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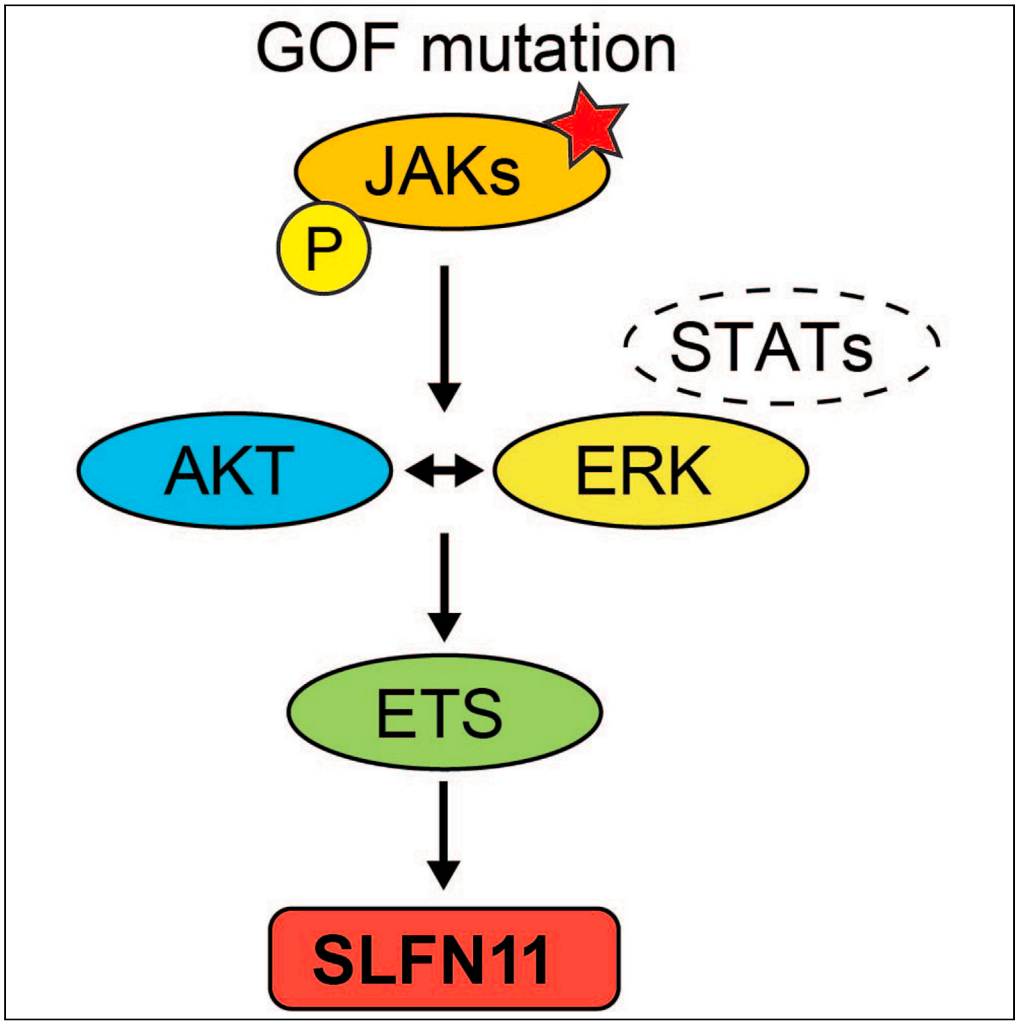

Yasuhisa Murai, Ukhyun Jo, Junko Murai, Shinsaku Fukuda, Naoko Takebe, Yves Pommier

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Highlights

SLFN11 expression is high in leukemia and confers response to DNA targeted agents

SLFN11 expression is driven by the innate immune IFN-JAK signaling pathway

Leukemia cell lines with JAK-GOF-mutations express high SLFN11 downstream from the AKT/ERK-ETS pathway

JAK and ETS inhibitors suppress SLFN11 expression

Murai et al., iScience *24*, 103173
October 22, 2021
https://doi.org/10.1016/j.isci.2021.103173

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Schlafen 11 expression in human acute leukemia cells with gain-of-function mutations in the interferon-JAK signaling pathway

Yasuhisa Murai,[1,2] Ukhyun Jo,[1] Junko Murai,[3] Shinsaku Fukuda,[2] Naoko Takebe,[1,4,5] and Yves Pommier[1,5,6,*]

SUMMARY

Schlafen11 (SLFN11) is referred to as interferon (IFN)-inducible. Based on cancer genomic databases, we identified human acute myeloid and lymphoblastic leukemia cells with gain-of-function mutations in the Janus kinase (JAK) family as exhibiting high SLFN11 expression. In these cells, the clinical JAK inhibitors cerdulatinib, ruxolitinib, and tofacitinib reduced SLFN11 expression, but IFN did not further induce SLFN11 despite phosphorylated STAT1. We provide evidence that suppression of SLFN11 by JAK inhibitors is caused by inactivation of the non-canonical IFN pathway controlled by AKT and ERK. Accordingly, the AKT and ERK inhibitors MK-2206 and SCH77284 suppressed SLFN11 expression. Both also suppressed the E26 transformation-specific (ETS)-family genes ETS-1 and FLI-1 that act as transcription factors for SLFN11. Moreover, SLFN11 expression was inhibited by the ETS inhibitor TK216. Our study reveals that SLFN11 expression is regulated via the JAK, AKT and ERK, and ETS axis. Pharmacological suppression of SLFN11 warrants future studies.

INTRODUCTION

SLFN11 is one of six human Schlafen genes involved in various cellular functions including cell quiescence, cell-cycle arrest and immune response to viral infections (Mavrommatis et al., 2013; Murai et al., 2019). SLFN11 is an increasing focus of basic and translational research as it drives the cytotoxicity of multiple and widely used anticancer drugs targeting DNA replication (Murai et al., 2019). SLFN11 irreversibly blocks replication and kills proliferating cancer cells by binding to stressed replication forks via the single-stranded binding protein replication protein A1 (RPA1) (Mu et al., 2016; Murai et al., 2018), interfering with the replicative helicase CMG complex (Murai et al., 2018), causing chromatin opening with activation of stress response and immediate-early response genes (Murai et al., 2020) and depleting the replication initiation factor CDT1 (Jo et al., 2021). Recently, SLFN11 was also reported to promote extensive fork degradation in FANCD2− cells, which is mediated by the nucleases MRE11 or DNA2 (Okamoto et al., 2021). In addition, SLFN11 regulates tRNA levels and protein translation (Li et al., 2012, 2018) and was recently found to control the unfolded protein response and steady-state cellular protein ubiquitylation (Murai et al., 2021).

SLFN11 expression is suppressed in approximately 50% of cancer cells (Murai et al., 2019), primarily by epigenetic mechanisms including promoter hypermethylation at CpG islands (Moribe et al., 2021; Nogales et al., 2016; Reinhold et al., 2017), histone deacetylation, and histone methylation by the polycomb repressor complex (Gardner et al., 2017; Tang et al., 2018). However, little is known on how SLFN11 expression is activated. SLFN11 is consistently high in Ewing sarcomas (Garnett et al., 2012; Tang et al., 2015), due to the expression of the chimeric transcription factor EWS-FLI1, which binds to E26 transformation-specific (ETS) domains near the transcription start site of *SLFN11* (Tang et al., 2015). A correlation between SLFN11 and FLI-1 expression was also reported in leukemia, colon, breast, and prostate cancers (Tang et al., 2015).

Schlafen genes are commonly referred to as interferon (IFN)-inducible. Indeed, human SLFN5 has been shown to be activated by IFN in melanoma cells (Katsoulidis et al'., 2010); albeit tyrosine kinase (TYK) 2-deficient or Janus kinase (JAK1)-deficient 2FTGH fibrosarcoma cells showed no induction of SLFN5 after IFNα stimulation (Katsoulidis et al., 2010). SLFN11 is also induced in human foreskin fibroblasts treated with IFN-β, poly-IC or poly-dAdT (Li et al., 2012), and recent studies showed that SLFN11 expression is regulated

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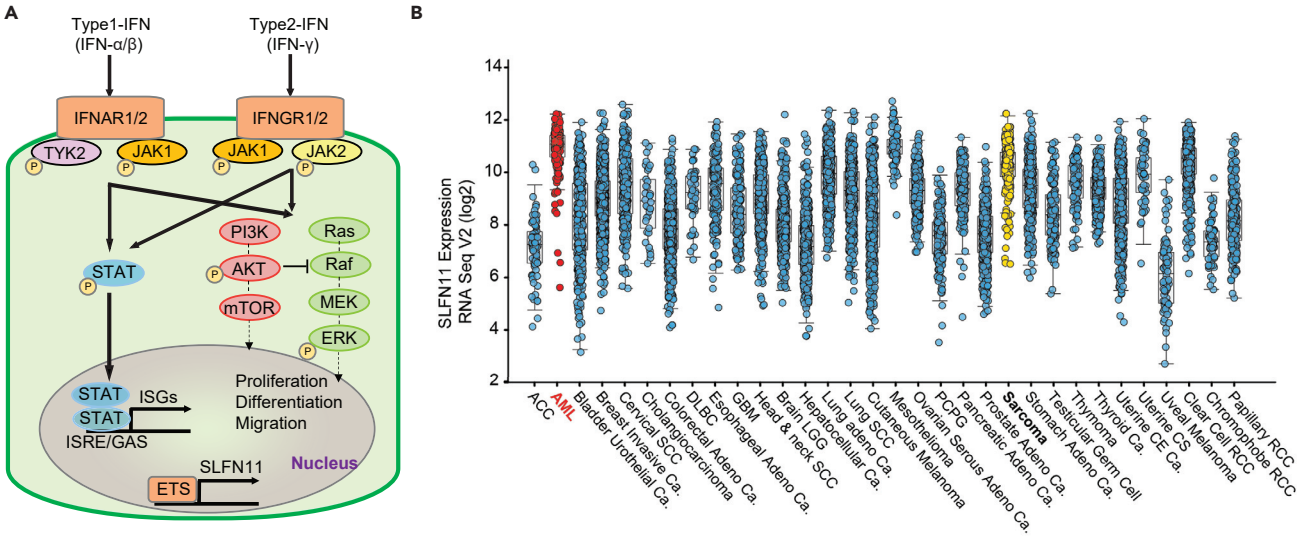
https://doi.org/10.1016/j.isci.2021.103173



