PHF24 is differentially expressed in the tumors of patients with glioblastoma.

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Epigenetic molecules represent attractive therapeutic targets in cancer (1, 2). We performed comparative transcriptome analysis of tumors from patients diagnosed with glioblastoma multiforme relative to the non-affected brain transcriptome using published microarray data (3, 4). The PHD-finger protein PHF24 was among the most differentially expressed genes in the glioblastoma transcriptome. Glioblastoma has a median survival of 15 months and there are zero available targeted treatment options for the disease (5, 6). Efforts should be prioritized towards understanding whether glioblastoma possesses any cancer-specific dependencies on PHF24.

Keywords: glioblastoma, differential gene expression analysis, systems oncology, targeted cancer therapeutics, tumor transcriptome analysis, PHF24, PHD fingers in cancer, cancer epigenetics

Introduction

Glioblastoma is the most common brain cancer in adults and one of the more deadly cancers a human being can develop (5, 6). Within 15 months, nearly half of those who had been diagnosed with the disease will have expired from it (5, 6). Treatment, other than surgical techniques to debulk the mass if operable, include temozolomide and an implantable wafer containing a mustard chemotherapy called polifeprosan 20 with carmustine (7-9). There are no targeted treatment options available for glioblastoma.

Systems-level interrogations of the genome, transcriptome, or proteome in an unbiased fashion have the potential to unleash critical details by which cancer cells function, survive, and evade treatment (10-12). We used differential gene expression analysis to compare the transcriptomes of 238 glioblastoma tumors in total obtained with that of benign brain tissue using published microarray data in two separate analyses (3, 4). Our analyses revealed that the epigenetic modulator and PHD-finger protein 24, PHF24, was one of the messages whose expression was most different between glioblastoma tumor and non-affected brain tissue.

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

PHF24 is differentially expressed in glioblastoma tumors

We compared the glioblastoma transcriptome to that of the "normal" brain by global differential gene expression analysis of a microarray dataset generated using tumors isolated from 17 patients diagnosed with glioblastoma multiform and 8 control, non-affected brain samples as reference transcriptome. PHF24 was the 42nd most differentially expressed gene in the glioblastoma transcriptome when compared to the genes expressed in control brain tissue, out of 47229 genes (Table 1). This was statistically significant (Table 1; $p=3.33E-15$).

We probed a second dataset (4), tumor microarray data from the REMBRANDT study, a collection of genomic data from patients with brain cancers, to determine whether differential expression of PHF24 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, PHF24 ranked 4322 out of 54613 transcripts. Differential expression of PHF24 in REMBRANDT study glioblastoma tumors was statistically significant (Table 2; $p=4.03E-15$).

PHF24 expression is significantly lower than in glioblastoma tumors than in the brain

We next extracted RNA expression values for the PHF24 transcript that was differentially expressed in the first dataset interrogated (3), for each individual tumor and in each control brain tissue sample. A statistical test was performed to determine if the difference between the expression level in tumor versus benign conditions was significant. The expression level of

PHF24 was lower in glioblastoma tumors than in control, non-affected brain tissue (Figure 1). This was statistically significant ($p < 0.0001$).

Discussion

The existing chemotherapeutic regimens for glioblastoma provide at best a 15 month median survival rate (1, 2). This includes an implantable wafer containing a nitrogen mustard-based drug (5, 6). Glioblastoma is the most common brain tumor in adults. There is an alarming need for a better understanding of the transcriptional behavior of glioblastoma tumors and how it differs from that of the brain in order to develop novel and targeted therapeutics for this disease. In this study we performed global differential gene expression analysis, comparing the total set of genes expressed in the glioblastoma tumors to the total set of genes expressed in healthy brain tissue (3, 4). In one dataset, PHF24 was the 42nd most differentially expressed gene out of 47229 transcripts whose expression was measured (3).

There is scarce literature regarding the gene product of PHD24, or PHD (plant homeodomain) finger-containing protein. In general, PHD fingers are nuclear interaction domains which function as “readers” of epigenetic marks (13, 14). In CHD4, the PHD-finger is a reader of the unmodified H3K4 and methylated H3K9 marks (15). In ING2, the PHD-finger is a reader of the trimethylated H3K4 mark. In RAG2, the PHD-finger is a reader for methylated H3K4 and H3R2 (16), and this connects reading of an epigenetic mark to VDJ recombination (17). The PHD-finger of NURF directly interacts with, or reads, the trimethylated H3K4 tail (18). In PHF2, the PHD-finger reads the trimethylated H3K4 mark and this interaction is important for the histone demethylation (19). PHF23, also known as JARID1A, is fused to nucleoporin 98 in acute myeloid and lymphoblastic leukemias (20, 21), and the gene product of the fusion results in acute myeloid leukemia formation in mouse models, and the PHD-finger is required for the leukemogenesis (22).

1 Epigenetic therapies are an emerging field in oncology pharmaceuticals (1, 2, 23, 24).
2 This is the first report of PHF24 perturbation in glioblastoma. The effect of activating PHF24
3 expression in glioblastoma cell lines should first be assessed by proliferation assays, as well as
4 in control glial cells. The effect of genetic over-expression of PHF24 in glioblastoma cell lines
5 xenografts into orthotopic mouse models of glioblastoma (25, 26) should then be evaluated. If
6 these results prove successful, and if the differential and significantly decreased expression of
7 PHF24 is validated in larger cohorts and on the protein level, PHF24 would be represent a
8 targeted treatment for glioblastoma multiforme.
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Rank	Gene symbol	Gene name	p-value	t	B	ID
42	PHF24	PHD finger protein 24	3.33E-15	17.1937409	24.797308	ILMN_3246625

Table 1: PHF24 is one of the most differentially expressed gene 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), as well as the Illumina probe ID.

Figure 1: PHF24 is expressed at significantly lower levels in glioblastoma tumors compared to non-affected control brain tissue.

The RNA expression value of PHF24 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
4311	213636_at	4.03E-15	8.3727552	23.69503	PHF24	PHD finger protein 24

Table 2: Statistically significant differential expression of PHF24 in glioblastoma tumors from the REMBRANDT study.

The rank of PHF24 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.