1 **Repression of RNA message for F-box proteins in the tumors of patients with**
2 **glioblastoma.**

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6 Glioblastoma is the most common brain cancer in adults and has a 15 month median
7 survival rate (1, 2). We performed differential gene expression analysis, comparing the
8 glioblastoma transcriptome from 17 patients to the transcriptome of 8 non-affected,
9 “normal” brain samples using a published dataset (3). Three separate genes encoding
10 F-box proteins (4), including FBXW7, FBXO41, and FBXL16 were differentially
11 expressed and at significantly lower levels in the tumors of patients with glioblastoma
than in the brain. Significant differential expression of FBXW7, FBXO41 and FBXL16
was also observed in glioblastomas from the REMBRANDT study (5).

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21 **Keywords:** glioblastoma, differential gene expression analysis, systems oncology,
22 targeted cancer therapeutics, tumor transcriptome analysis, FBXW7, FBXO41, FBXL16
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Introduction

Glioblastoma is the most common brain cancer in adults. Medical treatment includes temozolomide and polifeprosan 20 with carmustine, a wafer containing a mustard-based chemotherapy (6-10). These toxic treatments, considered the standard of care, afford a 15 month median survival rate (1, 2). Systems-level analysis of the genome, transcriptome, and proteome of glioblastoma patient tumors can provide an enhanced understanding of tumor biology (11-13).

We identified differentially expressed genes in glioblastoma by mining published microarray datasets, comparing global gene expression profiles from primary glioblastoma tumors to that of normal brain. Analysis of the first dataset (3) revealed that three genes encoding F-box containing proteins were among those most differentially expressed by glioblastoma tumors. Statistically significant differential expression of these genes was observed in a separate microarray dataset from the REMBRANDT study (5). Though F-box proteins have been implicated in cancer biology, there are limited studies regarding F-box proteins in glioblastoma.

Methods

GSE116520 (3) and GSE108474 (5) 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 compared the transcriptomes of glioblastoma tumors from 17 patients to reference brain transcriptomes from 8 non-affected individuals (3) by global differential gene expression analysis. We found that three genes encoding F-box proteins, including FBXW7, FBXO41, and FBXL16 were among the genes whose expression was most significantly different when comparing the transcriptomes of glioblastoma tumors to that of non-affected, control brain tissue (Table 1).

FBXW7 was the 80th most differentially expressed gene when comparing the transcriptomes of glioblastoma tumors to that of control, non-affected brain tissue, out of 47229 total transcripts detected and measured in the microarray data analyzed in this study. This was statistically significant (Table 1; $1.9E-14$).

FBXO41 was the 86th most differentially expressed gene when comparing the transcriptomes of glioblastoma tumors to that of control, non-affected brain tissue, out of 47229 total transcripts. This was also statistically significant (Table 1; $2.61E-14$).

1 FBXL16 was the 122nd most differentially expressed gene when comparing the
2 transcriptomes of glioblastoma tumors to that of control, non-affected brain tissue, out of
3 47229 total transcripts detected and measured in the microarray data analyzed in this
4 study. The differential expression of FBXL16 relative to all of the transcripts produced in
5 glioblastoma tumors was statistically significant (Table 1; $8.23\text{E-}14$).

7 FBXW7 expression was significantly lower in the tumors of patients with
8 glioblastoma compared to non-affected, “healthy” brain tissue used as reference control
9 (Figure 1). The decreased expression of FBXW7 in glioblastoma tumors was
10 statistically significant (Figure 1; $p<0.0001$).

12 FBXO41 was also expressed at lower levels in glioblastoma tumors when
13 compared to control, non-affected brain tissue (Figure 2). Decreased expression of
14 FBXO41 was statistically significant (Figure 2; $p<0.0001$).

16 FBXL16 expression was also lower in glioblastoma tumors when compared to
17 control, non-affected brain tissue. Again, the decreased expression of FBXL16
18 compared to brain tissue was statistically significant (Figure 3; $p<0.0001$).

20 We probed a second dataset to determine whether differential expression of each
21 of FBX71, FBXO41, or FBXL16 genes could be observed in glioblastoma tumors from a
22 separate group of patients. When sorting all of the genes expressed in glioblastoma
23 tumors based on change in mRNA levels between GBM tumor and normal brain,
24 FBXW7 ranked 9561 out of 54613 transcripts. Differential expression of FBXW7 in
25 glioblastoma tumors was statistically significant (Table 2; $p=3.46\text{E-}09$). When sorting all
26 of the genes expressed in glioblastoma tumors based on change in mRNA levels
27 between GBM tumor and normal brain, FBXO41 ranked 20458 out of 54613 transcripts.

Differential expression of FBXO41 in glioblastoma tumors was statistically significant (Table 2; $p=4.45E-04$). When sorting all of the genes expressed in glioblastoma tumors based on change in mRNA levels between GBM tumor and normal brain, FBXL16 ranked 28944 out of 54613 transcripts. Differential expression of FBXL16 in glioblastoma tumors was statistically significant (Table 2; $p=1.85E-02$).