C

Cell lines	Type	GOF mutations			SLFN11 mRNA expression GDSC	CCLE	NCI-60
MOLT4	ALL (T-cell precursor)	TYK2	E957D	0.94 *	8.59	8.86	8.36
CCRF-CEM	ALL (T-cell precursor)	TYK2	R1027H	0.50 *	7.82	—	7.52
HEL	AML (Erythroleukemia)	JAK2	V617F	1.00 **	7.72	8.62	—
HEL92.1.7	AML (Erythroleukemia)	JAK2	V617F	1.00 **	—	8.86	—
Set-2	AML (Megakaryoblastic)	JAK2	V617F	0.99 **	—	9.54	—
MUTZ-5	ALL (B-cell precursor)	JAK2	R683G	0.53 **	—	9.54	—
MHH-CALL-4	ALL (B-cell precursor)	JAK2	I682F †	0.44 **	—	9.33	—
OCI-M1	AML (Erythroleukemia)	JAK1	V658F	0.66 **	7.76	8.70	—
MOLM-6	CML (Ph+)	JAK1	R879C	0.55 **	—	9.15	—

† putative * GDSC ** CCLE

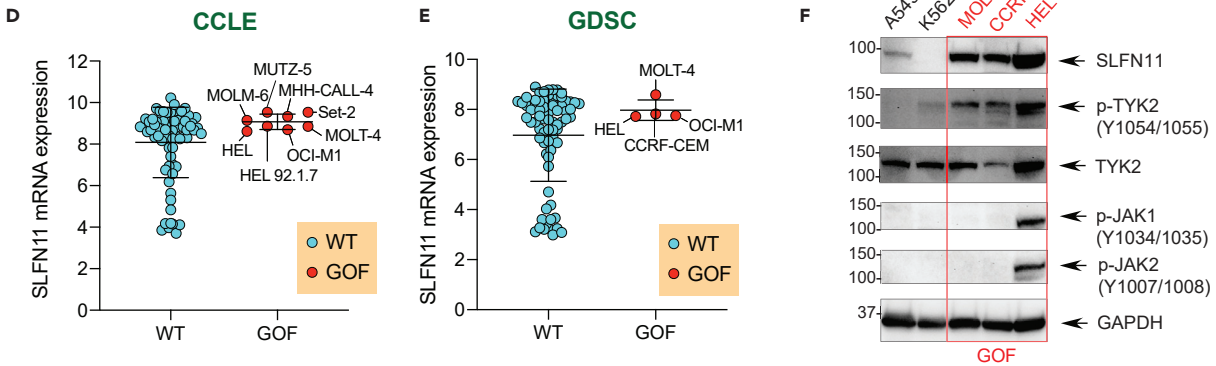


Figure 1. Leukemic cells with JAK GOF mutations overexpress SLFN11

(A) Diagram of proposed JAKs pathway. The canonical STAT pathway is shown in left side (blue). The non-canonical AKT and ERK pathways are shown in right side (red and green).

(B) High expression of *SLFN11* in AML (red) samples in the TCGA data set. Sarcomas samples are highlighted as a reference (yellow). ACC, adrenocortical carcinoma; AML, acute myeloid leukemia; SCC, squamous cell carcinoma; DLBC, diffuse large B-cell lymphoma; GBM, glioblastoma multiforme; LGG, low grade glioma; PCPG, pheochromocytoma and paraganglioma; CE, corpus endometrial; CS, carcinosarcoma; RCC, renal cell carcinoma.

Figure 1. *Continued*

(C) Leukemic cell lines with gain-of-function (GOF) mutations in TYK2/JAK1/JAK2 consistently express high SLFN11 transcripts. The GOF variants of JAKs were mined from cBioPortal. The values represent the probability of homozygous function-impacting mutation in the GDSC or CCLE data sets. SLFN11 mRNA expression was based on multi-platform microarray average $\log_2$ intensity in the GDSC, CCLE, and NCI-60 data sets. Ph, Philadelphia chromosome; †, putative GOF mutation; *, GDSC; **, CCLE.

(D and E) Relative SLFN11 mRNA expression of leukemic cells with GOF mutation in JAKs compared with other leukemic cells in the CCLE and GDSC data sets. SLFN11 mRNA expression were based on multi-platform microarray average $\log_2$ intensity.

(F) Activation of the JAK/TYK2 pathway detected by Western blotting. Leukemic cell lines with GOF are highlighted in red. Chronic myeloid leukemia K562 and lung adenocarcinoma A549 were used as control cells with wild-type JAKs.

by type I IFN-dependent and -independent pathways (Borrego et al., 2020). In addition, SLFN11 sensitizes leukemic HAP1 cells to IFN-γ-mediated T cell killing (Mezzadra et al., 2019) and is epigenetically regulated during B-cell differentiation (Moribe et al., 2021). SLFN11 has therefore been described as an IFN-stimulated gene (Borrego et al., 2020; Katsoulidis et al., 2010; Li et al., 2012; Mavrommatis et al., 2013; Mezzadra et al., 2019; Puck et al., 2015). However, there is as yet limited evidence for the relationships between SLFN11 and IFN in cancer or human somatic cells.

In the classical IFN-JAK-STAT pathway, type I or type II IFNs bind to the IFN receptors IFNAR1, IFNAR2, IFNGR1, and IFNGR2 on the surface of cells (Platanias, 2005) in association with JAK1, JAK2, and TYK2. JAK1/2-TYK2 activation by autophosphorylation and downstream tyrosine phosphorylation of STATs induce the formation of active homodimers or heterodimers that activate the downstream NF-κB and innate immune response pathways. In addition to the classical JAK-STAT signaling pathway, type I IFN-mediated signaling can activate the MAPK pathway including p38 and MEK-ERK pathway and PI3K-AKT pathway via JAKs (Figure 1A) (Hervas-Stubbs et al., 2011; Platanias, 2005).

In this study we investigated the molecular control mechanisms of SLFN11 expression in leukemic cell lines. We report that gain-of-function (GOF) mutations of the JAK receptor kinases (TYK2, JAK2 and JAK1) drive SLFN11 expression and that SLFN11 expression is inhibited by the clinical JAK inhibitors cerdulatinib, ruxolitinib, and tofacitinib at the mRNA and protein levels. We show that *SLFN11* expression is directly controlled via the ETS family transcription factors downstream of the non-canonical IFN-pathway involving AKT and ERK signaling. Accordingly, the AKT inhibitor MK-2206, the ERK inhibitor SCH772984, the PI3K inhibitor LY294002, and the recently developed ETS inhibitor TK216, as well as retinoic acid suppress SLFN11 expression in JAK GOF-driven leukemia cells.

RESULTS

Leukemic cell lines harboring TYK2/JAK1/JAK2 GOF mutations overexpress SLFN11

By analyzing the human TCGA transcriptome databases, we observed that acute myeloid leukemia (AML) cells are among the highest expressors for SLFN11 mRNA (Figure 1B) as was reported previously for Ewing sarcomas (Gao et al., 2013). Consistent with this observation, AML cell lines also show high SLFN11 expression across the large cancer cell line databases of the NCI-60, GDSC, and CCLE (Figures S1A and S1B) (CellMiner Cross Database: http://discover.nci.nih.gov/cellminercdb) (Luna et al., 2021). Acute lymphoblastic leukemia (ALL) cells also express high SLFN11, similar to AML while multiple myeloma cells have lower SLFN11 expression. As expected, AML and ALL cells with high SLFN11 expression have low SLFN11 promoter methylation (Figure S1C) (Nogales et al., 2016; Reinhold et al., 2017). ALL cells such as MOLT4 and CCRF-CEM and AML cells including HL-60 and HEL also have high SLFN11 protein expression (Figures S1D and S1E), consistent with the high correlation between SLFN11 transcript and protein levels (Winkler et al., 2021; Zoppoli et al., 2012).

