Cancer Therapy: Preclinical

Secretory Leukocyte Protease Inhibitor Antagonizes Paclitaxel in Ovarian Cancer Cells

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Abstract

Purpose: Ovarian cancer recurrence with the development of paclitaxel resistance is an obstacle to long-term survival. We showed that secretory leukocyte protease inhibitor (SLPI) is a survival factor for ovarian cancer. We hypothesize that SLPI may antagonize paclitaxel injury.

Experimental Design: Differential SLPI induction in response to paclitaxel and in response to stable forced expression of SLPI was shown in A2780-1A9 cells and their paclitaxel-resistant sublines, PTX10 and PTX22, and confirmed with HEY-A8 cells. SLPI-mediated survival was reduced by the MAP/extracellular signal-regulated kinase (ERK) kinase inhibitor, U0126, and a humanized neutralizing monoclonal anti-SLPI antibody, CR012. OVCAR3 xenographs tested the role of CR012 in vivo.

Results: SLPI expression was lower in A2780-1A9 ovarian cancer cells than in PTX10 and PTX22, and SLPI was induced by paclitaxel exposure. Stable SLPI expression yielded a proliferation advantage ($P = 0.01$); expression of and response to SLPI in OVCAR3 cells were abrogated by exposure to CR012. SLPI reduced the paclitaxel susceptibility of 1A9 and HEY-A8 cells ($P \leq 0.05$), and SLPI expression did not increase the resistance of PTX10 and PTX22 cells. Both paclitaxel and SLPI overexpression induced ERK activation. Inhibition of MAP/ERK kinase with U0126 increased paclitaxel injury and overcame SLPI-mediated cell protection. It did not reinstate PTX10 sensitivity to paclitaxel, which was associated with AKT activation. Significant inhibition of OVCAR3 xenograft growth was observed with CR012 and paclitaxel, over single agents ($P \leq 0.001$).

Conclusions: A two-pronged approach confirmed that SLPI overcomes paclitaxel in part through activation of ERK1/2. These results credential SLPI as a molecular target for ovarian cancer and suggest CR012 as a tool for proof of concept. Clin Cancer Res; 16(2); 600–9. ©2010 AACR.

Ovarian cancer is the most lethal gynecologic malignancy and the fifth most common cause of cancer-related deaths in women. The estimated number of new ovarian cancer cases in 2008 in the United States was 21,650 with an estimated 15,520 deaths (1). The standard treatment for epithelial ovarian cancer consists of cytoreductive surgery followed by a paclitaxel- and platinum-based chemotherapy regimen (2, 3). Paclitaxel resistance develops leading to treatment failure and death. Mechanisms of drug resistance are complex and may differ between cancers; they may be due to altered pharmacokinetics, tumor microenvironment, and/or cancer cell–specific biology and biochemistry (4). Proteins that affect apoptosis, growth factor and cytokine signaling, and cell cycle behavior also have been implicated in drug resistance. Signaling proteins with suggested roles in paclitaxel resistance include p53, cyclins, mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinase (MEK/ERK), and AKT. We sought to examine the role of a novel ovarian cancer growth and survival factor, secretory leukocyte protease inhibitor (SLPI; refs. 5–11), in the paclitaxel resistance of ovarian cancer cells.

SLPI is an 11.7-kDa whey acidic protein that is both genomically and transcriptionally upregulated in ovarian cancer at the 20q12-13 whey acidic protein locus (12), and many breast cancers (13–16). It is secreted by the mucosal surfaces of the respiratory, gastrointestinal, and reproductive tracts. Its most well-known activity is related to its alarm antiprotease (17, 18) and anti-inflammatory properties protecting normal mucosal tissues from the degradative actions of serine proteases, such as elastase, trypsin, and chymotrypsin (19–23). SLPI has an important function in carcinogenesis (7) and has been associated with aggressive and malignant ovarian tumors (15). We have recently described SLPI as a growth and survival factor for ovarian cancer cells in a manner not dependent on its antiprotease activity (10, 11).
Translational Relevance

