PEX5L is differentially expressed in the tumors of patients with glioblastoma.

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Understanding the cell biology of cancers and how it differs from that of benign tissue (1, 2, 3) is important for designing chemotherapeutic treatments with limited off-target effects and for rational identification of specific therapeutic targets (4). We performed global differential gene expression analysis using published datasets (5, 6) containing tumor transcriptome data from patients diagnosed with glioblastoma multiforme. Comparison of the glioblastoma patient transcriptome to that of non-affected control brain revealed that PEX5L (7, 8), a molecule involved in transport of cargoes into the peroxisome was among the most differentially expressed genes in the tumors of patients with glioblastoma. This is the first report documenting differential expression of PEX5L in glioblastoma, a cancer with a 15 month median survival rate and zero targeted therapeutic options (9, 10).

Keywords: glioblastoma, differential gene expression analysis, systems oncology, targeted cancer therapeutics, tumor transcriptome analysis, PEX5L

Chemotherapy generally functions by targeting the cell division rate of a cell, and no other property intrinsic to a cancer cell (11, 12). Targeted cancer therapeutics function by addressing a specific dependency or mutation that a cancer cell harbors, and in this manner increases efficacy of the treatment and generally decreases off-target effects commonly known as “side effects” (13). Understanding the basic cell biology of the cancer cell is critical for this endeavor. We used global differential gene expression analysis to understand the most significant differences between glioblastoma tumors and brain tissue using published microarray data (5, 6) generated from the tumors of patients diagnosed with glioblastoma. PEX5L (9, 10), a peroxisome-specific machinery functioning in import of cargoes into that organelle, was among the genes whose expression was most different between glioblastomas and non-affected brain tissue. This represents an advance in our understanding of the transcriptional makeup of the glioblastoma tumor.

Methods

GSE116520 (5) and GSE108474 (6) were also used for this differential gene expression analysis, in conjunction with GEO2R. The transcriptional data from GSE116520, GBM tumor from 17 patients diagnosed with glioblastoma multiform and normal brain from 8 controls, was generated by Kruthika et al. using Illumina HumanHT-12 V4.0 expression beadchip technology. The transcriptional data from GSE108474, GBM tumor from 221 patients diagnosed with glioblastoma multiform and normal brain tissue from 28 controls, was generated by Gusev et al. using Affymetrix Human Genome U133 Plus 2.0 Array technology. The *p*-value adjustment option was set to “Benjamini and Hochberg”, application of log transformation to data was set to “Auto-detect” and the NCBI generated category of platform annotation was used. Statistical

tests to compare the significance of RNA expression values between tumor and control groups was performed using a two-tailed, unpaired t-test with Welch's correction (PRISM 8.1.2) (227).

Results

PEX5L is among the most differentially expressed genes in glioblastoma tumors compared to control, non-affected brain tissue.

We performed global differential gene expression analysis to discover the genes whose expression was most different when comparing the transcriptomes of glioblastoma (GBM) tumors and "normal" brain tissue. We found that the Pex5-like gene PEX5L was ranked 43 out of 47229 total transcripts detected and measured by the microarray data used in this study (5) (Table 1). Differential expression of PEX5L was statistically significant (Table 1; $p=3.57E-15$).

We probed a second dataset (6), tumor microarray data from the REMBRANDT study, a collection of genomic data from patients with brain cancers, to determine whether differential expression of PEX5L could be observed in glioblastoma tumors from a separate group of patients. When sorting all of the genes expressed in 221 glioblastoma tumors based on change in mRNA levels between GBM tumor and normal brain, PEX5L ranked 26189 out of 54613 transcripts. Differential expression of PEX5L in REMBRANDT study glioblastoma tumors was statistically significant (Table 2; $p=7.11E-03$), albeit less so than in the first dataset analyzed.

PEX5L is expressed at significantly lower levels in glioblastoma tumors compared to control, non-affected brain tissue.

Next we extracted the individual RNA expression values for PEX5L for each individual tumor and control brain sample, and assessed whether the difference was statistically significant. PEX5L was expressed at significantly lower levels in the brain tumors of patients with glioblastoma than in control, non-affected brain tissue (Figure 1; $p<0.0001$).

