FBXW11 is differentially expressed in diffuse intrinsic pontine glioma and associates with survival.

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Diffuse intrinsic pontine glioma (DIPG) has the lowest median survival rate of any cancer. 99% of patients will expire within 5 years (1). The poor treatment options for this brain cancer (2) demand understanding of the basic manner in which DIPG function at the level of gene expression. By comparing the tumor transcriptomes of patients with DIPG that survived more or less than six months using a published dataset (3), we found that four of the genes whose expression was most different between these patients were genes encoding Fbox proteins. Moreover, the expression of one of these genes, FBXW11, was significantly associated with patient survival. This is the first report documenting differential expression of Fbox proteins in the tumors of patients with DIPG and their association with patient outcomes.

Keywords: DIPG, diffuse intrinsic pontine glioma, Fbox proteins, FBXW11, FBXL18, FBXO9, FBXO10, systems oncology, differential gene expression analysis, rational identification of therapeutic targets.

Introduction

The brain cancer diffuse intrinsic pontine glioma (DIPG) is the cancer with the worst prognosis (1). 99% of patients will expire within 5 years. 100% of patients will expire within 10 years (1). There is a need for new, targeted treatments in this disease (2). Understanding the transcriptional behavior of DIPG and how it differs most from the tissue in which it arises - the brain - is critical for the ability to identify therapeutic targets and pathways and design novel and specific treatments. Toward this goal, we performed global differential gene expression analysis of the transcriptomes of 35 tumors from patients with DIPG (3), stratifying patients based on their survival greater than 6 months using a published dataset. We found that multiple Fbox proteins (4) were among the genes whose expression was most different between DIPG tumors and the non-affected brain. Moreover, the expression of one of these genes, FBXW11 was significantly associated with the amount of time in which the patient expired. These data suggest that FBXW11 may be a therapeutic target or prognostic indicator in DIPG.

Methods

Dataset GSE50021 (3) was utilized for this analysis, in conjunction with GEO2R, to perform differential gene expression analysis on tumor samples from patients diagnosed with DIPG (n=35; n=21 for patients surviving greater than 6 months, and n=14 for patients surviving less than six months). There was one patient in this dataset who survived 6 months exactly and was included in the group of patients who survived less than 6 months. Buczkowicz et al. (3) used Illumina HT-12 microarray analysis to obtain transcriptome data from these tumors and control brain samples. These samples were all fresh frozen paraffin embedded tumors from pediatric patients, or normal healthy brain tissue. The p-value was not adjusted by any method. Log transformation was set to "No" and the submitter supplied category of platform

annotation was used. For statistical analysis to compare the RNA expression values between patient groups, an unpaired, two-tailed t -test with Welch's correction was used (PRISM 8.1.2) (227). Linear regression analysis and a Pearson correlation, comparing the mRNA expression level of each patient and the time amount of time they survived, were also performed using PRISM.

Results

Genes encoding Fbox proteins are differentially expressed when comparing patients who survived more or less than 6 months.

We performed global differential gene expression analysis based on patient survival in the deadliest cancer known to man, diffuse intrinsic pontine glioma. To understand in a systematic fashion the biological mechanisms of survival in this disease, we compared the transcriptomes of patients who survived greater than or less than 6 months. We found that four separate transcripts from the family of Fbox proteins were among the genes whose expression was most different between these two groups (Table 1).

FBXW11 was ranked 46th most differentially expressed out of 29285 total transcript detected and measured by the microarray dataset utilized in this study. The differential expression of FBXW11 relative to the rest of the transcriptome was statistically significant (Table 1; 0.010853). FBXL18 was ranked 65th most differentially expressed out 29285 total transcripts, and this was statistically significant (Table 1: $p=0.012733$). FBXO18 was ranked 96th most differentially expressed out of 19285 total transcripts. This differential expression was statistically significant (Table 1; $p=0.015818$). FBXO9 was ranked 170th most differentially expressed out of 29285 total transcripts. This was also statistically significant (Table 1; $p=0.022062$).

FBXW11 and FBXO9 are expressed at higher levels in the tumors of patients who survive greater than 6 months, while FBXL18 and FBXO10 are expressed at lower levels in the tumors of patients who survive greater than 6 months.

Next, we extracted the RNA expression levels of each Fbox transcript, for each individual patient. We then compared the expression levels of each differentially expressed Fbox gene between the two groups of patients in this analysis, those surviving greater than 6 months and those surviving less than 6 months. FBXW11 was expressed at significantly higher levels in the tumors of patients surviving greater than 6 months (Figure 1; $p=0.0036$), as was FBXO9 (Figure 3; $p=0.0089$). The two other differentially expressed Fbox genes, FBXL18 and FBXL10 were expressed at significantly lower levels in the tumors of patients surviving greater than 6 months (FBXL18: Figure 1: $p=0.0516$; FBXL10: $p=0.0386$). Thus, two of the Fbox genes differentially expressed when comparing the tumor transcriptomes of patients surviving greater than or less than six months were expressed at higher levels in the tumors of patients with greater survival outcomes, while two of these genes were expressed at lower levels in the tumors of patients with greater survival outcomes.