Epithelial ovarian cancer presents in late stage, and nearly all late-stage patients will suffer disease recurrence death. Molecular therapeutics requires credentialed and validated targets. Secretory leukocyte protease inhibitor, SLPI, is an alarm antiprotease genomically upregulated in a large proportion of ovarian cancer. We have shown previously that SLPI functions as a prosurvival factor, stimulates growth, inhibits apoptosis, and may augment dissemination of disease. We hypothesized that the survival function of SLPI was responsible in part for growth and loss of susceptibility to paclitaxel, a mainstay of ovarian cancer treatment. Our findings indicate that SLPI is upregulated in response to paclitaxel; SLPI forced expression reduces the effectiveness of paclitaxel in an extracellular signal-regulated kinase–mediated fashion. A humanized neutralizing monoclonal anti-SLPI antibody, CR012, confirmed these findings and augmented the antitumor effect of paclitaxel in ovarian cancer xenografts. These findings credential SLPI as an ovarian cancer molecular target, suggesting CR012 as a mechanism for clinical proof of concept.

Materials and Methods

Cells, culture, transfection. OVCAR3, OVCAR4, OVCAR8, IGROV1, and SKOV3 human ovarian cancer cells were obtained from American Type Culture Collection. A19-A2780 human ovarian cancer parental cell line and its two paclitaxel-resistant sublines, PTX10 and PTX22, were generous gifts from Dr. A. Fojo (National Cancer Institute, Bethesda, MD; ref. 27). The HEYA-8 cells were from Dr. G. Mills (MD Anderson, Houston, TX). The IC50 of paclitaxel in PTX10 and PTX22 cells was confirmed at 47 and 48 nmol/L, respectively, compared with 2 nmol/L for their parental 1A9 line. A2780 cell lines were grown in serum and 25 mmol/L HEPES-supplemented RPMI, and OVCAR3, OVCAR 4, OVCAR 8, IGROV1, and SKOV3 cells were grown in serum-supplemented DMEM, unless otherwise indicated. PTX10 and PTX22 cells were maintained in added 15 ng/mL paclitaxel and 5 μg/mL verapamil with drug removal 5 to 7 d before the start of an experiment. 3′ HA-tagged SLPI plasmid was generated and transfected as described in ref. (11). Cells were propagated in bulk and maintained in a medium containing G418 sulfate 1 mg/mL except for the passage immediately before experimental use. Cells from at least two separate independent transfection series were studied. Stable expression was monitored by immunoblot against the HA-tag. Reduced protease inhibitory activity of the clones has been reported (11). Treatment doses and durations of paclitaxel and U0126 (EMD), controlled with DMSO (±0.01%), and/or rhSLPI (R&D Systems) are indicated. SLPI concentration was measured using the Quantikine Human SLPI Immunoassay kit (R&D Systems) per manufacturer's instructions after 30× concentration.

Human monoclonal anti-SLPI. The human IgG2–bearing XenoMouse strain was immunized twice weekly by footpad injection with soluble human SLPI (Abgenix). Antigen-specific antibodies were selected from up to 2.5 million antibody-producing B cells per immunized mouse. Then, B cells producing antibodies were recovered as described in ref. (28) after applying a microplate-based assay to measure and rank antibodies according to their binding affinity to hSLPI and the measurement of their trypsin-neutralizing activity.

Western blot and immunoprecipitation. Total cell lysates were prepared with modified radioimmunoprecipitation assay buffer and subjected to immunoprecipitation as described in ref. (10). Conditioned medium (CM) was collected, centrifuged to remove cellular debris, aliquoted and frozen at −80°C after the addition of protease inhibitors (10 μg/mL aprotinin, 10 μg/mL leupeptin, and 1 mmol/L phenylmethylsulfonyl fluoride), and, where indicated, were concentrated using centrifugal filtration. Lysates and CM underwent no more than one freeze/thaw cycle. Protein samples were resolved by SDS-PAGE electrophoresis and were immunoblotted as described. SLPI rabbit antipeptide antibodies have been reported previously (10). ERK, phospho (p)-ERK, Akt, and p-Akt antibodies were from Cell Signaling, and p-p38 was from Santa Cruz.

hypothesized that the progrowth and survival behavior of SLPI would provide protection against paclitaxel-mediated cell injury.

