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Sub-chronic administration of LY294002 sensitizes cervical cancer cells to chemotherapy by enhancing mitochondrial JNK signaling

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ABSTRACT

Chemo-sensitization is used to improve the efficacy of chemotherapeutic agents against cancers, and understanding the precise molecular mechanisms of chemo-sensitization could lead to safer and more effective approaches to treat cancer. We have previously demonstrated that mitochondrial c-Jun N-terminal Kinase (JNK) signaling is a critical component of cell death. Mitochondrial JNK signaling is coordinated on the scaffold protein Sab. In this work, we developed a sub-chronic chemo-sensitization model by exposing HeLa cells to low-dose (2 μM) LY294002. We found that this treatment increased Sab expression on mitochondria, an effect not observed in acute exposures. To examine the role of Sab in chemo-sensitization, we ectopically expressed and silenced Sab in HeLa cells. We found that elevating Sab levels in HeLa cells increased the efficacy of chemotherapeutic agents, paclitaxel and cisplatin, while silencing Sab decreased the sensitivity of cells towards these agents. The effect of Sab-mediated signaling appeared to be dependent upon mitogen dependent protein kinases (MAPKs) as ablation of Sab's MAPK-binding motifs prevented chemo-sensitization. These results suggest that mitochondrial JNK signaling is an adaptable signaling pathway that can be enhanced or restored in cancer cells to improve therapeutic efficacy.

1. Introduction

Resistance to chemotherapy develops in many types of cancer; consequently, chemo-sensitizing agents, chemicals or biologics capable of improving chemotherapeutic responses, are of paramount interest for cancer drug discovery [1–6]. The mechanisms of chemo-resistance vary, and may involve perturbations in cellular processes such as DNA repair, cellular transport, survival responses and apoptotic processes [2,7,8]. Targeting these aberrant mechanisms with chemo-sensitizing agents may increase the efficacy of chemotherapies ultimately limiting the exposure of patients to toxic concentrations of these treatments.

Inhibition of Phosphoinositide 3-kinase (PI3K) has been used to reduce cell survival responses and proliferation in human cancers [9]. LY294002, an inhibitor of PI3K signaling, has been used extensively to improve the efficacy of many chemotherapeutics in cell culture [10–12]. Specifically, LY294002 has been shown to improve the sensitivity of cervical cancer cells to taxols and platinum drugs; two chemotherapies commonly used in clinical settings [13–15]. However, the doses used in previous studies appear to be high (20–50 μM) when compared to ideal drugs, which have efficacious impacts in the pico- and nano-molar ranges. Thus, dissecting the precise physiological consequences of PI3K inhibition may identify new and more effective targets to improve chemotherapeutic efficacy.

The pro-survival and anti-apoptotic mechanisms of chemo-resistance converge on mitochondria, the organelle responsible for regulating metabolism and viability [2,7,16]. We have recently described a signaling pathway on the outer mitochondrial membrane that engages mitochondrial dysfunction and cell death

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[17–20]. The c-Jun N-terminal kinase (JNK) was found to localize to mitochondria in HeLa cells following treatment with anisomycin. This localization and subsequent mitochondrial dysfunction required the protein scaffold Sab (or SH3BP5) [17]. Selective inhibition of the JNK-Sab interaction by gene silencing or use of a small cell permeable peptide was sufficient to prevent mitochondrial dysfunction and apoptosis induced by JNK [17]. Our studies and others have demonstrated that mitochondrial JNK signaling is a critical component of early apoptotic signaling in distinct tissues under a variety of stresses [17–25].

Scaffold proteins, such as Sab, are essential for organizing signal transduction pathways in the crowded cellular environment [26,27]. The abundance of particular scaffold proteins can concentrate distinct signal transduction events at precise locations to alter cell physiology [26,27]. Specifically, we propose that enhancing mitochondrial JNK signaling by increasing the concentration of Sab on mitochondria is an effective means to polarize cancer cells toward apoptosis. In our current work, we examine the impact of Sab concentration on the chemo-sensitizing ability of LY294002 in HeLa cells. Our studies reveal that increasing Sab expression enhanced the effects of low-dose LY294002 on the efficacy of paclitaxel and cisplatin. Further, silencing Sab prevented LY294002 chemo-sensitization of HeLa cells and reduced cell death in the presence of paclitaxel and cisplatin. This work suggests that altering outer mitochondrial signaling is an effective strategy for improving chemotherapeutic efficacy.

