Tox is over-expressed in hepatosplenic T-cell lymphoma.

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Hepatosplenic T-cell lymphoma is a rare cancer with a median survival of 8 months (1). By systems-level analysis of transcription and methylation in HSTCL patients (2, 3), I found that the thymocyte selection assisted high mobility group box gene *Tox* was differentially expressed at higher levels in the cells of patients with HSTCL, and that the *Tox* genomic locus was significantly hypomethylated in the cells of patients with HSTCL. The hypomethylation of the *Tox* locus was more significant in cells from patients of the gamma delta subtype. These combined data suggest that *Tox* may play a role in the biology of HSTCL.

Keywords: hepatosplenic, T-cell lymphoma, HSTCL, *Tox*

Introduction

Hepatosplenic T-cell lymphoma is a rare cancer with a median survival of eight months (1). By comparing the transcriptomes of spleens from patients with HSTCL with that of non-malignant spleen tissue (2) I found that the transcription factor *Tox* was one of the most differentially expressed genes in HSTCL tumor cells. In a separate patient cohort dataset (3), by comparing global methylation of the genomes of tumor cells from patients with HSTCL with that of benign CD4, CD8 and gamma delta T-cells, I found that the *Tox* genomic locus was among the most differentially methylated regions in the genomes of patients with HSTCL. *Tox* was significantly hypomethylated in the tumor cells of patients with the gamma delta disease subtype and to a lesser degree in tumor cells of patients with the alpha beta disease subtype. These combined data suggest that *Tox* may play a significant role in the biology of hepatosplenic T-cell lymphomas.

Methods

Comparative analysis of transcriptomes and methylomes was performed using GEO2R. Datasets GSE57944 (GPL570) (2) and GSE110081 (3) were used. Dataset GSE57944 is genome-wide RNA expression data obtained by microarray analysis of three control spleens and the splenic tissue from four patients with hepatosplenic T-cell lymphoma (2). Dataset GSE110081 is a genome-wide methylation dataset obtained by bisulfite sequencing combined with Illumina Infinium 450k Human DNA methylation beadchip technology of HSTCL cells from 4 patients with HSTCL $\gamma\delta$, HSTCL cells from 6 patients with HSTCL $\alpha\beta$, and $\alpha\beta$ T- (CD4+ or CD8+) or $\gamma\delta$ T-cells isolated from 4 healthy male donors (3). The 10 HSTCL patient samples were peripheral blood (1 patient), bone marrow (1 patient) or spleen (8 patients). The two hundred and fifty most differentially expressed genes in a given comparison were identified. Statistical significance at systems level was determined by GEO2R (p-value). Statistical analysis to compare expression values of each group was performed using a Welch's t test

(GSE57944) or a one-way ANOVA with multiple comparisons (GSE110081) based on n using PRISM 8.0.

Results

I performed a systems-level analysis of HSTCL using two publicly available datasets (2, 3).

First, I used global gene expression profiling to determine the genes whose expression was most different in the tumor cells of patients with HSTCL when compared to normal tissue (2). In this analysis, data was obtained from genome-wide microarray expression profiles of splenic tumors of 4 patients with HSTCL. Normal splenic tissue ($n=3$) was used for control comparison. The top two hundred and fifty most differentially expressed genes were identified by comparing the transcriptomes of control splenic tissue to the transcriptomes of splenic tumors from patients with HSTCL. This analysis identified the transcription factor *Tox* as one of the most differentially expressed genes in tumors from patients with HSTCL ($p=0.0000244$). *Tox* was significantly over-expressed in the tumors of patients with HSTCL when compared to control tissue (Figure 1, $p<0.0001$).

Next, I used a second dataset to perform genome-wide methylation profiling analysis to determine the genomic loci most differentially methylated in the tumor cells of patients with HSTCL, either peripheral blood (1 patient), bone marrow (1 patient) or spleen (8 patients). The top two hundred and fifty most differentially methylated loci in cells from patients with HSTCL $\alpha\beta$ and HSTCL $\gamma\delta$ were identified by comparing their genome-wide methylation profiles to that of $\alpha\beta$ and $\gamma\delta$ T-cells from healthy donors (3). This analysis identified the transcription factor *Tox* as one of the most differentially methylated loci in the genomes of patients with HSTCL $\gamma\delta$ ($p=0.000000000000813$). The *Tox* genomic locus contained less methyl marks in patients with HSTCL $\alpha\beta$ when compared to T-cells from healthy donors, as well (Figure 2, $p=0.0173$). Interestingly, the *Tox* locus was significantly less methylated in the cancer cells of

patients with HSTCL $\gamma\delta$ compared to the cancer cells of patients with HSTCL $\alpha\beta$ (Figure 2, $p < 0.0001$).

Discussion

HSTCL is a rare, aggressive cancer of T-cells that has a median survival of eight months (1). By integrating systems-level data from patients with a rare cancer, HSTCL, I found that the *Tox* gene was differentially expressed at higher levels and differentially methylated at lower levels when compared to healthy tissue or cells, respectively.

Interestingly, the *Tox* genomic locus was significantly less methylated in the cells of patients with HSTCL $\gamma\delta$ when compared to the cells of patients with HSTCL $\alpha\beta$.

Tox was recently identified as being critical for the exhaustion program in CD8+ T-cells (4), for T-cell exhaustion in viral infection (5), and as a regulator of “dysfunctional” tumor-specific T-cells (6).

The findings here suggest that *Tox* may play a role in the initiation or maintenance of the transformed state in hepatosplenic T-cell lymphoma, and that therapeutic targeting of *Tox* and/or modulation of *Tox* expression may be clinically advantageous in HSTCL.

References

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Figure 1: The Tox gene is differentially over-expressed in the cells of patients with hepatosplenic T-cell lymphoma.

The RNA expression value of the Tox gene in healthy spleen tissue (blue; $n=3$) or splenic tumors from patients with HSTCL (red; $n=4$) is shown. The bar represents the mean RNA expression value.

Figure 2: The *Tox* genomic locus is hypomethylated in the cancer cells of patients with HSTCL $\gamma\delta$, and to a lesser extent, in the cancer cells of patients with HSTCL $\alpha\beta$.

The methylation status at the *Tox* locus in control T-cells (CD4+, CD8, and $\gamma\delta$ T-cells) from healthy donors (blue; $n=12$), from patients with HSTCL $\alpha\beta$ (green; $n=4$) or from patients with HSTCL $\gamma\delta$ (red; $n=6$) is shown. The bar represents the mean methylation value.