FBXW11 expression at the mRNA level significantly correlates with patient survival

Finally, we performed linear regression analysis to attempt to correlate the expression level of each differentially expressed Fbox gene with the amount of time the patient survived. In the case of FBXW11, we found a linear and statistically significant correlation between the expression of the differentially expressed gene in the patient tumor and the overall survival of the patient (Figure 5A; $p=0.0192$). The expression of FBXO9, FBXL10, and FBXL18 in patient tumors were not correlated with patient survival (Figure 5B-D).

Thus, FBXW11 is differentially expressed when comparing the tumor transcriptomes of DIPG patients surviving greater or less than six months, it is expressed at significantly lower levels in the tumors of patients who survive greater than six months, and its expression significantly correlates with the amount of time within which the patient will expire.

Discussion

In this study, we compared the tumor transcriptomes of patients diagnosed with diffuse intrinsic pontine glioma (3), stratifying patients into two groups: those surviving greater than six

months and those surviving less than six months. We found that out of four genes from the Fbox family that were differentially expressed between these two groups, FBXW11 expression significantly correlated in a linear fashion with overall survival of the patient. FBXW11 was expressed at significantly higher levels in the tumors of patients who survived greater than six months.

FBXWs are Fbox genes that contain a WD40 domain (4, 5). FBXW11 functions, as a receptor subunit, together with SKP1 and CUL1 in an SCF complex. SCF is one of six CRLs, or cullin ring E3 /ligases in mammals. SCFs can control the degradation of a variety of substrate proteins, include proteins of the cell cycle by targeting them for proteasomal degradation via K48-ubiquitination (4, 5). In that manner, an SCF can control the degradation of an entire network of proteins through its K48-ubiquitin E3 ligase function. It is possible that since FBXW11 is expressed at significantly higher levels in patients surviving greater than six months, and that its expression significantly correlates in a linear fashion with patient survival, that it might function like a tumor suppressor in DIPG. In this case, it would be interesting to determine the E3-ligase substrates of FBXW11 in glioma cells and in DIPG tumors.

There is limited information regarding FBXW11 in other cancers, and it is clear that FBXW11 has tissue-specific properties as it functions more like in an oncogene in some cancers (4, 5). FBXW11, also known as β -TRCP2, is over-expressed in some breast cancers (6), and one study of gastric cancer and gastric cancer cell lines found a nucleotide substitution of FBXW11 in one of its WD-repeat domains (7). One study found that in non-small cell lung cancer, FBXW11 is repressed by miR-182 (8). A separate study found that a non-coding RNA, PCGEM1 promoted proliferative and aggressive properties in cervical cancer through dysregulation of the miR-182/FBXW11 axis (9). In another study of leukemias, FBXW11 expression significantly decreased in patients who achieved complete remission, was higher in the hematopoietic stem cells of mice with Notch10-induced leukemias, and in a lymphocytic leukemia cell line L12, high expression of FBXW11 promoted proliferation (10, 11).

With respect to the function of FBXW11 in non-transformed cells, a study that used a proteomic approach to identifying substrates of FBXW11 found that RAPGEF2 was a target of FBXW11 function, and that proteasome inhibition increased levels of RAPGEF2, indicating that FBXW11 controlled RAPGEF2 levels and through its K48-ubiquitin E3 ligase function could target RAPGEF2 for degradation (12). RAPGEF2 is able to induce multi-nucleation, a form of polyploidy (13). In patients with castration-resistant prostate cancer, treatment with docetaxel leads to resistance via multi-nucleation (14). It would be interesting to assess whether multi-nucleation is a phenomenon seen in patients with DIPG, before or after treatment, and if RAPGEF2 functions in such a manner in DIPG.

Regardless of whether RAPGEF2 functions in DIPG, or if multi-nucleation can be observed in the tumors of patients with DIPG, FBXW11 has at least 44 identified substrates (12). FBXW11 expression should be assessed in larger and separate patient datasets in DIPG, at the protein level, and it should be determined whether FBXW11 is required for DIPG cell proliferation or survival. FBXW11 function *in vivo* can also be assayed in xenograft mouse models of DIPG to assess whether FBXW11 over-expression could delay or prevent tumor progression (15-17).

FBXL18 has previously been implicated in glioma progression by K63-ubiquitination of Akt; depleting glioma cells of FBXL18 resulted in compromised proliferation and supported apoptosis (18). In addition, FBXL18 was found to mediate N-terminal ubiquitination of PUMA, a pro-apoptotic protein (19). PUMA functions with and without p53 in mediating cell death, by directly binding and antagonizing the BCL family of anti-apoptotic protein (e.g., Bcl-XL), and in this manner activating caspases and dysfunction of the mitochondria (20). By ubiquitinating PUMA, FBXL18 reduced the half-life of a pro-apoptotic protein from 3 hours to 1 hour (20). However, it should be noted that the results here are not in support of increasing levels of FBXL18 in DIPG as a therapeutic strategy as it is expressed in significantly lower levels in the tumors of patients who survived greater than 6 months.