2. Materials and methods

2.1. Materials

HeLa cells were obtained from American Type Tissue Culture (Manassas, VA), and the wild type (WT) and JNK1$^{-/-}$/JNK2$^{-/-}$ murine embryonic fibroblasts (MEFs) were generous gifts from Dr. Roger Davis (University of Massachusetts, Howard Hughes Medical Institute) [28]. General laboratory media, reagents, and chemicals were acquired through Fisher Scientific (Waltham, MA). LY294002 was purchased from Cell Signaling Technologies (Danvers, MA). Paclitaxel was supplied by LC Laboratories (Woburn, MA). Cisplatin was purchased from Sigma–Aldrich (St. Louis, MO). Antibodies were obtained from multiple vendors as indicated in the methods below.

2.2. Cell culture

HeLa cells, WT, and JNK1$^{-/-}$/JNK2$^{-/-}$ were cultured in Dulbucco's Modified Essential Media supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 10 μg/mL streptomycin, and 5 μg/mL plasmocin. Cells were maintained at 37 °C and under 5% CO_2 for no more than 25 passages after thawing.

2.3. LY294002 treatment

LY294002 was solubilized in dimethylformamide (DMF). We exposed HeLa cells to the indicated doses of LY294002 over 24 h for acute studies. We determined the 24-h IC_{10} for LY294002 in HeLa cells to be ~2 μM. For sub-chronic exposure, cells were dosed with 2 μM LY294002 for seven days. The media was exchanged every 48 h to sustain the drug concentrations.

2.4. Calculation of paclitaxel and cisplatin IC_{50}s

The IC_{50}s for paclitaxel and cisplatin in HeLa cells were determined using the TO-PRO-3 near infrared dye (Invitrogen). Briefly, 1×10^4 cells were plated in a black-walled optically clear bottom 96-well plate. Cells were then exposed to increasing concentrations of paclitaxel (0–100 μM) or cisplatin (0–1 μM) for 24 h. The cells were fixed in 4% paraformaldehyde for 25 min at room temperature, and stained with TO-PRO-3 (1 μM) in 1X PBST (150 mM NaCl, 8 mM Na_2HPO_4, 3 mM KCl, 2 mM KH_2PO_4 pH 7.4, 0.05% Tween 20). The cells were then washed five times in 1X-PBST while gently rocking for 5 min at room temperature. Samples were protected from light during incubations. Assays were visualized on the Li-Cor Odyssey CLx imager, and IC_{50}s were calculated using Graphpad® Prism.

2.5. Cell lysis and western blotting

Following 72 h of transfection, cells were lysed and proteins were harvested as described in our prior studies [17,19]. Protein concentrations of the supernatant was determined using the by Pierce BCA Assay kit protocol. Proteins were resolved by SDS-PAGE, and transferred onto PVDF membranes. Membranes were incubated with Li-Cor Biosciences Odyssey Blocking for at least 1 h at room temperature. The membranes were then incubated with primary antibodies specific for Sab (Novus Biologicals, H00009467-M01), JNK (Cell Signaling Technology, 9252), p44/42 MAPK (Erk1/2) (Cell Signaling Technology, 4695), p38 (Cell Signaling Technology, 9212), GAPDH (Cell Signaling Technology, 5174), Actin (Cell Signaling Technology, 4970), Calnexin (Cell Signaling Technology, 2679), TOM20 (Abcam, ab115746), or α-tubulin (Cell Signaling Technology, 2144) at dilutions of 1:1000 in blocking buffer. Membranes were washed three times for 5 min in 1 × PBST. Membranes were incubated with secondary antibodies in blocking buffer at 1:20,000 for fluorescently conjugated secondary antibodies purchased from Li-Cor Biosciences. Membranes were again washed three times for 5 min in 1 × PBST. Western blots were developed using the Odyssey CLx imager (Li-Cor Biosciences).

