Aquaporin 4 is differentially expressed in malignant mesothelioma.

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Malignant mesothelioma is a cancer of the mesothelial cells that is often caused by exposure to asbestos (1, 2). There are no targeted treatments available for the treatment of mesothelioma and current first-line chemotherapy provides a 7 month median overall survival (3). We studied the total transcriptome of mesothelial tumors from two groups of patients using two independent datasets (4, 5). Across both datasets, the water channel AQP1 was among the genes whose expression was most different between mesothelial tumors and normal lung and peritoneal tissues. AQP4 was expressed at significantly lower levels in tumors from patients with mesothelioma when compared to normal lung tissue. This is the first report of differential expression of AQP4 in mesotheliomas.

Keywords: malignant mesothelioma, mesothelioma, aquaporin 4, AQP4, differential gene expression analysis, tumor transcriptome analysis, systems oncology, targeted therapeutics in mesothelioma.

Introduction

Malignant mesothelioma is an aggressive cancer (1) that affects the pleura and the peritoneum, the linings of the pleural cavity of the lungs and the peritoneal cavity of the gut (2). Treatment of malignant mesothelioma with first-line chemotherapy results in a 7 month median overall survival. Novel therapeutics for the treatment of malignant mesothelioma are needed.

Understanding the basic transcriptional behavior of tumors and how they arise can reveal fundamental insights into the pathways and genes that tumors use to survive and thrive. We studied tumors from patients with malignant mesothelioma by comparing the transcriptomes of mesothelioma tumors to the transcriptomes of normal lung parenchyma tissues using two independent published datasets (4, 5). Using this systems-level, whole transcriptome analysis, we found that the water channel aquaporin 4 (AQP4) was among the genes most differentially expressed in mesothelioma tumors isolated from patients diagnosed with malignant mesothelioma.

Methods

Datasets GSE51024 (4) and GSE112154 (5) were utilized in this study. Investigators that created GSE51024 utilized Affymetrix Human Genome U133 Plus 2.0 Array technology. Investigators that created GSE112154 utilized Illumina HumanHT-12 V4.0 expression beadchip. In GSE51024, n=41 normal lung parenchyma (paired) samples and n=55 mesothelioma (malignant pleural). In GSE112154, n=3 normal peritoneum samples and n=45 mesothelioma samples (diffuse malignant peritoneal).

The Benjamini and Hochberg method of p-value adjustment was utilized, and p-value adjustment was set to “Auto-detect”. The NCBI generated category of platform annotation was utilized. To evaluate the statistical significance of differences in RNA expression values in control versus tumor groups, a two-tailed, unpaired t test with Welch’s correction was performed to obtain a p-value. P-values less than 0.05 were considered statistically significant.

Results

We performed whole transcriptome analysis of malignant mesothelioma by comparing the global gene expression profiles of tumors isolated from patients diagnosed with malignant mesothelioma to normal lung and peritoneal tissue.

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We found that aquaporin 4 (AQP4) was one of the genes whose expression changed the most significantly between mesothelioma tumors and normal lung tissue. AQP4 was the second most differentially expressed gene out of 54675 total transcripts; this was statistically quite significant (Table 1; $1.87\text{E-}37$). In a separate dataset, AQP4 differential expression was ranked 1611 out of 47323 total transcripts, placing AQP4 as among the top 3.4% of genes in terms of expression change between tumor and normal tissue in this dataset. This was also statistically significant (Table 1; $p=1.74\text{E-}04$).

Aquaporin 4 (AQP4) is expressed at significantly lower levels in mesothelioma than in normal lung tissue.

We extracted the exact mRNA expression levels of AQP4 from the dataset where it was ranked 2nd out of 54675 total transcripts. AQP4 was expressed at significantly lower levels in patient tumors in those diagnosed with mesothelioma than in normal lung tissue (Figure 1; $p<0.0001$).

Thus, we found that AQP4 was among the most differentially expressed genes in malignant mesothelioma and expressed at significantly lower levels in tumor tissue compared to control tissue (normal lung).

Discussion

Malignant mesothelioma is an aggressive cancer with poor outcomes (1). Treatment with first-line chemotherapy provides a 7 month median survival rate (3). Novel, targeted therapeutics are needed for patients diagnosed with mesothelioma.

To understand the transcriptional landscape of malignant mesothelioma tumors as they arise in human patients we compared the transcriptomes of mesothelioma tumors to that of normal lung and peritoneum (4, 5). We identified AQP4 as one of the most differentially expressed genes in mesothelioma.

As a transmembrane water channel, aquaporin 4 displays strict selectivity for water molecules as opposed to other aquaporins which can facilitate channel-mediated transport of hydrophilic molecules such as glycerol (6).

Aquaporin 4 has been implicated in the invasive behavior of lung cancer cells (7), reported as lost in non small cell lung cancer (8) and differentially expressed in gastric cancer (9). This is the first report of differential expression of AQP4 in malignant mesothelioma. We previously reported that AQP4 was differentially expressed in brain metastases in patients with breast cancer but contrarily, AQP4 was expressed at significantly higher levels in the brain metastases as compared to the primary breast tumor (10). Thus, aquaporin 4 is differentially expressed in multiple cancer types and between normal and tumor tissue as well as between tumor and metastasis.

We identified AQP4 as among the most differentially expressed genes in malignant mesothelioma and found that it was expressed at significantly lower levels in mesothelioma tumors than in normal lung tissue. Modulation of AQP4 expression by enforced over-expression in xenograft mouse models (11) should be evaluated in order to determine whether decreased expression of AQP4 is required to support tumor survival or tumor growth *in vivo*.

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Table 1

Rank	p-value	t	B	ID	Gene	Gene name
GSE51024						
2	1.87E-37	-20.8235549	74.523132	226228_at	AQP4	aquaporin 4
GSE112154						
1611	1.74E-04	-4.07	0.36037	ILMN_1747683	AQP4	aquaporin 4

Table 1: Aquaporin 4 is differentially expressed in malignant mesothelioma.

The rank of differential expression globally out of 54675 total transcripts (GSE51024) or 47323 total transcripts (GSE11254), the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between tumor and control groups, probe or transcript ID, gene symbol and gene name are shown in this chart (extracted from GEO2R).

Figure 1: Aquaporin 4 (AQP4) is expressed at significantly lower levels in mesothelioma than in normal lung tissue.

The mRNA expression levels of AQP are graphically represented as a bar graph with the error bars denoting the standard error of the mean (top), as a violin plot with the dotted horizontal black line representing the median value of each group and the finer colored lines representing the 25th and 75th quartiles (middle), and as dots representing the expression of AQP4 in each individual patient tumor and control lung tissue sample with the horizontal black line denoting the mean value in each group (below). A statistical test was performed to evaluate the significance of difference in expression of AQP4 between tumor and control groups; the result of that test is shown on each graph as a p-value.