The alarm anti-protease, secretory leukocyte protease inhibitor, is a proliferation and survival factor for ovarian cancer cells

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Alarm anti-proteases are secreted locally in response to inflammation and have been shown to be elevated in cancers. Secretory leukocyte protease inhibitor (SLPI), an alarm anti-protease, is amplified in ovarian carcinoma and is induced and binds to and protects progranulin (prgn) in inflammation. We reported prgn is a survival protein in ovarian cancer and now hypothesize that SLPI/prgn would promote proliferation and survival. Neutralizing anti-SLPI antibody treatment of HEY-A8 and OVCAR3 ovarian cancer cells decreased cell number ($P < 0.001$), induced apoptosis and reduced prgn quantity. This was confirmed using SLPI small interfering RNA. Prgn and SLPI were co-immunoprecipitated and co-localized by confocal microscopy. Prgn is a substrate of the serine protease elastase and SLPI is an inhibitor of elastase. Elastase reduced prgn expression, inhibited proliferation in a dose-dependent manner ($P \leq 0.01$) and was pro-apoptotic. SLPI protected prgn from elastase-mediated degradation and restored its survival and proliferative function ($P < 0.04$). SLPI also reversed elastase’s pro-apoptotic effects ($P \leq 0.03$), yielding recovery of S-phase fraction ($P < 0.001$) and increased cyclin D1. Treatment with a general serine protease inhibitor increased prgn, but did not reverse elastase-mediated prgn loss or apoptosis. These data demonstrate that inappropriate over-expression of the alarm anti-protease, SLPI, creates a pro-survival milieu for ovarian cancer.

Ovarian cancer is the fifth most common cause of death from cancer in women (7). SLPI has been described as amplified and over-expressed in ovarian carcinoma (1.8–11). Its messenger RNA and protein have been reported up-regulated in ovarian tumors compared with normal surface epithelium (12). SLPI and other whey acidic protein alarm anti-proteases, such as elafin and HE-4, have been proposed as putative serum biomarkers for malignant ovarian masses (13–16). HE-4 has been shown to be expressed in ovarian inclusion cysts lined by metaphlastic epithelium and to be expressed highly in serous and endometrioid ovarian cancers (13). Together these data suggest that SLPI, as an alarm anti-protease, may have context-dependent function. We hypothesize that SLPI has a pro-cancer function in ovarian cancer, independent of its anti-protease activity.

We identified progranulin (prgn), a 68 kDa protein with multiple polyglycosylated higher molecular weight isoforms, to be up-regulated in invasive serous ovarian carcinoma (17–19). It was present selectively in stage III epithelial ovarian cancer compared with serous borderline tumor. Blocking prgn expression by transfection of OVCAR3 human ovarian cancer cells with anti-sense prgn reduced monolayer and density-independent cell growth (19) and caused apoptosis (20). Prgn has also been shown to be induced in fibroblasts and endothelial cells after injury to promote proliferation, migration and formation of capillary-like structures, indicating a role for prgn in angiogenesis (21). It was also shown to be present in activated blood vessels in ovarian cancers, co-localizing with perlecain (22). The results indicate a role for prgn in growth and survival of ovarian cancer. Zhu et al. (3) describes a balanced interaction between prgn and SLPI necessary for wound healing, where SLPI, prgn and elastase elaborated by activated neutrophils come together to regulate the pro-inflammatory microenvironment. That both SLPI and prgn are over-expressed in ovarian cancer led us to propose that these two proteins may function to support the tumorigenic and malignant activity of this cancer. We now report SLPI promotes proliferation and survival of ovarian cancer cells. It furthers their survival through partnering with and protection of prgn.

Introduction

Anti-proteases are produced and secreted to function as systemic monitors, or acute alarm responders, increasing in response to local cytokine production (1,2). They have been described as being altered in wound-healing and metastatic cancer. SLPI over-expression in Lewis lung cancer cells demonstrated to be a pro-inflammatory protein, in response to and inducing cytokine production and local inflammation (3,4). Alarm anti-proteases have also been described as increased in a fashion not directly related to anti-protease activity in malignancy and metastasis (1). SLPI over-expression in Lewis lung cancer cells was shown to promote tumorigenic and metastatic potential in a syngeneic mouse model; this activity was dependent upon its anti-protease activity (5). However, SLPI was shown also to reduce the production of pro-inflammatory cytokines, E-selectin and TNF-α, tumor necrosis factor-α; XTT, 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide, yielding recovery of S-phase fraction ($P < 0.001$) and increased cyclin D1. Treatment with a general serine protease inhibitor increased prgn, but did not reverse elastase-mediated prgn loss or apoptosis. These data demonstrate that inappropriate over-expression of the alarm anti-protease, SLPI, creates a pro-survival milieu for ovarian cancer.

