Differential expression of protein phosphatase PPM1B and multiple protein phosphatase regulatory subunits in glioblastoma.

Shahan Mamoor
shahanmamoor@gmail.com
San Diego, CA 92901

Glioblastoma multiforme is an aggressive brain cancer with few treatment options and poor survival outcomes (1, 2). We used a public dataset (3) containing gene expression data from tumors of patients diagnosed with glioblastoma and compared it to that of the healthy brain understand the most significant differences in the transcriptional behavior of glioblastoma tumors relative to the tissue from which it arises. We integrated those findings with a similar analysis of glioblastoma tumors from the REMBRANDT brain cancer database (4). We found that protein phosphatase PPM1B and three protein phosphatase regulatory subunits were among the genes whose expression was most different between glioblastoma tumors and “normal” brain tissue. The fact that multiple phosphatase regulatory genes are expressed at significantly lower levels in glioblastoma tumors suggests that alteration of substrate phosphorylation might be an important event in glioblastoma formation, maintenance or progression.

Keywords: glioblastoma multiforme, systems oncology, differential gene expression analysis, PPM1B, PPM1R16B, PPP4R4, PPP1R3F

Introduction

Glioblastoma multiforme is a cancer of the brain with a dismal prognosis (1, 2). Standard treatment consists of temozolomide and polifeprosan 20 with carmustine, an implantable wafer containing a mustard-based chemotherapy, as well as radiation therapy (5-8). The lack of targeted chemotherapeutic options demands continued discovery of the basic manner in which glioblastoma tumors function (8-11), and this includes understanding the gene expression profiles of the glioblastoma tumor in humans. In this study, we used a microarray dataset (3, 4) containing transcriptional data from tumors of patients diagnosed with glioblastoma as well as total transcriptional data from the brain tissue of non-affected subjects as control for reference comparison. We identified three independent protein phosphatases regulatory subunits and one protein phosphatase that were differentially expressed in glioblastoma tumors and uniformly expressed at significantly lower levels that of healthy brain tissue. Upon replication in multiple cohorts and validation at the protein level, if these differentially expressed protein phosphatase family members are cancer-specific dependencies, these data can be used towards the development of novel therapeutics for glioblastoma multiforme.

Methods

GSE116520 (3) and GSE108474 (4) 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

Protein phosphatase PPM1B and three protein phosphatase regulatory subunits are among the genes most differentially expressed in the tumors of patients with glioblastoma multiform.

We performed differential gene expression analysis to understand the transcriptional behavior of glioblastoma tumors and how it differs from that of normal brain tissue. We compared the transcriptomes of 17 glioblastoma tumors from human patients to that of 8 control human brain tissue samples (3). This analysis revealed that a protein phosphatase, PPM1B, and three separate protein phosphatase regulatory subunits were among the genes most differentially expressed by glioblastoma tumors (Table 1).

Out of 47229 genes, PPM1R16B, protein phosphatase 1 regulatory subunit 16B, was ranked 103rd most differentially expressed in glioblastoma tumors (Table 1; $p=4.67E-14$). The protein phosphatase 4 regulatory subunit 4, PPP4R4, was ranked 117th out of 47229 total transcripts detected and measured (Table 1; $p=6.65E-14$). Protein phosphatase 1 regulatory subunit 3, PPP1R3F, was ranked 147th most differentially expressed between glioblastoma tumors and normal brain tissue, out of 47229 total transcripts (Table 1; $p=2.04E-13$).

Protein phosphatase Mg^{2+}/Mn^{2+} dependent 1B, PPM1B, was ranked 150th out of 47229 transcripts measured and detected in the dataset analyzed in this study (Table 1; $p=2.08E-13$).

We probed a second dataset (4), data from the REMBRANDT study, to determine whether differential expression of each of protein phosphatase genes could be observed in glioblastoma tumors from a separate group of patients. When sorting all of the genes expressed in glioblastoma tumors based on change in mRNA levels between GBM tumor and normal brain,

PPM1B ranked 15225 out of 54613 transcripts. Differential expression of PPM1B in glioblastoma tumors was statistically significant (Table 2; $p=7.62E-06$). When sorting all of the genes expressed in glioblastoma tumors based on change in mRNA levels between GBM tumor and normal brain, PPP1R16B was ranked 9471 out of 54613 transcripts. Differential expression of PPP1R16B in glioblastoma tumors was statistically significant (Table 2; $p=2.99E-09$). When sorting all of the genes expressed in glioblastoma tumors based on change in mRNA levels between GBM tumor and normal brain, PPP1R3F was ranked 20148 out of 54613 transcripts. Differential expression of PPP1R3F in glioblastoma tumors was statistically significant (Table 2; $p=3.71E-04$). PPP4R4 was not significantly differentially expressed in the REMBRANDT patient tumor dataset.

