Fas ligand is differentially expressed in a hepatosplenic T-cell lymphoma cell line.

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Hepatosplenic T-cell lymphoma is an aggressive cancer of T-cells that has a median survival of 8 months (1). A hallmark of cancer cells is dysregulation of survival signaling (2). The Fas receptor and Fas ligand are critical molecules in the transduction of death signals (3). By comparing the transcriptomes of 61 hematopoietic cancer cell lines I found that the FASLG gene encoding the protein Fas ligand (CD95L) is significantly and specifically over-expressed in the DERL-2 HSTCL cell line. Protein-protein interaction network analysis revealed a potential interaction between Fas ligand and Stat5b, a gene frequently mutated in hepatosplenic T-cell lymphoma (5). The Fas pathway may represent an attractive avenue for discovery and research of therapeutic targets in HSTCL.

Keywords: HSTCL, hepatosplenic T-cell lymphoma, Fas, Fas ligand, differential gene expression analysis, systems oncology

Introduction

Cancers are marked by their inability to stop growing, or proliferating (2). This can be the result of an inability to stop proliferating or a propensity to keep proliferating. The Fas pathway is a key signaling pathway by which T-cells execute death signaling to cease proliferation and induce apoptosis (3). Hepatosplenic T-cell lymphoma, or HSTCL is a rare, aggressive peripheral T-cell lymphoma that has a median survival of eight months (1). There are only two HSTCL cell lines in use, both derived from the same individual (4). To determine the key gene expression features that distinguish HSTCL from other blood cancers I used differential gene expression analysis to compare the transcriptome of the DERL-2 cell line to 60 other hematopoietic cancer cell lines. I found that the Fas ligand, encoded by FASLG, is differentially expressed by the DERL-2 cell line, at higher levels than each of 60 cell lines it was compared to. The specific and significant up-regulation of FASLG at the level of RNA may represent an artifact of adaptation to HSTCL growth *in vitro*, or it may represent a bona fide characteristic of HSTCL.

Methods

Dataset GSE57083 (Astra Zeneca cell line expression library; U133 Affymetrix Human Genome array) was used for comparative transcriptome analysis for identification of differentially expressed genes using GEO2R. The top two hundred and fifty most differentially expressed genes between all 61 cell lines were identified using “Benjamini and Hochberg” p-value adjustment and “auto-detect” log transformation and NCBI generated category of platform annotation. The name of each cell line is included in Table 1. The statistical significance of the difference between mean RNA expression values of each group was calculated by Ordinary one-way ANOVA with Dunnett’s multiple comparisons test (PRISM 8.1.2). Protein-protein interaction network analysis was performed using STRING. Confidence level was set to medium, with 50 1st shell interactors and 10 2nd shell interactors.

Confidence-based network edge was chosen (thickness of line represents confidence of interaction), and k-means clustering was set to 5.

Results

I performed differential gene expression analysis of an HSTCL cell line, DERL-2, alongside 60 hematopoietic cancer cell lines using GEO2R and transcriptome data from the Astra Zeneca cell line library. The global gene expression profile of DERL-2 was compared to that of seventeen acute myeloid leukemia (AML) cell lines, four chronic myelogenous leukemia (CML) cell lines, fifteen diffuse large B-cell lymphoma (DLBCL) cell lines, five B-cell non-Hodgkin's lymphoma (B-NHL) cell lines, 2 Burkitt's lymphoma cell lines, a T-cell cutaneous leukemia cell line, four B-cell chronic lymphoid leukemia (B-CLL) cell lines, ten Myeloma cell lines, one Sezary syndrome cell line and one B-cell acute lymphoblastic leukemia (B-ALL) cell line (Table 1).

The transcript produced by the FASLG gene, encoding Fas ligand, was significantly differentially expressed by the HSTCL DERL-2 cell line ($p=3.59 \times 10^{-16}$). Transcript for Fas ligand was expressed at significantly higher levels in DERL-2 HSTCL cells than any other hematopoietic cancer type studied (Figure 1; $p<0.0001$ for all comparisons) and was expressed at higher levels in DERL-2 cells than each of 60 other cell lines (Figure 2).

