Oncogenic signal transduction pathway activity in glioblastoma and relation to therapy response

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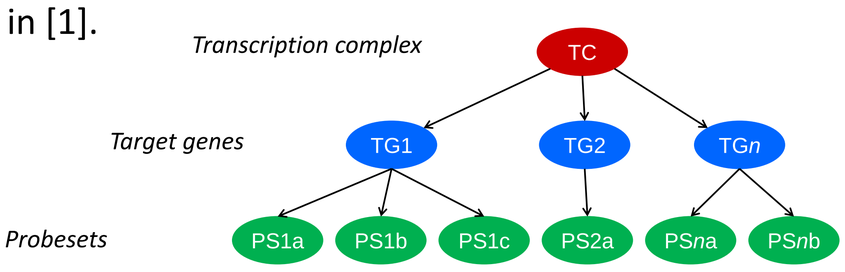
Summary

- **We developed computational models to assess *functional* activity of the ER, PI3K-FOXO, AR, Wnt, HH, NFκB and TGFβ pathways in *individual* tissue samples, using mRNA expression data, for future diagnostic applications**
- **We confirmed that our models, calibrated on non-brain tissues, are able to pick up known Wnt and HH pathway activity in brain tumors**
- **In glioblastoma we observe remarkable differences in pathway activity compared to normal brain and within glioblastoma subgroups**
- **TGFβ pathway activity is associated with worse prognosis and resistance**
- **These pathway activity results provide highly interesting leads for targeted therapy selection**
- **We expect pathway analysis to have clinical utility in predicting therapy response in a neoadjuvant setting and possibly also in an adjuvant setting**

Materials & methods

We have modeled the transcriptional programs of the ER, PI3K-FOXO, AR, Wnt, HH, NFκB and TGFβ pathways, to infer functional pathway activity from mRNA levels of their direct target genes, measured on Affymetrix HG-U133Plus2.0 arrays (fRMA preprocessed). Details of the approach are described in [1].



PI3K activity is in principle the inverse of FOXO activity.

None of the brain samples showed any AR or ER pathway activity.

Quality control. Quality control (QC) was performed on each public data set using 12 existing independent parameters before pathway analysis was done.

Statistical tests. Significance was calculated using the Mann-Whitney *U* test

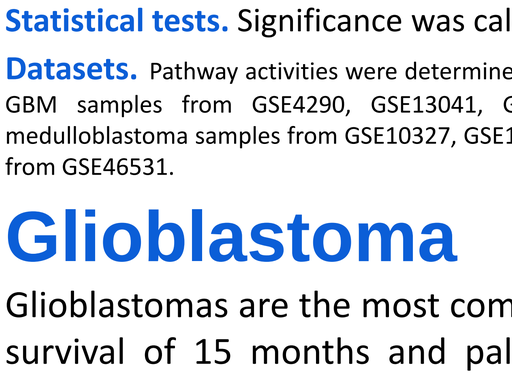
Datasets. Pathway activities were determined on 8 control brain samples from public data set GSE16011; 408 GBM samples from GSE4290, GSE13041, GSE16011, GSE36245, GSE43289, GSE51062, GSE60184; 234 medulloblastoma samples from GSE10327, GSE12992, GSE37418 and GSE49243; 12 GBM stem cell clone samples from GSE46531.

Glioblastoma

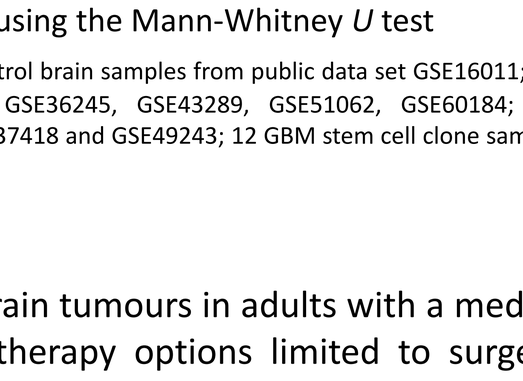
Glioblastomas are the most common brain tumours in adults with a median survival of 15 months and palliative therapy options limited to surgery, radiotherapy and chemotherapy. Four subtypes of glioblastoma have been identified: Classical, Proneural, Neural and Mesenchymal. A role for HH, TGFβ and Wnt pathways has been suggested based on tumor biopsies and/or on in vitro experimental work.

Knowledge on signaling pathway activity in individual tumors may enable stratification for targeted drug therapy.

Model validation: medulloblastoma



Wnt pathway. *CTNNB1* mutations in medulloblastoma activate the Wnt signaling pathway [2]. In data sets GSE10327 and GSE12992 Wnt pathway activity was significantly higher in the samples with a Wnt-driving CTNNB1 mutation.

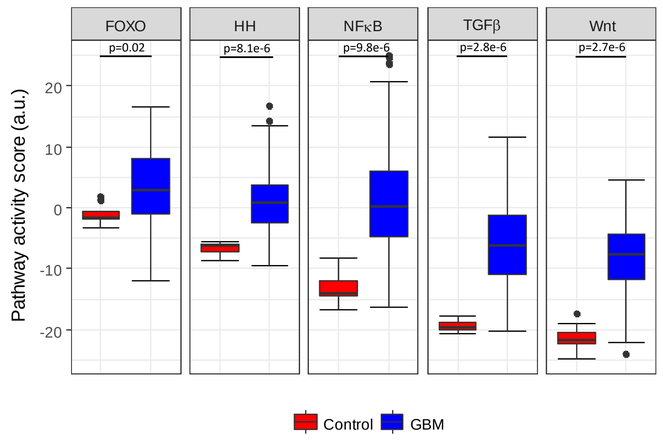


HH pathway. The SHH subgroup is characterized by aberrant SHH (Sonic HedgeHog) signaling [3]. Our model identifies higher Hedgehog activity in the SHH samples compared to other subgroups in data sets GSE37418 and GSE49243.