2.6. Mitochondrial isolation

The protocol for mitochondrial isolation in HeLa cells was described in detail in our previous studies [17,19]. Mitochondrial samples with greater than 80% purity were used for experiments. A sample size of 50 μg mitochondrial protein was used for each protein analysis.

2.7. Site-directed mutagenesis

To generate a variant of Sab incapable of supporting signaling, we used site-directed mutagenesis to alter the kinase interacting motifs 1 and 2 (KIM1/2) in order to prevent the binding of mitogen activated protein kinases (MAPKs), specifically JNK. We converted leucines 347 and 349 of the KIM1 motif and leucines 434 and 436 of KIM2 to alanines within Sab using a two reaction mutagenesis of the pLOC:Sab plasmid. First, KIM1 was mutated using sense (5′-GTGAGGCCTGGCAGCGCGGATGCGCCCAGCCCTGTGTC-3′) and antisense (5′-GACACAGGGCTGGGCGCATCCGCGCTGCCAGGCCTCAC-3′) harboring the leucine to alanine mutations. The reaction was assembled using the Phusion site-directed mutagenesis kit (Thermo Scientific). The KIM1 mutations were confirmed by sequencing. Next, the KIM2 mutations were introduced using sense (5′-GAGAACCGGATGAAGCAGGCCTCCGCACAGTGCTCAAAGGGAAG-3′) and antisense (5′GACACAGGGCTGGGCGCATCCGCGCTGCCAGGC CTCAC-3′) containing the appropriate mutagenic sequences. Following mutagenesis, single clones containing the double mutation of KIM1 and KIM2 were confirmed by sequencing. The resulting plasmid was named pLOC:Sab:KIM1/2 L-A. Similarly, silent mutations were introduced into Sab expression constructs to confer resistance to Sab shRNA #2 (TCRN0000139619 – 5′-

CCTGTCAGAGTTTGGGATG-3′). Sense (5′-TGTG TCCCTTTCGGAATTT-3′) and anti-sense (5′-AACACTGGGAACATCATCCC-3′) primers were used to introduce the desired mutations using the Phusion site-directed mutagenesis kit. The mutated plasmid was named pLOC:Sab:shRNA^r.

2.8. Ectopic expression and silencing

Transient transfections with pLOC plasmids for expression and pLKO.1 plasmids for silencing (Open Biosystems) were used to modulate Sab levels. HeLa cells were plated at a density of 2.0×10^5 cells per 35-mm dish the day prior to transfections. Plasmids were mixed with FugeneHD (Promega) at a ratio of 3:1 according to the manufacturer's protocol. Protein levels were monitored by western blot analysis.

2.9. Biological replicates and statistics

A minimum of eight biological replicates were measured for cell-based studies. Biochemical assays, fluorometric detection of superoxide, and other cellular measures were performed with a minimum of six experimental replicates. To determine statistical significance, Student's paired t test was employed for significance between treatments. Statistical significance is indicated by an asterisk (*) in figures in which the p-value is less than 0.05, and p-values less than 0.01 are indicated by two asterisks (**). Non-significant results are unlabeled or indicated by n.s. Data are displayed as means with error bars representing plus and minus one standard deviation from the mean.

