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Novel synthetic superenhancers for drug screening in cancer stem cells

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Introduction: Glioblastoma (GBM) is the most fatal malignant brain tumour. It is typically treated with a combination of surgery, radiotherapy and chemotherapy but is invariably fatal. A major driver of tumour growth is SOX2, a lineage specific transcription factor that is expressed in the GBM stem cells[1]. To date SOX2 is not amenable to structure-based drug design, as it is small transcription factor. We are able to use freshly isolated patient-derived GBM stem cell culture in cell based phenotypic screening to find new lead compounds that can disrupt GBM stem cell self-renewal. Our goal is to develop novel neural-specific transcriptional reporters with improved sensitivity and cell type specificity that can be used to monitor SOX2 activity. These would be highly desirable and could be engineered directly into GBM stem cells with CRISPR/Cas9 knockin.

Method: SOX2 binding peaks within enhancer regions and unique for tumour-initiating cells have been identified from published ChIP-Seq datasets[2]. We have deployed the latest tools of DNA assembly and synthetic biology to create a new platform for combinatorial screening of novel clusters of SOX2 enhancer tethered to a downstream Luciferase-mNeogreen cassette. A minimal promoter was chosen based on low background expression and high inducibility. Enhancers were cloned and tested for their ability to drive reporter gene expression of NanoLuc and mNeogreen in GBM cell lines as well as in HEK, a SOX2 negative cell line.

Results and Conclusions: Individual enhancers have been identified that are able to drive reporter gene expression in a cell type specific manner ($n \geq 3$). Clusters of between 4 and 8 of these enhancers ('synthetic superenhancers') are now being screened in combinatorial manner for optimal performance in GBM stem cells. These cell-type specific reporters of SOX2 activity will be valuable in future drug screening and development projects. Our overall approach provides a generic strategy that can be re-used for other transcription factors.

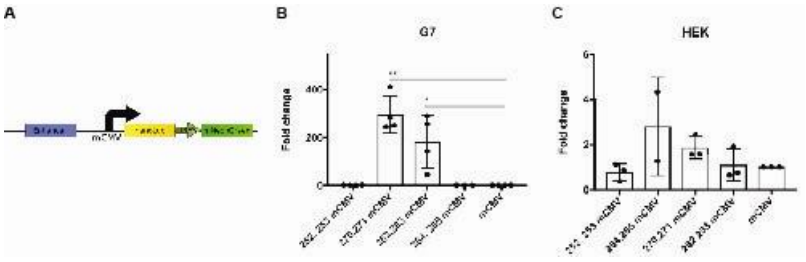

Figure 1: Enhancers are able to drive reporter gene expression in a cell type specific manner. (A) Schematic representation of a enhancer reporter activity construct. (B) Enhancers 272, 273 and 282, 283 show an up to 300-fold change in a GBM cell line. (C) Small fold change was observed in the SOX2 negative non-neural cell line. $p \leq 0.05$.

References

1. Gangemi, R et al(2009). *STEM CELLS*, 27(1):40-48.
2. Suvà, M. L et al (2013). *Cell*, 157(3):580-594.