Materials and methods

Reagents

Recombinant human SLPI (rhSLPI) and goat anti-rhSLPI antibody were purchased from R&D Systems (Minneapolis, MN). Human neutrophil elastase was acquired from Calbiochem (La Jolla, CA). ELISA kits measuring SLPI and elastase were from Cell Sciences (Canton, MA). Precast PAGE gels were from Invitrogen (San Diego, CA). XTT (3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium)-bis-(4-methoxy-6-nitro) benzene sulfonic acid hydrate (Cedars Sinai Cancer Center, Los Angeles, CA). All ovarian cancer cell lines were cultured in RPMI 1640 with 10% fetal bovine serum unless otherwise
indicated. Cells were cultured in serum-free medium for at least 6 h, washed and treated with indicated reagents, including neutralizing antibodies, in serum-free medium for 24 h unless otherwise indicated. Sodium acetate 50 mM, NaCl 200 mM (pH 5) and Tris 100 mM, CaCl2 10 mM, 0.1% fetal bovine serum (pH 7.5) were used as vehicle controls for elastase and rhSLPI, respectively. A rabbit polyclonal IgG control was included when cells were treated with peptide-purified anti-peptide antibodies; control IgG and anti-SLPI concentrations added were 30 μg and up to 22 μg, respectively, based on an anti-SLPI concentration of 1.1 μg/μl. Conditioned medium (CM) was clarified by centrifugation and then protease inhibitors (PI: aprotinin 10 μg/ml, leupetin 10 μg/g and phenylmethylsulfonyl fluoride 1 mM) were added. CM was then frozen and thawed used. Where indicated, SLPI concentration in CM was measured using Quantikine Human SLPI Immunoassay kit (R&D Systems) according to the manufacturer’s instructions. Incubations with p-toluene sulfonil-L-arginine methyl ester (TAME) mirrored those for the neutralizing antibodies or elastase.

Gene silencing
SKOV3 cells, expressing the highest amount of SLPI, were selected for these experiments. In some experiments, cells were plated on gelatin-coated circular cover glass slides for later immunofluorescence. Dharmafect 1 transfection reagent was added to a final concentration of 1.0 μl/ml with a non-targeting siRNA control or anti-SLPI siGENOME SMARTpool siRNA at concentrations of 20 nM in antibiotic-free medium. Cells were incubated at 37°C for 96 h and subjected to analysis.

Antibody production, immunoblot and immunoprecipitation
SLPI rabbit anti-peptide antibody was generated, purified and validated as described for anti-prgn antibodies (19,23). Peptide competition confirmed recognition. A rabbit polyclonal anti-SLPI and a dimer of 28 kDa (27). Total cell lysates were prepared using modified RIPA buffer, sonicated and aliquoted for use as reported (20). Proteins and CM underwent no more than one freeze/thaw cycle. Unconcentrated CM (1 ml) or lysate (500 μg) was immunoprecipitated overnight with anti-prgn Ab737 as described (20,23) at 4°C and then captured with protein A/G beads. Beads were washed three times with NP-40 lysis buffer (PI: 20 mM Tris–HCL, pH 8, 137 mM NaCl, 10% glycerol, 1% NP-40, 2 mM ethylenediaminetetraacetic acid) and twice with phosphate-buffered saline + PI. Immunoprecipitates were subjected to reducing gel electrophoresis followed by immunoblot with anti-SLPI. The protein bands of interest were visualized using Super Signal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL). All blots are representative of at least three replicate experiments. Where shown, parallel gels were immunoblotted with antibody to GAPDH as a putative housekeeping protein to show balance for loading.