To further ascertain whether any systems-level insights on the role of Fas ligand in HSTCL could be ascertained, I used STRING (6) to predict protein-protein interactions between Fas ligand. This analysis revealed a potential interaction between Fas ligand and Stat5b (Figure 3); Stat5b is recurrently mutated in patients with hepatosplenic T-cell lymphoma and is thought to function as a driving mutation in the context of T-cell neoplasia (7).

Discussion

I used global differential gene expression analysis to compare the transcriptomes of 61 cancer cell lines of hematopoietic origin. I found that the gene encoding the Fas ligand, FASLG, was one of the most differentially expressed genes among these cell lines, and that the

hepatosplenic T-cell lymphoma cell line DERL-2 expressed Fas ligand at higher levels than each of the other 60 cancer cell lines. DERL-2 is one of only two established HSTCL cell lines (4). Interestingly, protein-protein interaction network analysis (6) revealed that Fas ligand may potentially interact with Stat5b. Stat5b is recurrently mutated in patients with HSTCL (5) and mutation of Stat5b has been shown to function as a driver of T-cell neoplasia (7).

Cancer cells are characterized by their inability to properly transduce death signaling, or to “resist” cell death (2) and the Fas pathway induces apoptotic signaling in T-lymphocytes (3). HSTCL is an aggressive peripheral T-cell lymphoma with a median survival of less than one year (1).

As the Fas pathway is intimately involved with T-lymphocyte survival (3), the specific and significant up-regulation of Fas ligand in the DERL-2 HSTCL cell line, if validated as a general feature of HSTCL, may represent an attractive avenue for therapeutic intervention in hepatosplenic T-cell lymphoma and demonstrates the ability of systems-level transcriptome data combined with differential gene expression analysis to illustrate key features of a rare cancer.

References

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Figure 1: Fas ligand is differentially expressed in the HSTCL cell line DERL-2 and at higher levels than a panel of 60 hematopoietic cancer cell lines.

The expression value of the FasLG message from 61 cancer cell lines is shown. The bar represents the mean RNA expression value. AML: $n=17$, CML: $n=4$, DLBCL: $n=15$, B-NHL: $n=5$, Burkitt's Lymphoma: $n=2$, TCL: $n=1$, B-CLL: $n=4$, Myeloma: $n=10$, HSTCL: $n=1$, Sezary Syndrome: $n=1$, B-ALL: $n=1$.

Figure 2: Expression of Fas ligand in each individual cell line.

The expression value of the FasLG message from 61 cancer cell lines is independently shown.

Cancer Type	Sample	Sample Title (NCBI)	Cell line name
AML	GSM1374458	CTV-1 ghp221105	CTV1
AML	GSM1374527	EA10068_111346	HEL9217
AML	GSM1374536	HL 60 ghp221105	HL-60
AML	GSM1374591	EA10068_111342	KASUMI3
AML	GSM1374597	KG-1 ghp231105	KG-1
AML	GSM1374598	KG-1a ghp231105	KG-1a
AML	GSM1374683	ML-2 ghp231105	ML-2
AML	GSM1374689	MOLM13	MOLM13
AML	GSM1374694	MV-4-11 ghp231105	MV411
AML	GSM1374782	OCI-AML5 ghp280606	OCI-AML5
AML	GSM1374792	EA10068_111343	OCIM2
AML	GSM1374868	EA10068_111345	SET2
AML	GSM1374880	SKM-1 ghp231105	SKM-1
AML	GSM1374961	TF-1 ghp231105	TF-1
AML	GSM1374962	THP-1 ghp231105	THP-1
AML	GSM1374976	EA10068_111344	UKE1
AML	GSM1374979	EA10068_111341	UT7
CML	GSM1374474	EA10068_111347	EM2
CML	GSM1374583	K562 ghp231105	K562

Table 1: Identifying information for cell lines used in this study.