IFNs stimulation causes auto-phosphorylation of JAK family members including JAK1, JAK2, and TYK2 (Figure 1A). In hematological malignancies, beside AML, some ALL and myeloproliferative disease also have GOF mutations in JAKs or fusion genes with JAKs such as JAK2-TEL (ETV6) (Bercovich et al., 2008; Cerami et al., 2012; Flex et al., 2008; Gao et al., 2013; Gordon et al., 2010; Hornakova et al., 2011; James et al., 2005; Lacronique et al., 1997; Sanda et al., 2013). Accordingly, we found that GOF mutation in JAKs was seen in AML and ALL with high SLFN11 expression but less frequently in other leukemic cell lines (Figures 1C–1E, S1A, and S1B) (Cerami et al., 2012; Gao et al., 2013; Murai et al., 2018; Reinhold et al., 2015). CCRF-CEM and MOLT4 cells harboring the GOF mutation showed tyrosine phosphorylation (Y1054/1055) of TYK2 at steady-state conditions (Figure 1F) (Sanda et al., 2013). Also, HEL cells, an erythroleukemia cell

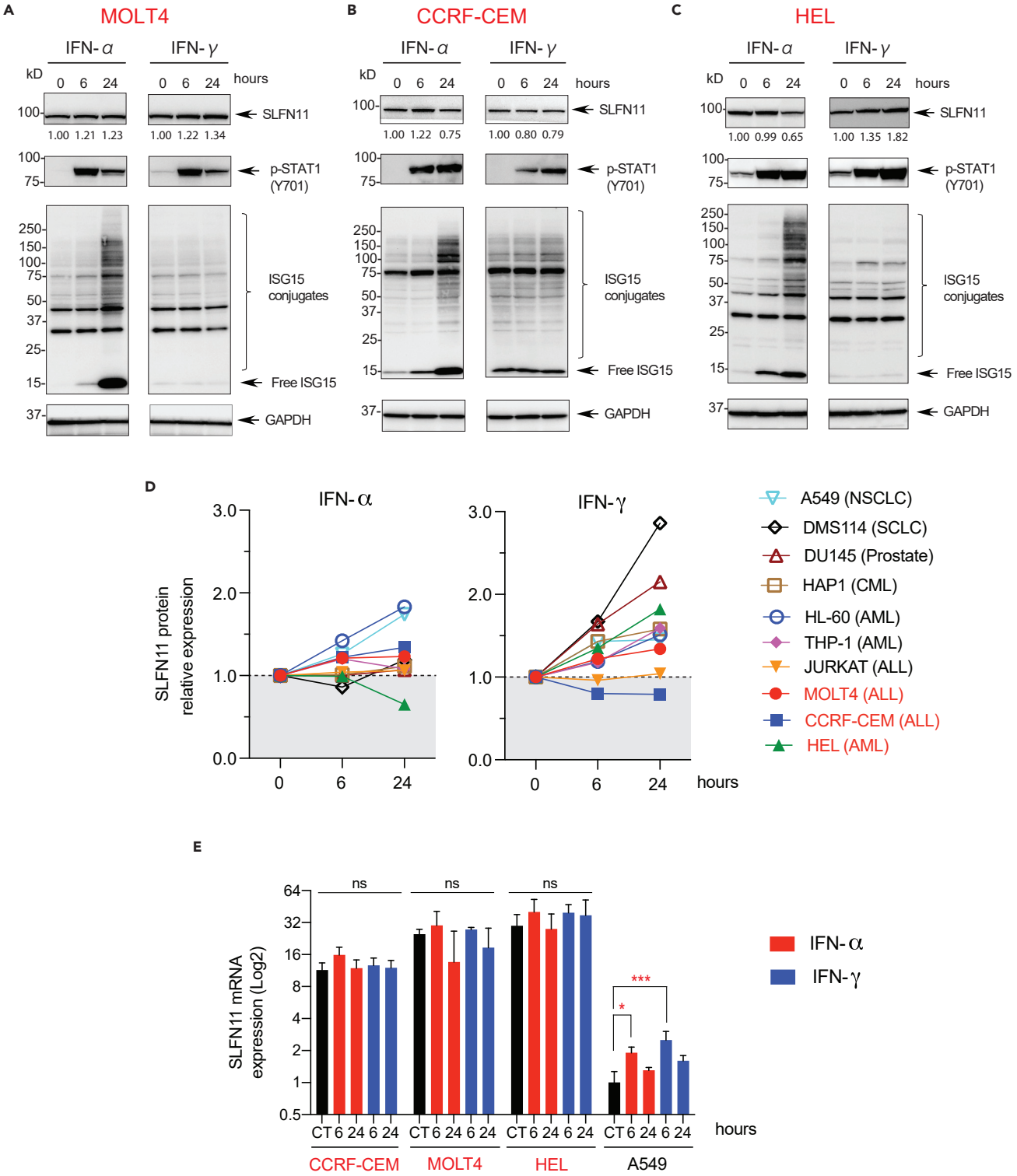


Figure 2. Baseline SLFN11 expression in JAK GOF mutant leukemia cells is independent of the classical IFN-STAT pathway and SLFN11 induction by IFN is cell line-dependent

(A–C) MOLT4 (A), CCRF-CEM (B) and HEL (C) cells were treated with IFN-α (10,000 U/mL) or IFN-γ (100 ng/mL) for 6 or 24 h.

(D) Relative expression profiling of SLFN11 in multiple cell lines in the response to IFN-α (10,000 U/mL) or IFN-γ (100 ng/mL) for 6 or 24 h. The original images of Western blotting were listed in Figure S2C.

Figure 2. *Continued*

(E) Transcriptional induction of *SLFN11* mRNA by IFNs in CCRF-CEM, MOLT4, and HEL cells. Relative $2^{-\Delta\Delta CT}$ changes of SLFN11 mRNA expression following treatment with IFN-α (10,000 U/mL) or IFN-γ (100 ng/mL) for 6 or 24 h in MOLT4 (N = 2), CCRF-CEM (N = 2) and HEL (N = 2). Non-small-cell lung cancer A549 cells were used as an IFN-inducible control cells (N = 3). Error bars represent SD; *P = 0.03, ***P < 0.001 with ordinary one-way ANOVA test and Tukey's multiple comparisons test.

line known to have GOF mutation in JAK2 (Quentmeier et al., 2006), showed additional autophosphorylation of JAK1 and TYK2. These results led us to conclude that AML and ALL leukemic cells with GOF mutations in JAKs express high SLFN11 compared with other leukemic cells.

SLFN11 expression in JAK-GOF-mutant leukemic cells is independent of the classical IFN-STAT pathway

Because IFN stimulation phosphorylates STATs, mainly STAT1 and STAT2 through IFN receptors and JAKs (Hervas-Stubbs et al., 2011; Platanias, 2005) (Figure 1A), we examined whether the cells with GOF mutation in JAKs show phosphorylation of STATs under steady-state conditions. We tested the CCRF-CEM and MOLT4 cells with GOF mutation in TYK2 and the erythroleukemia HEL cell line with GOF mutation in JAK2. Unexpectedly, only HEL cells displayed phosphorylated STAT1 (Y701) while MOLT4 and CCRF-CEM cells did not show detectable STAT1 activation under steady-state conditions (Figures 2A–2C, and S2A).

To further explore this unexpected lack of baseline STAT activation in the JAK GOF cells, we tested the response of MOLT4 and CCRF-CEM cells to exogenous IFNα stimulation. JURKAT T-ALL cells with wild-type JAKs were used as control (Figure S2B). Short-time exposure to IFNα induced hyper-phosphorylation of STAT1 (Y701) in all three cell lines, while SLFN11 expression was not changed (Figure S2B). Time course stimulation with IFNα and IFNγ in MOLT4, CCRF-CEM, and HEL cells showed that the intensity of phosphorylated STAT1 remained high at 24 h, along with induction of ISG15 conjugates (Loeb and Haas, 1992; Perng and Lenschow, 2018). However, SLFN11 expression remained unchanged (Figures 2A–2C). In HEL cells, phosphorylated STAT1 was further induced after IFNα and IFNγ treatment while IFNα did not increase SLFN11, and only IFNγ induced a weak induction of SLFN11 (Figure 2C). To further explore the SLFN11-IFN connection, we tested the inducibility of SLFN11 in additional cancer cell lines (Figure 2D) including some with high SLFN11 levels (Figure S1C). DU145, prostate cancer, and DMS114, small cell lung cancer, showed reproducible induction of SLFN11 in response to IFNγ (Figure 2D). However, the numerical changes were limited with around 2-3-fold at the protein levels (Figure 2D).

At the transcription level, basal mRNA expression of SLFN11 was notably high in MOLT4, CCRF-CEM, and HEL, and was not increased further by IFNα or IFNγ (Figure 2E). By contrast, A549 lung adenocarcinoma cells, which have relatively low SLFN11 expression (Figure S1D) demonstrated 1.9–2.5-fold SLFN11 mRNA induction in response to IFNα and IFNγ exposure for 6 h (Figure 2E). A possible reason for the lack of SLFN11 induction upon exogenous IFN stimulation in MOLT4, CCRF-CEM, and HEL cells may be a saturation of the SLFN11 promoter at steady-state as these cells express much higher SLFN11 transcripts than A549 cells (Figure 2E). We conclude that IFN-dependent SLFN11 expression depends on the cell line background and is generally much less intense than the classical IFN-inducible genes such as ISG15 (Figures 2A–2C).

Together, these results show that the two TYK2-GOF-mutant leukemia cell lines MOLT4 and CCRF-CEM with high SLFN11 expression do not activate STATs under steady-state conditions, suggesting that high SLFN11 expression in the TYK2-GOF-mutant cells is not regulated by the classical IFN-JAK-STAT signaling pathway.