Fluorescence microscopy
Cells were grown overnight on 0.1% gelatin-coated cover slips, serum starved for 6 h, and then treated with indicated reagents in a serum-free background for up to 24 h or transfected with SLPI or control siRNA as described. Cells were then fixed in 3.7% formaldehyde and permeabilized with 0.1% Triton X-100. Cover slips were stained with anti-prgn and/or anti-SLPI antibodies where indicated and mounted with DAPI stain mounting medium. Immunofluorescence images were acquired on a Zeiss 510 LSM confocal microscope at ×63/1.4 NA with an oil Differential interference contrast-Nomarski objectives and scan zoom of 1. The results presented are representative of at least three independent experiments.

Flow cytometric analyses of apoptosis
Cells were cultured and treated as described for microscopy. Floating and adherent cells were collected, pooled and fixed with 70% ethanol overnight, then RNaseA 1000 U/ml final volume was added. Fixed cells were stained with propidium iodide 50 μg/ml and subjected to flow cytometric analysis using CellQuest (BD Biosciences, San Jose, CA). FlowJo (Tree Star, San Carlos, CA) and ModFit software (Verity Software House, Topsham, ME) were used for cell cycle analysis.

Statistical analysis
Unpaired Student’s t-tests were applied to replicate experiments (Microsoft Excel, Seattle, WA). Two-sided P values are reported.

Results
SLPI protein is essential for ovarian cancer cell growth
SLPI protein was present in concentrations of 2–7 ng/ml in OVCAR3 and SKOV3 cell lysates and CM; concentrations were below the level of detection (<0.5 ng/ml) in HEY-A8 cell lysates and CM. Immunoblot shows undetectable SLPI protein in human ovarian surface epithelium primary culture, minimal presence in HEY-A8 cells and highest in SKOV3 (Figure 1A). The treatment of HEY-A8 and OVCAR3 cells with rhSLPI showed insignificant increase in proliferation (not shown). However, immunoneutralization of SLPI resulted in a net loss of both cell types in a dose-dependent fashion (P < 0.001; Figure 1B). SKOV3 cells, which secrete larger concentrations SLPI, are inhibited by neutralizing antibody but to a lesser extent (20% reduction; P ≤ 0.03; data not shown). This dose–response effect is consistent with the competitive nature of neutralizing antibodies. Anti-SLPI antibody induced nuclear degeneration, blebbing and apoptosis in a 16 h exposure (Figure 1C; blank IgG negative control not included). Apoptosis was confirmed by flow cytometry demonstrating a 10-fold and 2–3-fold increase in DNA fragmentation in HEY-A8 (0.2 versus 2.2% subG0G1 fraction; P = 0.005) and OVCAR3 cells (2 versus 5% subG0G1 fraction; P = 0.02). We have reported previously that treatment with anti-sense prgn and anti-prgn antibody caused decreased proliferation and induced apoptosis, indicating that prgn is a survival factor in ovarian cancer (19,20). Both anti-prgn and anti-SLPI antibodies cause loss of prgn protein, with accumulation of a previously confirmed 50 kDa fragment (Figure 1D). Concomitant exposure to SLPI protects cells from anti-prgn mediated loss of prgn. The mechanism through which SLPI protects prgn from loss due to immunoneutralization with anti-SLPI or anti-prgn is not clear. It may be related to binding site competition between the antibody and either prgn or SLPI.

RNA interference of SLPI reduces ovarian cancer cell survival and prgn quantity
RNAi was used to confirm the role of SLPI on ovarian cancer cell prgn and survival. Figure 2 shows marked reduction of SLPI (red) and prgn (green) in cells exposed to SLPI siRNA with some relocation. Co-localization of SLPI and prgn is overlapping in the control siRNA-treated cells, but altered and reduced in cells treated with SLPI siRNA, also shown by the merge and the higher power insets. The two sets of conditions for each panel show different fields of view from replicate experiments. These cells have reduced quantity and globular organization of SLPI, and appear to have more nuclear disruption and apoptotic changes by DAPI stain. The general co-localization of SLPI and prgn also is reduced in late apoptotic cells (arrow heads). These cells are shown to have initiated DNA fragmentation, nuclear blebbing and chromatin condensation. This confirms SLPI-mediated protection of prgn and cell survival. The specificity of silencing of SLPI to SLPI and prgn were tested by examination of expression of BAG3, a stress co-chaperone protein under investigation in our laboratory (24–26) and of GAPDH (in part as a loading control; Figure 2B). Neither BAG3 nor GAPDH were reduced under conditions in which both SLPI and prgn were diminished.

