

Putative DNA/RNA helicase Schlafen-11 (SLFN11) sensitizes cancer cells to DNA-damaging agents

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Edited* by Allan H. Conney, Rutgers, State University of New Jersey, Piscataway, NJ, and approved July 27, 2012 (received for review April 23, 2012)

DNA-damaging agents (DDAs) constitute the backbone of treatment for most human tumors. Here we used the National Cancer Institute Antitumor Cell Line Panel (the NCI-60) to identify predictors of cancer cell response to topoisomerase I (Top1) inhibitors, a widely used class of DDAs. We assessed the NCI-60 transcriptome using Affymetrix Human Exon 1.0 ST microarrays and correlated the in vitro activity of four Top1 inhibitors with gene expression in the 60 cell lines. A single gene, Schlafen-11 (SLFN11), showed an extremely significant positive correlation with the response not only to Top1 inhibitors, but also to Top2 inhibitors, alkylating agents, and DNA synthesis inhibitors. Using cells with endogenously high and low SLFN11 expression and siRNA-mediated silencing, we show that SLFN11 is causative in determining cell death and cell cycle arrest in response to DDAs in cancer cells from different tissues of origin. We next analyzed SLFN11 expression in ovarian and colorectal cancers and normal corresponding tissues from The Cancer Genome Atlas database and observed that SLFN11 has a wide expression range. We also observed that high SLFN11 expression independently predicts overall survival in a group of ovarian cancer patients treated with cisplatin-containing regimens. We conclude that SLFN11 expression is causally associated with the activity of DDAs in cancer cells, has a broad expression range in colon and ovarian adenocarcinomas, and may behave as a biomarker for prediction of response to DDAs in the clinical setting.

To date, DNA-damaging chemotherapeutic agents constitute the backbone of treatment for most solid and hematological tumors, despite the introduction of targeted therapies, which have changed the outcome of a minority of cancers, including chronic myelogenous leukemia (1), gastrointestinal stromal tumors (GISTs) (imatinib) (2), HER2+ breast tumors (trastuzumab) (3, 4), and CD20+ non-Hodgkin's lymphomas (5) (rituximab).

In the case of DNA-damaging chemo-radiotherapy regimens, no individual biomarker has been shown to be superior to tumor clinical stage and pathological features in predicting treatment response. With the exception of cell proliferation biomarkers, such as Ki-67 (6), none has yet entered clinical oncology practice to predict response to DNA-damaging agent (DDA)-based therapy and outcome, although promising results are expected for molecular markers such as γ -H2AX (7). It is thus clear that robust system pharmacology models are needed to identify factors that may predict sensitivity or resistance to DDA-based treatment of human neoplasia.

The NCI-60 encompasses 60 human cancer cell lines from nine different tissues of origin. It has been tested since the 1980s for more than 400,000 compounds of natural and synthetic origin (8–10). The NCI-60 panel has also been extensively characterized for gene expression using six different microarray platforms (11–13) and copy-number variation by array-based comparative genomic hybridization (aCGH) (9, 14) and has recently been sequenced for the entire exome at the National Cancer Institute (National Institutes of Health).

The aim of the present study was to identify genes that, at the transcript expression level, could predict cancer cell response to

DDAs such as topoisomerase I (Top1) inhibitors, topoisomerase II (Top2) inhibitors, or alkylating agents, with potential clinical relevance for the development of predictive biomarkers.

Results

SLFN11 Expression Correlates with the Antiproliferative Activity of Top1 Inhibitors in the NCI-60. To identify genes that, at the expression level, could predict the in vitro cytotoxicity profiles of Top1 inhibitors in the NCI-60, we correlated the expression profiles of more than 17,000 genes with the activity of four different Top1 inhibitors. We found that 294 genes were correlated with the cellular response to camptothecin (CPT), 217 with topotecan, 699 with irinotecan, and 734 with a non-CPT derivative in clinical trial, NSC724998 (15) (Pearson's $r > |0.33|$). By intersecting these four sets of data, we identified 42 genes, 23 of which were positively and 19 negatively correlated with the response to the four Top1 inhibitors (Table 1 and Fig. S1). Notably, the expression of a single gene, Schlafen-11 (SLFN11), showed a Pearson's $r > 0.613$ with the response to all of the four compounds [$P < 2.5 \times 10^{-7}$, false discovery rate (FDR) < 0.001 ; Fig. 1]. SLFN11 mRNA expression in the NCI-60 Panel is shown in Fig. S2 and Dataset S1. To assess the relationship between SLFN11 transcript and SLFN11 protein levels, we measured SLFN11 expression by Western blotting in several NCI-60 cell lines. High SLFN11 protein levels were detected in cell lines overexpressing the SLFN11 transcript, such as DU-145 or HOP-62 (Figs. S2 and S3A). Conversely, SLFN11 protein expression was very low in cell lines with low SLFN11 mRNA levels (e.g., MDA-MB-231 and HCT-116) (Figs. S2 and S3A).

SLFN11 Expression Correlates with the Antiproliferative Activity of Top1 Inhibitors, Top2 Inhibitors, and Alkylating Agents. We next expanded our correlation analysis to include a total of 1,444 compounds with a known mechanism of action, which have been tested independently in the NCI-60 more than twice (9, 12, 13). The high positive correlation between SLFN11 expression and cytotoxicity was not limited to Top1 inhibitors, but was also strongly significant for different categories of DDAs (Dataset S2). These included alkylating agents such as cisplatin (Pearson's $r = 0.619$, $P = 1.7 \times 10^{-7}$, FDR < 0.001) and Top2 inhibitors such

Author contributions: G.Z., M.R., E.L., and Y.P. designed research; G.Z., M.R., E.L., and S.V. performed research; G.Z., S.V., W.C.R., and J.H.D. contributed new reagents/analytic tools; G.Z., M.R., E.L., W.C.R., S.V., A.B., J.H.D., and Y.P. analyzed data; and G.Z., M.R., E.L., J.H.D., and Y.P. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Data deposition: The gene expression microarray data and compound cytotoxicity data reported in this paper are available in the relational database CellMiner, <http://discover.nci.nih.gov/cellminer>.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1205943109/-DCSupplemental.