Inhibitors of the JAK pathway suppress SLFN11 expression

To elucidate how leukemic cells with GOF mutation in JAKs constitutively up-regulate SLFN11 expression, we tested three JAK inhibitors, cerdulatinib, ruxolitinib, and tofacitinib. Cerdulatinib reduced SLFN11 protein expression by 70% within 6 h while, as expected, it also depleted phosphorylated TYK2 in both MOLT4 and CCRF-CEM (Figures 3A–3D). In addition, cerdulatinib decreased *SLFN11* mRNA expression (Figure 3E), and its suppressor effect was enhanced upon 24-h exposure both in MOLT4 and CCRF-CEM (Figures S3A and S3B). The expression of SLFN11 recovered within 4 h after removing cerdulatinib (Figure 3F), indicating that JAK activation is critical for SLFN11 expression and that the inhibitory effect of cerdulatinib is not

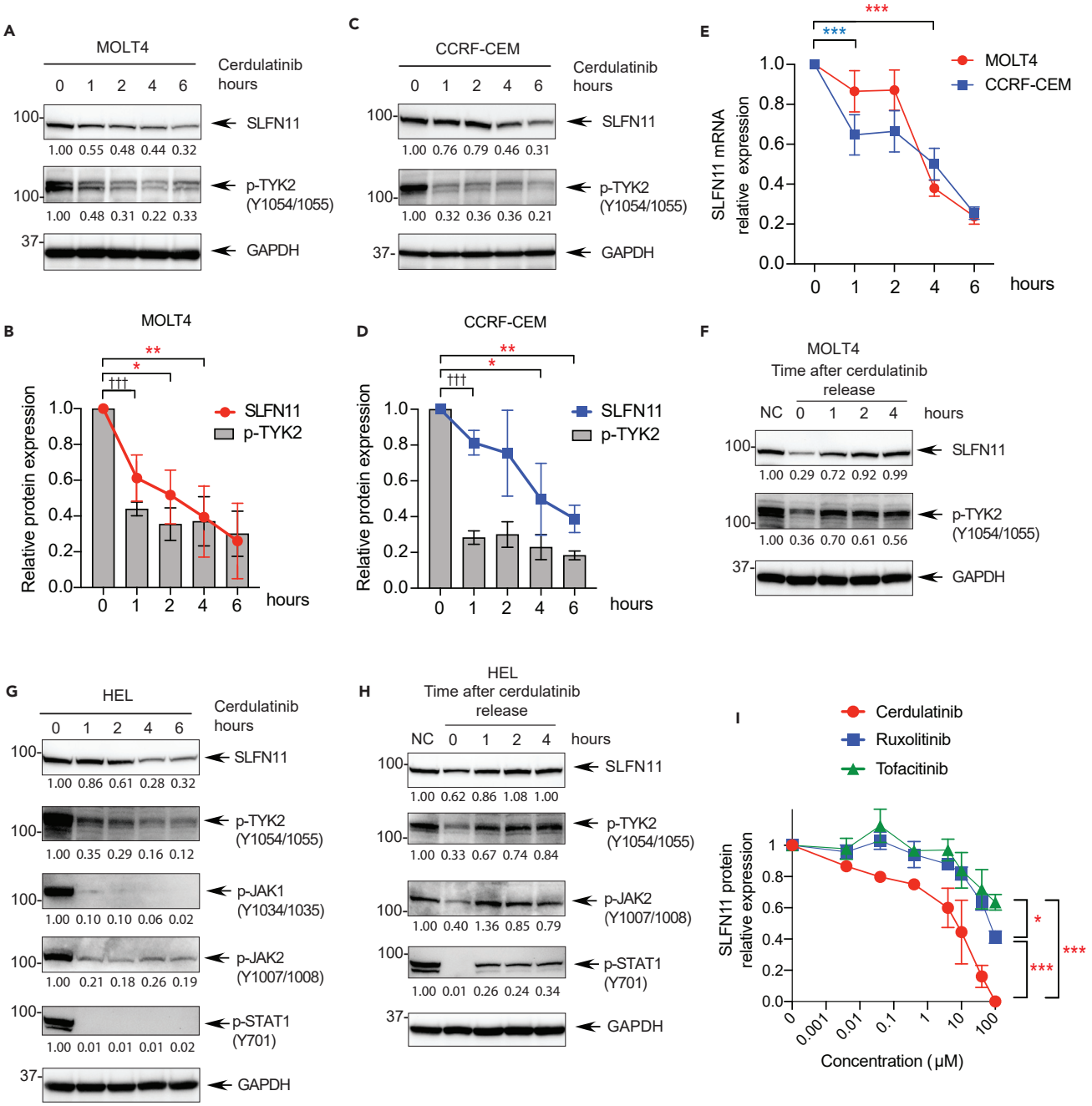


Figure 3. Inhibitors of JAK pathway decrease SLFN11 expression

(A and B) SLFN11 and phosphorylated TYK2 expression detected by Western blotting after cerdulatinib treatment. MOLT4 was treated with cerdulatinib (40 μM) for the indicated times. Quantitation of SLFN11 and phosphorylated TYK2 by 3 independent experiments as shown in panel A. Error bars represent SD; *p = 0.032, **p = 0.008, ***p < 0.001 (SLFN11), †††p < 0.001 (p-TYK2) with ordinary one-way ANOVA test and Tukey's multiple comparisons test.

(C and D) SLFN11 and phosphorylated TYK2 expression in CCRF-CEM treated with cerdulatinib (40 μM). Quantitation of SLFN11 and phosphorylated TYK2 in 3 independent experiments as shown in panel D. Error bars represent SD; *p = 0.013, **p = 0.003, ***p < 0.001 (SLFN11), †††p < 0.001 (p-TYK2) with ordinary one-way ANOVA test and Tukey's multiple comparisons test.

(E) Relative changes of SLFN11 mRNA expression treated with cerdulatinib (40 μM) for the indicated times in MOLT4 and CCRF-CEM cells (N = 3). Error bars represent SD; ***p < 0.001 with two-way ANOVA test and Tukey's multiple comparisons test.

(F) Restoration of SLFN11 expression after release of cerdulatinib treatment (40 μM, 24 h) in MOLT4 cells.

Figure 3. *Continued*

(G) SLFN11 and phosphorylated TYK2, JAK1, JAK2, and STAT1 expression detected by Western blotting in HEL cells treated with cerdulatinib (40 μM) for the indicated times.

(H) Restoration of SLFN11 expression in HEL cells after release of cerdulatinib treatment (40 μM, 24 h).

(I) Quantitation of the relative SLFN11 protein expression in 2 independent experiments as shown in Figures S3A, S3C, and S3D. Error bars represent SD; *p = 0.039, ***p < 0.001 with two-way ANOVA test and Tukey's multiple comparisons test.

merely due to cell death as it is reversible. We also tested the response to cerdulatinib in HEL cells with JAK2 GOF mutation. Consistent with the results in MOLT4 and CCRF-CEM cells, cerdulatinib inhibited SLFN11 expression while decreasing TYK2, JAK1, JAK2, and STAT1 phosphorylation (Figure 3G). After removal of cerdulatinib, SLFN11 expression also recovered in HEL cells (Figure 3H). These experiments demonstrate that cerdulatinib is a pharmacological inhibitor of SLFN11 expression, implying a regulatory role of the JAK pathway on SLFN11 expression.

To confirm the results obtained with cerdulatinib, we extended our experiments to two other clinical JAK inhibitors, ruxolitinib and tofacitinib. Both also suppressed SLFN11 expression (Figures 3I, S3C, and S3D) with cerdulatinib being the most potent SLFN11 expression inhibitor (Figure 3I).

AKT and ERK mediate SLFN11 expression in GOF mutant leukemic cells

Given that the classical IFN-JAK-STAT signaling pathway may not be activated in steady-state conditions in MOLT4 and CCRF-CEM, we hypothesized that the non-classical JAK pathway including MAPK, CRK, and PI3K-AKT might be involved in the regulation of SLFN11 expression. Consistent with this possibility, previous studies showed that cerdulatinib inhibits not only the STAT pathway but also the phosphorylation of AKT and ERK and the NF-κB pathway (Guo et al., 2017; Ishikawa et al., 2018).

To test whether the activation of AKT and MEK-ERK pathway by GOF mutation in JAKs could contribute to the high expression of SLFN11, we determined the phosphorylation status of AKT and ERK in MOLT4 and CCRF-CEM. As shown in Figure 4A, both cell lines showed high phosphorylation of AKT (S473) and a weak signal for phosphorylated ERK. Next, we investigated the effects of cerdulatinib on these phosphorylations. Cerdulatinib depleted phosphorylated AKT (S473) within 4 h and subsequently both AKT and ERK phosphorylation were suppressed within 6 h, although ERK was transiently phosphorylated at the early time points of cerdulatinib treatment (Figures 4B and S4A). Cross-talks are plausible between the Raf-MEK-ERK and PI3K-AKT pathways. For example, AKT-mediated phosphorylation of Raf has been shown to inhibit the activation of the Raf-MEK-ERK pathway (McCubrey et al., 2008; Moelling et al., 2002; Zimmermann and Moelling, 1999). Therefore, one interpretation of our findings is an AKT-mediated ERK regulation mechanism where inhibition of AKT leads to the transient activation of ERK. As was observed for phosphorylation of TYK2 (see Figure 3F), the removal of cerdulatinib restored the phosphorylation of AKT (Figure 4C), which is consistent with AKT signaling downstream from JAK activation. In contrast to MOLT4 and CCRF-CEM cells, HEL cells exhibited hyperphosphorylated ERK dominantly in parallel to AKT phosphorylation under steady state conditions, and cerdulatinib suppressed both ERK and AKT phosphorylation (Figure 4D).

Next, we investigated whether the combination of PI3K-AKT inhibitors and ERK inhibitor could reduce the expression of SLFN11. Combination treatment significantly suppressed SLFN11 expression while single treatments with either LY294002, a PI3K inhibitor, MK-2206, an AKT inhibitor or SCH772984, an ERK inhibitor, only modestly decreased SLFN11 expression (Figures 4E–4G, S4B, and S4D). Taken together, our results indicate that the activation of both AKT and ERK regulates SLFN11 expression in cells with GOF mutations in JAKs.