SLPI and prgn are binding partners
We next asked if both SLPI and prgn interact in ovarian cancer cells and their local microenvironment. A SLPI/prgn complex was shown by co-immunoprecipitation of both lysates and CM from OVCAR3 and SKOV3 cells, those containing the greatest quantity of SLPI (Figure 3A). More SLPI/prgn complex was present in CM consistent with the secretion of both proteins. Binding was confirmed by reverse order immunoprecipitation (data not shown). Co-localization was further demonstrated using fluorescence confocal microscopy (Figure 3B). Prgn and SLPI are both cytosolic, found in the region of the golgi, typical of secreted proteins.

SLPI reverses both anti-proliferation and pro-apoptotic effects of elastase
Prgn has been reported to be susceptible to cleavage by serine proteases such as elastase, which was shown to be necessary for the balance of prgn and SLPI in a wound-healing model (3). The ovarian cancer cell lines produce no detectable elastase measurable by ELISA (data not shown). Therefore, purified elastase was introduced to cultures for 24 h causing a marked and dose-dependent decrease in prgn protein in both HEY-A8 and OVCAR3 cells (Figure 4A, left panel). Addition of SLPI protected prgn from elastase shown both by
immunoblot (Figure 4A, right panel) and confocal imaging (Figure 4B). These data demonstrate the ability of SLPI to protect prgn from serine protease-mediated degradation. The functional consequence of SLPI protection of prgn was investigated next. Figure 4B shows loss of apoptosis in cells exposed to elastase concomitantly with SLPI. This was further measured in a dose-dependent fashion in all three cell lines using an XTT assay ($P \leq 0.01$; Figure 4C). Cell cycle analysis was done to determine whether this was due to the anti-proliferative and/or pro-apoptotic effects of elastase. Treatment with elastase significantly decreased the S-phase fraction in HEY-A8 and OVCAR3 cells (Table I). Addition of SLPI to elastase partially reversed the loss of DNA synthesis, with little proliferative activity of its own.

Fig. 1. Neutralizing anti-SLPI antibody decreases ovarian cancer cell proliferation and prgn production and induces apoptosis. (a) SLPI is present in ovarian cancer cell lines. Primary culture HOSE cells do not produce SLPI, while it is detectable in lysate of malignant cells. Lanes 1: recombinant huSLPI; 2: HOSE; 3: HEY-A8; 4: OVCAR3; 5: SKOV3. (b) Neutralizing polyclonal anti-SLPI antibody inhibits proliferation of Hey-A8 and OVCAR3. Cell viability was measured using the XTT assay for cells starved for 24 h then exposed to control IgG or anti-SLPI antibody for 48 h. Data shown represents the mean ± SEM of at least three independent experiments; $P$ value is comparison to control. (c) Apoptosis is demonstrated by DAPI immunofluorescence. Reduced cell number, nuclear degradation and apoptotic bodies are present when cells were exposed to neutralizing anti-SLPI antibody for 16 h. A shorter time was selected due to cell loss. Inset: apoptotic bodies. (d) Prgn is protected by SLPI. The left panel shows loss of prgn in HEY-A8 cells in the presence of anti-prgn antibody and recovery when SLPI is added. Prgn is lost when the anti-SLPI antibody is used in HEY-A8 cells (right panel). The 50 kDa prgn band is a previously documented prgn fragment (20).

Fig. 2. SLPI siRNA selectively reduces SLPI and prgn protein and ovarian cancer cell survival. (a) siSLPI reduces expression of both SLPI (red) and prgn (green). Control or SLPI siRNA were introduced and cells incubated for 96 h prior to fixation and staining. The right most panel shows the confocal overlay with yellow indicating presence of both proteins. Apoptosis is shown by loss of cells and nuclear changes (DAPI, blue). There is a dissociation of SLPI from prgn seen in apoptotic cells shown by arrow heads. Two independent fields of siRNA-treated cells are shown by arrow heads. (b) Silencing of SLPI does not reduce BAG3 quantity. Cells were exposed to siSLPI as in A, lysed and subjected to immunoblot for SLPI, prgn and BAG3 as indicated. No loss of BAG3 or GAPDH (as loading control) is seen under conditions where SLPI and prgn are markedly reduced.
This effect was further evaluated by assessing cyclin D1 (Figure 4D). In parallel with the other findings, there is a reduction in cyclin D1 expression in elastase-exposed cells that is restored when SLPI is added with elastase. Thus, SLPI reversed both the negative effects of elastase on proliferation and its pro-apoptotic activity.

