HLF is differentially expressed in malignant mesothelioma.

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Malignant mesothelioma is cancer of the mesothelial cells (1) that form linings of the lungs and the gut (2) often caused by exposure to asbestos (1). First-line chemotherapy treatment results in 7 month overall survival (3). There are zero targeted treatment options available for patients diagnosed with mesothelioma. To understand what genes make mesotheliomas different from the tissues from which they arise, we performed whole transcriptome analysis of tumors isolated from patients diagnosed with mesothelioma using two independent published datasets and compared them to control tissues from both the lung parenchyma and peritoneum (4, 5). In both cases, the transcription factor HLF (hepatic leukemia factor) was among the genes most differentially expressed by mesothelioma tumor tissue. HLF was expressed at significantly lower levels in mesotheliomas than in normal lung and peritoneal tissues. Mesothelioma cancer cells should be evaluated for potential dependency on decreased HLF expression and HLF modulation at the level of gene expression or through one of its regulators should be assessed as a novel therapeutic strategy in mesothelioma.

Keywords: malignant mesothelioma, thoracic cancers, differential gene expression, systems oncology, tumor transcriptome analysis, HLF, targeted therapeutics in mesothelioma.

Introduction

Malignant mesothelioma is a cancer that can affect the mesothelium (1), the tissue that forms the linings of the the gut as the peritoneum and the lungs as the pleural tissue (2). The most common first-line chemotherapeutic regimen consists of pemetrexed and cisplatin or carboplatin with a median overall survival of 7 months after treatment (3, 6, 7). Bevacizumab provides a survival advantage when added to this combination (8). There are zero targeted treatment options available for patients diagnosed with mesothelioma.

The development of targeted therapeutics requires an understanding of the genes and pathways that make tumor cells most different from the cells and tissues from which they arise. We have previously performed systems-level analyses, comparing tumor to tissue of origin, comparing tumor to metastasis, or comparing the tumors of patients based on patient survival in cancers of the pancreas, head and neck, breasts, ovaries, brain, and gamma delta T-cells of the blood (9-15). Here, we extended our systems analysis of human cancers by comparing the transcriptomes of tumors isolated from patients diagnosed with mesothelioma with the transcriptome of healthy lung and peritoneum using published datasets (4, 5). We found that the HLF transcription factor was among the genes most differentially expressed between mesothelioma tumors and normal lung and peritoneal tissues. This is the first report of HLF differential expression in 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 a systems-level analysis of differential gene expression in mesothelioma by comparing the transcriptomes of tumors from patients with mesothelioma against the transcriptomes of normal lung and peritoneum using a published dataset (4, 5).

HLF is one of the genes whose expression changes most significantly between the transcriptomes of mesothelioma tumor and normal tissues of the lung and peritoneum.

We found that the transcription factor hepatic leukemia factor HLF was among the genes whose expression changed the most between malignant pleural mesothelioma tumor tissue and benign lung tissues when comparing expression in 55 tumors and 41 normal lung parenchyma samples. When all of the transcripts detected by microarray were sorted based on amount of difference in expression between mesothelioma tumor and normal lung parenchyma, differential expression of HLF was ranked 144 out of 54675 total transcripts. This was statistically significant (Table 1; $p=2.62E-24$).

Using a separate dataset (5), we again asked what the most differentially expressed genes were when comparing the transcriptomes of diffuse malignant peritoneal mesothelioma tumors from human patients and normal tissue. Again, we found that HLF was among the most differentially expressed genes in the mesothelioma transcriptome relative to benign control peritoneum, ranking 89 in differential expression out of 47323 total transcripts. This was statistically significant (Table 1; $p=1.51E-14$).

HLF mRNA is produced in significantly lower quantities in mesothelioma tissues than in normal lung and peritoneum

Next, we extracted exact mRNA expression values for HLF from each patient tumor samples and each control tissue sample. Whether comparing mesothelioma tumors to the normal lung parenchyma or to the normal peritoneum, HLF was expressed at significantly lower levels in mesothelioma tumors (Figure 1, $p < 0.0001$ and $p < 0.0001$, respectively).

Thus, we identified HLF as one of the most differentially expressed genes in the mesothelioma transcriptome when compared to normal lung parenchyma and peritoneum, and found that HLF was expressed at significantly lower levels in mesothelioma tumors than in the lung and peritoneum.

Discussion

We performed whole transcriptome profiling of mesotheliomas using independent datasets (4, 5) to understanding the major transcriptional differences between normal lung and peritoneal tissues and mesothelioma tumor tissues from humans. We identified the hepatic leukemia factor transcription factor HLF as among the genes most differentially expression in the tumors of patients with mesothelioma.

The HLF, PAR bZIP transcription factor (HLF) is expressed in the liver and is known for its role in t (17:19) acute lymphoblastic leukemia (ALL) in the context of a fusion protein with E2A (16). It is also found as a fusion to TCF3 in ALL (17). Interestingly, HLF, like other PAR-domain basic leucine zipper transcription factors, accumulates in tissues in a circadian manner (18).

We found that HLF was expressed at significantly lower levels in mesothelioma tumors than in normal, benign lung parenchyma and peritoneal tissues. Mesothelioma cell lines should be assessed for their dependency on HLF down-regulation by enforced expression of HLF, and HLF over-expression should be evaluated in xenograft mouse models of

mesothelioma (19) for its effect on tumor initiation, tumor maintenance, and tumor progression.

This is the first report of HLF differential expression in mesothelioma.

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Table 1

Rank	p-value	t	B	ID	Gene	Gene name
GSE51024						
144	2.62E-24	-13.6772976	44.809221	204755_x_at	HLF	HLF, PAR bZIP transcription factor
GSE112154						
89	1.51E-14	-1.09E+01	22.92283	ILMN_1722829	HLF	HLF, PAR bZIP transcription factor

Table 1: HLF is one of the genes whose expression changes most significantly between the transcriptomes of mesothelioma tumor and normal tissues of the lung and peritoneum.

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: HLF mRNA is produced in significantly lower quantities in mesothelioma tissues than in normal lung and peritoneum.

The expression of HLF in normal peritoneum (top row) or lung (bottom row) (green) and mesothelioma tumor tissues (red) is shown by bar graph with the error bars denoting the standard error of the mean (left) by a violin plot representing minimum to maximum expression with the dotted horizontal line denoting the median mRNA expression value and the finer lines denoting the 25th and 75th percent quartiles of expression (middle), and with dots representing the expression level in each individual patient and the dotted horizontal line representing the mean mRNA expression value (right). Data from GSE112154 is shown in the top row, while data from GSE51024 is shown in the bottom row. A statistical test was performed to evaluate the significance of expression between tumor (mesothelioma) and control (peritoneum or lung) groups, and the results of those tests, a p-value, is shown on each graph.