The ETS pathway drives SLFN11 expression in GOF mutant leukemic cells

Previous studies reported that EWS-FLI1 is a transcription inducer of SLFN11 in Ewing sarcoma (Barretina et al., 2012; Tang et al., 2015) and the existence of cross talk between ERK and/or AKT and ETS family genes (Giorgi et al., 2015; Hervas-Stubbs et al., 2011; Mut et al., 2012; Plotnik et al., 2014; Selvaraj et al., 2014; Smith et al., 2012). Examination of the expression of ETS family genes in leukemic cell lines using the CellMiner (Luna et al., 2021) showed that most of *SLFN11*-positive leukemic cells expressed high ETS-1 and/or FLI-1 (Figures S5A and S5B). MOLT4 and CCRF-CEM cells also showed high expression of ETS-1 at the protein level (Figure S5C).

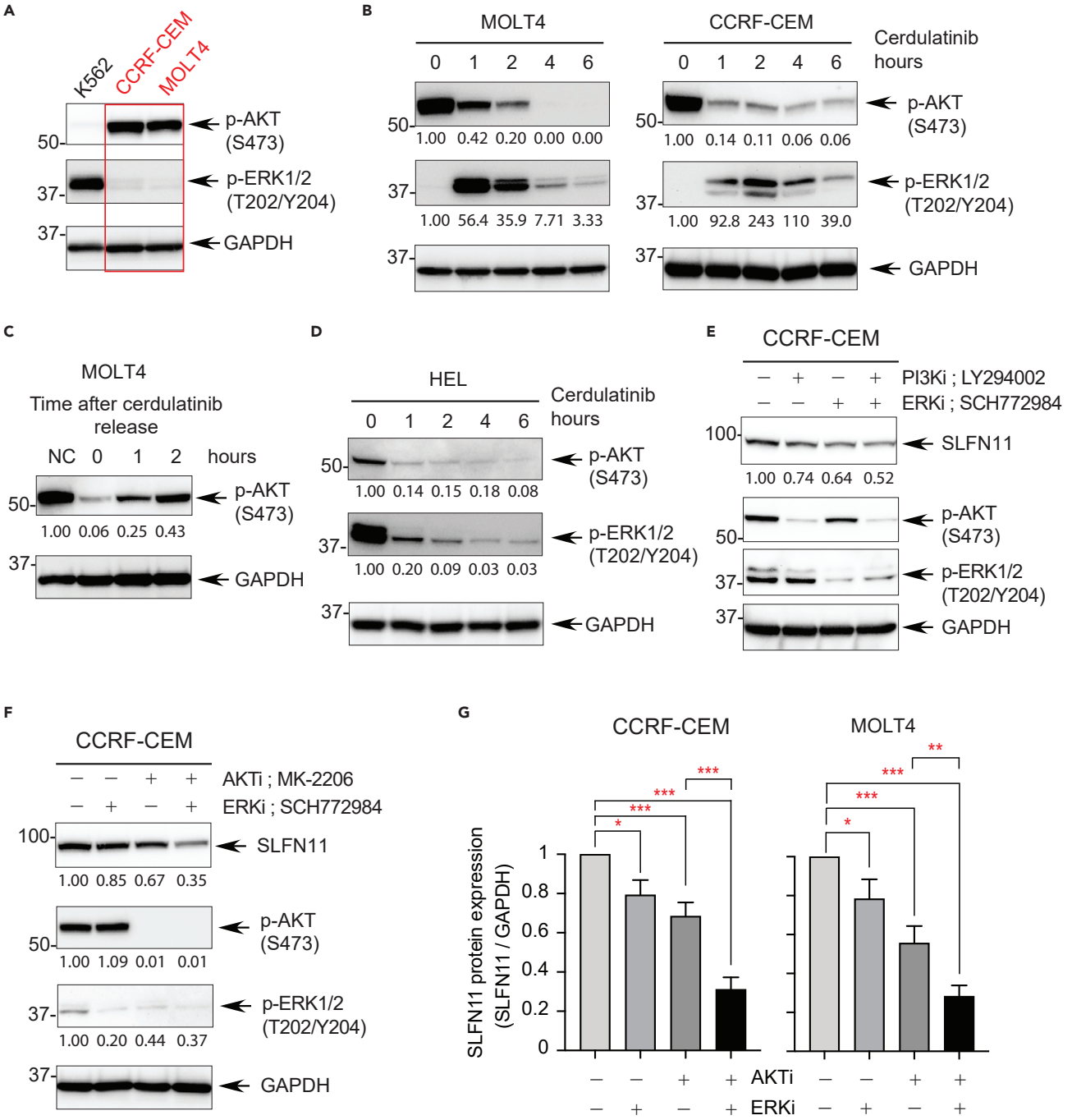


Figure 4. AKT and ERK critically mediate SLFN11 expression in GOF mutant leukemic cells

(A) Phosphorylation status of AKT and ERK detected by Western blotting on the steady-state condition. The red rectangle indicates the leukemic cells with GOF mutation in TYK2.

(B) Alteration of phosphorylated-AKT and -ERK by cerdulatinib (40 μM) was measured by Western blotting at the indicated time points.

(C) Phosphorylation status of AKT after release of cerdulatinib (40 μM, 24 h) in MOLT4 cells.

(D) Alteration of phosphorylated-AKT and -ERK in HEL cells by cerdulatinib (40 μM) was measured by Western blotting at the indicated time points.

(E) SLFN11 protein expression detected by Western blotting in CCRF-CEM cells treated with LY294002 (20 μM, PI3K inhibitor) and/or SCH772984 (20 μM, ERK inhibitor) for 24 h.

(F) SLFN11 protein expression in CCRF-CEM cells treated with MK-2206 (20 μM, AKT inhibitor) and/or SCH772984 (20 μM, ERK inhibitor) for 24 h.

(G) Quantitation of SLFN11 protein expression in 3 independent experiments as shown in Figure 4F (CCRF-CEM) and S4B (MOLT4). The error bars represent SD; *p = 0.012 (CCRF-CEM), *p = 0.026 (MOLT4), **p = 0.007, ***p < 0.001 with ordinary one-way ANOVA test and Tukey's multiple comparisons test.

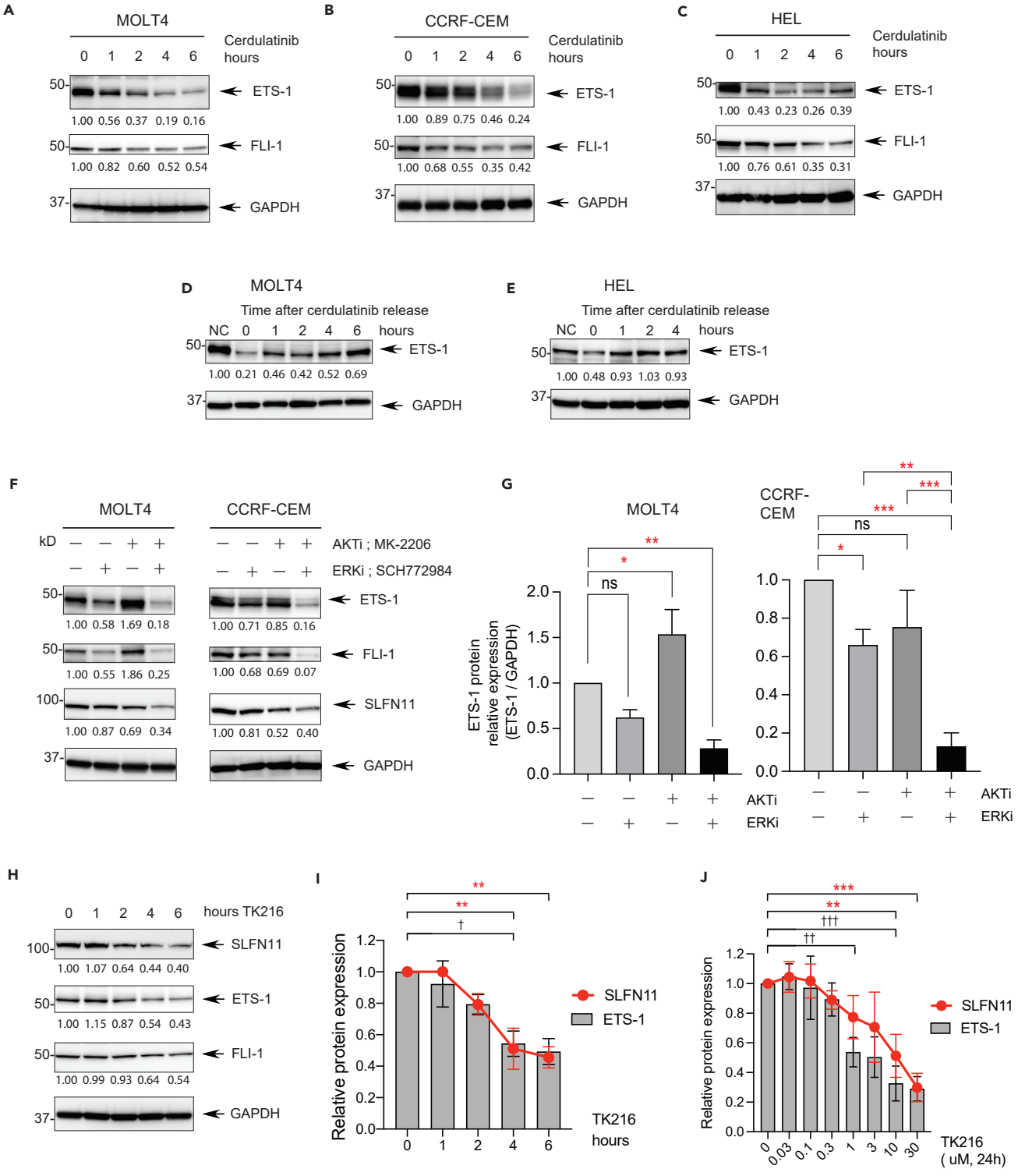


Figure 5. ETS pathway is a key regulator for SLFN11 expression in GOF mutant leukemic cells

(A–C) ETS family proteins in MOLT4, CCRF-CEM, and HEL cells treated with cerdulatinib (40 μM) for the indicated times.