**SLPI reverses elastase effects through prgn interaction and not by protease inhibition**

SLPI has been shown to bind both prgn and elastase, providing two opposing mechanisms for its protection of prgn (3). TAME, a general serine protease inhibitor, was used to address the protease inhibition function. Exposure of HEY-A8 and OVCAR3 cells to increasing concentrations of TAME under the same experimental conditions in which the elastase or SLPI were included caused small but reproducible increases in prgn in a dose-dependent manner, suggesting the involvement of a cellular serine protease in local prgn regulation (Figure 5A). However, TAME treatment did not protect prgn from elastase-mediated degradation (Figure 5B), strongly suggesting that SLPI protects prgn through a mechanism unrelated to its protease inhibitory activity. TAME also did not protect cells from elastase-mediated inhibition of proliferation although SLPI significantly overcame elastase-mediated injury (Figure 5C). This demonstrates that the

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**Fig. 3.** SLPI and prgn are binding partners. (a) SLPI and prgn co-immunoprecipitate. Both cell lysates and CM were subjected to immunoprecipitation with anti-prgn and probed for SLPI. Similar results were seen with anti-SLPI immunoprecipitation followed by immunoblot with anti-prgn (not shown). (b) Confocal imaging demonstrates cytosolic co-localization of prgn and SLPI.

**Fig. 4.** SLPI protects cells from elastase-mediated apoptosis. (a) Prgn is markedly reduced when cells are exposed to elastase in a dose-dependent fashion. This loss is reversed upon concomitant exposure to SLPI (right panel). (b) Elastase-mediated injury is reversed by SLPI. The left panel confirms the loss and recovery of prgn upon elastase treatment in the absence and presence of SLPI. Elastase apoptotic injury and SLPI-associated protection from injury is seen under the same conditions. (c) SLPI reverses elastase injury in a dose-dependent fashion in HEY-A8 cells. XTT assay is used to quantitate cell injury with elastase and recovery with concomitant SLPI (mean and SEM, n = 3; similar results are shown for OVCAR3 and SKOV3). (d) Cyclin D1 expression is reduced when cells are exposed to elastase and restored with co-treatment with SLPI.
protective role of SLPI on prgn and on cell proliferation is independent of its protease inhibitory activity. Thus, both SLPI and prgn have key roles in ovarian cancer but may synergize to make a locally permissive microenvironment.

**Discussion**

Genomic analysis of ovarian cancer identified amplification of SLPI and the whey acidic protein locus in a large proportion of epithelial ovarian cancers (28,29). Multiple functions of SLPI have been reported previously, including function as a protease inhibitor and as a regulator of local inflammatory responses downstream of chemokines and tumor necrosis factor-α (TNF-α) (5,30). One mechanism of its anti-inflammatory activity is through inhibition of cleavage of prgn into component pro-inflammatory granulins (3). We identified overexpression of prgn in epithelial ovarian cancer and demonstrated that it is both a proliferation and survival factor for ovarian cancer cell lines (19,20). The findings linking prgn and SLPI in inflammation led to the hypothesis that they would interact in malignancy to promote stabilization of the pro-survival prgn form. We now show that SLPI itself has pro-survival function, that by direct interaction it stabilizes prgn, and that SLPI protects prgn from serine protease-mediated degradation. This interaction makes a more potent anti-apoptotic and pro-growth environment for the ovarian cancer cells. Further, SLPI is an independent pro-survival factor for ovarian cancer, as shown by the stimulation of apoptosis in response to its neutralization and silencing. Thus, these results identify prgn and SLPI as putative molecular therapeutic targets in ovarian cancer.

Zhu et al. (3) proposed a balance necessary between SLPI-prgn and elastase for normal tissue growth. Williams et al. (1) describe SLPI as an alarm anti-protease secreted in response to inflammatory cytokines, such as the interleukins and TNF-α. Ovarian cancer is known to produce large amounts of and a large variety of pro-inflammatory cytokines that also behave as pro-invasive and pro-angiogenic factors (31–33). Thus, we proposed in our ovarian cancer model that there is an imbalance in SLPI caused by the cancer and not the alarm response to local inflammation. SLPI over-expression is caused both by amplification and over-expression (11) as well as its production in response to changes in the local microenvironment (1). The behavior of SLPI in this setting would be expected to be independent of its anti-protease activity. This is confirmed by the pro-survival activity of SLPI by itself and its serine protease-independent protection of prgn. These results suggest that interruption of the prgn/SLPI interaction may be targeted for therapeutic gain without altering its immunomodulatory role.