Cancer Type	Sample	Sample Title (NCBI)	Cell line name
CML	GSM1374606	KU812 ghp231105	KU812
CML	GSM1374669	EA10068_111348	MEG01
DLBCL	GSM1374481	EA10068_203557_HG-U133_PLUS_2_FARAGE	FARAGE
DLBCL	GSM1374489	EA10068_203542_HG-U133_PLUS_2_HBL1	HBL1
DLBCL	GSM1374539	EA10068_203543_HG-U133_PLUS_2_HLY-1	HLY-1
DLBCL	GSM1374775	EA10068_203561_HG-U133_PLUS_2_NU-DHL-1	NU-DHL-1
DLBCL	GSM1374845	EA10068_203554_HG-U133_PLUS_2_RC-K8	RC-K8
DLBCL	GSM1374849	EA10068_203546_HG-U133_PLUS_2_RI-1	RI-1
DLBCL	GSM1374904	EA10068_203559_HG-U133_PLUS_2_SUDHL10	SUDHL10
DLBCL	GSM1374905	EA10068_203547_HG-U133_PLUS_2_SUDHL2	SUDHL2
DLBCL	GSM1374909	EA10068_203555_HG-U133_PLUS_2_SUDHL8	SUDHL8
DLBCL	GSM1374963	EA10068_203548_HG-U133_PLUS_2_TMD8	TMD8
DLBCL	GSM1374971	EA10068_203549_HG-U133_PLUS_2_U2932	U2932
DLBCL	GSM1374972	EA10068_203550_HG-U133_PLUS_2_U-2940	U-2940
DLBCL	GSM1374973	EA10068_203562_HG-U133_PLUS_2_U-698-M	U698M
DLBCL	GSM1374989	EA10068_203552_HG-U133_PLUS_2_WILL-1	WILL-1
DLBCL	GSM1374990	EA10068_203553_HG-U133_PLUS_2_WILL-2	WILL-2
B-NHL	GSM1374467	DOHH-2 ghp221105	DOHH2
B-NHL	GSM1374558	HT ghp280606	HT
B-NHL	GSM1374577	JEKO ghp280606	JEKO

Cancer Type	Sample	Sample Title (NCBI)	Cell line name
B-NHL	GSM1374585	Karpas1106 ghp280606	Karpas1106
B-NHL	GSM1374861	SC1 ghp280606	SC1
Sezary Syndrome	GSM1374569	HUT78	HUT78
Lymphoma (Burkitt's)	GSM1374841	Raji ghp231105	Raji
Lymphoma (Burkitt's)	GSM1374844	Ramos ghp231105	Ramos
TCL	GSM1374580	Jurkat ghp280606	Jurkat
B-CLL	GSM1374392	AMO1 ghp280606	AMO1
B-CLL	GSM1374393	ARH77 ghp280606	ARH77
B-CLL	GSM1374572	IM9 ghp280606	IM9
B-CLL	GSM1374579	JJN3 ghp280606	JJN3
Myeloma	GSM1374589	Karpas620 ghp280606	Karpas620
Myeloma	GSM1374601	KMS12BM ghp280606	KMS12BM
Myeloma	GSM1374615	L363 ghp280606	L363
Myeloma	GSM1374631	LP1 ghp280606	LP1
Myeloma	GSM1374691	MOLP8 ghp280606	MOLP8
Myeloma	GSM1374767	NCLH929 ghp280606	NCLH929
Myeloma	GSM1374799	OPM-2 ghp280606	OPM-2
Myeloma	GSM1374855	RPMI8226 ghp280606	RPMI8226
Myeloma	GSM1374882	SKMM2 ghp150606	SKMM2
Myeloma	GSM1374970	U266B1 ghp150606	U266B1
HSTCL	GSM1374460	Bx030_020 HG2 DERL2	DERL-2
B-ALL	GSM1374587	KARPAS-231 ghp231105	KARPAS231

Figure 3: Protein-protein interaction network analysis reveals a potential interaction between Fas ligand and Stat5b. A protein-protein interaction network map generated by STRING analysis (6) is shown, with FASLG as the center node and STAT5B clustered with FASLG in blue.