(D and E) Expression of ETS-1 after release of cerdulatinib treatment (40 μM, 24 h) in MOLT4 and HEL cells.

(F) Protein expressions of ETS-1 and FLI-1 detected by Western blotting in MOLT4 and CCRF-CEM cells treated with MK-2206 (20 μM, AKT inhibitor) and/or SCH772984 (20 μM, ERK inhibitor) for 24 h.

Figure 5. *Continued*

(G) Quantitation of SLFN11 protein expression in 3 independent experiments as shown in panel (F). The error bars represent SD; *p = 0.01 (MOLT4), *p = 0.023 (CCRF-CEM), **p = 0.002, ***p < 0.001 with ordinary one-way ANOVA test and Tukey's multiple comparisons test.

(H) Protein expression of SLFN11 and ETS proteins in MOLT4 cells treated with TK216 (30 μM, ETS inhibitor) as the indicated time points.

(I) Quantitation of SLFN11 and ETS-1 protein expression as shown in panel (H). Error bars represent SD (N = 2); **p = 0.006 (SLFN11; 0 vs. 4 h), **p = 0.004 (SLFN11; 0 vs. 6 h), †p = 0.018 with ordinary one-way ANOVA test and Tukey's multiple comparisons test.

(J) Quantitation of SLFN11 and ETS protein expression in MOLT4 treated with the indicated concentrations of TK216 for 24 h as shown in Figure S5I. Error bars represent SD; **p = 0.006 (SLFN11), ***p < 0.001 (SLFN11), ††p = 0.004 (ETS-1), †††p < 0.001 (ETS-1) with ordinary one-way ANOVA test and Tukey's multiple comparisons test.

To examine whether the ETS transcription factor pathway is downstream of JAK activation in the cells harboring GOF mutations in JAKs, we treated the MOLT4 and CCRF-CEM cells with cerdulatinib. Cerdulatinib markedly reduced both ETS-1 and FLI-1 in time- and dose-dependent manners (Figures 5A, 5B, and S5F). Of note, protein expression of ETS-1 was decreased by 70–80% in 6 h (Figure S5D). ETS-1 mRNA expression in CCRF-CEM was also suppressed by cerdulatinib treatment (Figure S5E). Treatment of HEL cells with cerdulatinib also reduced the expression of ETS-1 and FLI-1 (Figure 5C). In addition, removal of cerdulatinib restored the expression of ETS-1 in both of MOLT4 and HEL (Figures 5D and 5E), which extends the connection between ETS activation and JAK1-AKT activation. To confirm these results, we tested the other JAK inhibitor, ruxolitinib, and found consistent reduction in the expression of ETS-1 accompanied with the depletion of phosphorylated TYK2 and SLFN11 (Figure S5G). Also, the combination of AKT and ERK inhibitors suppressed ETS-1 and FLI-1 expression while the inhibition of either AKT or ERK alone partially decreased ETS-1 and FLI-1 expression in MOLT4, CCRF-CEM, and HEL (Figures 5F–5G and S4C).

Finally, to further establish the dependency of SLFN11 expression on the ETS pathway in leukemia cells, we treated MOLT4 cells with the new clinical ETS inhibitor TK216 (NCT02657005). TK216 suppressed the expression of both SLFN11 and ETS-1 both at the protein and transcription levels (Figures 5H–5J and S5H) and in a dose-dependent manner (Figures 5J and 5S5I). Moreover, since it was reported all-trans-retinoic-acid (atRA) suppressed the activity of ETS family genes including ETS-1 and FLI-1 (Darby et al., 1997; Lulli et al., 2010), we explored the effect of SLFN11 expression by atRA. High-dose atRA moderately decreased SLFN11 expression along with the reduction of ETS-1 (Figures S5J and S5K).

Together, these results demonstrate that the ETS family is an important transcription factor for SLFN11 expression through the JAKs-AKT and -ERK activation in GOF mutant leukemic cells.

DISCUSSION

Our study demonstrates that leukemic cells with GOF mutations in JAKs overexpress SLFN11, and that, in these cells, SLFN11 expression is controlled by a non-classical JAK signaling pathway involving JAK-AKT/ERK (Figure 6A). We extend to leukemia our prior molecular explanation made in Ewing sarcomas (Tang et al., 2015) that ETS family genes drive the transcription of *SLFN11*. Accordingly, we show that JAK inhibitors, in particular cerdulatinib, combination of AKT or PI3K and ERK inhibitors and ETS inhibition reduce SLFN11 expression (Figure 6A). While the classical JAK-STAT signaling pathway in MOLT4 and CCRF-CEM is not further activated upon exogenous IFN stimulation, we confirm that SLFN11 acts as an IFN-responsive gene in other cancer cell lines through the classical IFN-JAK-STAT signaling pathway with induction of ISG15, one of the classical IFN stimulated genes (Perng and Lenschow, 2018) (Figure 6B).

While SLFN11 was previously described as an anti-viral molecule against human immunodeficiency virus 1 (HIV-1) and flaviviruses (Li et al., 2012; Valdez et al., 2019), its regulation and mechanisms of action from an immunological standpoint are still under investigation. The murine Slfn family genes including Slfn1, Slfn2, Slfn3, Slfn5, and Slfn8 are regulated by STAT1 and STAT3 (Katsoulidis et al., 2009). However there have been no reports about human SLFN11 and STATs. Our study suggests that classical IFN-JAK-STAT signaling is not a main regulator of SLFN11 expression in leukemic cells with GOF mutation in JAKs, and therefore that the inducibility of SLFN11 by exogenous IFNs is dependent on cell types. Puck et al. also reported that the inducibility of SLFN11 by Human rhinovirus 14, IFN-α and LPS was lower than that of MxA, one of the classical ISGs (Puck et al., 2015). They analyzed transcription factor binding sites using MatInspector (Cartharius et al., 2005) and found only few canonical IFN-stimulated response element (ISRE) sites in most human SLFN genes while MxA had 6 ISRE sites, inferring that this numerical difference may lead to the relatively low IFN inducibility of the SLFN genes. Additionally, because SLFN11 expression

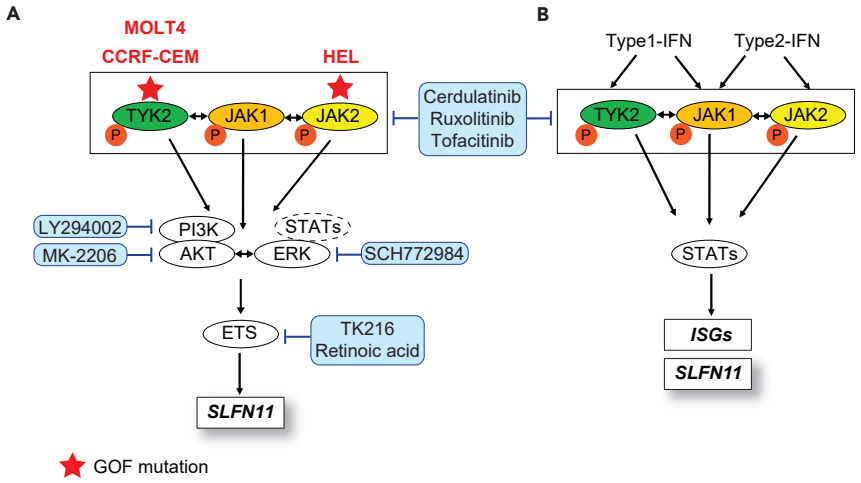


Figure 6. Proposed model for the mechanism of SLFN11 induction in GOF mutant leukemic cells

(A) In leukemic cells with GOF mutation in JAKs, JAKs are auto-phosphorylated without IFN stimulation. These cells promote phosphorylation of AKT while the activation of AKT may suppress the activity of ERK. Both of AKT and ERK induce SLFN11 expression via ETS family proteins.

(B) In leukemic cells with wild-type JAKs, SLFN11, and classical IFN stimulated genes can be induced in response to exogenous IFNs by the classical IFN-JAK-STATs signaling pathway.

is already activated fully via non-classical IFN pathways in the GOF mutant leukemic cells (MOLT4, CCRF-CEM, and HEL in our study), this may explain why JAK GOF leukemic cells do not respond to additional stimulation upon exposure to exogenous IFNs.

The JAK2 V617F mutation is a common GOF mutation in myeloproliferative disorders (Bercovich et al., 2008; Cerami et al., 2012; Gao et al., 2013; James et al., 2005). Other TYK2 and JAK1 GOF mutations are also detectable in human leukemic cells (Flex et al., 2008; Gordon et al., 2010; Hornakova et al., 2011; Sanda et al., 2013). Therefore, we connect here the GOF mutations in JAKs with overexpression of SLFN11 in leukemia cell lines. The ability of clinically used JAK inhibitors to suppress SLFN11 expression in JAKs GOF-mutated leukemic cells while reducing phosphorylated TYK2 demonstrates the functionality of the JAK GOF mutations in activating SLFN11. Additionally, we observed that most leukemic cell lines with WT JAKs also overexpress SLFN11. Approximately 50% of AML patients have acquired genetic abnormalities (McCubrey et al., 2008; Vey et al., 2004). Especially, FMS-like tyrosine kinase 3 - internal tandem duplication (FLT3-ITD) mutations are detected in 20-30% of AMLs and may cause activation of Ras-Raf-MEK-ERK, PI3K-AKT and JAK-STAT pathways (McCubrey et al., 2008). Moreover, CML cells express the BCR-ABL oncoprotein, which also activates JAK and Ras-Raf-MEK-ERK pathways (Desterke et al., 2018; Sanda et al., 2013), suggesting that BCR-ABL may be a positive regulator of SLFN11. At this point, we do not have data on whether inhibitors against BCR-ABL such as imatinib, nilotinib, or dasatinib can reduce SLFN11 expression, and further studies are warranted to investigate this possibility.