PPP1R16B, PPP4R4, PPP1R3F, and PPM1B are all expressed at significantly lower levels in the tumors of patients diagnosed with glioblastoma compared to control brain tissue.

Next, we extracted the RNA expression values of each differentially expressed gene, for each independent patient and control brain tissue sample, and performed statistical analyses to determine whether their mean expression levels were significantly different between tumor and control state. The expression of the phosphatase or phosphatase regulatory subunit was lower in tumors than in control brain tissues for all four genes, PPM1B (Figure 1), PPP4R4 (Figure 2), PPP1R3F (Figure 3), and PPP1R16B (Figure 4), and this was statistically significant in each case (PPM1B: Figure 1: $p<0.0001$; PPP4R4: Figure 2: $p<0.0001$; PPP1R3F: Figure 3: $p<0.0001$; PPP1R16B: Figure 4: $p<0.0001$).

Discussion

In this study, we used differential gene expression analysis of two transcriptome datasets to compare global gene expression profiles of tumors from patients with glioblastoma multiforme to that of healthy, control brain tissue. We found that the protein phosphatase

4 OF 15

1 PPM1B, as well as two protein phosphatase regulatory subunits, PP1R3F and PPP1R16B were
2 among the genes whose expression was most significantly different when comparing the
3 transcriptomes of glioblastoma tumors to that of “normal” brain tissue.

4
5 If decreased expression of PPM1B were to be validated in larger cohorts, it would be of
6 value to determine if increasing PPM1B expression would be of therapeutic use in glioblastoma
7 - whether the proliferation or viability of glioblastoma cells would be negatively affected by
8 such PPM1B modulation. PPM1B activity in cells requires N-myristoylation (13), a post-
9 translational modification, wherein a 14-carbon fatty acid known as myristic acid is added by
10 covalent linkage to the amino-terminus of the target protein (14). Myristoylation is performed
11 by the N-myristoyltransferase NMT, and there are many target substrates for NMT in eukaryotic
12 cells (14). Because of its pleiotropic function, NMT inhibition would not likely be a viable option
13 for therapeutic targeting in the case that PPM1B activation proved beneficial in glioblastoma,
14 but targeting the interface between PPM1B and NMT with small molecules or peptides may
15 represent an attractive option.
16

17
18 The fact that two regulatory subunits of protein phosphatases were differentially
19 expressed and among the genes most significantly differentially expressed between
20 glioblastoma tumor tissue and healthy brain tissue suggests that whatever targets that they
21 modulate may benefit from the decreased expression of their regulatory partners. There is little
22 published information available on PPP4R4 (15). PP1R3F, or R3R is a astrocyte cell
23 membrane-associated regulatory subunit for PP1 (16), which has glycogen synthase as one of
24 its targets for dephosphorylation, is involved in the regulation of glycogen metabolism
25 response signals. Decreasing glucose concentration causes increased phosphorylation of
26 PP1R3F, inhibiting glycogen synthase activity and thus decreasing glycogen synthesis, which is
27 a source of glucose for astrocytoma cells (16). PPP1R16B (also known as TIMAP) is another
28 regulatory subunit for protein phosphatase 1 (PP1); it is produced in endothelial and

1 hematopoietic cells in response to TGF-beta signaling (17) and like PP1R3F is also involved in
2 glycogen metabolism through glycogen synthase kinase-3beta (GSK-3B) (18). It has also been
3 shown to play role a in regulating PP1 activity towards the laminin receptor (19), as well as in
4 enhancing barrier function in pulmonary endothelial cells through myosin phosphatase and
5 moesin via forskolin-mediated cAMP/PKA signaling which dephosphorylates moesin at the cell
6 edges (20). In the absence of PP1R16B, forskolin does not affect moesin phosphorylation, and
7 agents like thrombin have a greater affect on barrier compromise than in PP1R16B-sufficient
8 states (20).
9

