Histone deacetylase 5 (HDAC5) is differentially expressed in the tumors of patients with glioblastoma.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

Histone deacetylases are epigenetic enzymes that are increasingly being developed as targets in oncology (1, 2). Glioblastoma is the most common brain tumor in adults and offers a 15 months median survival to those diagnosed with it (3, 4). We performed global differential gene expression analysis on tumors isolated from the brains of patients with glioblastoma using published microarray data (5, 6). We used benign brain tissue as a comparison for the glioblastoma transcriptome (5, 6). With these analysis, we found that the Class IIa histone deacetylase HDAC5 (7-9) was among the most differentially expressed genes in the glioblastoma tumor transcriptome. We observed decreased expression of HDAC5 in the primary tumors of patients with glioblastoma (10). HDAC5 can be assessed as a therapeutic target in glioblastoma multiforme.

Keywords: glioblastoma multiforme, glioblastoma, systems oncology, HDAC5, targeted cancer therapeutics, tumor transcriptome analysis.

1 Glioblastoma multiforme is the most common brain cancer in adults (3, 4). Diagnosis
2 with glioblastoma carries a 15 month median survival prognosis (3, 4). Standard of care
3 includes the chemotherapy temozolomide and an implantable wafer containing a nitrogen-
4 mustard, polifeprosan 20 with carmustine, in addition to radiation therapy and surgery if the
5 tumor is operable (10-13). The dismal outlook for those diagnosed with glioblastoma demands
6 a thorough and basic understanding of the transcriptional nature of glioblastoma tumors and
7 how they most differ from the tissue in which they arise, the brain. Towards this end, we
8 performed global differential gene expression profiling by comparing the transcriptomes of
9 tumors from patients with glioblastoma with that of control brain tissues using published
10 microarray data (5, 6). We identified HDAC5 as one of the genes whose expression was most
11 different between glioblastoma and benign brain tissue.
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13

14 **Methods**

15 GSE116520 (5) and GSE108474 (6) were also used for this differential gene expression
16 analysis, in conjunction with GEO2R. The transcriptional data from GSE116520, GBM tumor
17 from 17 patients diagnosed with glioblastoma multiform and normal brain from 8 controls, was
18 generated by Kruthika et al. using Illumina HumanHT-12 V4.0 expression beadchip technology.
19 The transcriptional data from GSE108474, GBM tumor from 221 patients diagnosed with
20 glioblastoma multiform and normal brain tissue from 28 controls, was generated by Gusev et al.
21 using Affymetrix Human Genome U133 Plus 2.0 Array technology. The *p*-value adjustment
22 option was set to “Benjamini and Hochberg”, application of log transformation to data was set to
23 “Auto-detect” and the NCBI generated category of platform annotation was used. Statistical
24 tests to compare the significance of RNA expression values between tumor and control groups
25 was performed using a two-tailed, unpaired t-test with Welch’s correction (PRISM 8.1.2) (227).
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Results

We performed global differential gene expression analysis by comparing the transcriptomes of glioblastoma tumors to the transcriptome of non-affected, benign control brain tissue (5).

HDAC5 is differentially expressed in the tumors of patients with glioblastoma when compared to non-affected benign brain tissue.

Of 47229 genes whose expression was compared between the reference brain transcriptome and glioblastoma tumor transcriptome, HDAC5 was the sixth most differentially expressed gene. Statistically, this was significant (Table 1; $p=2.64E-18$).

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 HDAC5 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, HDAC5 ranked 9885 out of 54613 transcripts. Differential expression of HDAC5 in REMBRANDT study glioblastoma tumors was statistically significant (Table 2; $p=6.12E-09$).

HDAC5 is expressed at significantly lower levels in glioblastoma tumors when compared to the brain.