Table 1. Top1 inhibitors share common correlating genes

Gene symbol*	GO biological process [†]	Camptothecin [‡]	Topotecan [‡]	Irinotecan [‡]	NSC724998 [‡]
SLFN11	—	0.707	0.714	0.613	0.613
STRADA	Protein amino acid phosphorylation	0.556	0.450	0.440	0.518
EP400	Chromatin modification	0.513	0.404	0.516	0.417
SFPQ	Alternative nuclear mRNA splicing via spliceosome	0.402	0.428	0.496	0.395
MST4	Protein amino acid phosphorylation	0.390	0.333	0.517	0.465
DDX23	cis-assembly of precatalytic spliceosome	0.365	0.369	0.548	0.419
RAB39B	Small GTPase-mediated signal transduction	0.380	0.348	0.456	0.496
PFAS	Purine nucleotide biosynthetic process	0.406	0.396	0.410	0.446
CUTC	Copper ion transport	0.455	0.389	0.398	0.389
ASB3	Intracellular signaling cascade	0.392	0.394	0.439	0.399
ZNF764	Regulation of transcription, DNA-dependent	0.452	0.392	0.373	0.400
ARHGEF6	Apoptosis	0.366	0.406	0.423	0.408
SPAG5	Cell cycle	0.390	0.365	0.444	0.395
CHD1L	DNA repair	0.360	0.393	0.387	0.445
METT10D	—	0.357	0.420	0.412	0.394
POLE3	DNA replication	0.354	0.353	0.454	0.390
ZFP1	Regulation of transcription, DNA-dependent	0.351	0.355	0.428	0.411
DCP1B	Nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	0.352	0.357	0.460	0.370
ELMOD1	Phagocytosis	0.384	0.338	0.431	0.336
SAFB2	Regulation of transcription	0.364	0.379	0.350	0.384
GIN53	DNA replication	0.361	0.357	0.354	0.402
HTATSF1	Regulation of transcription from RNA polymerase II promoter	0.345	0.364	0.353	0.406
DHX38	Nuclear mRNA splicing, via spliceosome	0.383	0.356	0.352	0.360
NBEAL1	—	-0.335	-0.343	-0.347	-0.382
FUCA1	Carbohydrate metabolic process	-0.366	-0.388	-0.403	-0.359
NPAS2	Regulation of transcription, DNA-dependent	-0.382	-0.354	-0.446	-0.368
FGD4	Apoptosis	-0.355	-0.374	-0.453	-0.370
NBEAL1	—	-0.336	-0.357	-0.421	-0.440
KLF3	Multicellular organismal development	-0.345	-0.341	-0.422	-0.451
TMEM38A	Ion transport	-0.409	-0.442	-0.358	-0.354
MYO1D	—	-0.395	-0.395	-0.366	-0.441
ASAP2	Regulation of ARF GTPase activity	-0.363	-0.362	-0.417	-0.455
PPFIA1	Cell-matrix adhesion	-0.407	-0.396	-0.377	-0.418
TES	—	-0.426	-0.475	-0.370	-0.346
SHROOM3	Cell morphogenesis	-0.359	-0.446	-0.380	-0.441
CHKA	Lipid metabolic process	-0.476	-0.372	-0.400	-0.384
GIPC1	Protein targeting	-0.419	-0.342	-0.444	-0.455
SLC44A3	—	-0.421	-0.465	-0.345	-0.460
JAG1	Angiogenesis	-0.394	-0.408	-0.452	-0.541
BAIAP2L1	Signal transduction	-0.468	-0.473	-0.480	-0.476
FBNP1L	Endocytosis	-0.458	-0.406	-0.486	-0.553
TGFBR3	Epithelial to mesenchymal transition	-0.540	-0.412	-0.483	-0.485

GI₅₀ cytotoxicity profiles of CPT, topotecan, irinotecan, and the indenoisoquinoline NSC724998 were correlated with the expression of 17,866 gene transcripts obtained from the NCI-60 panel tested with the Affymetrix Human Exon 1.0 ST microarray chips. The lists of genes whose transcription profile correlated with these drugs with a Pearson's $r > |0.33|$ were intersected to obtain the present list, which represents the 42 genes that correlate, positively or negatively, with all four TOP1 inhibitors. SLFN11 is the top common positively correlating gene and also the top positively correlating gene for all four drugs.

*Official gene symbol according to the Human Gene Organization (HUGO) nomenclature.

[†]Gene Ontology (GO) Biological Process annotation.

[‡]Pearson's correlation coefficient.

as etoposide (Pearson's $r = 0.533$, $P = 1.4 \times 10^{-6}$, $FDR < 0.01$), which are both widely used in clinical oncology. Overall, 34 genes had significant correlations at a $P < 0.01$ with the three prototypic drugs, CPT, cisplatin, and etoposide (Fig. S4 and Table S1). Of the human Schlafen family of genes for which expression profiles were available in the NCI-60, SLFN11 was the only one showing a significant correlation with all of the aforementioned DDAs (for the relative expression of SLFN11, SLFN12, SLFN13, SLFN5, and SLFNL1, see Fig. S5). Interestingly, although significantly correlated with the in vitro cytotoxicity profiles of several DDAs used in oncological practice, SLFN11 expression did not exhibit any association with the response to protein kinase

inhibitors or drugs targeting other components of the cell, such as tubulin poisons (Table 2 and Dataset S2).

SLFN11 Expression Is Causally Associated with the Activity of DDAs in Human Cancer Cells. Our statistical observations were surprising because the Schlafen family of genes has been studied mainly in the context of immune processes, mouse embryonic lethality, meiotic drive, and orthopoxvirus virulence (16).

