Stress Hormones Regulate Interleukin-6 Expression by Human Ovarian Carcinoma Cells through a Src-dependent Mechanism*

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Recent studies have demonstrated that chronic stress promotes tumor growth, angiogenesis, and metastasis. In ovarian cancer, levels of the pro-angiogenic cytokine, interleukin 6 (IL-6), are known to be elevated in individuals experiencing chronic stress, but the mechanism(s) by which this cytokine is regulated and its role in tumor growth remain under investigation. Here we show that stress hormones such as norepinephrine lead to increased expression of IL-6 mRNA and protein levels in ovarian carcinoma cells. Furthermore, we demonstrate that norepinephrine stimulation activates Src tyrosine kinase and this activation is required for increased IL-6 expression. These results demonstrate that stress hormones activate signaling pathways known to be critical in ovarian tumor progression.

Chronic stress, which results in increased production of catecholamines such as norepinephrine (NE)3 and epinephrine from the adrenal medulla and sympathetic neurons, has long been believed to adversely influence health (1). While elevated levels of such stress mediators are known to be associated with consequences such as increased risk of heart disease and infection (2–4), several studies have indicated that chronic stress can also promote tumor development and progression (5). For example, chronic stress has been associated with an increased incidence of mammary tumors in female mice carrying the Bittner oncogenic virus (6), and experimentally stressed mice exhibited accelerated development of ultraviolet-induced cutaneous tumors compared with non-stressed animals (7, 8). We have recently demonstrated that chronic stress resulted in greater tumor burden and markedly increased vascularization in orthotopic models of ovarian cancer (9). These studies demonstrate that chronic stress influences tumor progression, but to date, the specific underlying mechanisms responsible for this outcome are poorly understood.

A growing number of studies have demonstrated that chronic stress and other behavioral factors have specific effects on the immune system of cancer patients (10). These effects include decreased natural killer (NK) cell cytotoxicity, weakened response of NK cells to recombinant interferon γ, and reduced T-cell response to mitogen stimulation (10, 11). However, additional factors are known to play a major role in tumor growth; therefore, we asked whether stress mediators could directly alter tumor cell production of cytokines known to contribute to tumor progression.

Angiogenesis, the formation of new blood vessels from the preexisting vasculature, is necessary for tumor expansion and ascites formation and is regulated by the equilibrium of proangiogenic and antiangiogenic molecules (12, 13). Ovarian cancer cells have been shown to express β-adrenergic receptors, and stimulation of these cells with catecholamines results in enhanced expression of an important pro-angiogenic factor, vascular endothelial growth factor (9, 14). These findings suggest that stress hormones may promote tumor angiogenesis. Interleukin-6 (IL-6) has been implicated as an important molecule in tumor progression and angiogenesis (15). Elevated levels of IL-6 are frequently detected in the serum of ovarian cancer patients and are associated with a poor prognosis and increased tumor burden (16, 17). IL-6 has been shown to be secreted by ovarian cancer cells and to facilitate tumor cell proliferation (18), migration (19), and chemotherapy resistance (20, 21). The receptor for IL-6 (IL-6R) is expressed on endothelial cells, and stimulation of ovary and mesentery-derived endothelial cells with exogenous IL-6 results in enhanced cell migration in vitro (15). Furthermore, IL-6 is a potent angiogenic cytokine in vivo (15).

Previously, IL-6 has been linked to chronic stress, in that individuals experiencing chronic stress have been shown to exhibit elevated circulating levels of IL-6 (22). Moreover,
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Behavioral factors have been associated with circulating and ascites IL-6 levels in ovarian cancer patients (23). Epinephrine has been shown to increase IL-6 gene expression in adipose tissue (24), and norepinephrine infusion increases IL-6 expression in myocytes (25). However, whether tumor cell expression of IL-6 is influenced by chronic stress is unknown. In the current study, we evaluated the effect of stress-related hormones on IL-6 production by ovarian cancer cells and found that catecholamines enhance IL-6 promoter activity and protein levels. Moreover, the effect of norepinephrine on IL-6 expression is mediated through β-adrenergic receptors and requires the subsequent activation of the Src proto-oncogene product.

