Transcriptional Profiling of FET-Rearranged Sarcomas in Response to SP-2577 (Seclidemstat)

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Background

FET-Rearranged Sarcomas and LSD1

Ewing sarcoma (EW) and myxoid liposarcoma (ML) are characterized by the expression of oncogenic fusion proteins containing the N-terminal portion of a FET (FUS/EWSR1/TAF15) family protein with a transcription factor. EW is most commonly characterized by EWS/FLI and the most common fusion in ML is FUS/CHOP[1,2]. LSD1 is a coregulator of EWS/FLI in EW critical for tumor development and progression[3], and recently published data suggest that LSD1 may play an important role in ML[4]. Both genetic disruption (shRNA) and pharmacological blockade (SP-2509) of LSD1 reverse the transcriptional activity of EWS/FLI in Ewing sarcoma[3,5].

Rationale

SP-2577 (seclidemstat) is an oral, first-in-class, noncompetitive reversible LSD1 inhibitor that is an analog of SP-2509. SP-2577 was previously shown to reduce the *in vitro* viability of EW and ML cells[6]. SP-2509 treatment blocks the oncogenic transcriptional profile of EWS/FLI and mimics LSD1 depletion in EW cells[3,5]. We therefore hypothesized that SP-2577 treatment would display similar transcriptional activity in EW cells. We then aimed to evaluate the transcriptional activity of SP-2577 in ML and its similarities to SP-2577 treatment in EW.

Methods

We treated A673 (EW), 1765-92 (ML), and 402-91 (ML) cells with SP-2577 at their respective IC50 and IC90 doses for 24 and 48 hours, collected cells, extracted RNA, and analyzed samples by RNA-sequencing. DMSO (0.3%) treated samples were collected as a control at each time point and samples were collected in triplicate. Reads were trimmed with TrimGalore! and aligned to hg38 with STAR v2.7. Fusion detection was performed with EnFusion[7]. Gene counts were input into DESeq2 to determine differentially expressed genes (DEGs), using a multiple-hypothesis adjusted p-value < 0.05 as a cutoff. DEGs were then evaluated with Venn, correlation, and gene set enrichment analysis (GSEA).

Conclusions

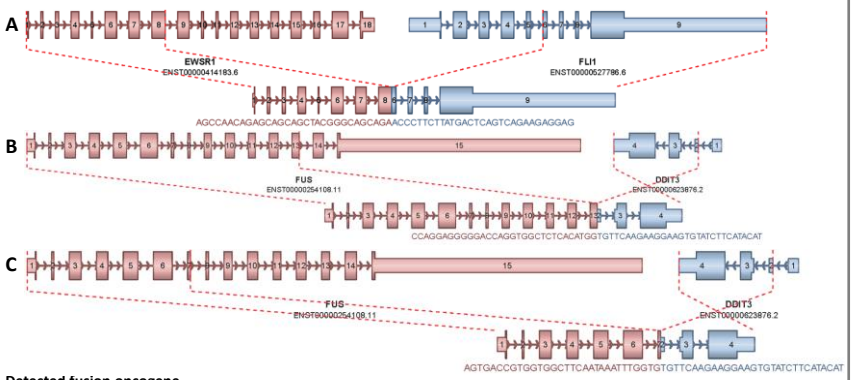
- SP-2577 induces dose- and time-dependent transcriptional changes in Ewing sarcoma and myxoid liposarcoma cells.
- Treatment with SP-2577 mimics knockdown of EWS/FLI and LSD1, recapitulating previous data with SP-2509 in EW.
- Treatment of ML cells with SP-2577 functionally correlates with treatment of EW cells with SP2509 and LSD1 shRNA.

Future Directions

- Define mechanisms of SP-2577 mediated gene regulation
- Identify biomarkers for sensitivity and response to SP-2577

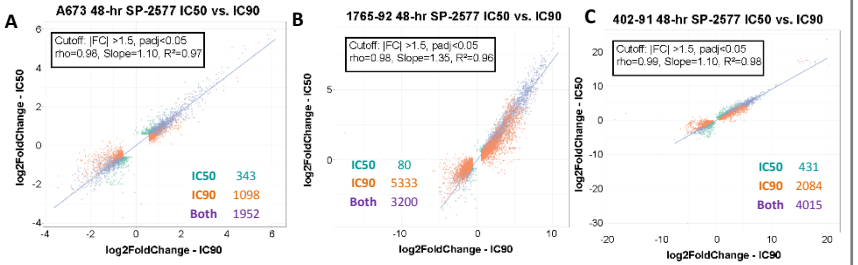
Results

Oncogenic Fusions were detected by RNA-Seq



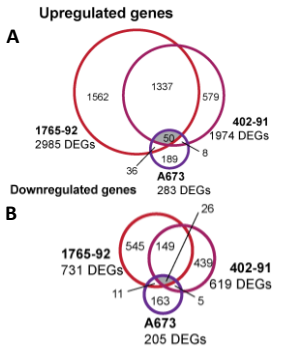
Detected fusion oncogene.
EWS/FLI, type I fusion in A673 (**A**), FUS-DDIT3, type I in 1765-92 (**B**), and FUS-DDIT3, type II in 402-91 (**C**).