To test the causal relationship between SLFN11 expression and sensitivity to DDAs, we transfected SLFN11-targeting siRNAs in two different human cancer cell lines, DU-145 (prostate cancer) and HOP-62 (non-small-cell lung cancer). Both express

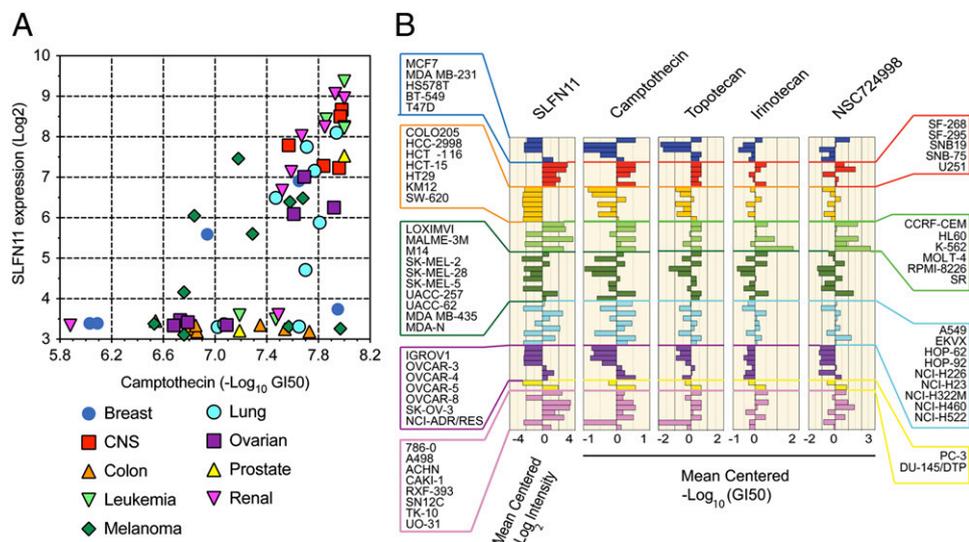


Fig. 1. SLFN11 expression is highly correlated with the in vitro antiproliferative activity of Top1 inhibitors. (A) Scatterplot showing the correlation between SLFN11 expression (y axis, Log₂ intensity) and CPT antiproliferative activity (x axis, negative Log₁₀ growth inhibitory molar concentration 50%, GI50) in the NCI-60. (B) Mean centered bar charts representing SLFN11 expression in the NCI-60 and the antiproliferative activities of CPT, topotecan, irinotecan, and the indenoisoquinoline Top1 inhibitor in clinical trial NSC724998. Color codes correspond to tissues of origin.

high SLFN11 levels and are highly sensitive to Top1 inhibitors (Fig. 1B). In both cell lines, siRNA-mediated silencing proved to be highly efficient and was sustained for 3 d or more (Fig. 2A, Lower Right, and Fig. S6.4). SLFN11-silenced cells exhibited at least a fivefold reduced sensitivity to CPT, etoposide, and cisplatin compared with mock-silenced cells ($P \leq 0.01$, Fig. 2A, Upper, and Fig. S6.4). These results demonstrate that SLFN11 confers sensitivity to Top1 inhibitors, Top2 inhibitors, and alkylating agents. In agreement with our bioinformatic predictions, SLFN11 silencing did not affect the cellular response to either the tubulin poison paclitaxel or the broad-spectrum protein kinase inhibitor and apoptosis inducer staurosporine (17) (Fig. 2A, Lower, and Fig. S6.4).

The influence of SLFN11 on long-term cell survival after CPT treatment was further tested by clonogenic assays. Three days after SLFN11 knockdown, DU-145 cells were treated with 100 nM CPT for 24 h and then released in fresh medium and grown for 15 d to allow colony formation. SLFN11-silenced cells were able to form multiple growing colonies (representative image in Fig. 2B). Similar results were obtained after treatment with different concentrations of CPT (20–500 nM for 24 h) or after a 1-h incubation with 1 μ M CPT followed by a 15-d drug-free growth ($P < 0.01$; Fig. 2B, Right, and Fig. S6C).

To rule out silencing-related off-target effects, we transfected SLFN11-targeting siRNAs in two cell lines expressing low SLFN11 levels (MDA-MB-231 breast cancer and HCT-116 colon carcinoma cells) (Fig. 1B). SLFN11 silencing did not modify the response to CPT, etoposide, and cisplatin in these two cell lines (Fig. 2C and Fig. S6B), demonstrating the specificity of SLFN11-targeting siRNAs. Together, these results demonstrate that SLFN11 is a determinant of the cellular responses to DNA damage.

To better understand how SLFN11 modulates cellular sensitivity to DDAs, we examined the effect of SLFN11 silencing on cell cycle distribution after DNA damage. Cell cycle profiles were determined by flow cytometry in control and SLFN11-silenced DU-145 cells treated with 100 nM CPT. As expected, after CPT treatment, control cells were arrested in early S phase with significant cellular fragmentation (27.4% sub-G1 cells) (Fig. 2D). On the contrary, SLFN11 silencing reduced CPT-induced S-phase arrest with a fraction of cells still progressing into S and G2/M (Fig. 2D). In addition, SLFN11 silencing prevented sub-G1 induction (Fig. 2D). It is noteworthy that SLFN11 silencing had no effect on cell cycle distribution in the absence of drug

(Fig. 2D). Together, these results suggest that SLFN11 plays a role in cell cycle arrest and/or induction of apoptosis in response to exogenously induced DNA damage. Consistent with a role of SLFN11 in the DNA damage response, immunostaining of DU-145 cells with the anti-SLFN11 antibody showed that the endogenous SLFN11 protein localizes in the nucleus (Fig. S3B).

SLFN11 Expression Varies Widely in Clinical Colon and Ovarian Adenocarcinoma Specimens and May Predict Overall Survival in Ovarian Cancer Patients Treated with Cisplatin-Containing Regimens.

We next evaluated the expression of SLFN11 in randomly chosen microarray data obtained from patients affected by colon adenocarcinoma or ovarian cystadenocarcinoma and from corresponding healthy tissues available from The Cancer Genome Atlas database (<http://cancergenome.nih.gov>) (Fig. 3A and Dataset S3), as Top1 inhibitors and platinum derivatives play an important role in the therapeutic management of those tumors. Tumor samples showed a greater than fivefold SLFN11 expression range in the ovarian cystadenocarcinoma and sixfold in the colon adenocarcinoma samples analyzed. Although mean SLFN11 expression in cancer specimens was lower than in normal tissues ($P < 0.01$ for colon adenocarcinoma and $P = 0.06$ for ovarian cystadenocarcinoma, unpaired t tests with Welch correction), expression variability tended to be greater in tumors than in normal counterparts, with several cancer samples showing SLFN11 expression higher or lower than the interquartile range for its expression in normal tissues ($P = 0.057$ for colon adenocarcinoma and $P < 0.05$ for ovarian cystadenocarcinoma, F test for equality of variance, one-tailed).