3. Results

3.1. Sub-chronic administration of low dose LY294002 induces chemo-sensitization

Studies using LY294002 have employed high doses (often >10 μM) [29,30]. To determine if a lower dose of LY294002 could achieve the same effects as high doses, we examined the chemo-sensitivity in HeLa cells treated with acute (24 h) and sub-chronic (7 day) exposures to LY294002. For acute treatments, cells were dosed with 0.1% DMF or increasing concentrations of LY294002 (2 μM, 10 μM, and 50 μM) for 24 h. The IC_{50}s for paclitaxel and cisplatin were measured for each dose of LY294002 (Fig. 1A and Table 1). LY294002 improved chemo-sensitivity to both paclitaxel and cisplatin, as cells treated with 50 μM LY294002 had IC_{50}'s of 1.9 μM and 0.45 nM, respectively. Meanwhile, 10 μM LY294002 had an IC_{50} of 6.3 μM for paclitaxel and 0.78 nM for cisplatin, and 2 μM LY294002 had a paclitaxel IC_{50} of 14.1 μM and a cisplatin IC_{50} of 2.9 nM, which was similar to untreated and vehicle controls) (Fig. 1A and Table 1). We then performed the experiment for seven days, and while high doses of LY294002 (10 μM and 50 μM) were lethal to HeLa cells, 2 μM LY294002 had little impact on HeLa cell viability (data not shown). Treatment of HeLa cells with 2 μM LY294002 for 7 days produced IC_{50}s for paclitaxel (5.1 μM) and cisplatin (0.54 nM) that were comparable to treatment with 10 μM and 50 μM for 24 h (Fig. 1B and Table 1). These data demonstrate that sub-chronic treatment of low dose LY294002 can induce chemo-sensitivity.

Table 1
IC_{50} values for HeLa cells treated with LY294002 or with ectopic expression of Sab.

	Paclitaxel IC_{50} (μM)	Cisplatin IC_{50} (nM)
Untreated	14.7 μM ± 4.1 μM	3.2 nM ± 1.3 nM
Acute (24 h)		
0.1% DMF	13.6 μM ± 3.2 μM	2.7 nM ± 1.1 nM
2 μM LY294002	14.1 μM ± 3.2 μM	2.9 nM ± 1.3 nM
10 μM LY294002	6.3 μM ± 4.8 μM	0.78 nM ± 0.4 nM
50 μM LY294002	1.9 μM ± 1.1 μM	0.45 nM ± 0.4 nM
Sub-chronic (7 days)		
0.1% DMF	13.9 μM ± 2.6 μM	2.9 nM ± 1.4 nM
2 μM LY294002	5.1 μM ± 3.9 μM	0.54 nM ± 0.3 nM
Ectopic expression		
RFP	13.4 μM ± 3.5 μM	2.9 nM ± 1.0 nM
Sab	4.7 μM ± 2.8 μM	0.42 nM ± 0.3 nM
Sab:KIM1/2-L/A	13.8 μM ± 3.3 μM	3.1 nM ± 1.4 nM
Gene silencing		
Control shRNA	15.3 μM ± 3.5 μM	3.9 nM ± 1.0 nM
Sab shRNA#1	61.8 μM ± 7.1 μM	12.1 nM ± 1.9 nM
Sab shRNA#2	73.4 μM ± 9.3 μM	18.8 nM ± 3.2 nM
Sab shRNA#2 + Sab: shRNA^r	28.0 μM ± 12.8 μM	7.3 nM ± 4.4 nM

±: represents the relative standard deviation from the mean.

