

# Obtaining the Genetic Fingerprint of Resistance to Glioblastoma Through a Novel Multigenerational Xenograft Model

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## Introduction

Despite positive pre-clinical/clinical trials, a major hurdle in the clinical application of bevacizumab, an anti-angiogenic therapy, in glioblastoma is the development of resistance and progression following a transient response period.

## Methods

We established a multi-generational glioblastoma xenograft model of acquired bevacizumab resistance through subcutaneous implantation of U87-cells, bevacizumab treatment, and selection and reimplantation of the fastest growing tumor in each generation to new mice. Whole human genome microarray (Illumina) was performed on 3 tumor samples from generations 1, 4 and 9, and bioinformatic analysis of gene expression data was performed in Matlab2014a.

## Results

Utilizing published statistical methods; we identified a set of genes exhibiting significant inter-generational variance. Protein-protein interaction(PPI) scores were extracted of String database(v10); subsequent spectral clustering revealed 13 gene subnetworks of closely interrelated genes. Gene set over-representation (GSO) analysis via ConsensusPathDB suggested biologically meaningful subnetworks mediating distinct functions, including inflammation, extracellular-matrix remodeling, cell-cycle, metabolism, and cytoskeletal dynamics.

Gene set enrichment analysis revealed significant overexpression across generations of previously identified gene expression signatures of the mesenchymal subtype. Important markers, including putative tumor-stemness marker CD44 and critical epithelial-mesenchymal-transition transcription factors SNAI2, and ZEB2, were upregulated across generations. These results suggest tumor progression under bevacizumab to be accompanied by a gene expression shift towards the mesenchymal subtype, associated with enhanced invasiveness, resistance, and worse outcomes.

Our analysis revealed expression changes in angiogenesis-related pathways. Genes identified via GSO suggested a tumor pro-angiogenic response to bevacizumab, composed of converging pathways involving inflammation, hypoxia, ECM remodeling, upregulation of alternative pro-angiogenic pathways

## Conclusions

Using microarray analysis of a model of bevacizumab resistance in glioblastoma, we found development of resistance to be accompanied by a gene expression shift towards the mesenchymal subtype, as well as activation of alternative pro-angiogenic pathways. These findings shed light on the mechanisms of resistance to anti-angiogenic therapy in glioblastoma.

## Learning Objectives

By the end of this presentation, attendees should have a clear understanding of the design and necessity of models required in order to study the progression of resistance to anti-angiogenic therapy in preclinical glioblastoma xenograft models. Furthermore, they should take away that under the stress of anti-angiogenic therapy with bevacizumab, tumor cells shift towards the mesenchymal subtype associated with enhanced invasiveness, resistance, and poor outcomes.

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## References

1. Batchelor, T. T., Reardon, D. a., de Groot, J. F., Wick, W. & Weller, M. Antiangiogenic Therapy for Glioblastoma: Current Status and Future Prospects. *Clin. Cancer Res.* 20, 5612–5619 (2014).
2. Ebos, J. M. L. & Kerbel, R. S. Antiangiogenic therapy: impact on invasion, disease progression, and metastasis. *Nat. Rev. Clin. Oncol.* 8, 210–221
3. Gilbert, M. R. et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. *N. Engl. J. Med.* 370, 699–708 (2014).
4. Jahangiri, A. et al. Gene expression profile identifies tyrosine kinase c-Met as a targetable mediator of antiangiogenic therapy resistance. *Clin. Cancer Res.* 19, 1773–1783 (2013).
5. McLachlan, G. J., Bean, R. W. & Jones, L. B. T. A simple implementation of a normal mixture approach to differential gene expression in multiclass microarrays. *Bioinformatics* 22, 1608–1615
6. Marczyk, M., Jaksik, R., Polanski, A. & Polanska, J. Adaptive filtering of microarray gene expression data based on Gaussian mixture decomposition. *BMC Bioinformatics* 14, 101 (2013).
7. Dean, N. & Raftery, A. E. Normal uniform mixture differential gene expression detection for cDNA microarrays. *BMC Bioinformatics* 6, 173
8. Von Mering, C. et al. STRING: Known and predicted protein-protein associations, integrated and transferred across organisms. *Nucleic Acids Res.* 33, (2005).
9. Franceschini, A. et al. STRING v9.1: Protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res.* 41,
10. Nepusz, T., Sasidharan, R. & Paccanaro, A. SCPS: a fast implementation of a spectral method for detecting protein families on a genome-wide scale. *BMC Bioinformatics* 11, 120 (2010).
11. Kamburov, A., Wierling, C., Lehrach, H. & Herwig, R. ConsensusPathDB--a database for integrating human functional interaction networks. *Nucleic Acids Res.* 37, D623–D628 (2009).
12. Phillips, H. S. et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell* 9, 157–173 (2006).
13. Verhaak, R. G. W. et al. Integrated Genomic Analysis Identifies Clinically Relevant Subtypes of Glioblastoma Characterized by Abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* 17, 98–110 (2010).