IC90 induces more transcriptional changes than IC50



Scatterplots of the log2 (FoldChange) in the IC50 dose with respect to the IC90 dose. (**A**) A673 IC50 = 621nM, IC90 = 1μM. (**B**) 1765-92 IC50 = 600nM, IC90 = 2.7uM. (**C**) 402-91 IC50 = 850nm, IC90 = 1.4uM. Values indicate number of unique differentially expressed genes detected in samples. Both treatments induce similar sets of genes, though increased dosing yields a greater number of changes.

Common Genes Across Cell Lines



More commonly regulated genes within tumor types than across tumor types. LSD1 inhibition results in more de-repression than downregulation, consistent with LSD1's role as a corepressor.

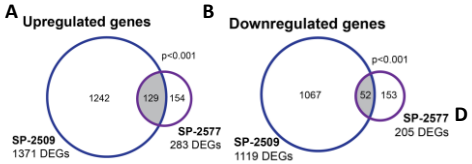
C

	ID	Name	pValue
Upregulated	GO:0046208	Spermine catabolic process	6.383E-6
	GO:1990399	Epithelium regeneration	1.275E-5
Downregulated	GO:0006334	Nucleosome assembly	2.149E-25
	GO:0034728	Nucleosome organization	5.899E-24
	GO:0031497	Chromatin assembly	1.542E-23

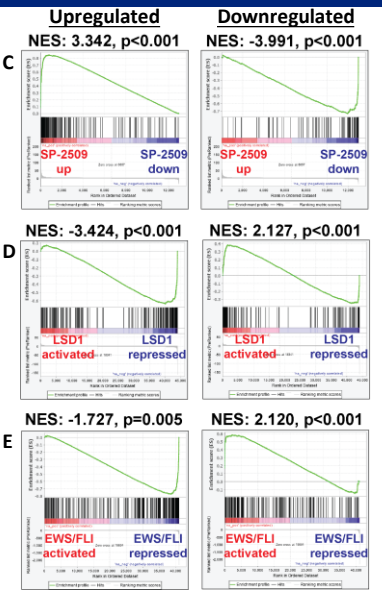
Differentially expressed genes across cell lines. 3-way Venn overlap of DEGs across cell lines. for SP-2577 induced (**A**) and downregulated (**B**) DEGs. DEGs meeting both a p<0.05 and a |FC|>3 cutoff for subsequent analyses. Pathway analysis of common DEGs (**C**)

SP-2577 in Ewing Sarcoma

Treatment with SP-2577 mimics that of SP-2509, blocks the oncogenic transcriptional profile and mimics LSD1 depletion in EW cells.

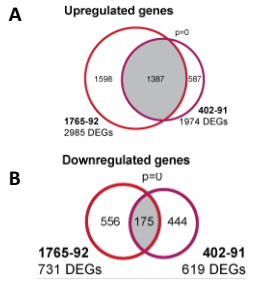


A,B) Venn overlap shows significant overlap between DEGs identified in both SP-2509 and SP-2577 treatment. **C,D,E**) GSEA showing that SP-2577 treatment functionally correlates with previously published SP-2509 treatment (**C**), LSD1 knockdown (**D**), and EWS/FLI knockdown (**E**) in EW cells.

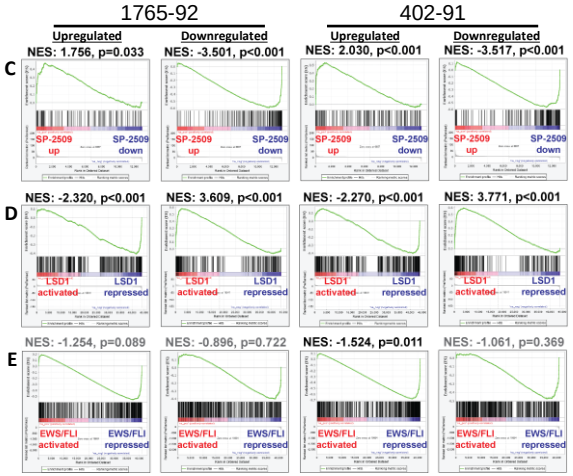


SP-2577 in Myxoid Liposarcoma

SP-2577 treatment in ML functionally correlates with SP-2509 treatment and LSD1 depletion in EW but does not recapitulate EWS/FLI KD. This suggests SP-2577 disrupts common LSD1 functions, but that LSD1 also has unique regulatory roles defined by distinct fusion oncogenes.



A,B) Venn overlap shows significant overlap between DEGs identified in both ML cell lines. **C,D,E**) GSEA showing that SP-2577 treatment in ML cells functionally correlates with SP-2509 treatment (**C**), LSD1 knockdown (**D**), but not EWS/FLI knockdown (**E**) in EW cells.



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