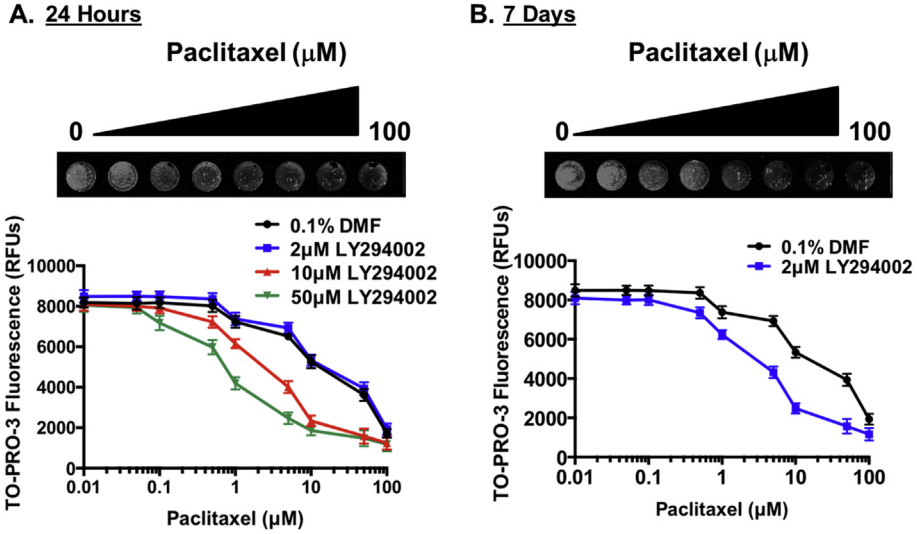

Fig. 1. Sub-chronic administration of low dose LY294002 promotes chemo-sensitivity. HeLa cells were treated with 0.1% DMF, 2 μM, 10 μM or 50 μM LY294002 for 24 h (A) or 7 days (B). After the indicated amount cells were treated with increasing concentrations of paclitaxel. IC_{50}s were then calculated based on TO-PRO-3 staining (tops panels). Fluorescent signals were plotted on GraphPad® Prism.

3.2. Sub-chronic LY294002 increases Sab expression and mitochondrial JNK signaling

To determine if LY294002 impacted the concentration of JNK signaling on mitochondria, we measured the expression of Sab in HeLa cells in both acute and sub-chronic administration of LY294002. HeLa cells were treated with 2 μM, 10 μM and 50 μM LY294002 over 24 h, and neither condition increased Sab expression above that of 0.1% DMF and untreated controls (Fig. 2A, quantified in Supplemental Figure S1A). To ascertain if prospective changes in Sab levels were due to a change in mitochondrial number, COX-IV was employed as a mitochondrial loading control, and Fig. 2A demonstrates equivalent mitochondrial amounts were present. α-tubulin was employed as a cellular loading control (Fig. 2A). To determine if Sab expression changed during sub-chronic treatment with 2 μM LY294002, HeLa cells were treated with 2 μM LY294002 or DMF for 7 days. As time increased, there was a noticeable increase (~8-fold) in Sab expression in the LY294002 compared to DMF-treated and untreated cells (Fig. 2B and quantified in Supplemental Figure S1B). Since Sab expression increased during LY294002 treatment, we examined JNK activation (phosphorylation) during the time course as well (Fig. 2B). Analysis of the protein lysates from our time-course experiment revealed that phospho-JNK levels increased (over 10-fold) between days 2 and 4 before diminishing after day 4 (Fig. 2B and quantified Supplemental Figure S1C). No changes were noted in the level of total JNK (Fig. 2B). To determine if mitochondrial translocation of JNK occurred during the sub-chronic treatment, we analyzed mitochondrial isolates for the presence of JNK on days 3 and 4 (the days of maximal JNK activation). Analysis of the LY294002 exposure revealed that JNK levels increased on mitochondria between days 3 and 4 (Fig. 2C). Moreover, the JNK migrating to mitochondria was active JNK (Phospho-JNK) (Fig. 2C). ERK1/2 or p38 were not observed in the mitochondria isolated at the selected times of the LY294002 time course (Fig. 2C). Our mitochondrial preparations (COX-IV and TOM20) were shown to have low contamination from nuclear (histone H3), cytosolic (enolase), and microsomal fractions (calcineurin) (Fig. 2C). There was some contamination observed from the peroxisomes (PEX19) (Fig. 2C). To determine if JNK could be propagating its own mitochondrial signaling, we examined Sab levels in WT and JNK1$^{-/-}$/JNK2$^{-/-}$ MEFs. Compared to WT MEFs, JNK1$^{-/-}$/JNK2$^{-/-}$ MEFs had markedly decreased Sab expression (3–4 fold on average) (Fig. 2D). These data suggest that low-grade induction of JNK signaling may be sufficient to promote mitochondrial JNK signaling during chemo-sensitization.