The Ras/Raf/MAPK pathway couples signals from cell surface receptors to transcription factors, regulating gene expression and cellular activity. This cascade triggers or induces many proteins involved in proliferation, differentiation, and apoptosis (24). ERK1/2 are serine/threonine kinases that are activated upon phosphorylation by MEK1/2. Paclitaxel has been shown to transiently activate ERK and AKT in CaOV3 ovarian cancer cells (25). More activated ERK1/2 was shown in paclitaxel-resistant hematopoietic cells (26). These observations suggest that ERK activation enhances the survival of paclitaxel-exposed cells. We further hypothesized that the survival function of SLPI would yield protection against paclitaxel through activation of the ERK pathway.

We report that SLPI is upregulated in human ovarian cancer cells upon exposure to paclitaxel. Furthermore, overexpression of SLPI decreases susceptibility of A2780 and HEY-A8 human ovarian cancer cells to paclitaxel in a MEK/ERK-dependent fashion. Conversely, exposure of OVCAR3 human ovarian cancer xenografts to neutralizing anti-SLPI antibody increased susceptibility to paclitaxel. These results indicate that high levels of endogenous SLPI and further SLPI induction by paclitaxel are associated with paclitaxel resistance, and further credentials SLPI as a molecular target in ovarian cancer.
Biotechnology. The HA-tag antibody was from Sigma Aldrich. Blots were replicated at least thrice.

**Elastase assay.** EnzChek Protease Assay kit (Molecular Probes) was used to measure elastase and trypsin activity. Briefly, 30 nmol/L elastase (Calbiochem) were incubated with or without recombinant 170 nmol/L SLPI, with or without CR012 (0-240 nmol/L), in 100 μL of 1x digestion buffer [10 mmol/L Tris-HCl (pH 7.8), 0.1 mmol/L sodium azide]. The substrate BODIPY casein was then added and samples were incubated for 1 h, and were protected from light according to the manufacturer’s instructions. Fluorescence as a measure of cleavage was read at excitation of 480 ± 25 nm and emission of 530 ± 25 nm.

**Flow cytometry.** Flow cytometry was done using standard procedure without permeabilization on cells harvested with buffered EDTA and with permeabilization for cell cycle analysis with propidium iodide. Cells were stained for 1 h with 240 nmol/L CR012 or IgG2 isotype and were counterstained with peroxidase-conjugated donkey anti-human IgG (1:500; H+L; Jackson Immunolabs). After 30 min, cells were washed with fluorescence-activated cell sorting buffer and fixed with 1% formaldehyde in PBS. Analysis was done using a FACs Calibur flow cytometer (Becton Dickinson).

**Proliferation and clonogenic assays.** Three proliferation assays were done—2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT; Roche Diagnostics), trypan blue exclusion, and clonogenic assays. Trypan blue-excluding cell number was determined at successive 24-h time points. For XTT assays, cells were pulsed with paclitaxel for 6 h and cellular proliferation was assessed 96 h later. XTT reagent was added for 6 h before the quantification of absorbance. Note that the susceptibility to paclitaxel with this pulse approach is different between PTX10 and PTX22 cells. Cells were pretreated with the ERK inhibitor UO126 for 1 h before paclitaxel treatment; drugs were maintained through the assay. OVCAR3, OVCAR4, OVCAR8, IGROV1, and SKOV3 cells were used for the CR012 clonogenic assays. Recombinant SLPI 170 nmol/L was added to a serum-containing medium 24 h after plating, concomitant with CR012 or control IgG. Cells were harvested with trypsin, mixed 1:1 with growth medium and then an aliquot transferred to a culture dish for 7 days. Colonies were stained with crystal violet solution. The data were presented as percentage of untreated control; data represent mean of three separate studies.

