SLFN11 expression in castration resistant prostate cancer and response to platinum--based chemotherapy

CONQUER CANCER® THE ASCO FOUNDATION | MERIT AWARD RECIPIENT

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Background

Schlafen family member 11 (SLFN11) is a DNA/RNA helicase, first described for its role in normal thymocyte development, immune response, and cell proliferation. Later, SLFN11 expression was assessed in several cancer cell lines, including Ewing's sarcoma, small cell lung, ovarian, and colon cancers.

SLFN11 expression has been associated with response to DNA damaging chemotherapy agents. In addition, SLFN11 expression has been associated with sensitivity to PARP inhibitors.

To date, clinical studies investigating the role of SLN11 as a potential biomarker to predict sensitivity to DNA damaging agents has been largely investigated in small cell lung cancer.

Objective

We sought to identify SLFN11 expression in tissue and blood from advanced prostate cancer patients and to elucidate its potential predictive and prognostic role in platinum (PLT)-treated CRPC patients.

Methods

1. Patient cohort
Patients with metastatic CRPC treated with PLT were retrospectively identified. Patients had a metastatic biopsy of adenocarcinoma (CRPC-Adeno) or mixed or pure neuroendocrine prostate cancer (CRPC-NE).
The protocol was approved by the Institutional Review Board. Clinical and demographic information was collected by medical record review. PCWG3 criteria was used to assess clinical, biochemical and radiographic response to different therapies.

2. Tissue biopsies
In cases with adequate fresh/frozen tissue, RNA sequencing (RNA-Seq) data was evaluated to assess the expression of SLFN11. In addition, when available, metastatic tumor genomic status of select genes (i.e., AR, TP53, RB1, PTEN, BRCA2, BRCA1, ATM) was collected from review of whole-exome sequencing (WES) data from a CLIA/CLEB approved clinical assay.

3. CTC collection and characterization
Whole blood samples (10 ml) from CRPC patients were collected in Streck tubes and shipped to Epic Sciences for processing. In the Epic Sciences platform, slides were stained for DNA (DAPI), whole blood cell linage marker (CD45), epithelial cells marker (CK), and SLFN11. CTC enumeration was performed, the slides were evaluated by immunofluorescence (IF), and the images of nucleated cells were assessed using a multi-parametric digital pathology algorithm.

4. *In vitro* studies
A dose response curve with PLT was performed in patient-derived organoids using Cell Title Glo according to the manufacturer's protocol.

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Results

1) Patient cohort characteristics

- ✓ 41 patients with metastatic CRPC (21 CRPC-Adeno, 20 CRPC-NE) were identified between May 2012 and June 2018 with a median age of 67.1 years (range 50.6-90.7).
- ✓ All patients underwent PLT [38 in combination with etoposide (N=21) or taxanes (N=17), and 3 monotherapies with carboplatin]
- ✓ Specimens were collected with a median of 85 days (range 0-707 days) before PLT.
- ✓ All patients received prior ADT and a median of 2 therapeutic lines (range 1-7) for CRPC before PLT, including in 23 (56.1%) cases AR-directed therapies (abiraterone or enzalutamide)

2) SLFN11 expression by RNA-Seq

- ➢ We evaluated *SLFN11* mRNA expression in prostate cancer cancer and compared with other tumor types (Fig.1) and disease states (Fig.2), and identified a subset of CRPC cases with SLFN11 overexpression (lower in CRPC-NE).

Figure 1. SLFN11 expression across different types of cancers RNA-Seq data from our internal cohorts (N=562)

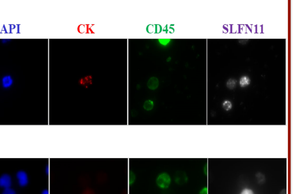
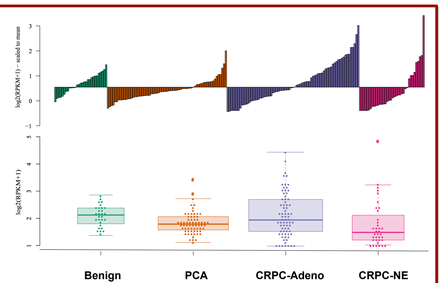


Figure 2. SLFN11 expression in Benign, localized prostate cancer (PCA), CRPC-Adeno, CRPC-NE, using RNA-Seq data from our previously published studies *[Beltran, Cancer Discov 2011; Robinson, Cell 2015; Beltran, Nat Med 2016].*



Comparison class (by Wilcoxon test)	PCA	CRPC-Adeno	CRPC-NE
Benign	0.002	0.651	0.002
PCA		0.079	0.019
CRPC-Adeno			0.009

5) SLFN11 and sensitivity to PLT *in vitro*

- ➢ Knockdown of SLFN11 in organoids derived from patient tumors reduces sensitivity to PLT *in vitro*.