On an exploratory basis, we evaluated a recent, well-annotated microarray dataset of 110 ovarian cancer patients treated with a cisplatin-containing regimen (18). SLFN11 greater or lower than mean expression stratified patient data using univariate analysis for overall survival (OS), with a median of 80 mo [95% confidence interval (CI) = 55–105] for high SLFN11 expressers vs. 49 mo (95% CI 38–60) for low SLFN11 expressers ($P = 0.016$, log-rank Mantel–Cox analysis, Fig. 3B). Moreover, SLFN11 was still significant for OS prediction in a multivariate model retaining only predictors with a $P < 0.1$, together with optimal (i.e., complete resection) surgery at diagnosis [$P = 0.05$, hazard ratio 1.35 (95% CI = 1.00–1.83) for SLFN11, and $P = 0.04$, hazard ratio 1.91 (95% CI = 1.03–3.55) for optimal surgery, Cox regression analysis, backward stepwise Wald].

Table 2. Clinically used anticancer agents and their correlations with SLFN11 transcript

NSC*	Chemical name [†]	Mechanism of action	<i>r</i> [‡]	<i>P</i> value [§]	FDR [¶]
Compounds with significantly positive correlation with SLFN11 transcript					
609699	Topotecan	Topoisomerase I inhibitor	0.714	2.2×10^{-10}	1.9×10^{-9}
616348	Irinotecan	Topoisomerase I inhibitor	0.613	2.5×10^{-7}	7.3×10^{-6}
301739	Mitoxantrone	Topoisomerase II inhibitor	0.624	1.3×10^{-7}	4.5×10^{-6}
141540	Etoposide	Topoisomerase II inhibitor	0.533	1.4×10^{-5}	2.2×10^{-10}
82151	Daunorubicin	Topoisomerase II inhibitor	0.399	0.002	0.018
v123127	Doxorubicin	Topoisomerase II inhibitor	0.387	0.002	0.024
3088	Chlorambucil	Alkylating agents at N7 guanine	0.750	8.1×10^{-12}	1.2×10^{-9}
8806	Melphalan	Alkylating agents at N7 guanine	0.680	3.2×10^{-9}	3.3×10^{-9}
119875	Cisplatin	Alkylating agents at N7 guanine	0.619	1.7×10^{-7}	5.4×10^{-6}
241240	Carboplatin	Alkylating agents at N7 guanine	0.488	8.9×10^{-5}	0.001
409962	Carmustine	Alkylating agents at O6 guanine	0.439	0.001	0.006
613327	Gemcitabine	DNA synthesis inhibitor	0.669	7.0×10^{-9}	7.0×10^{-9}
V63878	Cytarabine	DNA synthesis inhibitor	0.644	3.8×10^{-8}	1.5×10^{-6}
Compounds with nonsignificant correlation with SLFN11 transcript					
67574	Vincristine	Tubulin active antimetabolic agent	0.067	n.s.	n.s.
49842	Vinblastine	Tubulin active antimetabolic agent	0.054	n.s.	n.s.
125973	Taxol	Tubulin active antimetabolic agent	-0.100	n.s.	n.s.
718781	Erlotinib	Kinase inhibitor	0.147	n.s.	n.s.
732517	Dasatinib	Kinase inhibitor	0.041	n.s.	n.s.
750690	Sunitinib	Kinase inhibitor	0.001	n.s.	n.s.
747971	Sorafenib	Kinase inhibitor	-0.075	n.s.	n.s.
715055	Gefitinib	Kinase inhibitor	-0.090	n.s.	n.s.
745750	Lapatinib	Kinase inhibitor	-0.093	n.s.	n.s.
743414	Imatinib	Kinase inhibitor	-0.167	n.s.	n.s.
747599	Nilotinib	Kinase inhibitor	-0.212	n.s.	n.s.
733504	Everolimus	mTOR inhibitor	0.160	n.s.	n.s.
109229	L-asparaginase	Protein synthesis inhibitor	-0.086	n.s.	n.s.
681239	Bortezomib	Proteasome inhibitor	-0.123	n.s.	n.s.

Correlation is between the in vitro activities of commonly used FDA-approved anticancer drugs and SLFN11 transcript across the NCI-60. n.s., not significant.

*National Service Center (NSC) number assigned by the Developmental Therapeutics Program to compounds tested in the NCI-60.

[†]Commonly used chemical name.

[‡]Pearson's correlation coefficient.

[§]Two-sided *P* value.

[¶]Step-up False Discovery Rate (FDR). An extensive correlation analysis with 1,444 compounds tested in the NCI-60 (9, 10) is available in Dataset S2.

Taken together, these data indicate the wide expression range of SLFN11 in cancers (colon and ovarian carcinoma) that are routinely treated with DDAs, including cisplatin and Top1 inhibitors, and suggest that SLFN11 levels may be a predictive marker at least for ovarian cancer patients treated with DDA-based chemotherapy.

Discussion

By analyzing the NCI-60 Panel of cancer cell lines for the correlation of their transcriptome with the cytotoxicity profiles of four Top1 inhibitors, we identified one gene, SLFN11, that was highly correlated with their in vitro antiproliferative activity. By expanding our analysis to 1,444 compounds tested in the NCI-60 (10, 14), many of which with known mechanisms of action and of common use in clinical practice, we consistently observed significant positive correlations of SLFN11 expression with the cytotoxicity profiles of FDA-approved DDAs, including Top1 inhibitors (topotecan and irinotecan), Top2 inhibitors (doxorubicin, mitoxantrone, etoposide), DNA alkylating agents (chlorambucil, melphalan, cisplatin), and DNA synthesis inhibitors (gemcitabine and fludarabine), but not with drugs targeting other components of the cancer cell, such as protein kinases (erlotinib, sorafenib, dasatinib), tubulin (docetaxel, paclitaxel, vincristine), protein synthesis (L-asparaginase), or the proteasome (bortezomib). We provide evidence that SLFN11 expression is causative in determining sensitivity of human cancer cell lines from different tissues of origin to DDAs because silencing SLFN11 in cells expressing high SLFN11 levels results in resistance to CPT, cisplatin,

and etoposide. We also show that silencing SLFN11 enables DDA-treated cells to maintain cell cycle progression. Finally, we present evidence that SLFN11 expression exhibits a wider expression range in ovarian and colon adenocarcinoma samples from the The Cancer Genome Atlas database than in their corresponding healthy tissues. Most interestingly, SLFN11 high or low expression may be able to stratify patients affected by ovarian cancer and treated with cisplatin-containing regimens for overall survival.