Discussion

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2 Current treatment for glioblastoma, including temozolomide and polifeprosan 20 with
3 carmustine wafer provide a 15 month median survival (14-17). An enhanced understanding of
4 the cellular biology of glioblastoma tumors and how they function most differently from brain
5 cells is essential for advancing the development of novel therapeutics for glioblastoma. We
6 performed global differential gene expression analysis by comparing the transcriptomes (5) from
7 18 glioblastoma tumors to that of 7 control, non-affected brain tissue samples and found that
8 PEX5L was among the genes whose expression was most different in the primary glioblastoma
9 tumors of human patients.
10

11 The function of the Pex5-like gene is not completely understood but is thought to be a
12 receptor for the signal sequence (peroxisomal targeting sequences, or PTS) by which cargoes
13 designated for peroxisomal localization can be targeted to that organelle (7, 8, 18). Whether
14 PEX5L is differentially expressed in larger datasets generated using primary glioblastoma
15 tumors from humans, whether it is required for the proliferation or survival of glioblastoma cells,
16 and whether over-expression of PEX5L in xenograft mouse models of glioblastoma (19, 20)
17 confers any advantage with respect to tumor initiation, progression, metastasis or survival
18 should be assessed. Glioblastoma is a disease with zero targeted therapeutics available and a
19 dismal prognostic outlook (9, 10) and more resources must be focused on understanding the
20 basic transcriptional behavior of glioblastoma tumors.
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References

1. Levine, B., 2007. Cell biology: autophagy and cancer. *Nature*, 446(7137), p.745.
2. Vega, F.M. and Ridley, A.J., 2008. Rho GTPases in cancer cell biology. *FEBS letters*, 582(14), pp.2093-2101.
3. Slawson, C. and Hart, G.W., 2011. O-GlcNAc signalling: implications for cancer cell biology. *Nature Reviews Cancer*, 11(9), p.678.
4. Yarden, Y. and Pines, G., 2012. The ERBB network: at last, cancer therapy meets systems biology. *Nature Reviews Cancer*, 12(8), p.553.
5. Kruthika, B.S., Jain, R., Arivazhagan, A., Bharath, R.D., Yasha, T.C., Kondaiah, P. and Santosh, V., 2019. Transcriptome profiling reveals PDZ binding kinase as a novel biomarker in peritumoral brain zone of glioblastoma. *Journal of neuro-oncology*, 141(2), pp.315-325.
6. Gusev, Y., Bhuvaneshwar, K., Song, L., Zenklusen, J.C., Fine, H. and Madhavan, S., 2018. The REMBRANDT study, a large collection of genomic data from brain cancer patients. *Scientific data*, 5, p.180158.
7. Kunze, M., Malkani, N., Maurer-Stroh, S., Wiesinger, C., Schmid, J.A. and Berger, J., 2015. Mechanistic Insights into PTS2-mediated Peroxisomal Protein Import THE CO-RECEPTOR PEX5L DRASTICALLY INCREASES THE INTERACTION STRENGTH BETWEEN THE CARGO PROTEIN AND THE RECEPTOR PEX7. *Journal of Biological Chemistry*, 290(8), pp.4928-4940.
8. Harper, C.C., Berg, J.M. and Gould, S.J., 2003. PEX5 binds the PTS1 independently of Hsp70 and the peroxin PEX12. *Journal of Biological Chemistry*, 278(10), pp.7897-7901.
9. Davis, M.E., 2016. Glioblastoma: overview of disease and treatment. *Clinical journal of oncology nursing*, 20(5), p.S2.
10. Alexander, B.M. and Cloughesy, T.F., 2017. Adult glioblastoma. *Journal of Clinical Oncology*, 35(21), pp.2402-2409.
11. DeVita, V.T. and Chu, E., 2008. A history of cancer chemotherapy. *Cancer research*, 68(21), pp.8643-8653.
12. Chabner, B.A. and Roberts Jr, T.G., 2005. Chemotherapy and the war on cancer. *Nature Reviews Cancer*, 5(1), p.65.
13. Kitano, H., 2007. A robustness-based approach to systems-oriented drug design. *Nature reviews Drug discovery*, 6(3), p.202.

