RNA helicase DDX52 is produced in significantly higher quantities in the tumors of males with DIPG.

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Diffuse intrinsic pontine glioma (DIPG) is a pediatric cancer with the worst prognosis of any cancer known to man. 99% of patients will expire within 5 years (1). The poor outcomes in this disease demand a basic understanding of the transcriptional behavior of DIPG tumors, how they differ from the tissue of origin and how they differ between patients. We compared the transcriptomes of tumors from men and women diagnosed with the disease using a published dataset (2) to more thoroughly understand sex-specific transcriptional differences in cancer and found that RNA helicase DDX52 (3) was among the genes most differentially expressed based on the sex of the patient. DDX52 is not located on a sex chromosome. Multiple DDX genes have been found to be dysregulated in a variety of cancers (4-16); this is the first report of dysregulation of a DDX gene in males with diffuse intrinsic pontine glioma.

Keyword: diffuse intrinsic pontine glioma, DDX52, DIPG, RNA helicase, sex-specific transcriptional differences in cancer, systems oncology, differential gene expression

Introduction

Diffuse intrinsic pontine glioma is a pediatric cancer that arises out of the glial cells of the brainstem. Of all the cancers known to man, it carries the worst prognosis, with 99% of patients expiring within 5 years. The only treatment that presents even a transient benefit to patients with DIPG is radiation therapy (17-19). Targeted chemotherapies are of dire need for patients with this fatal disease. To more thoroughly understand the transcriptional behavior of tumors in patients with DIPG, we performed global comparative transcriptional profiling of DIPG tumors from males and females using a published dataset. When comparing the tumors transcriptomes of men and women with DIPG, we found that the RNA helicase DDX52 was among the genes whose expression was most different in tumors based on sex of the patient. DDX genes are perturbed in a variety of cancers (4-16) and this is the first report of differential, sex-specific expression of a DDX gene in diffuse intrinsic pontine glioma.

Methods

Dataset GSE50021 (2) was utilized for this analysis, in conjunction with GEO2R, to perform differential gene expression analysis on tumor samples from patients diagnosed with DIPG ($n=35$; $n=19$ males and $n=16$ females) . Buczkowicz et al. (2) used Illumina HT-12 microarray analysis to obtain transcriptome data from DIPG tumor samples. These samples were all fresh frozen paraffin embedded tumors from pediatric patients. The Benjamini and Hochberg method of p -value adjustment was used. Log transformation was set to “Auto-detect” and the submitter supplied category of platform annotation was used. For statistical analysis to compare the RNA expression values between male and female tumor 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

We performed global differential gene expression analysis to compare the transcriptomes of tumors from males and females with diffuse intrinsic pontine glioma (DIPG) (2). This analysis revealed that among the genes whose expression was most different between tumors based on sex of the patient was the RNA helicase Ddx52 (Table 1).

DDX52 is among the genes most differentially expressed in the tumors of males with DIPG.

Out of 29285 total transcripts detected and measured by the microarray data utilized in this analysis. DDX52 was the 70th most differentially expressed gene when comparing the transcriptomes of males and females. This was statistically significant (Table 1; $p=3.43E-03$).

DDX52 is expressed in significantly higher quantities at the RNA level in the tumors of males with diffuse intrinsic pontine glioma.

We next extracted the exact RNA expression value for DDX52 in each patient tumor, for both males and females. DDX52 was expressed at significantly higher levels in the tumors of males than in the tumors of females with DIPG (Figure 1; $p=0.0083$).

Discussion

There are zero targeted treatments available for patients with diffuse intrinsic pontine glioma (DIPG). We performed a global differential gene expression analysis of tumors from patients with DIPG, comparing the tumor transcriptomes from men versus that of women with DIPG. This analysis revealed that the RNA helicase DDX52 was one of the most differentially expressed genes when comparing the transcriptomes of tumors from males with DIPG versus that of the tumors from females with DIPG. DDX52 was expressed at significantly higher levels in the tumors of males with DIPG than in the tumors of females.

There is limited published information on DDX52, but it has 44% identity to Rok1 in yeast (20) which is required for rRNA processing at sites A0, A1 and A2 which leads to generation of 18S rRNA (21). With respect to the involvement of Ddx genes in cancers, one study found that Ddx39 functioned as an invasion suppressor in bladder cancer (4). Another

study found that Ddx5 promoted the proliferation of non-small cell lung cancer cell lines through the β -catenin signaling pathway (5). In oral cancer, one study found that Ddx3 expression predicted poor prognosis in non-smokers (6). Ddx3x was also found to be over expressed in lung cancers and was associated with lower overall survival. Bol et al. designed a first-in-class inhibitor of Ddx3 and found that treatment of multiple mouse models of lung cancer with this inhibitor (RK-33) lead to regression of tumors *in vivo* (12). Another study found that Ddx4 was expressed in myeloma and leukemia cell lines, and that knockdown on Ddx4 in the myeloma cell line IM-9 compromised its proliferation capacity (9). An investigation of 176 breast cancer tumors using microarray data found that Ddx1 could function as an independent marker of breast cancer prognosis (10). Recurrent truncation mutations of Ddx3x were found in chronic lymphocytic leukemia (11). In ovarian cancer, decreased expression of Ddx10 by epigenetic mechanisms caused increased proliferation through the Akt and NF- κ B pathways (13). In two studies of colon cancers, Ddx1 was found to support tumorigenesis through LGR5 expression (15), and amplification of Ddx56 was found to lead to splicing abnormalities (16). Thus, across multiple cancer types, a variety of Ddx genes have been found to be perturbed and play a role in support of oncogenic gene expression programs and/or cell states.

In this study, we found through global differential gene expression analysis that Ddx52 was one of the genes whose expression was most significantly different between the tumors of men and women with DIPG, and that the expression of Ddx52 was significantly higher in the tumors of males with this disease. There are no targeted treatments available for patients with diffuse intrinsic pontine glioma, and small molecule inhibition of at least one Ddx gene in at least one cancer type has shown beneficial effects on tumor regression in mouse models *in vivo*. Whether Ddx52 represents a therapeutic target in DIPG should be assessed by studying the effect of Ddx52 inhibition on proliferation *in vitro*, and on tumor progression *in vivo* using xenograft mouse models of DIPG (22-24). Diffuse intrinsic pontine glioma presents the lowest

overall survival rate of all cancers and efforts should be directed and prioritized towards understanding the transcriptional landscape of this disease.

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Figure 1: DDX52 is expressed in significantly higher quantities at the RNA level in the tumors of males with diffuse intrinsic pontine glioma.

The expression of DDX52 in the tumors of males (A, B, and C; left) and in the tumors of females (A, B and C; right) is graphically represented as dots to denote the expression of DDX52 in each individual patient, with the black horizontal bar denoting the mean expression value of DDX52 in each sex (A), in a bar graph with the error bars representing the standard error of the mean (B); and as a violin plot with the dotted horizontal line denoting the median (C). A statistical test was performed to evaluate the significance of the difference between expression values between the tumors of each sex; the result of this test is shown as a *p*-value on each graph.

Table 1

Rank	Symbol	<i>p</i> -value	<i>t</i>	<i>B</i>	Chr.	GB_ACC	Transcript	ID
70	DDX52	3.43E-03	-3.0746413	-2.4341	17	NM_007010.2	ILMN_21446	ILMN_2366790

Table 1: DDX52 is among the genes most differentially expressed in the tumors of males with DIPG.

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 chromosome the gene is encoded on, the GenBank accession ID, as well as the transcript ID and probe ID from Illumina are displayed in this table (all provided by GEO2R).