1 **Differential expression of RAB11FIP2 and RAB11FIP4 in glioblastoma tumors.**

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6 Glioblastoma multiforme is the most common brain cancer in adults, and it offers a median
7 overall survival of 15 months to those diagnosed with it (1, 2). The development of novel and
8 targeted therapies to extend the survival of those diagnosed with glioblastoma requires a basic
9 understanding of the transcriptional behavior of these tumors compared to the tissue from
10 which it arises - the brain. In this study I compared the transcriptomes of 17 glioblastoma
11 tumors to that of brain tissues from 8 non-affected individuals using a published dataset (3). I
12 found that two genes encoding proteins involved in the cellular transport of endocytic vesicles,
13 RAB11FIP2 and RAB11FIP4 were differentially expressed in the tumors of patients with
14 glioblastoma. Both genes were expressed at significantly lower levels in glioblastoma tumors
15 when compared to non-affected brain tissues.

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23 **Keywords:** glioblastoma multiforme, glioblastoma, systems oncology, differential gene
24 expression analysis, RAB11FIP2, RAB11FIP4, tumor transcriptome analysis, targeted cancer
25 therapeutics.

Introduction

Glioblastoma is one of the deadliest maladies known to man and also the most common brain cancer in the United States (1, 2). Those diagnosed with glioblastoma will obtain a 15 month median survival prognosis (1, 2). Standard of care includes the chemotherapy temozolomide and polifeprosan 20 with carmustine, a nitrogen mustard-based implantable wafer, as well as radiation therapy (5-8). The lack of targeted treatments and poor outcomes demand that a basic understanding of the biology of glioblastoma tumors be obtained to identify novel therapeutic targets. With this in mind, we performed global differential gene expression analysis of 17 glioblastoma tumors and 8 non-affected brain samples using a published dataset (3). These data showed that two Rab interacting proteins, RAB11FIP2 and RAB11FIP4 were among the genes whose expression was most different when comparing the transcriptomes of glioblastoma tumors and of the “healthy” brain. Statistically significant differential expression of these genes was observed in a separate microarray dataset from the REMBRANDT study (4). RAB11FIP genes have been implicated in a multitude of cancers (8-19) and this is the first report of perturbation of RAB11FIP genes in glioblastoma.

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

RAB11FIP4 and RAB11FIP2 are differentially expressed in glioblastoma.

We performed differential gene expression analysis in glioblastoma by comparing the transcriptome of 17 glioblastoma tumors isolated from patients diagnosed with glioblastoma to the transcriptomes of 8 non-affected, control brain tissue samples (3). We found by these analyses that two Rab-interacting proteins, RAB11FIP4 and RAB11FIP2 were among the most differentially expressed genes in the glioblastoma transcriptome (Table 1). RAB11FIP4 was the 4th out of 47229 total transcripts detected and measured by the microarray data utilized in this analysis, the 4th most differentially expressed gene in the glioblastoma transcriptome. This was very statistically significant (Table 1; $p=5.79E-20$). RAB11FIP2 was the 197th most differentially expressed gene in the glioblastoma transcriptome, and this was also very significant ($p=6.89E-13$).

We probed a second dataset (4) to determine whether differential expression of each of RAB11FIP2 or RAB11FIP4 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, RAB11FIP2 ranked 2372 out of 54613 transcripts. Differential expression of RAB11FIP2 in glioblastoma tumors was statistically significant (Table 2; $p=3.53E-18$). When sorting all of the genes expressed in glioblastoma tumors based on change in mRNA levels between GBM tumor and normal brain, RAB11FIP4 was ranked 10131 out of 54613 transcripts. Differential expression of RAB11FIP4 in glioblastoma tumors was statistically significant (Table 2; $p=9.01E-09$).

RAB11FIP4 and RAB11FIP2 are expressed at lower levels in glioblastoma tumors than in the brain.

Next, we extracted the expression levels of each RAB11FIP gene in each of the glioblastoma tumors and in the control brain tissue samples, and performed statistical tests to evaluate if the difference in expression between the tumor and control groups was significant. RAB11FIP4 was expressed at lower levels in the tumors of patients with glioblastoma than in the brain, and this was very significant (Figure 2; $p < 0.0001$). RAB11FIP2 was also expressed at significantly lower levels in the tumors of patients with glioblastoma (Figure 1; $p < 0.0001$).

Thus, RAB11FIP2 and RAB11FIP4 were among the genes whose expression was most different between glioblastoma tumors and control, non-affected brain tissues, and the expression of both RAB11FIP2 and RAB11FIP4 was significantly lower in glioblastoma tumors than in non-affected control brain tissue.

Discussion

Glioblastoma presents a poor patient prognosis and there are no targeted treatments available for the most common brain cancer in adults. The identification of therapeutic targets and development of novel targeted chemotherapies requires understanding the basic transcriptional nature of these tumors in relation to the tissue from which it arises - the brain. In this study, we performed such an analysis, comparing the transcriptomes of 17 glioblastoma tumors to 8 control, non-affected brain tissue samples to understand the most significant differences in the global gene expression profiles of glioblastomas as they relate to the “normal”, non-affected brain. Two Rab11 interacting proteins, RAB11FIP2 and RAB11FIP4, were among the genes whose expression was most different in the tumors of patients with glioblastoma and they were both expressed at significantly lower levels in glioblastoma tumors than in the brain.