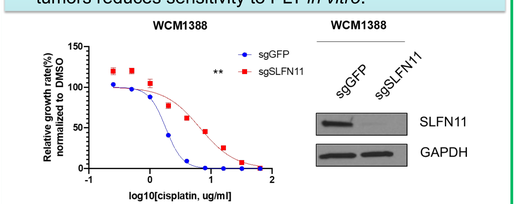
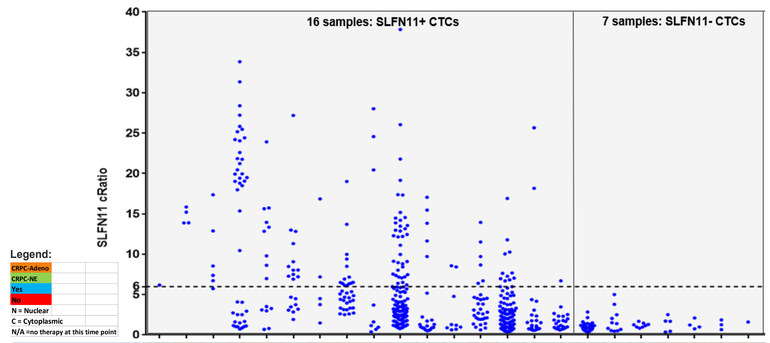


Figure 6. SLFN11 knockout using CRISPR-Cas9. Patient-derived prostate cancer organoids with either sgGFP (control, blue) or knockdown of SLFN11 (sgSLFN11, red) were plated in 96-well plate overnight and treated with indicated dose of cisplatin for 6 days. Cell growth rate was measured by CellTiter-Glo assay. **p-value <0.01, measured by 2-way ANOVA.

3) SLFN11 detection in circulating tumor cells

- ➢ SLFN11 expression in CTCs of patients was concordant with SLFN11 expression by RNA-Seq of matched metastatic tumor biopsies



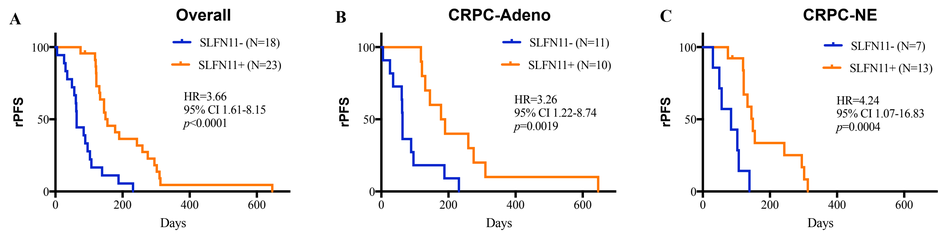
Pt ID	44	20_1	50	34	4	43	5	49_3	6_2	29	39	46	23	31	35	10_1	26	10_2	36	27	6_1	21	49.4
# Cells	1	4	6	42	15	20	5	35	9	136	21	9	32	102	21	23	64	11	8	5	4	3	1
# SLFN11+ Cells	1	4	5	26	9	12	2	14	3	37	5	2	6	11	2	1	0	0	0	0	0	0	0
% SLFN11+ Cells	100	100	83	62	60	60	40	40	33	27	24	22	19	11	10	4	0	0	0	0	0	0	0
SLFN11 Localization	C	N	C	N	N/C	C	N/C	C	N	N	N/C	N	N/C	N/C	N	N							
SLFN11 exp by RNAseq			+						+	+	+		-				-				-	-	
IF & RNAseq concordance																							
Days on platinum	122	121	348	118	178	313	131	243	145	149	122	145	155	155	120	N/A	109	75	30	63	N/A	26	N/A

Figure 3. SLFN11 Expression in CTCs.
23 patient samples (12 CRPC-NE and 11 CRPC-Adeno) were tested with the SLFN11 Epic 4-color immunofluorescence (IF) assay. SLFN11+ CTCs were detected in 16/23 (70%) patient samples. Patients with SLFN11+ CTCs exhibited a dynamic range in the proportion of SLFN11+ CTCs and the SLFN11 signal-to-noise ratio (cRatio). The dot plot above shows the SLFN11 expression in patient CTCs. Each dot represents a detected cell, and the dotted line at 6 along the y-axis indicates the analytical threshold of positivity for SLFN11. SLFN11 cRatio is plotted along the y-axis, and patient ID is plotted along the x-axis. The x-axis also includes a data table with additional patient specific information including pathology (CRPC-Adeno-green or CRPC-NE-orange), percentage of SLFN11+ CTCs, SLFN11 localization, SLFN11 exp by RNA-Seq, and IF/RNA-Seq concordance (concordant-blue or discordant-red), time on PLT response (days).