10 We conducted a global differential gene expression analysis to understand the most
11 significant transcriptional differences between glioblastoma tumors and the brain tissue from
12 which they arise. We found two protein phosphatase subunits,
13 PPP1R16B and PP1R3F, as well as the PPM1B protein phosphatase were among the most
14 significantly differentially expressed genes in the glioblastoma transcriptome. Assessing
15 whether activation of PPM1B, PPP4R4, PPP1R16B or PP1R3F function would be deleterious
16 to glioblastoma viability or proliferation in vitro, wether this effect possesses any sort of
17 specificity towards cancer cells or glioblastoma cells in specific, and whether the inhibition of
18 any of their gene products would have an effect on tumor maintenance or metastasis in
19 xenograft mouse models of glioblastoma (21, 22) is an important next step towards the
20 development of novel, targeted therapeutics for an aggressive brain cancer with few treatment
21 options and a median survival of 15 months (23).
22
23
24
25
26
27
28

References

1. Davis, M.E., 2016. Glioblastoma: overview of disease and treatment. *Clinical journal of oncology nursing*, 20(5), p.S2.
2. Alexander, B.M. and Cloughesy, T.F., 2017. Adult glioblastoma. *Journal of Clinical Oncology*, 35(21), pp.2402-2409.
3. 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.
4. 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.
5. 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.
6. McGirt, M.J., Than, K.D., Weingart, J.D., Chaichana, K.L., Attenello, F.J., Olivi, A., Lattera, 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.
7. 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.
8. National Comprehensive Cancer Network, 2015. Clinical Practice Guidelines in Oncology: Central nervous system cancers [v. 1.2015].
9. Brennan, C.W., Verhaak, R.G., McKenna, A., Campos, B., Nounmehr, H., Salama, S.R., Zheng, S., Chakravarty, D., Sanborn, J.Z., Berman, S.H. and

- 1 Beroukhim, R., 2013. The somatic genomic landscape of glioblastoma. *Cell*,
2 155(2), pp.462-477.
- 3 10. Parsons, D.W., Jones, S., Zhang, X., Lin, J.C.H., Leary, R.J., Angenendt, P.,
4 Mankoo, P., Carter, H., Siu, I.M., Gallia, G.L. and Olivi, A., 2008. An
5 integrated genomic analysis of human glioblastoma multiforme. *Science*,
6 321(5897), pp.1807-1812.
- 7 11. Ohgaki, H. and Kleihues, P., 2007. Genetic pathways to primary and
8 secondary glioblastoma. *The American journal of pathology*, 170(5), pp.
9 1445-1453.
- 10 12. Wang, J., Cazzato, E., Ladewig, E., Frattini, V., Rosenbloom, D.I., Zairis, S.,
11 Abate, F., Liu, Z., Elliott, O., Shin, Y.J. and Lee, J.K., 2016. Clonal evolution of
12 glioblastoma under therapy. *Nature genetics*, 48(7), p.768.
- 13 13. Chida, T., Ando, M., Matsuki, T., Masu, Y., Nagaura, Y., Takano-Yamamoto, T.,
14 Tamura, S. and Kobayashi, T., 2013. N-Myristoylation is essential for protein
15 phosphatases PPM1A and PPM1B to dephosphorylate their physiological
16 substrates in cells. *Biochemical Journal*, 449(3), pp.741-749.
- 17 14. Farazi, T.A., Waksman, G. and Gordon, J.I., 2001. The biology and
18 enzymology of protein N-myristoylation. *Journal of biological*
19 *chemistry*, 276(43), pp.39501-39504.
- 20 15. Huang, J., Jiang, W., Tong, X., Zhang, L., Zhang, Y. and Fan, H., 2019.
21 Identification of gene and microRNA changes in response to smoking in
22 human airway epithelium by bioinformatics analyses. *Medicine*, 98(38).
- 23 16. Kelsall, I.R., Voss, M., Munro, S., Cuthbertson, D.J. and Cohen, P.T., 2011.
24 R3F, a novel membrane-associated glycogen targeting subunit of protein
25 phosphatase 1 regulates glycogen synthase in astrocytoma cells in response
26 to glucose and extracellular signals. *Journal of neurochemistry*, 118(4), pp.
27 596-610.
- 28 17. Cao, W., Mattagajasingh, S.N., Xu, H., Kim, K., Fierlbeck, W., Deng, J.,
Lowenstein, C.J. and Ballermann, B.J., 2002. TIMAP, a novel CAAX box
protein regulated by TGF- β 1 and expressed in endothelial cells. *American*
Journal of Physiology-Cell Physiology, 283(1), pp.C327-C337.
18. Li, L., Kozlowski, K., Wegner, B., Rashid, T., Yeung, T., Holmes, C. and
Ballermann, B.J., 2007. Phosphorylation of TIMAP by glycogen synthase

kinase-3 β activates its associated protein phosphatase 1. *Journal of Biological Chemistry*, 282(35), pp.25960-25969.