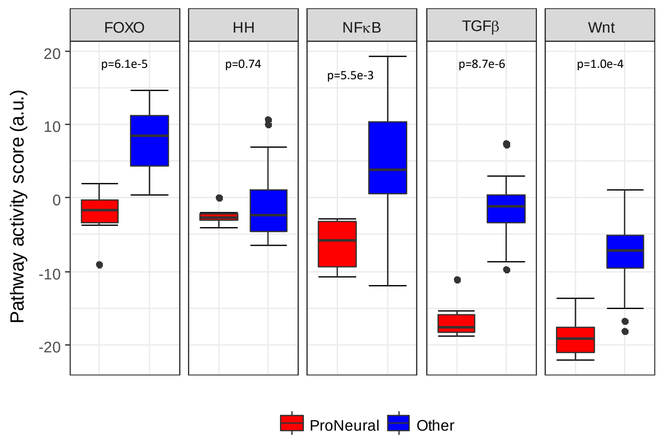
Pathway activity in glioblastoma

We compared 408 glioblastoma multiforme (GBM) samples from 7 public data sets to 8 control samples and found a significant increase in pathway activity for FOXO, HH, NFkB, TGFb and Wnt.

Variation in pathway activity in GBM is very high, agreeing with known heterogeneity of GBMs.

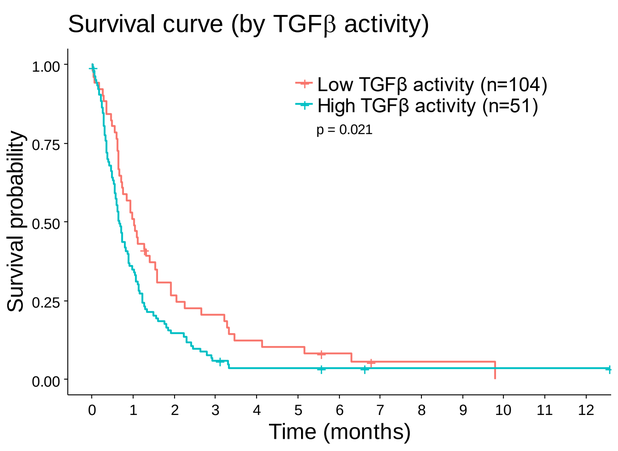


Glioblastoma subtypes: ProNeural vs. non-ProNeural



We compared GBM samples that were classified as ProNeural or non-ProNeural (GSE13041) and control samples and found a significant increase in pathway activity for FOXO, NFkB, TGFb and Wnt for non-ProNeural GBM. It is known that ProNeural have substantially longer survival times compared to other GBMs.

TGFβ activity negatively correlates with survival

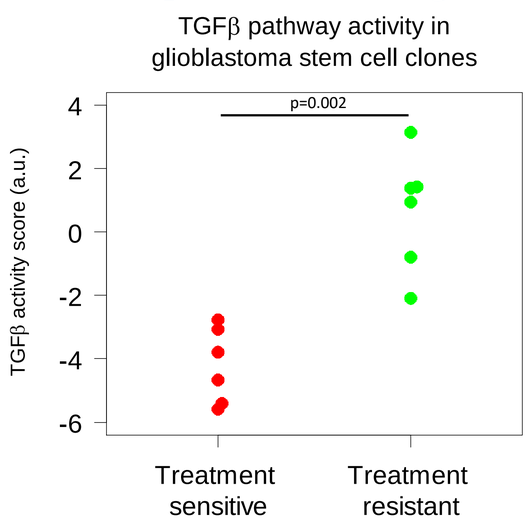


We focused on the role of TGFβ pathway activity in GBM and generated Kaplan-Meier curves for GBM samples in a public dataset (GSE16011). We calculated the optimal threshold between high/low activity and found that High TGFβ pathway activity corresponds to lower survival (p=0.021).

Treatment resistant stem cell clones have higher TGFβ activity

Ye et al [4] treated glioblastoma stem cell clones with (chemo)radiation. Surviving cells (treatment resistant) were compared to non-treated cells (treatment sensitive).

We ran our pathway analysis method and found a significant difference in TGFβ pathway activity between the 2 groups, pointing to a potential role of TGFβ in treatment resistance.



Conclusion

Our signal transduction pathway models can identify functional signaling pathway activity in brain tumor tissue samples based on mRNA analysis.

TGFβ pathway activity was associated with worse prognosis and resistance to (chemo)radiation.

Our pathway analysis is expected to have clinical utility, complementary to mutation analysis, in predicting glioblastoma therapy response to radio-chemotherapy and targeted drugs.

References

[1] W. Verhaegh et al. Cancer Res 2014;74(11):2936-45

[2] S. Fattet et al. J Pathol 2009; 218(1): 86-94

[3] G. Robinson et al. Nature 2012; 488 (7409):43-8

[4] F. Ye et al. PLoS One 2013; 8(11):e80397