1 In the cell biology of benign tissues, RAB11FIP2 is involved in transcytosis, the
2 transport of cargoes across the length of a cell (9) and interacts with Myosin 5b in the cellular
3 trafficking of recycling vesicles (10, 11). Rather than supporting the trafficking of these vesicles
4 per se, interaction of RAB11FIP2 with Myosin 5b appears to stabilize endocytic vesicles,
5 tethering them to the cytoskeleton (10). RAB11FIP2 has been shown to be perturbed in
6 colorectal cancers, gastric cancer, breast cancer, non-small cell lung cancer, and in
7 nasopharyngeal cancers (12-17).

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9 RAB11FIP4 was identified as a Rab11 interacting protein that lacked ability to interact
10 with Rab4. It possesses a Rab-binding domain (RBD) within a ERM motif, contained within a
11 coiled-coil secondary structural element (18). The interaction between RAB11FIP4 and Rab11
12 requires GTP and over-expression of RAB11FIP4 lead to condensation of the Rab11
13 compartment in the perinuclear region (18). RAB11FIP4 co-localizes with transferrin, and
14 association of RAB11FIP4 with the endosomal membrane requires its carboxy-terminus (18).
15 Wallace et al. proposed that it functioned as a “platform” in regulating membrane trafficking
16 events (19). In the model organism zebrafish, RAB11FIP4 is expressed in neural tissues and is
17 involved in the proliferation and differentiation of retinal tissues in the zebrafish retinal
18 development process (19, 20). RAB11FIP4 has also been shown to be perturbed in a variety of
19 cancers, including pancreatic cancer, colon cancer and hepatocellular cancer (21-23).

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21 In summary, we performed a systems-level, unbiased gene expression analysis of
22 glioblastoma tumors by comparing the transcriptomes of 17 glioblastoma tumors to 8 brain
23 tissue samples as reference control. We integrated these findings with comparative
24 transcriptome analysis using tumor data from the REMBRANDT study, the largest collection of
25 glioblastoma genomic data. We found that two of the genes whose expression was most
26 different between glioblastoma tumors and the non-affected brain was RAB11FIP2 and
27 RAB11FIP4, two molecules belonging to the same family, involved in the trafficking of cellular
28 cargoes, and both implicated as perturbed in a variety of cancers across tissue types. Of note,
in one dataset, RAB11FIP4 is the 4th most differentially expressed gene in the glioblastoma

transcriptome out of 47229 transcripts when compared to benign brain tissue. This is the first report of differential expression of RAB11FIP2 and RAB11FIP4 in the glioblastoma tumors. RAB11FIP2 over-expression *in vitro*, by proliferation assay, and *in vivo* on tumor maintenance and progression should be assessed using xenograft mouse models of glioblastoma (24, 25). RAB11FIP2 and RAB11FIP4 represent potential viable therapeutic targets in glioblastoma, the most common brain cancer in adults.

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Rank	Symbol	Gene name	p-value	t	B	ID
3	RAB11FIP4	RAB11 family interacting protein 4	5.79E-20	27.4853622	34.973997	ILMN_1681467
197	RAB11FIP2	RAB11 family interacting protein 2	6.89E-13	13.5355213	19.582035	ILMN_2072541

Table 1: RAB11FIP4 and RAB11FIP2 are among the most differentially expressed genes in the glioblastoma transcriptome when compared the non-affected, control brain transcriptome.

The rank of differential expression, 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.

Figure 1: RAB11FIP2 is expressed at significantly lower levels in the tumors of patients with glioblastoma.

The expression of RAB11FIP2 is shown as dots denoting the expression level in each individual patient, with the horizontal black bar representing the mean expression value in each group (A); as bar graph with the error bars denoting the standard of the mean (B); and as box and whisker plots from minimum to maximum value (C). A statistical test was performed to assess whether the difference in expression between the groups was statistically significant; the result of that test is shown as a *p*-value on each graph.

Figure 2: RAB11FIP4 is expressed at significantly lower levels in the tumors of patients with glioblastoma.

The expression of RAB11FIP4 is shown as dots denoting the expression level in each individual patient, with the horizontal black bar representing the mean expression value in each group (A); as bar graph with the error bars denoting the standard of the mean (B); and as box and whisker plots from minimum to maximum value (C). A statistical test was performed to assess whether the difference in expression between the groups was statistically significant; the result of that test is shown as a *p*-value on each graph.

Rank	ID	p-value	t	B	Gene	Gene name
2732	203883_s_at	3.53E-18	9.4029073	30.61632	RAB11FIP2	RAB11 family interacting protein 2
10131	224482_s_at	9.01E-09	5.9496082	9.40775	RAB11FIP4	RAB11 family interacting protein 4

Table 2: RAB11FIP4 and RAB11FIP2 are significantly differentially expressed genes in glioblastoma tumors from a separate 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.