14. Stupp, R., Mason, W.P., Van Den Bent, M.J., Weller, M., Fisher, B., Taphoorn, M.J., Belanger, K., Brandes, A.A., Marosi, C., Bogdahn, U. and Curschmann, J., 2005. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *New England Journal of Medicine*, 352(10), pp.987-996.
15. McGirt, M.J., Than, K.D., Weingart, J.D., Chaichana, K.L., Attenello, F.J., Olivi, A., Latta, J., Kleinberg, L.R., Grossman, S.A., Brem, H. and Quiñones-Hinojosa, A., 2009. Gliadel (BCNU) wafer plus concomitant temozolomide therapy after primary resection of glioblastoma multiforme. *Journal of neurosurgery*, 110(3), pp.583-588.
16. Affronti, Mary Lou, Christopher R. Heery, James E. Herndon, Jeremy N. Rich, David A. Reardon, Annick Desjardins, James J. Vredenburgh, Allan H. Friedman, Darell D. Bigner, and Henry S. Friedman. "Overall survival of newly diagnosed glioblastoma patients receiving carmustine wafers followed by radiation and concurrent temozolomide plus rotational multiagent chemotherapy." *Cancer: Interdisciplinary International Journal of the American Cancer Society* 115, no. 15 (2009): 3501-3511.
17. National Comprehensive Cancer Network, 2015. Clinical Practice Guidelines in Oncology: Central nervous system cancers [v. 1.2015].
18. Amery, L., Hideki, S.A.N.O., MANNAERTS, G.P., SNIDER, J., FRANSEN, M. and van VELDHoven, P.P., 2001. Identification of PEX5p-related novel peroxisome-targeting signal 1 (PTS1)-binding proteins in mammals. *Biochemical Journal*, 357(3), pp.635-646.
19. Saito, R., Bringas, J.R., Panner, A., Tamas, M., Pieper, R.O., Berger, M.S. and Bankiewicz, K.S., 2004. Convection-enhanced delivery of tumor necrosis factor-related apoptosis-inducing ligand with systemic administration of temozolomide prolongs survival in an intracranial glioblastoma xenograft model. *Cancer research*, 64(19), pp.6858-6862.
20. Giannini, C., Sarkaria, J.N., Saito, A., Uhm, J.H., Galanis, E., Carlson, B.L., Schroeder, M.A. and James, C.D., 2005. Patient tumor EGFR and PDGFRA gene amplifications retained in an invasive intracranial xenograft model of glioblastoma multiforme. *Neuro-oncology*, 7(2), pp.164-176.

Rank	Gene symbol	Gene Name	p-value	t	B	ID
43	PEX5L	peroxisomal biogenesis factor 5 like	3.57E-15	17.1407742	24.729498	ILMN_1722037

Table 1: PEX5L is among the most differentially expressed genes in the tumors of patients with glioblastoma multiform.

Rank of differential expression, gene symbol, name of the gene whose transcript was identified as differentially expressed, p-value with respect to global differential expression (relative to the transcriptome), t, a moderated t-statistic, and B, the log-odds of differential expression between the two groups is shown (all provided by GEO2R).

Figure 1: PEX5L is expressed at significantly lower levels in glioblastoma tumors compared to non-affected control brain tissue.

The RNA expression value of PEX5L is depicted graphically, by dots representing RNA expression in each patient tumor or control brain tissue (left), by bar graph with standard error of the mean (SEM) (middle), or by box and whisker plot (right). The significance of difference between the mean expression values in tumor versus control group is shown on each graph as a *p*-value, determined by a two-tailed unpaired t-test with Welch's correction.

Rank	ID	p-value	t	B	Gene	Gene name
26189	222910_s_at	7.11E-03	-2.7138841	-3.4733	PEX5L	peroxisomal biogenesis factor 5 like

Table 2: Statistically significant differential expression of PEX5L in glioblastoma tumors from the REMBRANDT study.

The rank of PEX5L differential expression, probe/transcript ID, *p*-value with respect to global differential expression, t-statistic, B, the log-odds of differential expression between the two groups (tumor and control), gene symbol and gene name are listed in this chart.