Discussion

There are limited treatment options for glioblastoma and a 15 month median survival for the glioblastoma patient. Probing the transcriptional behavior of glioblastoma tumors at the systems level to understand how they differ most significantly from their tissue of origin - the brain - can provide critical insights into glioblastoma tumor biology. In this study, we performed global differential gene expression analysis by comparing the tumor transcriptomes of 17 patients diagnosed with glioblastoma to the brain transcriptome, obtained from 8 non-affected control brain tissue samples (3). We also probed the Rembrandt glioblastoma tumor dataset (5). We found that three F-box containing proteins, FBXW7, FBXO41 and FBXL16 were differentially expressed by glioblastoma tumors, and that each of these genes were expressed at significantly lower levels than in the brain.

F-box proteins are canonically thought of as receptors, or substrate-recognition components for the SCF ubiquitin ligase, a cullin-based E3 ligase (10). SCF contains three major components: an E3 subunit composed of CUL1 and RBX1, SKP1, and a third component - an F-box protein (14). Dysregulation of F-box proteins has been reported in multiple cancers (15).

1 There is limited information regarding F-box proteins in glioblastoma. One
2 previous study has reported a role for FBXW7 in glioblastoma, and consistent with our
3 reporting here, found that FBXW7 was expressed at significantly lower levels in patient
4 tumors (16). However, the investigators compared FBXW7 expression between tumor
5 grades, and not between tissue of origin (the non-affected brain) and glioblastoma
6 tumor. This is the first report documenting differential expression of genes encoding F-
7 box proteins in glioblastoma compared to control brain tissue, and the first to document
8 perturbed expression of multiple genes encoding F-box proteins in the tumors of
9 patients with glioblastoma.

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12 FBXW7 has been suggested to possess a tumor-suppressive role, functioning by
13 targeted oncogenic proteins for degradation by the ubiquitin-proteasome system (11).
14 Our finding of decreased expression of FBXW7 in glioblastoma tumors is consistent
15 with such a tumor-suppressive role for FBXW7.

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18 FBXO41 has been reported to promote disassembly of the primary cilia in
19 neurons (13). There is otherwise limited information regarding the functions, tissue-
20 specific or otherwise, of FBXO41.

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22 FBXL16 has been reported to regulate stability of c-Myc (18), c-Myc is a
23 transcription factor implicated in multiple cancers as an oncogene (19, 20), is important
24 for embryonic stem cells (21) and is one of the four transcription factors Yamanaka
25 showed can be used to for transcriptional re-programming of induced pluripotent stem
26 cells (21). Connecting these two roles, c-Myc has been argued to be the link between
27 the transcriptional programs of stem cells and cancer cells (22). Remarkably, c-Myc
28 was reported to be required for maintenance of the glioma cancer stem cell (23).

FBXL16 has been reported to regulate the differentiation of embryonic stem (ES) cells through interactions with the protein phosphatase PP2A-B55 α (24). In this context, FBXL16 was found to negatively regulate PP2A activity, and thus inhibit differentiation of ES cells towards the FLK1+ cardiogenic lineage. Moreover, one study showed a reciprocal relationship between the gene products of the CDKN2A locus, p16INK4A and p14ARF and FBXL16, as knockdown of either p14ARF or p16INK4A both lead to transcriptional induction of FBXL16 (25). The investigators also found that silencing of FBXL16 in HeLa cells lead to an increase in proliferation (25).

We performed global differential gene expression analysis using transcriptome data from the glioblastoma tumors of 17 patients compared to the brain transcriptome from 8 non-affected individuals. We found differential and significantly decreased expression of FBXW7, FBXO41 and FBXL16 in the primary tumors of glioblastoma patients. Our findings merit study into the role of each of these F-box proteins in tumor maintenance and progression in glioblastoma. Experiments in glioblastoma mouse models using enforced expression of FBXW7, FBXO41 or FBXL16 can determine whether increasing expression of any of these genes has effects on tumor initiation or progression, or advantages in overall survival.

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**Table 1: FBXW7, FBXO41, and FBXL16 are among the most differentially
expressed genes in the tumors of patients with glioblastoma multiform.**

Rank	Gene Symbol	Gene Name	p-value	t	B
80	FBXW7	F-box and WD repeat domain containing 7	1.9E-14	15.9173456	23.104507
86	FBXO41	F-box protein 41	2.61E-14	15.6963783	22.798498
122	FBXL16	F-box and leucine rich repeat protein 16	8.23E-14	14.9079538	21.67338

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: FBXW7 is expressed at significantly lower levels in glioblastoma tumors compared to non-affected control brain tissue.

The RNA expression value of FBXW7 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.

Figure 2: FBXO41 is expressed at significantly lower levels in glioblastoma tumors compared to non-affected control brain tissue.

The RNA expression value of FBXO41 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.

Figure 3: FBXL16 is expressed at significantly lower levels in glioblastoma tumors compared to non-affected control brain tissue.

The RNA expression value of FBXL16 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
9561	222729_at	3.46E-09	6.1265785	10.3369	FBXW7	F-box and WD repeat domain containing 7
20458	221945_at	4.45E-04	-3.5593184	-0.93131	LOC101927826 ///FBXO41	uncharacterized LOC101927826///F-box protein 41
28944	232409_x_at	1.85E-02	-2.3713569	-4.31966	FBXL16	F-box and leucine rich repeat protein 16

Table 2: FBXW7, FBXO41, and FBXL16 are significantly differentially expressed in tumors from a separate group of patients with glioblastoma multiforme.

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).