**Confocal fluorescence microscopy.** Cells were grown overnight on 0.1% gelatin-coated slides, serum starved for 24 or 48 h, fixed in 3.7% formaldehyde, and permeabilized with 0.1% Triton X-100. Coverslips were stained with anti-SLPI antibodies and mounted with Vectashield mounting medium containing 4’,6-diamidino-2-phenylindole (Vector Laboratories). Immunofluorescent images were acquired on a Zeiss 510 LSM confocal microscope at 63×/1.4 numerical aperture with an oil differential interference contrast microscopy objective and a scan zoom of 1.

**Immunohistochemistry.** Formalin-fixed, paraffin-embedded mouse xenograft specimens (5-μm sections) were stained with CR012 or IgG2 isotype control after standard rehydration in graded ethanol and quenching of endogenous peroxides. Stain was developed using 3,3′-diaminobenzidine reagent, counterstained with hematoxylin, and dehydrated through alcohol dilution series and xylene prior to coverslip.

**Xenograft studies.** OVCAR3 tumors were passaged in female CB.17 severe combined immunodeficient mice at an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-certified facility (Piedmont Research Center, Morrisville, NC). Mice (n = 40) were implanted s.c. with 20- to 30-mg fragments of OVCAR3 tumor xenografts. Mice were randomized into four groups (from 31 mice with tumors) when the tumors reached 80 to 120 mm³. IgG2 control and CR012 (1 mg/kg) were given i.v. once every 4 d for four treatments; paclitaxel (7.5 mg/kg) was given i.v. every other day for a total of five treatments. Tumor length and width by caliper, and mouse body weight were measured twice weekly. Tumor volume (TV) was calculated as: 

\[ \text{TV} = \text{[(length) x (width)²}/2 \text{]} \]

Median tumor volumes were plotted as a function of days. Mice were euthanized when tumors reached ≥750 mm³. Tumor growth delay was calculated as increase in time-to-end point (TTE) expressed in days calculated as TTE (days) = [log₁₀ (end point volume) – b] / m, where b is the intercept and m is the slope of the line obtained by linear regression of a log-transformed tumor growth data set.

**Statistical analyses.** Experimental differences were tested for statistical significance using ANOVA and Student’s t-test. A two-sided P value of <0.05 was considered to be statistically significant.

**Results**

**SLPI increases cell proliferation.** SLPI is expressed in most ovarian cancer cells of the NCI-60 cell line screen by using the U133A expression array (Supplementary Fig. S1). We previously reported SLPI expression, proliferative, and survival activity in OVCAR3, HEYA-8, and SKOV3 ovarian cancer cell lines (10, 11); we now include the A2780-1A9 line and its paclitaxel-resistance sublines, PTX10 and PTX22. Forced expression of HA-tagged SLPI in the 1A9 and PTX10 lines resulted in a small but significant increase in proliferation (P ≤ 0.05; Fig. 1A). Cell cycle analysis confirmed increased cycling cells with a 50% increase in S-phase fraction for both 1A9- and PTX10-SLPI compared against controls (P < 0.01 and 0.03, respectively). CR012, a mouse monoclonal-neutralizing anti-SLPI antibody, recognized SLPI in OVCAR3 cell lysates and on cell membranes by flow cytometry (Fig. 1B). SLPI has been shown by our group and by others to inhibit serine protease activity. The neutralizing activity of CR012 was confirmed by demonstrating that CR012 plus SLPI abrogated the protease inhibition of SLPI against elastase (Fig. 1C). Antiproliferative activity of CR012 against the OVCAR3 cells was also demonstrated alone against endogenous SLPI and also...
when recombinant SLPI was included in the culture (Fig. 1D). SLPI expression was examined by immunohistochemistry in cell lines, and the qualitative expression was graded as ++, +, or − as high, medium, or no expression, respectively. The response of OVCAR3, OVCAR4, OVCAR8, IGROV1, and SKOV3 cells to CR012 was related to SLPI expression (Supplementary Table S1). High-SLPI-expressing OVCAR3 cells were most sensitive to CR012 (IC50,
90 nmol/L; maximum cell kill, 78%), whereas a SLPI-nonexpressing SKOV3 line was insensitive.