We previously showed that ETS binding domains are located near the transcription start site of *SLFN11*, and that FLI-1 and ETS-1 work as transcriptional factors for *SLFN11* (Tang et al., 2015). In this study, we extend this finding by showing that ETS family proteins drive *SLFN11* expression in JAK-GOF mutant leukemic cells. This conclusion is based on the suppression of SLFN11 expression by the new clinical ETS inhibitor TK216 and by retinoic acid, and on the suppression of both ETS and SLFN11 expression by the JAK inhibitors (Figure 6A). Raf-MEK-ERK pathway or PI3K-AKT pathway are reported as a regulator of ETS family genes (Giorgi et al., 2015; Hervas-Stubbs et al., 2011; Plotnik et al., 2014; Selvaraj et al., 2014; Smith et al., 2012). While E-twenty-six (ETS)-like transcription factor 1 (Elk-1) is controlled by both Raf-MEK-ERK and PI3K-AKT pathways (Mut et al., 2012), it is unknown whether ETS-1 and FLI-1 are regulated by both pathways. Our report suggests that the expression of ETS-1 and FLI-1 are under the control of both pathways, which leads to *SLFN11* expression.

Suppression of SLFN11 expression, which occurs in approximately 50% of cancer cells (Murai et al., 2019) is controlled epigenetically by CpG promoter hypermethylation (Nogales et al., 2016; Reinhold et al., 2017;

Tang et al., 2018) and is a dominant resistance factor to a broad range of widely used antileukemic agents targeting DNA replication including cytarabine, hydroxyurea, methotrexate, anthracyclines, etoposide, and topotecan (Murai et al., 2019). Hence, reactivation of SLFN11 by epigenetic modulators is being pursued to overcome global drug resistance. In addition to DNA methyltransferase inhibitors (Nogales et al., 2016; Tang et al., 2018), EZH2 and class I histone deacetylase inhibitors have also been shown to induce SLFN11 expression (Gardner et al., 2017; Nogales et al., 2016; Tang et al., 2018). Hence, reactivating SLFN11 is a rational approach for combination therapies with DNA replication inhibitors. Assuming that SLFN11 may also acts as a tumor suppressor gene, arresting cells with abnormal replication (Murai et al., 2019; Zhou et al., 2020), reactivation of SLFN11 may also act by itself to stop tumor growth.

It is striking that highly proliferative leukemia cells express high SLFN11 levels. This suggests that expressing SLFN11 provides a selective advantage in the context of those cells. Further studies are needed to elucidate how SLFN11 expression may benefit some tumor cells. For instance, SLFN11 has been shown to protect against viral infections (Li et al., 2012; Valdez et al., 2019) and reduce proteotoxic stress (Murai et al., 2021). Here we show that for the cancers that overexpress SLFN11 such as leukemia, Ewing sarcoma, and mesotheliomas (see Figure 1B), it is possible to effectively suppress SLFN11 expression. Targeted therapies including clinically used JAK kinase and ETS inhibitors effectively and reversibly suppress SLFN11 expression in leukemia (Figure 6). Although, it is unclear whether leukemia cells derive a growth advantage by overexpressing SLFN11, and would be affected by suppressing SLFN11 expression, it is now feasible to address this question by performing functional and clinical studies using JAK and ETS inhibitors. Notably, a recent study showed that a subset of hepatocellular carcinomas overexpresses SLFN11, and it has been proposed that suppressing SLFN11 expression may specifically affect such cancers by modulating mTOR signaling (Zhou et al., 2020).

Scoring SLFN11 by immunohistochemistry and transcriptome analyses are readily feasible and ongoing at multiple institutions (Buettner, 2021; Kagami et al., 2020; Knelson et al., 2021; Mao et al., 2021; Moribe et al., 2021; Murai et al., 2019; Takashima et al., 2021a, 2021b; Winkler et al., 2021; Zhou et al., 2020). Extending SLFN11 testing in hematologic malignancies should be readily feasible.

Limitations of the study

Potential limitations in this study include the possibility that other types of leukemic cells such as AML with GOF mutations in JAK1 or JAK2 behave differently from the leukemia cell lines used for molecular analyses in the present study. As T cell receptor (TCR) activation induces ERK and AKT phosphorylation (Hwang et al., 2020), whether TCR activation can induce SLFN11 expression needs to be studied further. An additional point may be the lack of data on SLFN11 expression in LOF mutations in JAKs. Future studies to investigate these points will complete our understanding of how SLFN11 expression is controlled by the Ras-Raf-MEK-ERK, PI3K-AKT and JAK-STAT pathways, and whether targeting SLFN11 could be used therapeutically.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103173.

ACKNOWLEDGMENTS

This project was supported by the National Cancer Institute Intramural Program, Center for Cancer Research, NIH (Z01-BC006150; to Y.P.). We wish to thank Dr. Peter D. Aplan (NCI/NIH) for providing HEL cells and scientific insights.

AUTHOR CONTRIBUTIONS

Y.M., U.J, N.T., and Y.P. planned the study design and wrote the manuscript with support from J.M. and S.F.; Y.M. and U.J. performed the experiments and analyzed the data with help from U.J., J.M., N.T., and Y.P.; J.M. generated *SLFN11*-KO cell lines; and Y.P., N.T., and S.F. supervised in this study.

DECLARATION OF INTERESTS

The authors declare no competing financial interest.

Received: May 25, 2021
Revised: August 16, 2021
Accepted: September 22, 2021
Published: October 22, 2021

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-TYK2	Cell signaling Technology	Cat# 9312S; RRID: AB_2256719
Rabbit monoclonal anti-phospho (Y1054/1055)-TYK2 (D7T8A)	Cell signaling Technology	Cat# 68790S; RRID: AB_2799752
Rabbit monoclonal anti-phospho (Y1034/1035)-JAK1 (D7N4Z)	Cell signaling Technology	Cat# 74129S; RRID: AB_2799851
Rabbit polyclonal anti-phospho (Y1007/1008)-JAK2	Cell signaling Technology	Cat# 3771S; RRID: AB_330403
Rabbit monoclonal anti-phospho (Y701)-STAT1 (58D6)	Cell signaling Technology	Cat# 9167S; RRID: AB_561284
Rabbit polyclonal anti-phospho (T202/Y204)-p44/42 MAPK (ERK1/2)	Cell signaling Technology	Cat# 9101S; RRID: AB_331646
Rabbit polyclonal anti-ETS-1 (C-20)	Santa Cruz Biotechnology	Cat# sc-350; RRID: AB_2100688
Rabbit polyclonal anti-FLI-1 (C-19)	Santa Cruz Biotechnology	Cat# sc-356; RRID: AB_2106116
Mouse monoclonal anti-ISG15 (F-9)	Santa Cruz Biotechnology	Cat# sc-166755; RRID: AB_2126308
Mouse monoclonal anti-SLFN11 (D-2)	Santa Cruz Biotechnology	Cat# sc-515071
Rabbit polyclonal anti-GAPDH	GeneTex	Cat# GTX100118; RRID: AB_1080976
ECL anti-mouse IgG, horseradish peroxidase linked whole antibody (from sheep)	GE Healthcare	cat# NA931; RRID: AB_772210
ECL anti-rabbit IgG, horseradish peroxidase linked whole antibody (from donkey)	GE Healthcare	cat# NA934; RRID: AB_772206
Chemicals, peptides, and recombinant proteins		
Recombinant human interferon-gamma	Thermo Fisher Scientific	Cat# PHC4031
Human interferon-alpha a protein (alpha 2a)	PBL assay science	Cat# 11101-2
Cerdulatinib	MedChemExpress	Cat# HY-15999
Ruxolitinib	MedChemExpress	Cat# HY-50856
Tofacitinib	MedChemExpress	Cat# HY-40354
MK-2206 dihydrochloride	MedChemExpress	Cat# HY-10358
SCH772984	MedChemExpress	Cat# HY-50846
TK216	Shelleckchem	Cat# S9718
LY294002 (InSolution)	Sigma-Aldrich	Cat# 440204
Retinoic acid	Sigma-Aldrich	Cat# R2625
Critical commercial assays		
TRIzol reagent	Invitrogen	Cat# 15596026
PureLink RNA Mini Kit	Invitrogen	Cat# 12183025
SuperScript II Reverse Transcriptase Kit	Invitrogen	Cat# 18064022
FastStart Universal SYBR Green Master (Rox)	Sigma-Aldrich	Cat# 4913850001
protease inhibitor cocktail (100x)	Cell signaling Technology	Cat# 5871S
Pierce phosphatase inhibitor mini tablets	Thermo Fisher Scientific	Cat# A32957
Novex tris-glycine SDS sample buffer	Invitrogen	Cat# LC2676
Tris/glycine/SDS buffer (10x)	BioRad	Cat# 1610732
Novex tris-glycine transfer buffer (25x)	Invitrogen	Cat# LC3675

