MUTYH is differentially expressed in glioblastoma.

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Glioblastoma is the most common brain cancer in adults (1, 2). The poor prognostic outlook for patients diagnosed with glioblastoma demands an enhanced understanding of the basic transcriptional nature of glioblastoma tumors (1, 2). In this study we compared global gene expression profiles of glioblastoma tumors to that of the brain in health using published microarray data (3, 4). We found that mutY DNA glycosylase, or MUTYH was among the genes whose expression was most different between the transcriptomes of glioblastoma tumors and non-affected brain tissue. Mutations in MUTYH have been identified in pediatric gliomas (5) and this is the first report of perturbed differential expression of MUTYH in primary human glioblastoma tumors.

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

Glioblastoma is a brain cancer that arises from the glial cells of the brain (1, 2). Standard of care treatment includes surgery if the tumor is operable, radiation therapy, and the drugs temolozide and polifeprosan 20 with carmustine, an implantable wafer with a mustard-based chemotherapy (6-9). Even with these toxic treatment regimens, the median survival for patients diagnosed with glioblastoma is 15 months (1, 2). Improving the quality of life for patients with glioblastoma requires an enhanced understanding of the basic biology of glioblastoma tumors. Systems-level transcriptome analysis can provide this understanding. In this study, we performed global differential gene expression analysis in human glioblastoma by comparing the transcriptomes of 238 tumors in total isolated from patients with glioblastoma to the brain transcriptome in health using independently published microarray data (3, 4). We found that MUTYH, a DNA glycosylase, was one of these genes whose expression was most different between the glioblastoma transcriptome and the brain in health.

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

We performed global differential gene expression analysis in humans with glioblastoma by comparing the transcriptomes of 17 tumors isolated from patients with glioblastoma with that of the brain transcriptome from 8 samples of non-affected, benign control brain tissue.

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

We found that MUTYH was the 217th most differentially expressed gene, out of 47229 total transcripts in the glioblastoma transcriptomes relative to the brain transcriptome in health. This was statistically significant (Table 1; $p=1.09E-12$).

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 MUTYH 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, MUTYH ranked 4271 out of 54613 transcripts. Differential expression of MUTYH in REMBRANDT study glioblastoma tumors was statistically significant (Table 2; $p=3.5E-15$).

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

Next, we extracted the exact mRNA expression levels for the MUTYH transcript for each patient tumor and for each control brain tissue sample from the published dataset that we utilized (3), to compare the mean expression level of MUTYH in tumor and control groups. We also performed a statistical test to determine whether the difference in MUTYH expression between tumor and control groups was statically significant. Expression of MUTYH was lower in glioblastoma tumors than in control brain tissue (Figure 1). The decreased expression of

MUTYH in the tumors of patients with glioblastoma was statistically significant (Figure 1; $p < 0.0001$).

In sum, in one microarray dataset (3), the MUTYH transcript was among the top 1% of transcripts differentially expressed by glioblastoma tumors as compared to “normal” brain tissue from non-affected individuals, and it was expressed at significantly lower levels in the tumors of patients with glioblastoma as compared to non-affected, benign brain tissue. We observed statistically significant differential expression of MUTYH in a second dataset (4).

Discussion

Glioblastoma is the most common brain cancer in adults (1, 2). There are zero targeted treatment options and even with the use of chemotherapy and radiation, there is a 15 month median survival. The lack of treatment options and a dismal prognostic outlook demand an enhanced understanding of the transcription nature of the glioblastoma tumor. We performed such an analysis here and found that the DNA glycosylase MUTYH was among the genes whose expression was most different between glioblastoma tumors and the non-affected, benign brain.

MUTYH is a base excision repair (BER) glycosylase that functions in repairs of oxidative damage, specifically oxidation of guanine residues leading to 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxoG) (10). 8-oxoG will pair with A, causing a mismatch, and in the following round of DNA replication, this mismatch causes a G:C to T:A transversion event. MUTYH is able to recognize the 8-oxoG mismatch, and excise the involved adenine base by base-flipping. Following the activity of DNA polymerases that generate an oxoG:C pair, a separate BER glycosylase, OGG1, can replace the oxidized guanine residue with guanine (10).

A mutation of MUTYH at codon 466, resulting in a truncated protein, was found in a 6-year old patient diagnosed with WHO grade IV, K3-K27M mutant, spinal cord glioblastoma on resection (11).

1 Mutation of MUTYH in MUTYH-associated polyposis, or MAP, results in a virtually
2 100% lifetime risk of developing colorectal cancer (12).

3 We found that MUTYH was expressed at significantly lower levels in the tumors of
4 patients with glioblastoma compared to the non-affected, benign brain. Mutation of MUTYH is
5 associated with colorectal cancers (12), it has been found to be mutated in pediatric gliomas
6 and in individual patients with spinal cord glioblastoma (11), and here, we have found
7 transcriptome-wide significant differential and decreased expression of MUTYH in glioblastoma
8 tumors in adults. The effect of MUTYH over-expression in glioblastoma cell lines *in vitro* by
9 proliferation assay, and *in vivo* using xenograft mouse models (13, 14) to assess the effect of
10 such over-expression on tumor maintenance and progression and overall survival should be
11 performed. Activation or over-expression of MUTYH may represent a novel and targeted
12 therapeutic option in glioblastoma.
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Figure 1: MUTYH is expressed at significantly lower levels in glioblastoma tumors when compared to the brain.

The mRNA expression level of MUTYH 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); as box and whisker plots from minimum to maximum value (C), and as a violin plot with the dotted black horizontal line representing the median value (D) .

Rank	ID	p-value	t	B	Gene symbol	Gene name
217	ILMN_2277537	1.09E-12	13.2521952	19.127753	MUTYH	mutY DNA glycosylase

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

The rank of 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.

Rank	ID	p-value	t	B	Gene	Gene name
4271	207727_s_at	3.5E-15	-8.3939989	23.83343	MUTYH	mutY DNA glycosylase

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

The rank of MUYTH 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.