Intra-Tumor Epigenetic Heterogeneity Is Associated With Tumor Grade and Epigenetic Drivers in Glioblastoma Multiforme

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Why Heterogeneity Matters

- Intra-tumor heterogeneity refers to cellular, genetic, and epigenetic variation in one tumor.
- GBM is well established to have high degrees of inter and intra tumor heterogeneity.
- First GBM genome atlas found 453 mutations in 223 genes (Guzman et al. 2017).
- Many mutations/proteins not found in every tumor section
- Critical need to define how and why heterogeneity contributes to malignant phenotype before epigenetic therapy can be applied
Methods

1. **Section tumors into 5 regions each**

2. **Neuropathology grading**

3. **PIXUL-MatrixChIP-qPCR to study epigenetic marks at 30 known oncogenes**

4. **(a) Calculate coefficient of variation of slices A-E for all tumors, epigenetic marks, and oncogenes. (b) Perform statistical analysis and PCA.**

5. **Principle Component Analysis**
1. Intra-tumor epigenetic heterogeneity increases with malignant phenotype

(a) Intra-tumor variation increases with tumor grade. Principle component analysis performed on CV values of tumor slices A-E shows heterogeneity of Pol II, H3K27Ac, K3K9,14Ac, H3K27me3, 5mC, and 5hmC at thirty oncogene sites studied.

(b) Scattergram plot analysis showing distribution of CV values for different glioma grades (**p<0.0001, Spearman ranked correlation, Mann-Whitney test).
2. Transcript heterogeneity matches epigenetic heterogeneity

Epigenetics

mRNA
3. What drives this heterogeneity?

**Figure 1.** Principle component analysis shows permissive marks (H3K27Ac/H3K9,14Ac) clustering together and repressive marks (H3K27me3/5mC) clustering together.

**Figure 2.** Relative to normal brain tissue, repressive marks show significantly higher intra-tumor heterogeneity than permissive marks, suggesting that variation is driven more by H3K27me3/5mC silencing rather than H3K27Ac/H3K9,14Ac activation at these oncogene loci (Mann-Whitney U Test).
4. Intra-tumor heterogeneity is oncogene dependent

- HIF1A and TLX1 showed greatest overall intra-tumor epigenetic heterogeneity
- ZEB2, SOX2, and HIF2A showed the lowest overall intra-tumor heterogeneity
Discussion

• Study purpose is to show how and why heterogeneity drives malignant gliomas
• Results show high degree of heterogeneity on phenotype, mark, and gene levels, suggesting that novel treatment strategies could exploit this variation.
• Mark-gene permutations could exist that combine information regarding aberrant expression and degree of heterogeneity.
• Here we identified SOX2 and ZEB2 as known GBM oncogenes with low intra-tumor epigenetic variation.
Conclusions

• Combining aberrant expression and gene heterogeneity to identify optimal mark-gene permutations is critical to epigenetic therapy design.
• Multiple biopsies needed per tumor to accurately describe transcript, genetic, epigenetic levels.
• Some repressive marks are responsible for driving heterogeneity more than activating H3 acetylation marks.
• Intra-tumor heterogeneity contributes to inter and intra study variability.
• High throughput epigenetic platforms needed to study GBM heterogeneity.