3.3. Increasing Sab expression confers chemo-sensitivity in HeLa cells

To determine if the chemo-sensitivity induced by sub-chronic, low dose LY294002 was dependent upon the level of mitochondrial JNK signaling, we examined chemo-sensitivity in HeLa cells ectopically expressing or silencing Sab. First, we transiently transfected HeLa cells with either pLOC:RFP or pLOC:Sab (Fig. 3A) for 48 h and then treated the cells with increasing concentrations of paclitaxel or cisplatin for 24 h. Increasing Sab expression resulted in a 5-fold decrease in the IC_{50} of paclitaxel in HeLa cells when compared to untreated or mock-transfected cells and HeLa cells expressing RFP (Fig. 3B and Table 1). To determine if this sensitization was directly due to the interaction between JNK and Sab, we mutated the KIM motifs of Sab to prevent JNK binding (pLOC:Sab:KIM1/2-L-A) [31,32]. Ectopic expression of this JNK-binding deficient version of Sab had no impact on the IC_{50}s for paclitaxel or cisplatin in HeLa cells (Fig. 3B and Table 1). To further validate the role of Sab in chemo-sensitivity, we treated cells with either a control shRNA or Sab-specific shRNAs for 72 h (Fig. 3C) and then exposed cells to increasing doses of paclitaxel or cisplatin. Silencing Sab increased the IC_{50}s for both paclitaxel and cisplatin when compared to mock-transfected and control shRNA-treated cells (Fig. 3D and Table 1). To determine if the knockdown

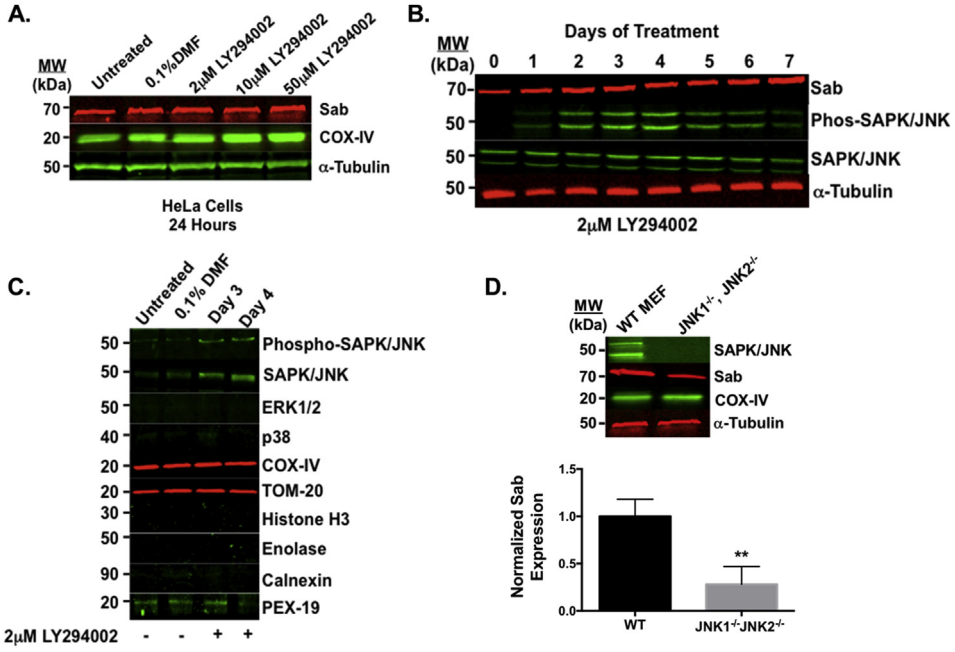

Fig. 2. Sub-chronic, but not acute, LY294002 treatment increases mitochondrial JNK signaling. (A) HeLa cells were exposed to 0.1% DMF, 2 μM, 10 μM or 50 μM LY294002 for 24 h, and cells were lysed and examined for Sab expression. (B) HeLa cells were treated with 0.1% DMF, 2 μM, 10 μM or 50 μM LY294002 for 7 days, and cells were lysed daily and examined for levels of Sab, phosphorylated (active) JNK, and total JNK. (C) Mitochondria were isolated from HeLa cells treated with either DMF or 2 μM LY294002. Isolated mitochondria were lysed and examined for MAPK signaling proteins and sub-cellular contaminants. (D) WT and JNK knockout MEFs were analyzed for Sab expression by western blot (top). Western blots were quantified using the LI-COR Odyssey CLx imager.

