Elucidating pediatric brain tumor pathophysiology by assessing signal transduction pathway activation

Laurent Holtzer, Monique Stoffels, Wim Verhaegh, Anja van de Stolpe

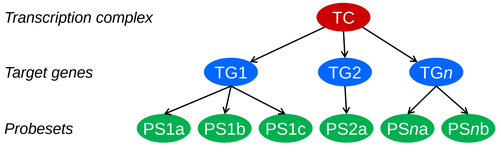
Philips Research, Eindhoven, The Netherlands, contact: anja.van.de.stolpe@philips.com

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 assessed activity of these pathways in normal pediatric brain samples and various pediatric brain tumors**
- **We confirmed that our models, calibrated on non-brain tissues, are able to pick up known Wnt and HH pathway activity**
- **In ependymoma we observe remarkable differences in pathway activity between different subgroups**
- **These pathway activity results provide highly interesting leads for targeted therapy selection**
- **We expect pathway analysis to have clinical utility 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].



A Bayesian network is used to infer the probability of activity of a specific pathway in an individual sample. The network has three types of nodes. Arrows indicate the dependencies between the nodes.

An active pathway is defined as having an inferred probability above 0.5.

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.

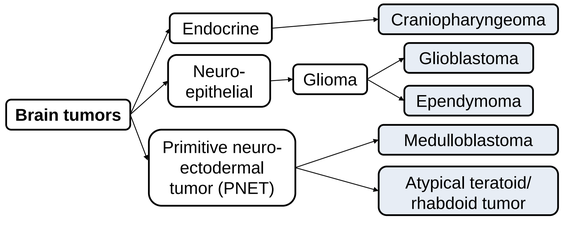
Datasets. Pathway activities were determined on 11 normal pediatric brain samples from public data set GSE50161; 460 normal adult brain samples from GSE3562, GSE4290, GSE7307, GSE11882, GSE15824, GSE44971, GSE68015 and GSE74195; 163 pediatric medulloblastoma samples from GSE10327, GSE12992 and GSE37418; 62 pediatric ependymoma samples from GSE66354; 24 pediatric glioblastoma samples from GSE36245; 16 pediatric atypical teratoid/rhabdoid tumor samples from GSE66354; 15 pediatric adamantinomatous craniopharyngioma samples from GSE68015.

Pediatric brain tumors

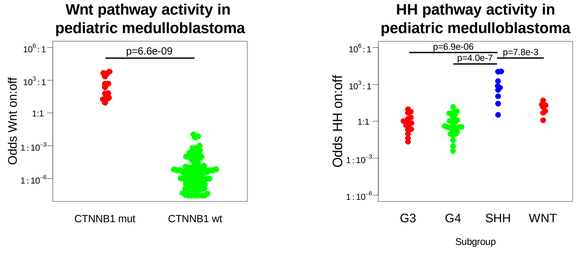
Treatment of children's brain tumors faces multiple challenges that differ from adult brain tumors: aside from an often bad prognosis, surgery is associated with relatively frequent and lifelong comorbidities. Tumor pathophysiology is often characterized by abnormal activation of developmental signaling pathway activity.

Driving mutations are scarce and epigenetic abnormalities prevail due to the stem cell origin, which makes it very difficult to identify patients for targeted therapies based on genomics analysis. For example, ependymoma is the third most common pediatric brain tumor, yet because of the paucity of effective therapeutic interventions, 45% of patients remain incurable.

Availability of diagnostic tests which can reliably identify signal transduction pathway activity in tumor tissue will enable new therapeutic approaches.



Model validation in medulloblastoma

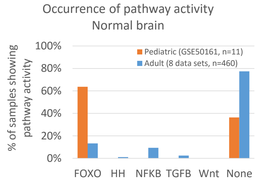


Wnt pathway. *CTNNB1* mutations in medulloblastoma activate the Wnt signaling pathway [2]. In data sets GSE10327 and GSE12992 our Wnt model successfully scores the samples with *CTNNB1* mutation as active.

