Investigating the Inhibition of MYBL2 for Therapeutic Intervention in Group 3 Medulloblastoma

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TBIOMD 496

BACKGROUND

- Medulloblastoma is the most common type of pediatric brain tumor accounting for approximately 20% of all pediatric central nervous system tumors.
- Out of all 4 subtypes, Group 3 medulloblastoma is the most lethal with a 39% 10-year survival rate.
- MYBL2 is a transcription factor necessary for cell proliferation, survival and differentiation.
- Demethylzeylasteral (T-96) has shown anti-tumor effects.
  - Utilized in glioma cells to target MYBL2
- We hypothesized that the use of T-96 could inhibit MYBL2 activity disrupting the cell cycle, reducing tumor cell proliferation, and potentially enhancing the sensitivity of medulloblastoma cells to existing treatments.

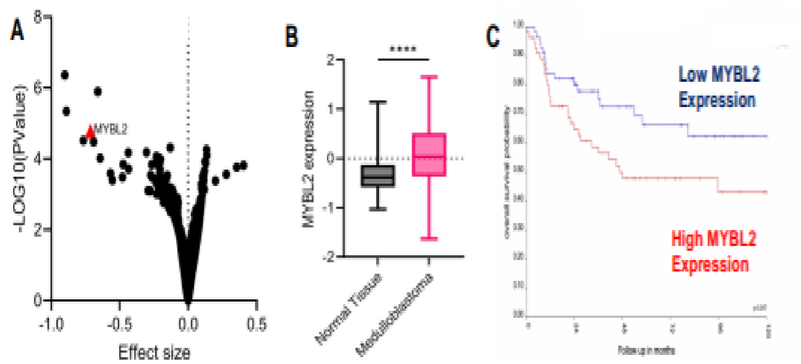
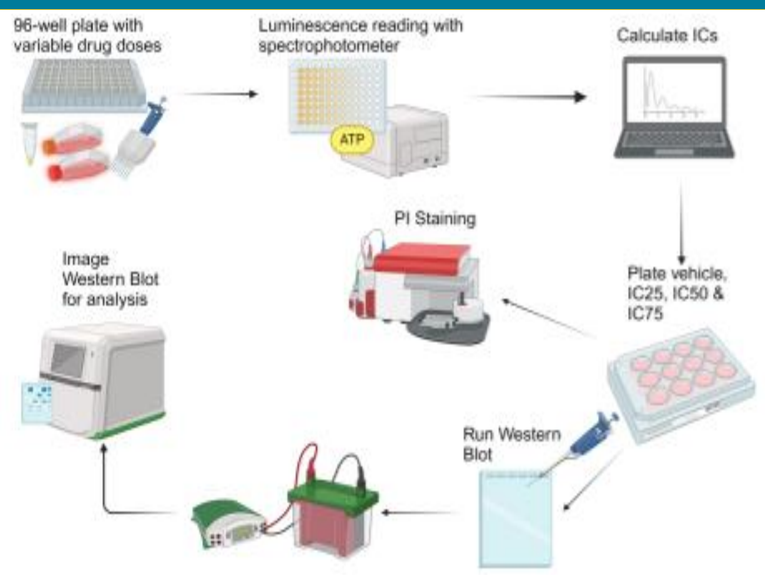


Figure 1: (A) Volcano plot of gene effect size in G3 MB vs pediatric CNS tumors. (B) Plot depicting MYBL2 expression in patients with normal brain tissue vs. Medulloblastoma tumors. (C) Kaplan-Meier survival plot of Group 3 medulloblastoma patients based on MYBL2 expression.

METHODS



RESULTS

Targeting *MYBL2* by RNAi decreases sphere area

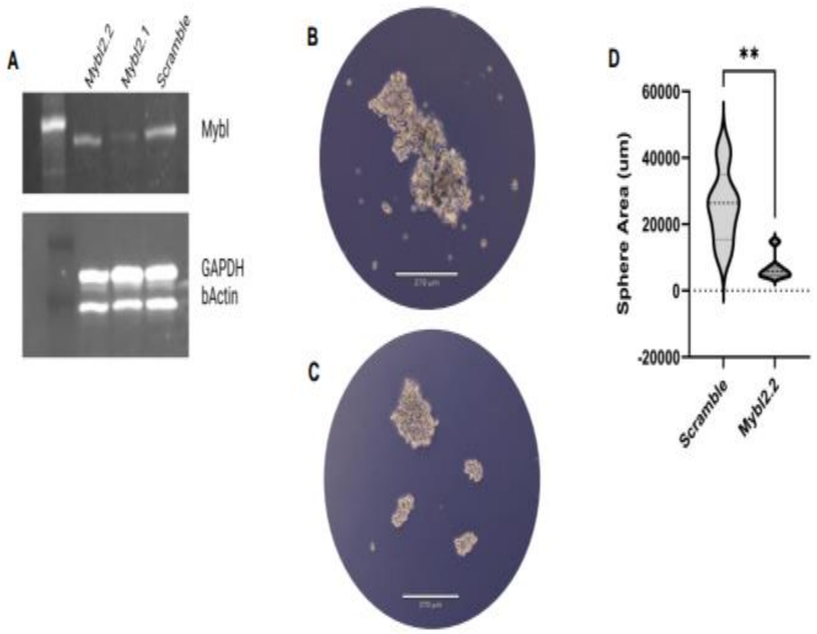


Figure 2: (A) Western Blot analysis of MYBL2, GAPDH, and B-Actin after MYBL2 knockdown (B) Image of 2112 scramble sphere. (C) Image of 2112 MYBL 2.2 knockdown sphere (D) Plot depicting sphere area of 2112 scramble and 2112 MYBL2.2. T-test revealed statistical significance between both groups.