While our data were being presented at the 2012 American Association for Cancer Research Annual Meeting, an independent confirmation of our results was published, which confirmed our findings and their importance for cancer research (19). In contrast to the paper by Barretina et al. (19), our results show that SLFN11 exerts a broader role in determining sensitivity to DDAs, as we find a highly significant association of SLFN11 with the cellular response not only to Top1 inhibitors, but also to Top2 inhibitors, alkylating agents, and DNA synthesis inhibitors in the NCI-60. Moreover, we demonstrate a causative effect of SLFN11 expression as a determinant of cancer cell sensitivity to the above-mentioned compounds; surprisingly, Barretina et al. (19) reported no significant effect of SLFN11 depletion by shRNA on the response to camptothecin derivatives in sarcoma cell lines. This may possibly be due to the choice of different cell lines for silencing experiments as we found that SLFN11 silencing has an impact only on cells with endogenously high SLFN11 expression. It is worth mentioning that sarcomas, which exhibit high SLFN11 levels, are sensitive not only

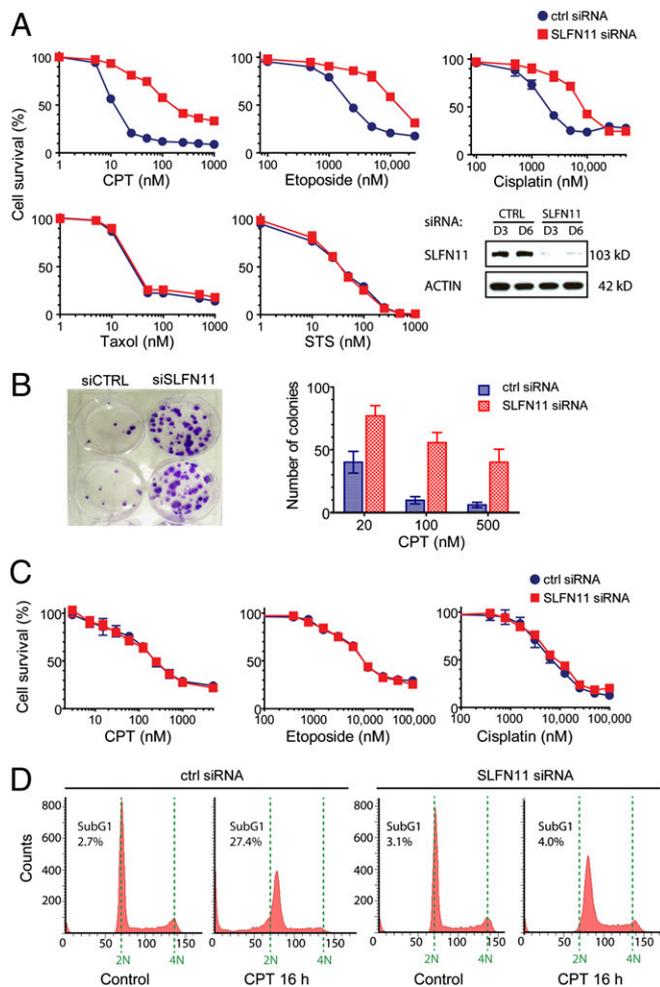


Fig. 2. Silencing SLFN11 significantly reduces sensitivity to different classes of DNA-damaging agents in cells expressing high endogenous SLFN11 levels. (A) Cytotoxicity curves of the prostate cancer cell line DU-145 (high SLFN11 expresser) transfected with nontargeting (ctrl) or SLFN11-targeting siRNAs and treated for 72 h with CPT, etoposide, cisplatin, taxol, or staurosporine (STS). Mean values \pm SD are shown (one representative experiment performed in triplicate). (Lower Right) Western blot showing SLFN11 knock-down 3 and 6 d after transfection with SLFN11-targeting siRNAs. (B) (Left) Representative image of a clonogenic assay (100 nM CPT for 1 d). (Right) Number of colonies formed after 24 h treatment with CPT followed by a 15-d release (average of three independent experiments). (C) Cytotoxicity curves of the breast cancer cell line MDA-MB-231 (low SLFN11 expresser) transfected with nontargeting (ctrl) or SLFN11-targeting siRNAs and treated for 72 h with CPT, etoposide, and cisplatin. Mean values \pm SD are shown (one representative experiment performed in triplicate). Whiskers in all charts represent SDs (when not visible they were within the symbol size). (D) FACS analysis showing sustained cell cycle progression and lack of apoptosis in SLFN11 knockdown DU-145 cells treated with 100 nM CPT for 16 h.

to Top1 inhibitors (20), but also to Top2 inhibitors such as doxorubicin and to alkylating agents such as ifosfamide [see ref. 21 and the National Comprehensive Cancer Network guidelines for soft tissue sarcoma (<http://www.nccn.org>)]. It must be noted, however, that these drugs show activity in a minority of sarcomas, so other molecular determinants must explain why response rates for most subtypes of sarcomas remain extremely low. Interestingly, Garnett et al. (22) have also recently screened a large panel of cancer cell lines to uncover biomarkers of sensitivity and resistance to cancer therapeutics, but have not identified SLFN11 as a marker of response to Top1 inhibitors. As pointed out by Weinstein in his editorial (23), the only partial overlap of cell lines between Barretina's and Garnett's studies, as well as different cell culture

conditions, methods for molecular profiling, and pharmacological assays among our analyses, may explain this apparent discrepancy.

Little is known regarding SLFN11 functions, especially in cancer cells. An important finding of our study is that SLFN11 enhances sensitivity to DDAs but not to other drugs (see Fig. 2 and Fig. S6 and above), suggesting that SLFN11 participates in the DNA damage response. Supporting this hypothesis, we found that SLFN11 is a nuclear protein (Fig. S3B) and that SLFN11-depleted cells fail to arrest in S phase after CPT treatment (Fig. 2D). How the effects on cell cycle relate to SLFN11's putative helicase function remains to be determined. We propose that SLFN11 is a component of the DNA damage response, enforcing cell cycle arrest and apoptosis in response to exogenous DNA injuries.