19. Kim, K., Li, L., Kozlowski, K., Suh, H.S., Cao, W. and Ballermann, B.J., 2005. The protein phosphatase-1 targeting subunit TIMAP regulates LAMR1 phosphorylation. *Biochemical and biophysical research communications*, 338(3), pp.1327-1334.
20. Csontos, C., Czikora, I., Bogatcheva, N.V., Adyshev, D.M., Poirier, C., Olah, G. and Verin, A.D., 2008. TIMAP is a positive regulator of pulmonary endothelial barrier function. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 295(3), pp.L440-L450.
21. 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.
22. 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.
23. Thakkar, J.P., Dolecek, T.A., Horbinski, C., Ostrom, Q.T., Lightner, D.D., Barnholtz-Sloan, J.S. and Villano, J.L., 2014. Epidemiologic and molecular prognostic review of glioblastoma. *Cancer Epidemiology and Prevention Biomarkers*, 23(10), pp.1985-1996.

Rank	Gene Symbol	Gene Name	p-value	t	B
103	PPP1R16B	protein phosphatase 1 regulatory subunit 16B	4.67E-14	15.2923106	22.228482
117	PPP4R4	protein phosphatase 4 regulatory subunit 4	6.65E-14	15.0512392	21.881815
147	PPP1R3F	protein phosphatase 1 regulatory subunit 3F	2.04E-13	14.3093678	20.782826
150	PPM1B	protein phosphatase, Mg2+/ Mn2+ dependent 1B	2.08E-13	14.2971655	20.764332

Table 1: Protein phosphatase PPM1B and three protein phosphatase regulatory subunits are among the genes most differentially expressed 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: Protein phosphatase PPM1B is expressed at significantly lower levels in the tumors of patients with glioblastoma compared to non-affected control brain tissue.

The RNA expression value of PPM1B is depicted graphically, by dots representing RNA expression in each patient tumor or control brain tissue (A), by bar graph with standard error of the mean (SEM) (B), or by box and whisker plot (C). 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.

Figure 2: Protein phosphatase regulatory subunit PPP4R4 is expressed at significantly lower levels in the tumors of patients with glioblastoma compared to non-affected control brain tissue.

The RNA expression value of PPP4R4 is depicted graphically, by dots representing RNA expression in each patient tumor or control brain tissue (A), by bar graph with standard error of the mean (SEM) (B), or by box and whisker plot (C). 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.

Figure 3: Protein phosphatase regulatory subunit PP1R3F is expressed at significantly lower levels in the tumors of patients with glioblastoma compared to non-affected control brain tissue.

The RNA expression value of PP1R3F is depicted graphically, by dots representing RNA expression in each patient tumor or control brain tissue (A), by bar graph with standard error of the mean (SEM) (B), or by box and whisker plot (C). 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.

Figure 4: Protein phosphatase regulatory subunit PPP1R16B is expressed at significantly lower levels in the tumors of patients with glioblastoma compared to non-affected control brain tissue.

The RNA expression value of PPP1R16B is depicted graphically, by dots representing RNA expression in each patient tumor or control brain tissue (A), by bar graph with standard error of the mean (SEM) (B), or by box and whisker plot (C). 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
9471	233813_at	2.99E-09	6.1533398	10.47916	PPP1R16B	protein phosphatase 1 regulatory subunit 16B
15225	213225_at	7.62E-06	4.5714957	2.91275	PPM1B	protein phosphatase, Mg2+/Mn2+ dependent 1B
20148	229365_at	3.71E-04	-3.6090198	-0.7624	PPP1R3F	protein phosphatase 1 regulatory subunit 3F

Table 2: PPM1B, PPP1R16B, and PP1R3F and RAB11FIP2 are significantly differentially expressed genes in glioblastoma tumors from the REMBRANDT patient dataset.

The rank of differential expression, probe/transcript ID, gene symbol, gene name, *p*-value with respect to global differential expression, t-statistic, B, the log-odds of differential expression between the two groups (tumor and control), as well as Illumina probe ID (all provided by GEO2R) are shown here.