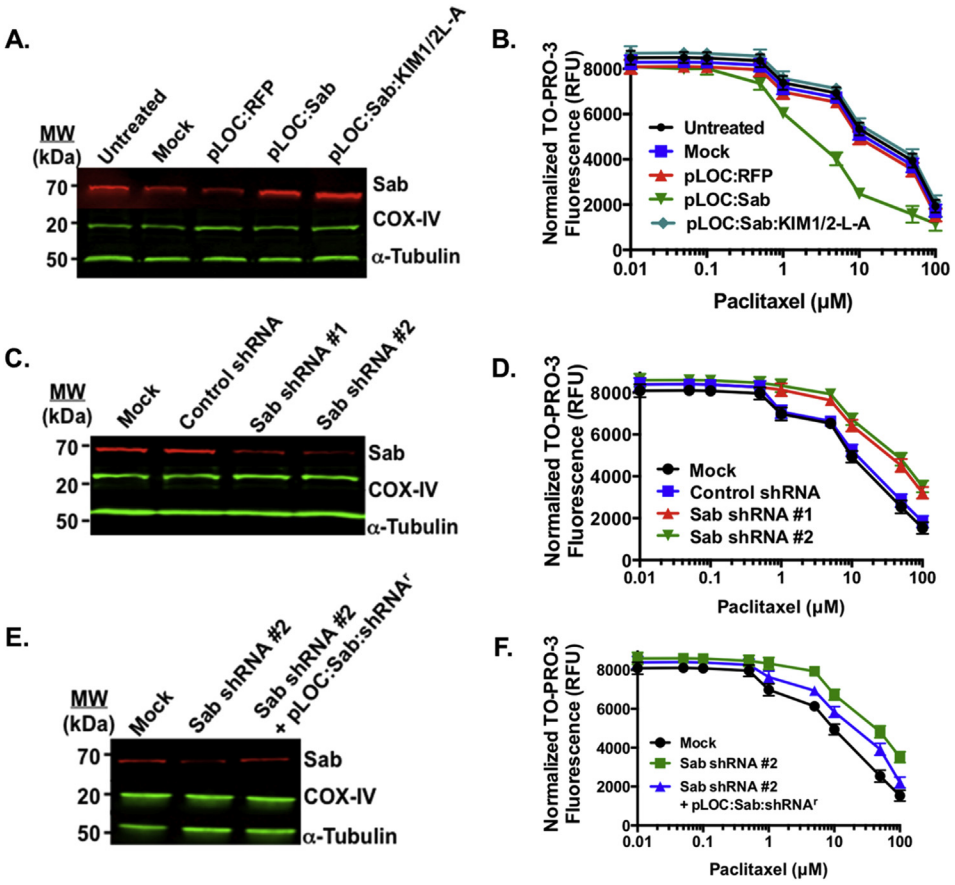

Fig. 3. Over-expression of Sab promotes chemo-sensitivity, while silencing Sab enhances chemo-resistance. (A) HeLa cells were transfected (or mock transfected) with plasmids expressing RFP, Sab, or a MAPK-binding deficient mutant (Sab:KIM1/2L-A). After 72 h expression was monitored by western blot analysis. (B) IC_{50}s were then calculated for paclitaxel and cisplatin. (C) HeLa cells were transfected with plasmids expressing shRNAs designed to silence Sab. Sab expression was assessed by western blot analysis after 72 h of transfection. (D) IC_{50}s again were calculated for paclitaxel and cisplatin. (E) An shRNA-resistant mutant (Sab:shRNA^r) was expressed in HeLa cells to rescue Sab-mediated chemo-sensitization. Cells were transfected 72 h prior to analysis western blot analysis. (F) IC_{50}s were calculated using TO-PRO-3 staining.

of Sab was indeed responsible for the chemo-resistance observed in the previous experiment, we expressed an shRNA-resistant mutant of Sab (pLOC:Sab:shRNA^r) for 72 h (Fig. 3E), and repeated the IC_{50} measurements for both paclitaxel and cisplatin. Fig. 3F demonstrates that recovery of Sab expression yields IC_{50}s of 28 μM and 7.3 nM for paclitaxel and cisplatin, respectively (Table 1). These data taken together demonstrate that the extent of mitochondrial JNK signaling may be a significant determinant for chemo-responsiveness of cancer cells.

