Pharmacological Inhibition of REST in Glioblastoma

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Introduction: RE1-silencing transcription factor (REST) silences neuronal differentiation genes. Its overexpression in an aggressive subset of gliomas is believed to support the enhanced tumor-initiating and self-renewal capacities of glioblastoma cancer stem cells (GSCs). Therefore, REST knockdown is hypothesized to inhibit tumor growth and recurrence. Because REST, as a large protein, is difficult to target directly with small molecules, our study focuses on knocking down REST by inhibiting one of its regulatory enzymes, small C-terminal domain phosphatase 1 (SCP1). Dephosphorylation of REST by SCP1 protects the former from degradation; consequently, SCP1 inhibition with an experimental drug, T62, is expected to reduce REST protein levels. This REST knockdown is hypothesized to induce the expression of neuronal differentiation genes, thereby forcing differentiation of GSCs and making them more vulnerable to standard treatments. We begin our study by validating patient-derived GSC lines and subsequently testing the efficacy of T62 drug in these cells. Our work supports an effort to understand various molecular pathologies of GBM and its intrinsic GSCs in order to develop novel therapeutic strategies.

Materials and Methods: Patient-derived GSCs were validated for having stem-like properties by immunocytochemistry. Cells were seeded into 8-well chamber slides and probed after 3 hours with mitogens (EGF and FGF) or after 4 days without mitogens. (Absence of mitogens directs GSC differentiation.) GSCs were then classified by western blotting for expression of neural lineage proteins, which correlate with tumor invasiveness and patient survival. In the current study, basal REST and SCP1 protein levels in non-cancerous human neural stem cells (hNSCs) and differentiated normal human astrocytes (NHAs) were quantified by western blotting (Figure 1A). Effective T62 drug dosage (ED50) was determined by treating hNSCs with different concentrations of drug for 30 hours and assaying for post-treatment REST and SCP1 protein levels (Figure 1B).

Results and Discussion: Two new patient-derived GSC lines, 105 and 107, were validated to possess stem-like properties. 105 was classified as Class III, which is characterized by high expression of astrocyte progenitor cell (APC) markers but low expression of neural progenitor cell (NPC) and oligodendrocyte progenitor cell (OPC) markers. 107 was classified as Class II, with low expression of APC/OPC markers but moderate expression of NPC markers. 105 and 107 GSC neural lineage expression profiles predicted invasiveness in brain tumor mouse xenograft models. Next, consistent with previous studies, we determined that the baseline expression of REST protein is higher in neural stem cells than in differentiated cells, e.g. NHAs (Figure 1A). The predicted reduction in REST protein levels in the presence of T62 was not observed after 30-hour drug treatment (Figure 1B). We predict that REST protein levels may decrease if drug treatment duration is extended to 48 or 72 hours. As expected, SCP1 protein levels are unaffected by T62, which competitively inhibits SCP1 but does not alter its protein expression.

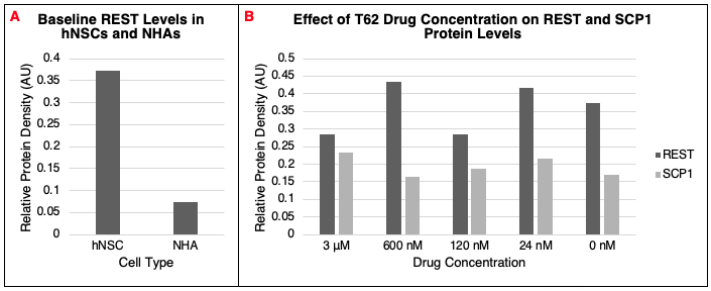

Figure 1. Assessing pharmacological inhibition of REST levels in hNSCs. A: Baseline REST protein levels in hNSCs and NHAs. B: Post-treatment REST and SCP1 protein levels at different drug concentrations. hNSCs were treated with T62 drug in stem cell media for 30 hours. *Protein levels are normalized to TUBB3 loading control.*

Conclusions: Future studies will assess the inhibitory effect of T62 drug on REST protein levels in GSCs by optimizing drug concentration (ED50) and treatment duration. We will also test the efficacy of T62 drug *in vivo* in mouse tumor models, with the goal of lowering REST protein levels and increasing tumor response to GBM standard therapy.