EXPERIMENTAL PROCEDURES

Cell Culture—SKOV3.ip1 is a variant derived from SKOV3 cells (ATCC, Manassas, VA) that was established from the ascites fluid of female nude mice following intraperitoneal injection of the parental line (26). The human ovarian cancer cell line Hey-A8 was a kind gift from Dr. Gordon B. Mills (The University of Texas M. D. Anderson Cancer Center, Houston, TX). EG cells have been described previously (14). Cell lines were grown as monolayers in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum, vitamins, sodium pyruvate, L-glutamine, and nonessential amino acids (Invitrogen).

To evaluate the effect of stress hormones on IL-6 secretion, 1.0 × 10⁵ SKOV3.ip1, Hey-A8, and EG cells were seeded into individual wells of a 24-well plate. Following a 24-h incubation, triplicate cultures (wells) were stimulated with 500 μl of media containing norepinephrine, epinephrine, or the synthetic β-adrenergic receptor agonist, isoproterenol (Sigma), at 0-, 0.1-, 1-, and 10-μM concentrations. After 1, 3, and 6 h, medium was collected, centrifuged, and tested for the presence of IL-6 by ELISA (R&D Systems, Minneapolis, MN). The doses of catecholamines used for our studies were selected to reflect physiologic conditions of these hormones at the level of the tumor. While circulating plasma levels of norepinephrine range from 10 to 1000 pm in a normal individual and may reach as high as 100 nm in conditions of stress (27), catecholamine levels in the ovary are at least 100 times higher. Studies suggest that within the parenchyma of the ovary, and thus the tumor microenvironment, concentrations may reach as high as 10 μM (28, 29).

IL-6 mRNA Assay by Real-time Reverse Transcription PCR—Total cellular RNA was isolated (Qiagen RNeasy) and treated with DNase-free DNase (Qiagen), both according to the manufacturer’s protocol. 10% of the resulting RNA was assayed using a one-step real-time reverse transcription PCR protocol (Bio-Rad) with SYBR Green fluorescence intensity data were analyzed using iCycler™ software, with gene induction measured as the change in glyceraldehyde-3-phosphate dehydrogenase-normalized threshold cycle numbers (Ct); fold change = 2^ΔΔCt experimental – Ct control

Western Blot Analysis—Cell lysate was prepared with modified radioimmune precipitation (RIPA) lysis buffer as previously reported (30). Protein concentrations were determined using a BCA protein assay reagent kit (Pierce). 50 μg of whole cell lysate protein was subjected to 15% SDS-PAGE separation and transferred to a nitrocellulose membrane via wet transfer (Bio-Rad Laboratories). Nonspecific sites were blocked with 5% nonfat milk and incubated with IL-6-specific antibody (1:500; R&D Systems, Minneapolis, MN) overnight at 4 °C. Primary antibody was detected utilizing anti-goat IgG (Roche Applied Science) and developed with a chemiluminescence detection kit (PerkinElmer). β-Actin antibody (1:3,000; Sigma) confirmed equal loading.

IL-6 Promoter Activity—SKOV3, SKOV3.ip1, and HEY-A8 cells were transfected with 1 μg of a luciferase reporter construct driving production of firefly luciferase from a 651-bp sequence upstream of the transcription start site for the human IL6 gene. Transfections were carried out using Lipofectamine 2000 reagent (Invitrogen) in OPTI-MEM-reduced serum according to the manufacturer’s protocol. 1 h after transfection, cells were treated with pharmacologic antagonists for 30 min, followed by NE exposure for 90 min. Cells were washed in phosphate-buffered saline, lysed, and assayed for firefly luciferase activity using standard substrate reagents (Promega, Madison, WI) and a Bacterial Systems BG-1 luminometer (GEM Biomedical, Sparks, NV). To determine potential regions of the IL-6 promoter responsible for mediating stress hormone-mediated transcriptional activation, we generated luciferase expression vectors driven by variants of the human IL-6 promoter bearing point mutations in the cAMP-response element (CRE) or C/EBP-β (NFIL6)-response element, as previously described (31).

