells were lysed with 50 uL (per well) mammalian cell lysis buffer and then added 50uL (per well) substrate buffer solution, mixed and incubated under dark conditions before measuring the luminescence in the Luminometer.

Results

A concentration-dependent decrease in the growth of both DIPG-IV-GFP and DIPG-IV-GFP-REST was observed with JQ1. At 1 µM concentration, cell growth in both cell lines was decreased to 75% compared to their respective controls (Fig 2.).

Summary of Results

Statistics:

• The experimental data was analyzed using student’s t test. The significance was taken when the p value between two experimental groups was less than *p<0.05, **p<0.01 and ***p<0.001.
• JQ1 treatment resulted in a significant decrease in cell growth with a 75% decrease in DIPG-IV (GFP and GFP-REST) at 1 µM concentration.
• UNC0638 resulted in a decrease in cell growth in a REST-dependent manner only in DIPG-IV cell lines, having very minimal effect on DIPG-VII. The DIPG-XIII cells (GFP and GFP-REST) exhibited a 50% decrease in cell growth at 1 µM concentration.
• The combination of UNC0638 and JQ1 treatments indicated that DIPG-GFP-REST cell growth was decreased to 38% when used the combination of drug doses of 0.25 µM of UNC0638 and 0.1 µM of JQ1 compared to 1% with DIPG-IV-GFP cells.

Conclusions

• UNC0638 treatment promoted a REST-dependent, statistically significant, reduction in cell growth only in DIPG IV cells.
• A decrease in cell growth following JQ1 treatment was seen in all cells but was REST-independent.
• The combination of UNC0638 and JQ1 did not result in a synergistic decrease in cell growth in high or low-REST DIPG cells compared to either drug alone.
• In parallel, a REST-context specific chemical screen was performed, which identified new therapeutic vulnerabilities. These will be investigated in future studies.

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Pediatrics Department, UT - MD Anderson Cancer Center, Houston, TX.