In conclusion, the discovery of a causative association between SLFN11 expression and the in vitro activity of DDAs in cancer cells, together with the observation of SLFN11's potential relevance in the prediction of the clinical outcome of human tumors treated with those drugs, concur in establishing SLFN11 as a protein with high relevance in both basic and translational cancer research. Although experiments to understand SLFN11 functions in cancer biology are ongoing, SLFN11's role in the clinical setting should be established in future clinical studies.

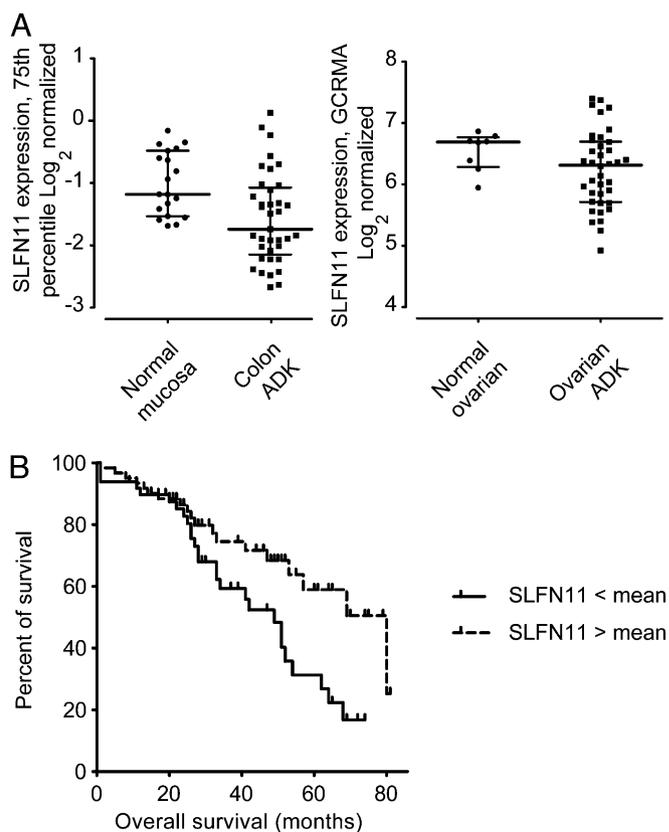


Fig. 3. SLFN11 expression varies over a wide range in human colon and ovarian carcinoma compared with healthy tissues and may predict overall survival in ovarian cancer patients. (A) SLFN11 expression in healthy colon mucosa ($n = 19$) and colon adenocarcinoma ($n = 37$, Left; y axis: Log₂ 75th percentile normalized data obtained using Agilent 244k expression microarrays), and ovarian healthy tissue ($n = 8$) and ovarian cystadenocarcinoma ($n = 38$; Right; y axis: Log₂ GC-robust multiarray normalized data from Affymetrix Human Exon 1.0 ST v2 expression microarrays). Medians are represented as horizontal lines, and whiskers show the interquartile expression ranges. (B) Kaplan-Meier curves of 110 patients affected by ovarian cancer and treated with a first-line cisplatin-containing regimen. Patients are stratified as having higher or lower than average SLFN11 expression levels in that cohort (y axis: percentage survival; x axis: overall survival in months from diagnosis).

Materials and Methods

Microarray Data. Whole-genome expression analysis of the NCI-60 has recently been described (13). Data concerning SLFN11 expression in the ovarian cancer dataset (18) were downloaded from the Gene Expression Omnibus website (<http://www.ncbi.nlm.nih.gov/geo>, accessed Nov. 30, 2010). SLFN11 expression preprocessed data were retrieved from the The Cancer Genome Atlas portal (<http://cancergenome.nih.gov>, accessed Dec. 16, 2011) for a randomly selected subset of 38 ovarian cystadenocarcinomas, 37 colon adenocarcinomas, and corresponding healthy tissues (19 samples from colon mucosa and 8 samples from ovarian tissues). See details in *SI Materials and Methods*.

NCI-60 Drug Cytotoxicity Data. In vitro cytotoxicity data were obtained from our database, CellMiner (9, 13). In addition, data for 15 recently tested drugs were obtained from the Developmental Therapeutics Program website (10). All cancer cell lines and compounds for further tests were obtained from the Developmental Therapeutics Program (National Cancer Institute) and the Drug Synthesis and Chemistry Branch (National Cancer Institute).

siRNA Transfection, Cell Viability, and Clonogenic Assays. Cells were transfected with 5 nM SLFN11-targeting or nontargeting siRNAs, and 72 h after transfection, cells were subjected to 72 h of continuous drug exposure, after which cell viability was measured by MTS assay (Promega). For clonogenic assays, cells were treated as above in six-well plates or T25 flasks and incubated 15 d to allow colony formation.

Protein Assessment, Cell Cycle Analysis, and Confocal Microscopy Experiments. Proteins were separated by SDS/PAGE and immunoblotted with anti-SLFN11 (sc-136891, Santa Cruz Biotechnology) or anti- β -actin (#A5441, Sigma-

Aldrich) antibodies. For cell cycle analysis, cells were prepared and analyzed with a LSRII Fortessa flow cytometer (BD Biosciences) using the FACSDiva software (BD Biosciences). Confocal images were acquired with the LSM 710 NLO microscope (Carl Zeiss Inc.).

Statistical Analysis. Correlations of gene expression with drug cytotoxicity were considered significant based on uncorrected two-tailed tests $P < 0.001$. Multiple correction testing was carried out with the Step-up False Discovery Rate Method (see *SI Materials and Methods* for details). Nonlinear regression was used to infer the IC50s for tested compounds, and t tests were used as appropriate. The F test for equality of variance was used to assess differences in the distribution of SLFN11 expression between The Cancer Genome Atlas cancer and normal samples, and the null hypothesis was rejected for a $P < 0.05$ (one-tailed). Comparison of OS Kaplan–Meyer curves based on higher- or lower-than-average SLFN11 expression in the Yoshihara dataset (18) was performed using the Mantel–Cox log-rank. Multivariate analysis of survival was used to observe independent outcome predictors in the analyzed dataset. All P values are from two-tailed tests, unless otherwise stated.