**Paclitaxel-resistant cells have increased basal expression of SLPI protein.** We hypothesized that SLPI would function as a survival factor against paclitaxel treatment, implying also that SLPI would be upregulated in paclitaxel-resistant cells. The paclitaxel-resistant sublines, PTX10 and PTX22, produce and secrete more SLPI in their CM than their 1A9 parental counterparts ($P \leq 0.01$; Fig. 2A). These results were reinforced by confocal microscopy, showing increased SLPI expression in PTX10 compared with 1A9 cells (Fig. 2B). We next examined whether short-term paclitaxel exposure induced SLPI. Secreted SLPI was induced by paclitaxel exposure in both 1A9 and PTX10 cells ($P \leq 0.001$; Fig. 2C). Immunoblot confirms changes in secreted SLPI with dose and time of exposure to paclitaxel. Prolonged paclitaxel exposure at either dose could not be done with the 1A9 cells due to profound cell loss. (Fig. 2D). These data show that SLPI is upregulated in response to treatment with or resistant to paclitaxel.

**SLPI overexpression confers paclitaxel resistance to wild-type but not paclitaxel-resistant cells.** The effects of forced SLPI overexpression on paclitaxel susceptibility in 1A9 and PTX10/22 cells was examined. SLPI-transfected 1A9 were statistically significantly less sensitive to a 6-hours paclitaxel pulse ($P = 0.05$; Fig. 3A), with 78% versus 36% survival at 20 nmol/L paclitaxel. This was tested in HeyA8 cells confirming increased paclitaxel resistance in SLPI-HeyA8 transfectants ($P = 0.009$; Fig. 3B). However, no effect was observed in SLPI-PTX10 cells (Fig. 3C). A paradoxical increased sensitivity was seen in SLPI-PTX22 cells ($P = 0.03$; Fig. 3D). PTX10 and PTX22 cells have a higher basal expression of SLPI, and further upregulation

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**Fig. 2.** Paclitaxel exposure and resistance stimulate SLPI in ovarian cancer cells. SLPI is upregulated in paclitaxel-resistant cells. A increased SLPI production and secretion in lysates (immunoblot) and CM (ELISA) in samples collected after 24 h of serum starvation. Immunofluorescence (B) confirms upregulation and similar cellular localization. C and D, exposure to paclitaxel stimulates cellular SLPI (D, immunoblot) and CM (E, ELISA).

PTX10 cells were exposed to 50 or 500 nmol/L paclitaxel over 24 and 48 h; 1A9 cells were only treated for 24 h. CM SLPI concentration is expressed as pg/10^6 cells to normalize for cell number. Representative data of at least three independent experiments are shown. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
of this protein did not confer additional protection from paclitaxel, suggesting a threshold effect.

**SLPI and paclitaxel treatment upregulate ERK activation.** Next, the A2780 cell series was pulsed with higher doses of paclitaxel for up to 4 hours and activation of ERK assessed. A transient activation of ERK was observed in the 1A9 cells as early as 10 min into exposure with a biphasic effect seen in resistant cells, concomitant with the known behavior of ERK with paclitaxel resistance (Fig. 4A). No clear activation of p38 was seen and no effect was observed on c-Jun-NH2-kinase (data not shown). In contrast, increased activation of AKT was seen only in the paclitaxel-resistant cells (Fig. 4B). OVCAR3 cells had upregulation of both pERK and pAKT upon SLPI treatment, which was reversed by coexposure to CR012 (Fig. 4C). CR012 treatment also reduced activation of pAKT, induced in the OVCAR3 cells exposed to SLPI. SLPI overexpression was not associated with AKT activation in the A2780 cell set.