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Blotting-Grade Blocker	BioRad	Cat# 1706404
Immun-Blot PVDF membranes	BioRad	Cat# 1620177
Experimental models: Cell lines		
Human: prostate cancer DU145 cells	Developmental Therapeutics Program (NCI/NIH)	N/A
Human: colon cancer HCT116 cells	Developmental Therapeutics Program (NCI/NIH)	N/A
Human: leukemia CCRF-CEM cells	Developmental Therapeutics Program (NCI/NIH)	N/A
Human: leukemia MOLT4 cells	Developmental Therapeutics Program (NCI/NIH)	N/A
Human: leukemia K562 cells	Developmental Therapeutics Program (NCI/NIH)	N/A
Human: leukemia JURKAT cells	Developmental Therapeutics Program (NCI/NIH)	N/A
Human: leukemia THP-1 cells	Developmental Therapeutics Program (NCI/NIH)	N/A
Human: leukemia HL-60 cells	Dr. T. Breitman NCI, NIH	N/A
Human: leukemia HAP-1 cells	Horizon Discovery	N/A
Human: lung cancer A549 cells	Developmental Therapeutics Program (NCI/NIH)	N/A
Human: lung cancer DMS114 cells	American Type Culture Collection	Cat# CRL-2066
Human: leukemia HEL cell	Dr. Peter D. Aplan NCI, NIH	N/A
CCRF-CEM SLFN11 knockout cells	Murai et al., 2016	N/A
MOLT4 SLFN11 knockout cells	Murai et al., 2016	N/A
Oligonucleotides		
Forward primer for qPCR of SLFN11: 5′-GGCCCAGACCAAGCCTTAAT-3′	IDT oligo	N/A
Reverse primer for qPCR of SLFN11: 5′-CACTGAAAGCCAGGGCAAAC-3′	IDT oligo	N/A
Forward primer for qPCR of ETS-1: 5′-GTTAATGGAGTCAACCCAGC-3′	IDT oligo	N/A
Reverse primer for qPCR of ETS-1: 5′-GGGTGACGACTTCTTGTTTG-3′	IDT oligo	N/A
Forward primer for qPCR of GAPDH: 5′-TCAACGACCACTTTGTCAAGCT-3′	IDT oligo	N/A
Reverse primer for qPCR of GAPDH: 5′-GTGAGGGTCTCTCTCTTCCTCTTGT-3′	IDT oligo	N/A
Software and algorithms		
GraphPad Prism 9 (software for drawing graphs and statistics analysis)	GraphPad	N/A
ImageJ (software for image analysis)	NIH	N/A
Image Lab software (software for image analysis)	BioRad	N/A
CellMinerCDB (web application for analysis of NCI-60, CCLE and GDSC database)	Genomics & Bioinformatics Group/ Developmental Therapeutics Branch/Laboratory of Molecular Pharmacology/CCR/NCI/NIH	https://discover.nci.nih.gov/rsconnect/cellminercdb/

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
cBioPortal (web application for analysis of TCGA database)	Cerami et al. (2012) and Gao et al. (2013)	https://www.cbioportal.org
Other		
QuantStudio 5 Real-Time PCR System	Thermo Fisher Scientific	N/A
ChemiDoc™ Touch MP	BioRad	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for reagents and resource sharing should be directed to and will be fulfilled by the lead contact, Yves Pommier (pommier@nih.gov).

Materials availability

This study did not generate new unique reagents.

Date and code availability

This paper analyzes existing, publicly available data. These accession numbers for the datasets are listed in the key resources table. All data reported in this paper will be shared by the lead contact upon request. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENT MODEL AND SUBJECT DETAILS

Cell lines and cultures

K562, DU145, CCRF-CEM and MOLT4 were obtained from the NCI Developmental Therapeutics Program (DTP) of the Division of Cancer Treatment and Diagnosis (DCTD). The HEL cell line was kindly provided by Dr. Peter D. Aplan (NCI, NIH). DU145 and HCT116 cell lines were grown in DMEM medium (11995065; GIBCO, ThermoScientific, Waltham, MA, USA) with 10% Fetal Bovine Serum (FBS; 100106; GeminiBio, West Sacramento, CA, USA) and 1% penicillin-streptomycin (15140122; GIBCO) at 37°C in 5%CO_2. CCRF-CEM, MOLT4, DMS114, A549, K562, THP-1, HL-60, HEL and JURKAT cell lines were grown in RPMI 1640 medium (11875093; GIBCO) with 10% FBS and 1% penicillin-streptomycin at 37°C in 5%CO_2. HAP1 cell line was grown in IMDM medium (12440053; GIBCO) added with 10% FBS and 1% penicillin-streptomycin at 37°C in 5%CO_2.

Generation of SLFN11-deleted cells

SLFN11-knockout cells in CCRF-CEM and MOLT4 cell lines were generated by CRISPR/Cas9 methods as described (Murai et al., 2016).

METHODS DETAILS

TCGA data analysis

mRNA expressions of SLFN11 in various cancer cell lines were obtained from TCGA data in cBioPortal (https://www.cbioportal.org) (Cerami et al., 2012; Gao et al., 2013).

CellMiner CDB analysis and gain-of-function mutation data

mRNA expressions or promoter methylation of SLFN11 and mutations of JAK family across NCI-60, CCLE and GDSC were available from the CellMiner website (https://discover.nci.nih.gov/cellminercdb/) (Murai et al., 2018; Reinhold et al., 2015). Biological effects of JAK kinase mutation were obtained from cBioPortal (https://www.cbioportal.org) (Cerami et al., 2012; Gao et al., 2013).

Western blotting

For preparing whole cell lysates, cells were lysed with RIPA buffer (150 mM NaCl, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1% NP40 (v/v), 0.1% SDS (v/v) and 0.5% sodium deoxycholate (w/v)), protease inhibitor cocktail

(5871; Cell Signaling Technology, Danvers, MA, USA) and phosphatase inhibitor (A32957; ThermoScientific). After mixing cell pellets with the buffer and incubation at 4°C for 40 min, lysates were centrifuged at 12,000 rpm at 4°C for 15 min, and supernatants were collected. Samples were mixed with Novex tris-glycine SDS sample buffer (LC2676; Invitrogen, ThermoScientific, Waltham, MA, USA) and heated at 95°C for 5 min. The mixtures were loaded into wells of Novex tris-glycine gels (Invitrogen). Gels were transferred into Immun-Blot PVDF membranes (1620177; BioRad, Hercules, CA, USA) and membranes were blocked with 5% non-fat milk using Blotting-Grade Blocker (1706404; BioRad) in PBST. The primary antibodies were diluted in 1% milk/PBST by 1:1000 for TYK2 (9312; Cell Signaling), pTYK2 (Y1054/1055; 68790; Cell Signaling), pJAK1 (Y1034/1035; 74129; Cell Signaling), pJAK2 (Y1007/1008; 3771; Cell Signaling), pSTAT1 (Y701; 9167; Cell Signaling), pAKT (S473; 4060; Cell Signaling), pERK1/2 (T202/Y204; 9101; Cell Signaling), ETS-1 (C-20; sc-350; Santa Cruz Biotechnology, Dallas, TX, USA) and FLI-1 (C-19; sc-356; Santa Cruz), 1:500 for ISG15 (F-9; sc-166755; Santa Cruz), and 1:2500 for SLFN11 (D2; sc-515071; Santa Cruz) and GAPDH (GTX100118; GeneTex, Irvine, CA, USA). The horseradish peroxidase (HRP)-conjugated secondary antibodies (NA931 and NA934; GE Healthcare, Boston, MA, USA) were diluted in 1% milk/PBST by 1:4000. Protein signals were visualized by ChemiDoc^{TM} Touch MP. Quantification of band intensity was done using ImageJ software and Image Lab software (BioRad). An appropriate square-shaped gating slightly larger than blot bands was set, and we measured the mean intensity of each band.

Quantitative reverse transcription PCR

Total RNAs were extracted with TRIzol reagent (15596026; Invitrogen) and their purification was performed by PureLink RNA Mini Kit (12183025; Invitrogen). Complementary DNA (cDNA) was generated using SuperScript II Reverse Transcriptase Kit (18064022; Invitrogen) according to the manufacturer's instructions. To amplify specific genes, primers were used in the following: *SLFN11* (forward 5′-GGCCCAGA CCAAGCCTTAAT-3′ and reverse 5′-CACTGAAAGCCAGGGCAAAC-3′), *ETS-1* (forward 5′-GTTAAT GGAGTCAACCCAGC-3′ and reverse 5′-GGGTGACGACTTCTTGTTTG-3′) and *GAPDH* (forward 5′-TC AACGACCACTTTGTCAAGCT-3′ and reverse 5′-GTGAGGGTCTCTCTCTTCCTCTTGT-3′). Quantitative PCR was performed with FastStart Universal SYBR Green Master (Rox) (4913850001; Roche, Sigma-Aldrich, St. Louis, MO, USA) and the amplification was detected by QuantStudio 5 Real-Time PCR System (Life sciences, ThermoScientific, Waltham, MA, USA) according to the manufacturer's instructions. ROX was used as a passive reference dye and *GAPDH* was used as an internal control. The relative mRNA expression was measured by $2^{(-\Delta\Delta Ct)\ method}$.

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were conducted with GraphPad Prism 9.0. Statistical significances were determined by the two-way ANOVA test (Figures 3E and 3I), the ordinary one-way ANOVA test (Figures 2E, 3B, 3D, 4G, 5G, 5I, 5J, S4D, S5D, S5E, and S5H) and Tukey's multiple comparisons test (Figures 2E, 3B, 3D, 3E, 3I, 4G, 5G, 5I, 5J, S4D, S5D, S5E, and S5H). The threshold for statistical significance was $P < 0.05$. For the quantitative data, the statistical parameters were shown in the figure legends.