ACKNOWLEDGMENTS. G.Z. thanks Dr. D. Bedognetti and P. Blandini for insightful suggestions. This work was supported by the Center for Cancer Research, Intramural Program of the National Cancer Institute; by the Division of Cancer Treatment and Diagnosis, Extramural Program of the National Cancer Institute (National Institutes of Health); and by an Associazione Italiana per la Ricerca sul Cancro “My First AIRC Grant” ID MFAG 10570 (to G.Z.). The results of the present work were presented in part as a poster at the American Association for Cancer Research Annual Meeting, March 31–April 4, Chicago.

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Supporting Information

Zoppoli et al. 10.1073/pnas.1205943109

SI Materials and Methods

Microarray Data. Whole-genome expression analysis of the National Cancer Institute Antitumor Cell Line Panel (NCI-60) has been described previously (1). In brief, the NCI-60 cell lines with the exception of SF-268 were tested in triplicate with the Affymetrix Human Exon 1.0 ST array chips, and the data were analyzed using the Partek Genomics Suite, version 6.5 (Partek Inc.). The data were processed according to default Partek specifications, corrected for GC content, quantile-normalized, and Log_2 -transformed. Triplicate experiments were then averaged for further analysis. Data concerning Schlafen-11 (SLFN11) expression in the ovarian cancer dataset described by Yoshihara et al. (2) were downloaded from the Gene Expression Omnibus website (<http://www.ncbi.nlm.nih.gov/geo>, accessed Nov. 30, 2010) and extracted using Agilent GeneSpring GX 10.0 (Agilent Technologies, Inc.). SLFN11 expression preprocessed data were retrieved from the The Cancer Genome Atlas portal (<http://cancergenome.nih.gov/>, accessed Dec. 16, 2011) for a randomly selected subset of 38 ovarian cystadenocarcinomas, 37 colon adenocarcinomas, and corresponding healthy tissues (19 samples from colon mucosa and 8 samples from ovarian tissues). Expression data were normalized to the 75th percentile for microarray expression, Log_2 -transformed according to Agilent specifications for colon samples (assayed using Agilent 244k gene expression microarrays), and processed with the GC-robust multiarray (GCRMA) algorithm, quantile-normalized, and Log_2 -transformed for the ovarian samples (tested with Affymetrix Human Exon 1.0 ST v2 microarrays).

NCI-60 Drug Cytotoxicity Data. In vitro cytotoxicity data for 1,429 compounds with known 2D structure, known mechanism of action, and tested at least four times were obtained from our database, CellMiner (3, 4). In addition, data for 15 recently tested drugs were obtained from the Developmental Therapeutics Program website (5). Cytotoxicity tests with each cell line and each drug were repeated in four independent experiments by the Developmental Therapeutics Program.

Cell Lines and Drugs. All cancer cell lines were obtained from the Developmental Therapeutics Program (National Cancer Institute). Cells were maintained in RPMI 1640 supplemented with 10% FBS. Camptothecin (CPT) was obtained from the Drug Synthesis and Chemistry Branch (National Cancer Institute). Cisplatin, etoposide, paclitaxel, and staurosporine were purchased from Sigma-Aldrich. Stock solutions of CPT, etoposide, paclitaxel, and staurosporine were prepared in DMSO. Cisplatin was dissolved in ultrapure water.

siRNA Transfection, Cell Viability, and Clonogenic Assays. Cells were transfected with 5 nM SLFN11-targeting or nontargeting siRNAs (#L-016764-01-0005 and #D-001810-10-05, Dharmacon) using Dharmafect I transfection reagent (Dharmacon). Seventy-two hours post transfection, cells were subjected to 72 h of continuous drug exposure, after which cell viability was measured by MTS assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-3H-tetrazolium, Promega). Cell survival was calculated as $\text{OD}_{490\text{nm-treated cells}}/\text{OD}_{490\text{nm-untreated cells}} \times 100$. Cytotoxicity assays were repeated in four independent experiments, each performed in duplicate or triplicate. For clonogenic assays, cells were treated as above in six-well plates or T25 flasks and in-

cultured 15 d to allow colony formation. Colonies were then fixed and stained with 0.05% (wt/vol) methylene blue (Sigma-Aldrich).

Western Blotting. Whole-cell extracts were obtained by incubation and brief sonication of cell pellets in cold lysis buffer [1% (wt/vol) SDS, 10 mM Tris-HCl (pH 7.4)] supplemented with proteases and phosphatases inhibitors (Roche Diagnostics). Protein determination was performed using the Micro BCA Protein Assay kit (Pierce). Twenty micrograms of proteins were separated on 10% (wt/vol) Tris-glycine gels and transferred to nitrocellulose membranes. Membranes were immunoblotted with anti-SLFN11 (sc-136891, Santa Cruz Biotechnology) or anti- β -actin (#A5441, Sigma-Aldrich) antibodies.

Cell Cycle Analysis by Flow Cytometry. Cells were harvested and fixed overnight in 70% (vol/vol) ice-cold ethanol. After two washes with PBS, cells were treated with 0.5 mg/mL RNase A and 50 $\mu\text{g}/\text{mL}$ propidium iodide. Samples were analyzed with a LSR II Fortessa flow cytometer (BD Biosciences) using the FACSDiva software (BD Biosciences).

Immunofluorescence Microscopy. DU145 cells transfected with control or SLFN11-targeting siRNAs were washed with PBS, fixed with 3% (vol/vol) formaldehyde in PBS, washed, postfixed, and permeabilized with cold 70% (vol/vol) ethanol. After blocking with 5% (vol/vol) goat serum, cells were incubated with anti-SLFN11 primary antibody (1:500 dilution) followed by incubation with anti-goat Alexa Fluor 568 antibody (Invitrogen, 1:1,000 dilution) and mounted using Vectashield medium (Vector Laboratories). Confocal images were acquired with LSM 710 NLO microscope (Carl Zeiss Inc.).