**Treatment with the MAPK inhibitor, UO126, reverses paclitaxel resistance caused by SLPI overexpression.** In the previous experiments, we showed that SLPI overexpression conferred paclitaxel resistance and ERK activation in 1A9 cells. This suggested that MEK inhibition would overcome SLPI effects. We examined this with the MAPK inhibitor, UO126 (Fig. 5). Cells were pretreated with 10 μmol/L UO126 before paclitaxel treatment and were continuously maintained during the assay duration. UO126 completely reversed SLPI-induced paclitaxel resistance in 1A9 SLPI.HA cells collapsing the 1A9-Neo and 1A9-SLPI growth curves below the basal 1A9 phenotype (P = 0.02; Fig. 5A). Immunoblot showed the loss of pERK in these UO126-treated cells (Fig. 5B). No significant changes were noted in PTX10 Neo and SLPI.HA cells exposed to UO126, suggesting that resistance in these cells is not dependent on the ERK pathway, although ERK activation is reduced (Fig. 5C and D).

**Downregulation of SLPI with CR012 augments paclitaxel effects.** OVCAR3 tumor fragments were xenografted as described. Treatment was initiated when tumors were on average 100 mm³, and mice were euthanized when tumors reached ∼750 mm³. Human SLPI produced by the xenografts was recognized by CR012 as shown by immunohistochemical staining in control IgG2-treated OVCAR3 xenografts (Fig. 6A). Tumor growth was monitored over time and median tumor volumes were plotted (Fig. 6B). Time to end for all treatments was compared against the IgG2 control and paclitaxel alone against CR012+paclitaxel. TTE for IgG2, paclitaxel, CR012, and paclitaxel+CR012 were 23, 39, 28, and 68 days, respectively; an ∼3-fold

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**Fig. 3.** Overexpression of SLPI confers paclitaxel resistance to wild-type but not to paclitaxel-resistant cells. Cells were pulsed with paclitaxel at 0 to 70 nmol/L (A and B) or 1,000 nmol/L (C and D) for 6 h and then allowed to grow for 4 d. A, 1A9; B, HeyA8; C, PTX10; D, PTX22. Representative data of at least three independent experiments are shown. P values represent the statistical significance for the entire curve.
Improvement in TTE was observed for CR012 + paclitaxel compared with IgG2 treatment. Single-agent paclitaxel afforded a 16-day improvement \( (P < 0.05) \) and the combination 45 days \( (P < 0.0001) \) over IgG2 alone. CR012 + paclitaxel resulted in a significant increase in TTE over paclitaxel alone of 29 days \( (P < 0.001) \). Limited weight loss (7-11%) was recorded in paclitaxel-treated groups and no treatment-related deaths were observed (data not shown). Thus, CR012 with paclitaxel delays recurrent tumor growth with the treatment schedule applied.

**Discussion**

SLPI is a serine protease inhibitor overexpressed in epithelial ovarian cancers and shown to be associated with aggressive and malignant phenotypes (15). In certain environments, SLPI functions as both a growth factor and a survival factor (10). We hypothesized that the prosurvival function of SLPI play a role in paclitaxel resistance in ovarian cancer cells. We showed that paclitaxel-resistant cells have a higher baseline expression of SLPI and that exposure to paclitaxel in wild-type cells upregulated SLPI. Forced expression of SLPI resulted in a reduced sensitivity to paclitaxel that was overcome by blockade of MEK activity. However, this was not observed in the paclitaxel-resistant cells, PTX10. These cells have a higher basal SLPI expression and further upregulation of SLPI did not result in further paclitaxel resistance. CR012, a humanized neutralizing anti-SLPI antibody, recognizes human SLPI produced in OVCAR3 xenografts and blocked the protease inhibitory activity of SLPI and reduced growth *in vitro*. Addition of CR012 to paclitaxel prolonged time to target tumor size \( \sim 3 \)-fold in xenografts. These data show the survival and proliferative activity of SLPI and further credentials SLPI as a molecular target for ovarian cancer therapy.