With respect to FBXO9, one study has shown that it can enhance survival of multiple myeloma by targeting Tel2 and Tti1, components of mTORC1 and mTORC2, respectively, for degradation (21). mTOR is an essential cellular growth factor and amino acid signaling pathway and important in the survival of cancer cells (22).

One study focusing on the mechanisms involving resistance to the chemotherapy targeting bruton's tyrosine kinase, ibrutinib, in mantle cell lymphoma implicated increased BCL2, and found that decreased or no expression of FBXO10 in mantle cell lymphoma contributed in part to this increased expression of BCL2 (23). FBXO10 has been reported to be a susceptibility gene in breast cancer (Mcs5a) (24) and to function through a mammary gland autonomous manner, causing a higher percentage abundance of gamma delta T cells in the spleen and mammary gland (25).

In conclusion, we used a systems-level transcriptomic approach to identify FBXW11, along with three other Fbox genes, FBXO9, FBXO10, and FBXL18, as some of the genes most differentially expressed between the tumors of patients that survived greater or less than 6 months (3) in the cancer with the worst survival prognosis of all cancers, diffuse intrinsic pontine glioma. We also found that FBXW11 was expressed at significantly higher levels in the tumors of patients who survived greater than 6 months, and of importance, in this dataset the expression of FBXW11 in patient tumors was correlated in a linear fashion with the amount of time in which the patient expired. Diffuse intrinsic pontine glioma is a deadly pediatric cancer with a complete lack of targeted therapeutic options, and efforts should be prioritized towards identification of novel therapeutic targets and tailored treatment approaches.

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Figure 1: FBXW11 is among the genes whose expression is most significantly different between patients who survive more or less than 6 months.

The expression of FBXW11 is graphically represented in A-D, as dots representing the mRNA expression level in each individual patient (A) with the horizontal black bar denoting the mean expression level; in a violin plot in (B); as a bar graph in (C) with the error bars denoting the standard error of the mean; and in X & Y format in, with each patient sorted across the X axis in (D). The statistical significance of the difference between the expression level of FBXW11 in those surviving more or less than 6 months is shown as a p -value on each graph.

Figure 2: FBXL18 is among the genes whose expression is most significantly different between patients who survive more or less than 6 months.

The expression of FBXL18 is graphically represented in A-D, as dots representing the mRNA expression level in each individual patient (A) with the horizontal black bar denoting the mean expression level; in a violin plot in (B); as a bar graph in (C) with the error bars denoting the standard error of the mean; and in X & Y format in, with each patient sorted across the X axis in (D). The statistical significance of the difference between the expression level of FBXL18 in those surviving more or less than 6 months is shown as a p -value on each graph.

Figure 3: FBXO9 is among the genes whose expression is most significantly different between patients who survive more or less than 6 months.

The expression of FBXO9 is graphically represented in A-D, as dots representing the mRNA expression level in each individual patient (A) with the horizontal black bar denoting the mean expression level; in a violin plot in (B); as a bar graph in (C) with the error bars denoting the standard error of the mean; and in X & Y format in, with each patient sorted across the X axis in (D). The statistical significance of the difference between the expression level of FBXO9 in those surviving more or less than 6 months is shown as a *p*-value on each graph.

Figure 4: FBXO10 is among the genes whose expression is most significantly different between patients who survive more or less than 6 months.

The expression of FBXO10 is graphically represented in A-D, as dots representing the mRNA expression level in each individual patient (A) with the horizontal black bar denoting the mean expression level; in a violin plot in (B); as a bar graph in (C) with the error bars denoting the standard error of the mean; and in X & Y format in, with each patient sorted across the X axis in (D). The statistical significance of the difference between the expression level of FBXO10 in those surviving more or less than 6 months is shown as a p -value on each graph.

Figure 5: Expression of FBXW11, not but FBXL18, FBXO8, and FBXO9 is significantly correlated with the length of patient survival.

The expression level, on the Y axis, of each Fbox gene was graphed against the amount of time that patient survived, on the X axis. Linear regression modeling was performed for each

mRNA/overall survival correlation, and a *p*-value was obtained as a statistical measure of how linear and non-zero that correlation (shown on each graph).

Table 1

Rank	Symbol	P.Value	t	B	GB_ACC	ID
46	FBXW11	0.010853	2.6837799	-4.52	NM_033644.2	ILMN_1735655
65	FBXL18	0.012733	-2.619252	-4.53	NM_024963.2	ILMN_1670920
96	FBXO10	0.015818	-2.5303188	-4.53	XM_933940.1	ILMN_1716952
170	FBXO9	0.022062	2.3906832	-4.54	NM_033481.2	ILMN_1702928

Table 1: Genes encoding Fbox proteins are differentially expressed when comparing patients who survived more or less than 6 months.

The rank of expression out of 29285 total transcripts, the gene symbol, the *p*-value of differential expression globally, a moderated *t*-statistic (*t*), *B*, the log-odds of differential expression between both groups, the GenBank accession ID, and the Illumina probe ID are displayed in this table (all provided by GEO2R). One patient survived exactly 6 months and was included in the group of patients who survived less than 6 months.