Statistical Analysis. Correlation analysis of gene expression and drug cytotoxicity was performed using the Pearson's correlation coefficient and considered significant with an uncorrected two-tailed $P < 0.01$ (i.e., showing a Pearson's $r > |0.33|$). Step-up False Discovery Rates (FDR) were calculated to correct for multiple tests (6). IC50s for the compounds tested in cell lines after SLFN11 or mock silencing were calculated using nonlinear regression assuming a sigmoidal log-linear Hill shape and compared by t tests. Significant differences between colonies counted during clonogenic assays were calculated by paired t tests. Such analyses were carried out using GraphPad Prism version 5.0 (GraphPad Software, <http://www.graphpad.com>). The F test for equality of variance was used to assess differences in the distribution of SLFN11 expression between The Cancer Genome Atlas cancer and normal samples, and the null hypothesis was rejected for a $P < 0.05$ (one-tailed). Comparison of overall survival (OS) Kaplan-Meier curves based on higher- or lower-than-average SLFN11 expression in the Yoshihara dataset (2) was performed using the Mantel-Cox log-rank test. For multivariate analysis of OS predictors, a backward stepwise Cox regression model including the variables "optimal surgery yes vs. no," "stage IIIc/IV vs. IIIa/IIIb," the interaction of stage and optimal surgery, and "SLFN11 expression greater vs. smaller than average" was calculated, and predictor variables were retained in the model if showing a P value < 0.1 . These analyses were performed using SPSS Statistics 20.0 (IBM). All P values are from two-tailed tests, unless otherwise stated.

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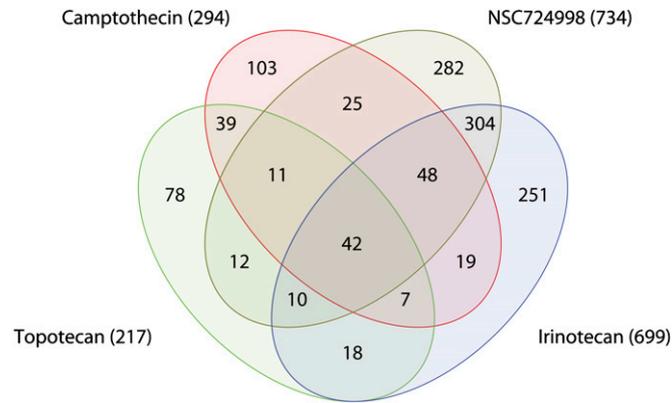


Fig. S1. Venn diagram of genes that correlate with Top1 inhibitors. The numbers of genes correlating with the in vitro cytotoxicity profiles of one or more of the four Top1 inhibitors used for gene expression correlation analysis (camptothecin, topotecan, irinotecan, NSC724998) are represented. Total numbers of correlating genes ($P < 0.01$) with each drug are indicated in parentheses next to the compound names.

Table S1. Genes commonly correlated between three DDAs

Symbol	GO biological process	CPT	Cisplatin	Etoposide
SLFN11	—	0.707	0.619	0.533
STRADA	Protein amino acid phosphorylation	0.556	0.418	0.422
ARHGEF6	Apoptosis	0.366	0.380	0.495
RAB39B	Small GTPase-mediated signal transduction	0.380	0.456	0.404
EP400	Chromatin modification	0.513	0.345	0.374
PFAS	Purine nucleotide biosynthetic process	0.406	0.422	0.394
FMNL1	Formin-related protein, cell shape	0.338	0.353	0.527
VAMP1	Vesicle-mediated transport	0.403	0.460	0.337
ZNF764	Regulation of transcription, DNA-dependent	0.452	0.372	0.360
CHD1L	DNA repair	0.360	0.462	0.348
ZNF225	Regulation of transcription, DNA-dependent	0.398	0.364	0.382
DOCK2	Membrane raft polarization	0.348	0.357	0.430
GSC	Negative regulation of transcription from RNA polymerase II promoter	0.345	0.412	0.375
ZNF26	Regulation of transcription, DNA-dependent	0.334	0.337	0.444
GIT2	Regulation of G-protein-coupled receptor protein-signaling pathway	0.340	0.339	0.419
MAST4	Protein amino acid phosphorylation	0.333	0.415	0.340
LMNA	Nuclear envelope organization	-0.334	-0.346	-0.388
PICK1	Protein amino acid phosphorylation	-0.342	-0.370	-0.361
PPFIA1	Cell-matrix adhesion	-0.407	-0.334	-0.338
GJB3	In utero embryonic development	-0.335	-0.384	-0.385
PCDH1	Cell adhesion	-0.358	-0.370	-0.378
CD9	Cellular component movement	-0.339	-0.396	-0.410
OSBPL2	Lipid transport	-0.469	-0.360	-0.339
C1orf126	Keratinization	-0.439	-0.363	-0.396
DOCK9	Biological process N.O.S.	-0.369	-0.475	-0.363
MYO1D	—	-0.395	-0.447	-0.370
KLF3	Multicellular organismal development	-0.345	-0.404	-0.482
ASAP2	Regulation of ARF GTPase activity	-0.363	-0.505	-0.365
SLC44A3	—	-0.421	-0.358	-0.564
CTDSPL	Biological process N.O.S.	-0.413	-0.464	-0.503
GIPC1	Protein targeting	-0.419	-0.444	-0.535
FBNP1L	Endocytosis	-0.458	-0.472	-0.480
TGFBR3	Epithelial to mesenchymal transition	-0.540	-0.355	-0.530
BAIAP2L1	Signal transduction	-0.468	-0.500	-0.517

Dataset S1. SLFN11 expression values and GI50s of Top1 inhibitors in the NCI-60[Dataset S1](#)

SLFN11 expression values are expressed as Log_2 from Affymetrix Human Exon arrays 1.0 ST, and GI50 values are expressed as negative Log_{10} of molar concentrations averaged over three or more experiments.

Dataset S2. SLFN11 expression correlation with 1,444 compounds tested in the NCI-60[Dataset S2](#)

All compounds were tested at least twice, and the majority of them four times or more.

*National Service Center number assigned by the Developmental Therapeutics Program to compounds tested in the NCI-60.

†Commonly used chemical name.

‡Main mechanism of action (if known).

§Pearson's coefficient between the negative Log_{10} of the GI50 of any compound across the NCI-60 panel, measured by sulforhodamine assay, and SLFN11 Log_2 intensity across the NCI-60.

¶Two-sided *P* value for Pearson's correlation.

||Step-up False Discovery Rate.

††Lower and upper confidence interval for Pearson's correlation coefficient.

†††Number of cell lines with good-quality GI50 values.

Dataset S3. SLFN11 expression values in randomly chosen samples from the The Cancer Genome Atlas database for colon and ovarian adenocarcinoma and corresponding normal tissues

[Dataset S3](#)

"Sample barcode" is the specimen ID, "Sample type" indicates whether the specimen is cancer or normal tissues, and "SLFN11" shows the expression for each sample.