4. Discussion

Chemo-resistance is typified by altered mitochondrial physiology that culminates in the inhibition of cell death [2,7]. The ability to restore apoptosis in resistant cancer cells hinges on the ability to re-establish death signaling to mitochondria. In our present study, we propose that this can be accomplished by increasing mitochondrial JNK signaling in cancer cells. To examine how PI3K inhibition may impact cancer cell physiology over a chemotherapy regimen, we employed a sub-chronic low dose treatment of LY294002 (2 μM) over the course of a week. We found that this approach yielded the same impact on chemo-therapeutic efficacy as acute doses of 10 μM and 50 μM LY294002. In the absence of these large doses, we were able to examine cellular changes that may occur during chemo-sensitization. In this context, we found that Sab expression increased during chemo-sensitization; further, mitochondrial JNK levels were elevated during the sub-chronic model (Fig. 2C). Our work has previously shown in HeLa cells that mitochondrial JNK was an essential component for apoptosis; however, prior to this study, changes in Sab concentrations have not been reported within cells. We have demonstrated that low dose sub-chronic cellular stress can lead to an increase in Sab expression. This result is intriguing as it suggests that Sab expression can be modulated by the cellular environment and influence mitochondrial physiology, in the case of this study apoptosis. Likewise, this pathway could be enhanced or inhibited to polarize apoptotic responses in cells or tissues affected by human disease.

Our subsequent analysis demonstrated that JNK was required for mitochondrial JNK signaling, and ablation of the JNK binding sites on Sab reduced the efficacy of chemotherapeutic agents (Fig. 3, Table 1). Contrariwise, silencing Sab expression did not enhance chemo-sensitization, but in fact promoted chemo-resistance. Our recent examination of expression studies performed on gynecological cancers reveal that Sab expression is decreased in late stage and resistant cancers at the mRNA level (data not shown, pending publication). This would suggest that inhibition of mitochondrial JNK signaling may be a mechanism by which gynecological cancers become resistant to chemotherapy and other treatments. We are currently investigating if restoring mitochondrial JNK signaling in chemically resistance gynecological cancers is a useful approach to recover chemo-sensitivity.

Mitochondrial JNK is necessary for sub-chronic chemo-sensitization by LY294002; however, we also discovered that JNK was partly responsible for Sab expression (Fig. 2). This may suggest that under physiological and certain stress conditions JNK signaling amplify mitochondrial dysfunction by inducing Sab expression. Therefore, JNK would create a positive feedback loop and enhance Sab-mediated events on mitochondria. Our previous studies showed that mitochondrial JNK signaling amplified mitochondrial reactive oxygen species generation and inhibited anti-apoptotic functions of Bcl-2 leading to mitochondrial depolarization [17–20]. The production of oxidants and loss of mitochondrial membrane potential have been shown to activate JNK signaling [19,21,28,33–35]. Thus, it is conceivable that nuclear JNK can increase Sab-mediated signaling leading to mitochondrial toxicity. As such, therapeutic strategies that enhance mitochondrial JNK signaling by increasing Sab expression may be highly effective in sustaining cell death in cancer cells. We also acknowledge that a potential side-effect of increasing Sab expression may be elevated toxicity in non-cancerous tissues. Thus, selectively targeting substances capable of inducing Sab expression to cancer cells would be the preferred approach to enhance early apoptotic responses.

In this study, we have described an inducible mechanism to exacerbate apoptotic responses in HeLa cells; further, our data suggests that enhancing mitochondrial JNK signaling is a viable approach to recover apoptotic capacity in chemo-resistant cancer cells. Further, combination chemotherapeutic regimens that couple chemo-sensitizing agents and enhanced mitochondrial JNK signaling may be useful strategies to lower the dose of toxic chemotherapeutic agents and improve treatment outcomes in cancer.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.05.075.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.05.075.

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