Next, we obtained the exact mRNA expression values for HDAC5 transcript in each of the glioblastoma tumor samples, as well as the reference brain tissue samples. We compared the mean expression values of HDAC5 between tumor and control groups, and performed a statistical test to determine if this difference in mRNA expression was statically significant. HDAC5 was expressed at lower levels in glioblastoma tumors than in control brain tissue, and this was statistically significant (Figure 1; $p=0.0001$).

Discussion

To understand the glioblastoma transcriptome in a systematic, unbiased fashion, we performed global differential gene expression analysis by comparing the transcriptomes of glioblastoma tumor samples to that of normal brain tissue as reference. We found that the class IIa histone deacetylase HDAC5 was among the most differentially expressed genes in the glioblastoma tumor transcriptome. In one dataset, out of 47229 genes, it was the sixth most differentially expressed gene when comparing the global gene expression profiles of glioblastoma tumors and brain tissue.

One previous report investigated HDAC5 as a potential gene of interest in glioblastoma but concluded that any association they found between HDAC5 and glioblastoma was not a statistically significant (10). Their statistically insignificant association was also one of higher HDAC5 expression in glioblastoma (10). Not only have we found that HDAC5 is expressed in lower levels in glioblastoma tumors than in brain tissue, but our findings are also statically significant.

HDACs are an emerging target in the field of oncology (1, 2). Though there is an FDA-approved HDAC inhibitor available (15), the biology of HDAC5 in glioblastoma that we have uncovered here would not be amenable to inhibition as it is expressed at lower levels in tumors than in benign tissue. If validated in separate patient datasets, over-expression, rather, might be an effective therapeutic strategy in glioblastoma. Theoretically, this could be delivered by lentiviral expression as in gene therapy (16) or as a CAR-styled viral vector (17). Another therapeutic strategy could be inhibiting a negative regulator of HDAC5 or HDAC5 activity (18). In myocytes, salt-inducible kinase SIK1 is a negative regulator of HDAC5 activity (19). If this proved to be the case in glioblastoma tissues as well, then inhibition of SIK1 as a means of activating HDAC5 could be an effective therapeutic strategy. This is predicated upon the basis that activation of HDAC5 would have a beneficial effect on tumor proliferation, survival or

1 progression. This could be tested *in vitro* by proliferation assay or *in vivo* using a glioblastoma
2 xenograft mouse model (20, 21).

3 We found that HDAC5 was among the genes most differentially expressed in
4 glioblastoma. It was the sixth most differentially expressed gene in the glioblastoma
5 transcriptome when compared to the brain transcriptome in one dataset. We used an
6 unbiased, systematic analysis of primary glioblastoma tumors from human patients to perform
7 this analysis. The viability of HDAC5 as a novel therapeutic target should be assessed by over-
8 expression *in vitro* and *in vivo*. HDAC5 may represent a promising pathway for the
9 development of novel and targeted therapeutics in glioblastoma multiforme.
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Rank	p-value	t	B	Gene symbol	Gene name	ID
6	2.64E-18	23.3934752	31.536721	HDAC5	histone deacetylase 5	ILMN_1810856

Table 1: HDAC5 is differentially expressed in the tumors of patients with glioblastoma when compared to non-affected benign brain tissue.

The rank of differential expression, 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, gene name, as well as Illumina probe ID (all provided by GEO2R) are shown here.

Figure 1: HDAC5 is expressed at significantly lower levels in glioblastoma tumors when compared to the brain.

The mRNA expression level of HDAC5 is graphically represented by dots denoting the expression level in each individual patient and the horizontal black bar representing the mean expression value (A); as a bar graph with the error bars denoting the standard error of the mean (B); and as box and whisker plots from minimum to maximum value (C).

Rank	ID	p-value	t	B	Gene	Gene name
9885	229408_at	6.12E-09	6.0216734	9.78367	HDAC5	histone deacetylase 5

Table 2: Statistically significant differential expression of HDAC5 in glioblastoma tumors from the REMBRANDT study.

The rank of HDAC5 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.