We have shown a prosurvival and confirmed here a pro-growth activity of SLPI. We have previously shown that this is independent of the protease inhibitory activity of SLPI (10, 11). SLPI has been characterized as an alarm antiprotease, a protease inhibitor–induced under stress conditions that may include exposure to pharmacotoxins, such as paclitaxel (17). This concept was confirmed when exposure to the microtubule-stabilizing agent paclitaxel induced SLPI expression and secretion, and was supported by the finding that paclitaxel-resistant cells had increased basal SLPI concentration. That SLPI would antagonize paclitaxel to promote survival in the wild-type cells was a logical extension of these findings. Demonstration that the neutralizing anti-huSLPI antibody was more than additive with paclitaxel also suggests that both may signal through common pathways, as we showed.

Paclitaxel is a mainstay of treatment for ovarian cancer and other cancers, such as lung cancer (29, 30), both of which highly express SLPI (7, 12, 16). Paclitaxel treatment causes the activation of numerous signal events, including activation of MEK resulting in phosphorylation of ERK.
Prolonged activation of ERK is proposed to be at least partially responsible for paclitaxel resistance (26). This role for phosphorylated ERK is controversial. Paradoxically, we observed that paclitaxel-resistant PTX10 cells had lower pERK expression compared with the parental 1A9 cells. ERK activation may serve as a prosurvival signal in our model, as evidenced by the initial upregulation of pERK seen in the first few hours of paclitaxel exposure and in the secondary induction later in the time course in the resistant cells. SLPI also contributed to the activation of ERK, which may have strengthened the survival signal and thus led to paclitaxel resistance. This would explain why initial upregulation of pERK induced by SLPI causes paclitaxel resistance in the 1A9 cells, but when cells have a persistent paclitaxel-resistant phenotype (i.e., PTX10 cells), overexpression of SLPI and upregulation of pERK has no added effect on chemoresistance. However, other studies have shown that the progressive loss of ERK activation can lead to the emergence of chemoresistance (32). This dichotomous finding may be interpreted as a model or selective cancer-specific event.

The development of MEK/ERK small-molecule inhibitors opens new directions for combination therapy approaches (33–35). Studies have shown that the use of ERK inhibitors enhances the cytotoxic effects of chemotherapeutic drugs (35, 36). We confirm that SLPI expression results in paclitaxel resistance in 1A9 and HeyA8 cells and also causes activation of ERK, which is reversed by the MAPK inhibitor, UO126. When 1A9 SLPI.HA cells are treated with UO126 before the treatment with paclitaxel, the paclitaxel resistance conferred by overexpression of SLPI is reversed. This does not hold true for the PTX10 cells, possibly because these cells have lost dependence on pERK and thus are not as susceptible to the effects of UO126. No effect on paclitaxel susceptibility was observed when inhibitors of c-Jun-NH2-kinase or p38 MAPKs were used (p38 inhibitor SB220025, or c-Jun-NH2-kinase inhibitor SP600125; data not shown). A direction for later clinical consideration is the combination of CR012 with a MEK inhibitor, affecting two potentially important and interacting targets for ovarian cancer.

We can also suggest that protection through the induction of SLPI may occur in response to alarms other than paclitaxel exposure, those that are more difficult to model in vitro. These events may be simple tumor presence, tumor injury by hypoxia or agents, or in response to local microenvironmental cues such as activated stroma. Furthermore, SLPI production by tumor may result in other survival effects, those that may be protective from paclitaxel or other chemotherapy injury. We reported protection/induction of the partner protein of SLPI, progranulin, with increased SLPI presence (11). Progranulin has survival activity for ovarian cancer; its ability to protect from paclitaxel is unknown. It has been suggested that the alarm antiproteases such as SLPI may also be part of the adaptive immune response. SLPI may be synthesized and secreted by cells local to the site of alarm, such as inflammation or in response to tissue injury (37, 38). Thus, it is...
possible that in an applied clinical approach, reduction of SLPI by CR012 may shift the behavior of the local inflammatory response and increase effectiveness of anticancer agents. Although these postulates will not explain a direct effect of SLPI against an M phase–specific regulator, such as paclitaxel, they do suggest further directions for preclinical and clinical investigation to support credentialing of SLPI for future clinical targeting.

Disclosure of Potential Conflicts of Interest

W. LaRochelle, consultant for CuraGen. No other potential conflicts of interest were disclosed.

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