Figure 4. Representative case showing CTC characteristics of one CRPC-Adeno patient analyzed at two different time-points suggesting a possible dynamic variation of SLFN11 during prostate cancer progression

4) Association of SLFN11 and response to platinum therapy

- ➢ SFLN11 expression in PLT treated CRPC patients was significantly associated with radiographic PFS and PSA response, but not with OS
- ➢ Multivariable analysis identified SLFN1 as an independent predictor of PFS in PLT-treated patients



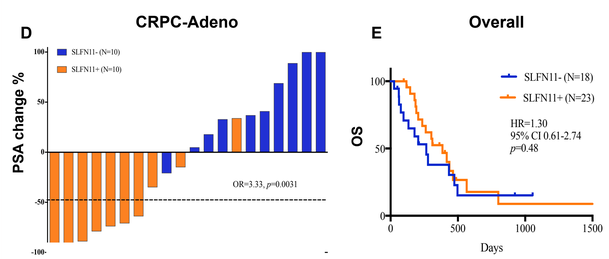


Figure 5. Association of SLFN11 expression and outcome in patient cohort. Radiographic progression-free survival (rPFS) in overall (A), CRPC-Adeno (B), CRPC-NE (C) patients according to SLFN11 expression by RNA-Seq and/or immunofluorescence (IF) in CTCs. Waterfall plot (D) showing PSA decline by SLFN11 status. Bars were clipped at maximum 100%. Overall survival (OS) (E) in overall patients according to SLFN11 expression

Table 1. Multivariable analysis in PLT-treated patients

	PFS		OS	
	HR (95% CI)	p	HR (95% CI)	P
SLFN11 (high vs low exp)	0.14 (0.03-0.70)	0.017	0.72 (0.12-4.45)	0.726
Age (continuous variable)	1.079 (0.964-1.206	0.185	1.101 (0.947-1.281)	0.211
CRPC-NE vs CRPC-Adeno	1.75 (0.47-6.56)	0.406	0.11 (0.02-0.57)	0.009
Visceral mets (yes vs no)	0.52 (0.11-2.41)	0.404	0.28 (0.04-1.98)	0.201
PSA (continuous variable)	1.001 (1.000-1.002)	0.121	1.002 (1.000-1.003)	0.054
CGA (continuous variable)	1.000 (0.998-1.002)	0.724	1.000 (0.997-1.002)	0.801
***AR* alterations** (yes vs no)	0.42 (0.10-1.74)	0.232	0.02 (0.01-0.24)	0.002
***TP53* alterations** (yes vs no)	0.62 (0.10-3.81)	0.602	0.09 (0.01-0.79)	0.030
***RB1* alterations** (yes vs no)	1.17 (0.17-7.96)	0.870	8.25 (0.68-100.01)	0.097
***DDR* alterations** (yes vs no)	0.74 (0.19-2.87)	0.669	0.34 (0.07-1.61)	0.172

*DDR alterations included *BRCA1* and *BRCA2* mutation or deletion, and *ATM* mutation

Abbreviations. *AR*, androgen receptor; *ATM*, ataxia telangiectasia mutated gene; *BRCA*, breast cancer gene; CGA, chromogranin A; CI, confidence interval; CRPC-Adeno, castration-resistant prostate adenocarcinoma; DDR, DNA defect repair; exp, expression; HR, hazard ratio; LDH, lactate dehydrogenase; mets, metastasis; CRPC-NE, castration-resistant prostate cancer with neuroendocrine features; PSA, prostate specific antigen; *RB1*, retinoblastoma 1; *TP53*, tumor protein p53

Conclusions

- ❖ SLFN11 overexpression may identify CRPC patients with a better response to platinum-based chemotherapy.
- ❖ Larger prospective studies are warranted to translate these findings into biomarker-informed clinical decision making for patients with advanced prostate cancer.