Knockdown of *MYBL2* arrests cells in S phase

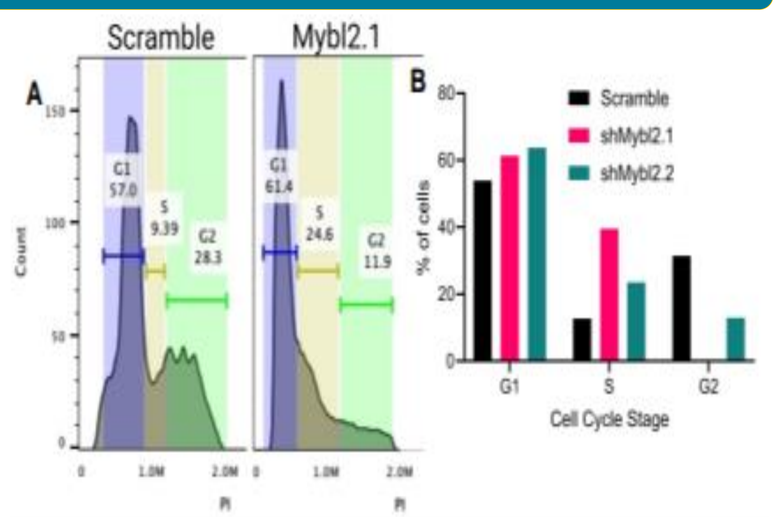


Figure 3: (A) Flow cytometry plot of scramble and MYBL2 knockdown cells with cell cycle phases highlighted. (B) Percent of cells in each phase measured by propidium iodide staining

Demethylzeylasteral inhibits MYLB2 expression

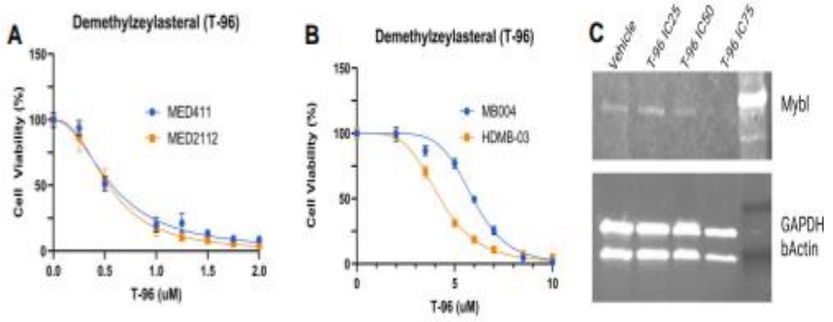


Figure 4:(A-B) Cell viability of (A) patient-derived xenograft (PDX) cell lines treated with increasing doses of T-96 vs (B) human G3MB cell lines treated with increasing doses of T-96.(C) Western Blot analysis of MYBL2, GAPDH, and B-Actin after cell treatment with T-96 inhibitory concentrations after 48 hours.

CONCLUSIONS AND FUTURE DIRECTIONS

- MYBL2 is a dependency in G3 MB cells.
- MYBL2 knockdown affects sphere formation size indicating that MYBL2 is essential for cell growth & proliferation.
- Knockdown of MYBL2 arrests cells in the S phase
- T-96 inhibited the proliferation of group 3 medulloblastoma cell lines.

- ➢ In-vivo experimentation with T-96
- ➢ Currently injecting knockdowns in mice
- ➢ Send treated cells for RNA sequencing
- ➢ Run Western Blot and PI staining for FTY720
- ➢ Test T-96 and FTY720 in combination with other therapies to see if a synergistic effect can be observed

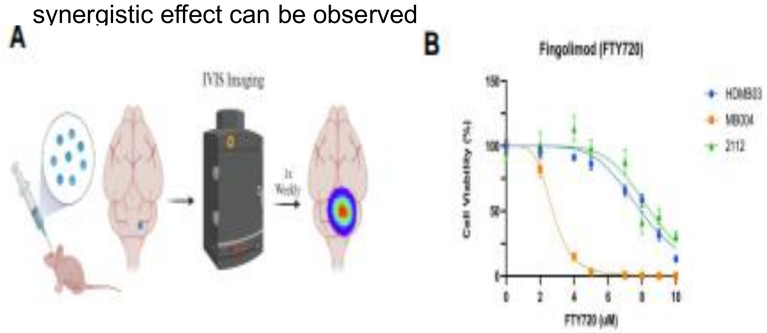


Figure 5: (A) Schematic of in-vivo experimentation. (B) Cell viability of PDX and HDMB cell lines treated with increasing doses of FTY720

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