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Glioblastomas Become More Stem Cell Enriched During Evolution of Resistance to Anti-Angiogenic Therapy

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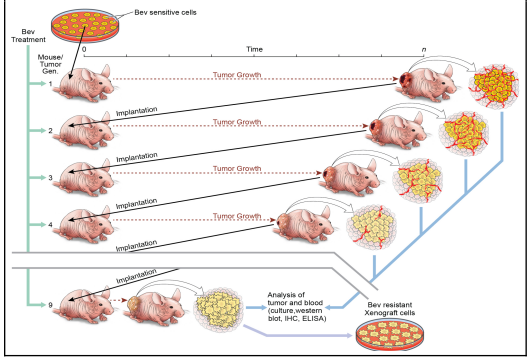
Introduction

While bevacizumab initially shows therapeutic response in glioblastoma (GBM), it quickly leads to a resistant and invasive phenotype with a mesenchymal gene expression profile. Here, we report stem cell enrichment in bevacizumab-resistant GBM using patient tumor samples and a novel xenograft mouse model.

Methods

Patient tumor samples (bevacizumab-naive and resistant) were collected and used to harvest neurospheres. To better understand the mechanisms of glioblastoma resistance to anti-angiogenic therapy, we developed a novel multi-generational xenograft model of resistance.

Figure 1: Novel Multigeneration model for bevacizumab resistant GBM



To study transcriptional changes, we collected tumor samples from successive generations of our model and performed a microarray gene expression and qPCR analysis in bevacizumab-resistant GBM tumors for glioma stem cell markers.

To confirm stem cell enrichment in bevacizumab-resistant GBM, total stem cell counts from patient tumor-derived neurospheres were performed. Neurosphere reformation assays were performed in multiple generations of xenograft-derived bevacizumab-resistant and responsive cells from which neurosphere diameter, neurosphere counts and absolute stem cell counts were analyzed.

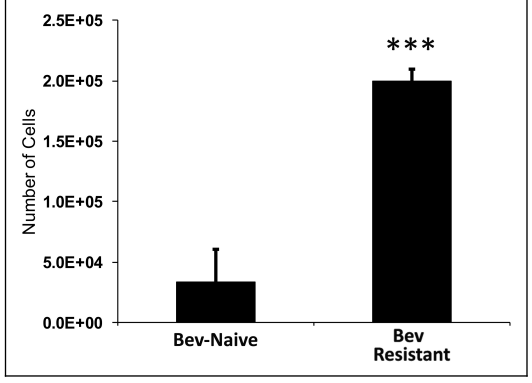
For all figures, *p<0.05; **p<0.01, ***p<0.001

Results

Patient tumor-derived neurospheres have a higher absolute stem cell count

On performing cell counts from glioma neurospheres derived from patient tumors, neurospheres from bevacizumab-resistant tumors (n=4) yielded a 4-time higher stem cell count as compared to neurospheres from bevacizumab-naive tumors (n=3) (p<0.001).

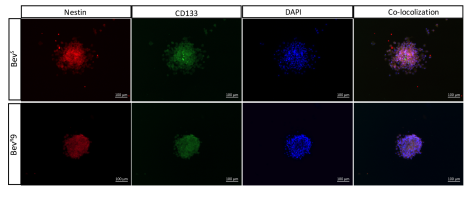
FIgure 2: Stem cell counts from patient tumor derived neurospheres



Validation of xenograft-derived neurospheres

Xenograft-derived neurospheres were stained with Nestin and CD133, putative glioma stem cell markers, to confirm for true glioma stem cell neurospheres.

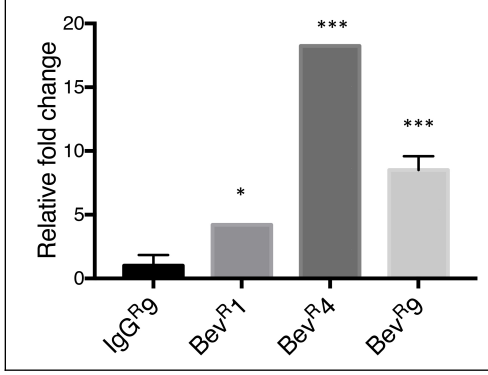
Figure 3: Immunocytochemistry for Glioma Stem Cell markers



Overexpression of glioma stem cell markers in late generations of bevacizumab-resistant GBM

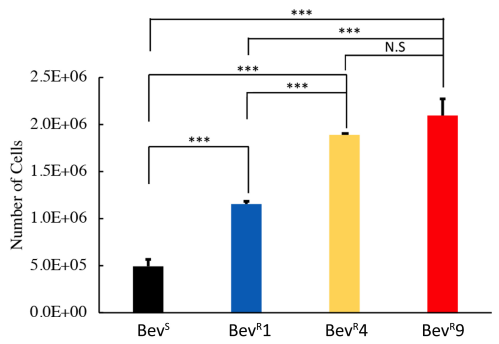
Glioma stem cell genes are signficantly upregulated at each generation of bevacizumab-resistant GBM tumors as compared to bevacizumab-naive GBM tumors.

Figure 4: Transcriptional analysis of Glioma Stem Cell genes



Xenograft-derived bevacizumab resistant neurospheres have a higher absolute stem cell count

Figure 5: Stem cell counts from xenograft-derived neurospheres



Stem cell counts were higher in bevacizumab-resistant neurospheres in a generation dependant manner, with generation 9 having almost four-times higher stem cell counts than neurospheres from bevacizumab-sensitive xenografts (p<0.001).

Xenograft-derived bevacizumab resistant neurospheres are larger yet fewer

Figure 6: Diameter of bevacizumab-naive and resistant neurospheres

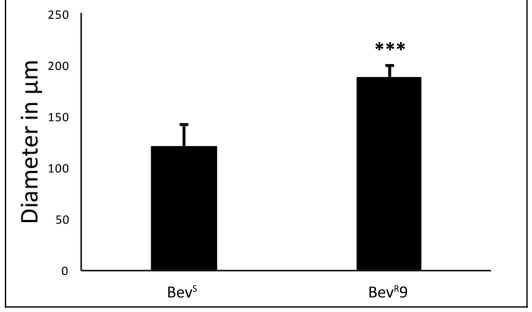
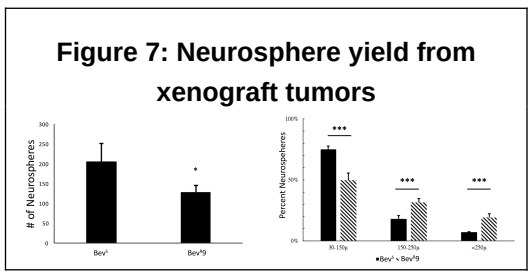


Figure 7: Neurosphere yield from xenograft tumors



Conclusions

These results suggest tumor progression under anti-angiogenic treatment is accompanied by a shift in genetic and phenotypic expression towards a more aggressive stem-cell enriched phenotype. Strategies perturbing the evolution of stemness characteristics should be developed and further validated to improve the durability of therapeutic response to anti-angiogenic therapies.