This theme is further supported by the observation that introduction of SLPI can markedly inhibit apoptosis caused by elastase, also independent of elastase protease activity. Immunoneutralization of SLPI caused apoptosis of the ovarian cancer cells similar to that seen on expression of prgn in epithelial ovarian cancer and demonstrated that it is both a proliferation and survival factor for ovarian cancer cell lines (19,20). The findings linking prgn and SLPI in inflammation led to the hypothesis that they would interact in malignancy to promote stabilization of the pro-survival prgn form. We now show that SLPI itself has pro-survival function, that by direct interaction it stabilizes prgn, and that SLPI protects prgn from serine protease-mediated degradation. This interaction makes a more potent anti-apoptotic and pro-growth environment for the ovarian cancer cells. Further, SLPI is an independent pro-survival factor for ovarian cancer, as shown by the stimulation of apoptosis in response to its neutralization and silencing. Thus, these results identify prgn and SLPI as putative molecular therapeutic targets in ovarian cancer.

**Table I. Elastase alters proliferation and apoptosis in a SLPI-sensitive fashion**

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*Average of three independent experiments.
*Control versus elastase.
*Elastase versus SLPI + elastase.

Fig. 5. The protective effects of SLPI are not caused by its protease inhibitory activity. (a) The general serine protease inhibitor, TAME, increases prgn quantity in a dose-dependent manner in both HEY-A8 and OVCAR3 cells. (b) Immunofluorescence demonstrates protection of prgn only when SLPI is added to elastase. (c) SLPI but not TAME protects cells from elastase-mediated inhibition of proliferation. Elastase versus SLPI + elastase: P = 0.01.
SLPI is a survival factor in ovarian cancer

with elastase and immunoneutralization of prgn (20). One mechanism of this may be the small but dose-dependent increase in prgn observed when cells are incubated with SLPI. It may be due to the protective role of SLPI on prgn, such that loss of SLPI may leave prgn open to cleavage by elastase or other, as yet undefined serine proteinases. The findings with recombinant SLPI also may be limited by the nature of the bacterial recombinant protein (11 kDa) that may not be recognized as physiologically as the endogenously produced glycosylated form (14 kDa). It is possible that there may be differential activity of the modified forms of SLPI; we found more expression of the higher molecular weight SLPI in the HEY-A8 cells which also seemed more susceptible to neutralization of SLPI than would be expected by their relatively lower secreted concentrations. These findings are supported by the increase in prgn quantity in the presence of TAME. SLPI appears to have its pro-malignant effects more through inhibition of cell death than promotion of cell proliferation in ovarian cancer, contrary to reports assessing its role in other cancers (6,8,30,34,35). Preliminary data indicates that SLPI has a limited direct proliferative drive on these ovarian cancer cells in the absence of elastase or neutralizing antibody, despite the marked protective effects observed when the injuring agent is present.

Findings in the literature indicate that the anti-protease-independent role for SLPI in cancer may be either context or cancer dependent. SLPI has been implicated in HIV disease in an anti-protease-independent fashion (36–38). Induction of SLPI in response to inflammatory cytokines has been shown in several systems (39,40). TNF-α, produced by many types of cancer stimulates SLPI production (41). Devoogdt et al. (5) showed previously that TNF-α producing macrophages enhanced resistance to TNF-α mediated lysis and promoted malignancy of 3LL cells associated with up-regulation of SLPI. Further studies have revealed that although induction of SLPI occurred during TNF-α treatment, the promotion of tumor progression by SLPI abrogated tumor advance in a TNF-α-independent fashion (5,30,41,42). Thus, induction of SLPI in the microenvironment whether from tumor or local inflammatory cells can shift the local balance to favor tumor progression.

SLPI and prgn have both been shown to be over-expressed in ovarian cancer. This over-expression is now reduced to function showing that these proteins have proliferative and anti-apoptotic activity independently, but that they work in concert to yield a stronger effect as a partnered complex to promote survival and proliferation of the malignant cells. Interruption of this complex is a logical molecular therapeutic target and one that may also be monitored as a biomarker due to the secreted nature of both proteins.

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