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. (GSE37418)

Pathway activity in normal brain

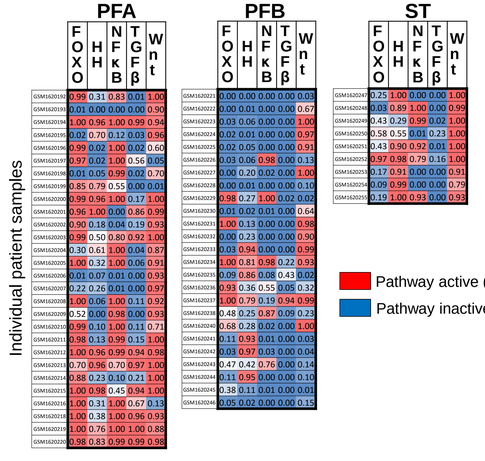
We assessed the probability of activity of each pathway in non-tumor brain. None of the pediatric samples showed oncogenic pathway activity. In 7 pediatric samples we found FOXO activity (meaning no PI3K activity).



Ependymoma subgroups have distinct pathway activity signatures

Data set GSE66354 contains 3 subgroups of pediatric ependymoma:

- Posterior Fossa A (PFA): young patients, bad prognosis, inflammatory phenotype.
- Posterior Fossa B (PFB): older patients, better prognosis than PFA.
- Supratentorial (ST): Relatively rare, different location, better survival rate than PF



We observe remarkable differences in pathway activity:

- PFA: Dominant NFκB-activity (>70% of samples), in agreement with inflammatory phenotype and Wnt-activity in almost all samples. TGFβ pathway activity in 40% of samples.
- PFB: Dominant PI3K activity (low FOXO activity).
- Supratentorial: high Wnt combined with HH-activity.

H3F3A mutant glioblastoma show significantly lower NFκB activity

One third of pediatric glioblastoma has a mutation in the *H3F3A* gene, which encodes the histone variant H3.3 [4].

***H3F3A* wt**

	FOXO	HH	NFκB	TGFβ	Wnt
GSM884998	0.72	0.63	0.01	0.01	0.02
GSM885009	0.99	0.99	1	0.52	0
GSM885009	0.11	0.81	0.38	0	0
GSM885021	0.13	0.7	0.19	0	0
GSM885024	1	0.92	0.88	0.04	0
GSM885028	1	0.03	1	0.81	0
GSM885031	0.96	0.09	0.51	0	0
GSM958494	0.96	0.9	0	0.03	0
GSM958495	0.15	0.23	0.85	0	0
GSM958496	0.91	0.99	0.53	0	0
GSM958499	1	0.1	0.86	0.73	0.35
GSM958500	0.73	0.94	0	0	0

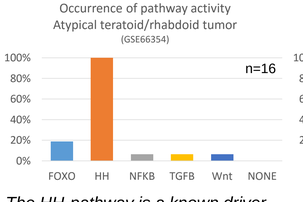
***H3F3A* mut**

	FOXO	HH	NFκB	TGFβ	Wnt
GSM884997	0.06	0.98	0	0	0
GSM884999	0	1	0	0	0.02
GSM885000	0.57	0.8	0.07	0	0
GSM885023	0.59	0.95	0.21	0	0
GSM885029	0.68	0.95	0	0	0.04
GSM885030	0.61	0.91	0.21	0	0.01
GSM958490	0.53	0.8	0	0	0
GSM958491	1	0.46	0	0.2	0.58
GSM958492	0.58	0.91	0.03	0	0.01
GSM958493	1	0.83	0.56	0.05	0
GSM958497	0.98	0.99	0.02	0.02	0.14
GSM958498	0.93	0.97	0.4	0	0

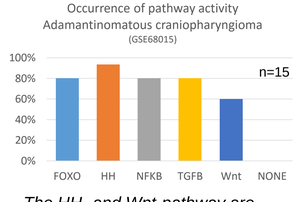
We observe a significant difference in NFκB activity between the 2 groups (p=0.03). (GSE36245)

The known heterogeneity of glioblastoma is clearly visible from our pathway analysis results.

Other pediatric tumors



The HH-pathway is a known driver of this tumor [5]



The HH- and Wnt-pathway are known drivers of this tumor [6,7]

Conclusion

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

This opens the way to new targeted therapy approaches for pediatric cancers, for example in a neo-adjuvant setting to reduce surgical brain resection and improve survival.

[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] D. Sturm et al. Cancer Cell 2012; 22(4): 425-37
[5] M.C. Frühwald et al. Neuro Oncol. 2016; 18(6): 764-78
[6] J.P. Martinez-Barbera NAN 2015, 41(6): 721-32
[7] D.C. Gomes, Eur J Endocrinol 2015, 172(5): 603-8

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