Defining Glioblastoma Stem Cell Heterogeneity

Nermin Sumru Bayin MS; Sheng Si; Rajeev Sen; Aram Modrek; Valerio Ortenzi; David Zagzag MD; Igor Dolgalev; Adriana Heguy; Dimitris G. Placantonakis MD, PhD

NYU School of Medicine

Introduction

Glioblastoma stem cells (GSCs) dominate the cellular hierarchy in GBM. They are able to self-renew and differentiate into tumor lineages, and are resistant to current therapies. It is unclear whether the GSC population in any given tumor is heterogeneous.

Methods

Using human GBM biospecimens engineered to express GFP upon activation of Notch signaling, we observed partial overlap between cells expressing cell surface CD133 and cells with activated Notch signaling, contrary to expectations based on prior literature. To further investigate heterogeneity within the GSC population, we isolated distinct GSC populations and characterized them.

Figure 1

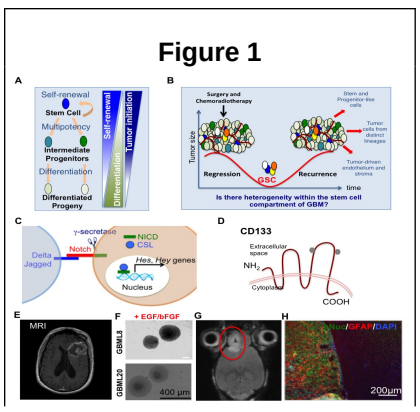

Figure 2

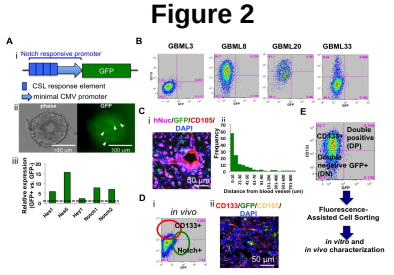

Figure 2. Experimental model for following Notch pathway activation and GSC Heterogeneity.

Figure 3

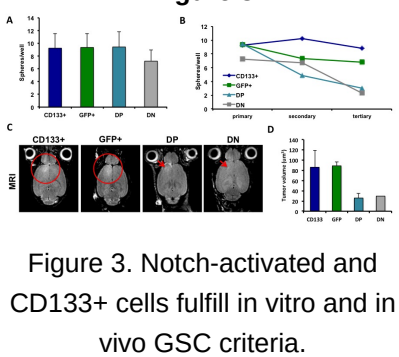

Figure 3. Notch-activated and CD133+ cells fulfill in vitro and in vivo GSC criteria.

Figure 4

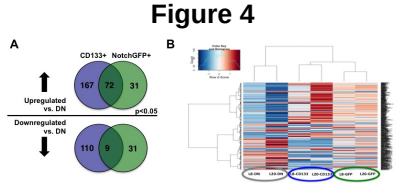

Figure 4. Characterization of distinct GSC populations by RNA-seq.

Figure 5

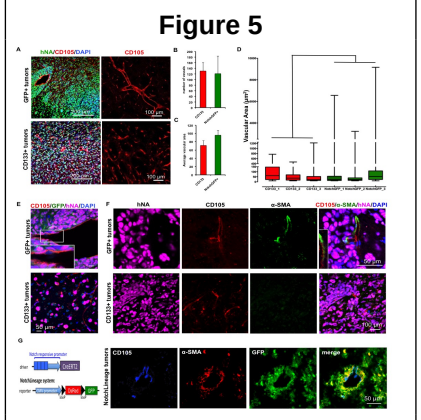

Figure 5. Notch-activated and CD133+ cells give rise to xenograft tumors with distinct vascular phenotypes.

Figure 6

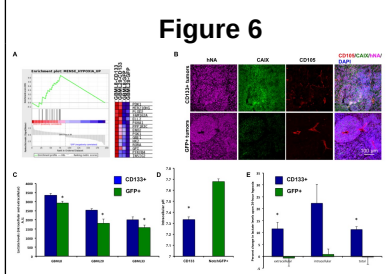

Figure 6. CD133+ cells are primed for hypoxic conditions by their enhanced glycolytic metabolism.

Figure 7

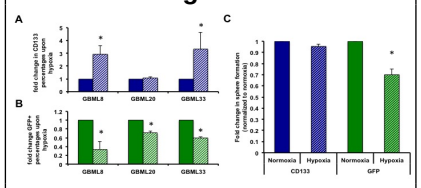

Figure 7. CD133+ GSCs are able to expand and continue to self-renew under hypoxic conditions.

Results

We found that CD133+ (CD133+/Notch-) and Notch+ (CD133-/Notch+) GSCs differ substantially in their transcriptome, metabolism and differentiation capacity, thus giving rise to histologically distinct tumors. CD133+ GSCs have increased expression of hypoxia-regulated genes, and expand under hypoxic conditions by activating anaerobic glycolysis. In contrast, Notch+ GSCs are unable to activate glycolysis under hypoxic conditions, leading to decreased tumorsphere formation ability. Furthermore, CD133+ GSCs give rise to histologically homogeneous tumors devoid of large tumor vessels. Tumors initiated by Notch+ GSCs are marked by large perfusing vessels enveloped by pericytes. Using a lineage tracing system, which allows tracking of the progeny of Notch+ GSCs, we showed that pericytes are derived from Notch+ GSCs. Notch+ cells are able to give rise to all tumor lineages, as opposed to Notch- populations *in vitro* and *in vivo*, which have restricted differentiation capacity and do not generate Notch+ lineages. These findings suggest that multipotent Notch+ GSCs lie at the apex of GBM's cellular hierarchy.

Conclusion

GBM's stem cell population is marked by cellular and metabolic heterogeneity. Discreet GSC subtypes are able to support tumor growth either by surviving in hypoxic conditions or supporting tumor angiogenesis by differentiating into pericytes.

Figure 8

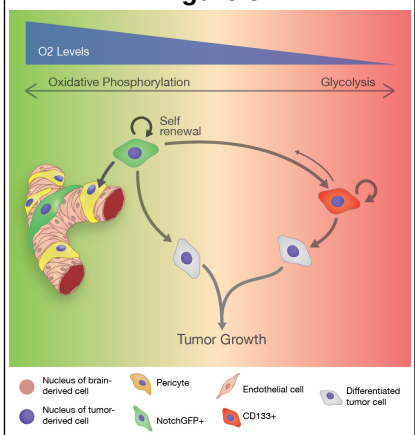

Figure 8. Illustration demonstrating metabolic and angiogenic heterogeneity in GBM's stem cell population.

References

Bayin et al. 2015. Non-uniform Notch signaling underlies intratumoral heterogeneity within the cancer stem cell population of glioblastoma (in review).