Receptor Inhibitors and cAMP Agonists—To inhibit activation of β-adrenergic receptors, SKOV3.ip1 and Hey-A8 cells were seeded into 24-well plates as described above. Cells were pretreated with the β2-adrenergic receptor inhibitor, DL-propranolol (Sigma-Aldrich) at a concentration of 1 μM. Following a 1-h incubation, medium was removed and cells were stimulated with 10% fetal bovine serum DMEM containing 10 μM NE with or without 1 μM propranolol for 3 h. Similarly, cells were incubated with the adenylyl cyclase activator forskolin (10 μM) (Sigma Aldrich) or 100 μM of an analogue of cAMP that specifically activates Epac (8-(4-chloro-phenoxythio)-2′,5′-O-methyladenosine-3′,5′-cyclic monophosphate [8CPT-2Me-cAMP]; (Biolog Life Science Institute, Bremen, Germany) for 3 h. Conditioned medium was collected, centrifuged, and tested for the presence of IL-6 by ELISA.

Detection of Src Phosphorylation—SKOV3.ip1 cells (1 × 10⁶) were seeded into 10-cm dishes in 10% fetal bovine serum/DMEM. One day later the cells were incubated in serum-free DMEM in the presence or absence of the Src inhibitor AP23846 (1 μM; Ariad Pharmaceuticals, Cambridge, MA) for 18 h. Cells were then stimulated for 5, 10, 15, or 45 min with serum-free DMEM containing 10 μM NE in the presence or absence of 1 μM...
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RESULTS

Adrenergic Stimulation of Ovarian Cancer Cells Enhances IL-6 Expression—To examine the effect of stress hormones on IL-6 expression in ovarian tumor cell lines, SKOV3.ip1, Hey-A8, and EG cells were stimulated with increasing concentrations of norepinephrine, epinephrine, or isoproterenol, and culture supernatants were assayed for IL-6 by ELISA (Fig. 1, A–C). In all three human ovarian carcinoma cell lines stimulated with isoproterenol or physiological stress levels of norepinephrine or epinephrine, a significant increase in IL-6 secretion resulted. Specifically, stimulation of SKOV3.ip1 cells with norepinephrine resulted in a peak >20-fold increase in IL-6 production at the 3-h time point (p = 0.004). The mean basal level of IL-6 secretion by SKOV3.ip1 cells at 3 h was 6.4 ± 2.25 pg/ml, and after treatment with 1 and 10 μM norepinephrine IL-6 levels increased to 152.38 ± 5.12 and 129.47 ± 8.70, respectively. Similarly, norepinephrine led to a 4-fold increase in IL-6 secretion by EG cells (p = 0.02) and a 120-fold increase in IL-6 secretion by Hey-A8 cells (p < 0.001). Stimulation of human ovarian cancer cell lines with epinephrine also increased IL-6 production. Specifically, Hey-A8 cells treated with 10 μM epinephrine produced 656.06 ± 151.02 pg/ml of IL-6, resulting in a 77-fold increase (p = 0.018) in comparison to controls. Epinephrine induced an ~5-fold increase in IL-6 secretion in both

FIGURE 1. Catecholamines enhance IL-6 secretion by tumor cells. Human ovarian cancer cell lines (SKOV3.ip1, Hey-A8, and EG) were stimulated with 0.1-, 1-, or 10-μM concentrations of norepinephrine (A), epinephrine (B), or isoproterenol (C). Medium was collected after 1 h (closed square), 3 h (open triangle), or 6 h (open oval) and tested for the presence of IL-6 by ELISA. Data are graphed as mean ±fold increase in IL-6 secretion relative to basal level of IL-6 secretion with error bars representing S.D.* p < 0.005. D, SKOV3.ip1 cells were stimulated with norepinephrine 10 μM or isoproterenol 10 μM for 3 h, and protein was obtained from cell lysate for Western blot analysis using an IL-6 antibody. The quantification of band intensity relative to β-actin intensity is shown on the right.

ADDITIONAL TABLES AND FIGURES

In Vivo Tumor Model—Female C.B-17/IcrHsd-Pkdcscid mice were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). The mice were housed and maintained under specific pathogen-free conditions in facilities approved by the American Association for Accreditation of Laboratory Animal Care in accordance with current regulations and standards of the U.S. Department of Agriculture, U.S. Department of Health and Human Services, and the National Institutes of Health. The mice were used according to institutional guidelines when they were 8–12 weeks of age. SKOV3.ip1 tumor cells were harvested from cultures by a brief exposure to 0.25% trypsin and 0.02% EDTA. Trypsinization was neutralized with fetal bovine serum-containing medium. The cells were then washed once in serum-free medium and suspended in serum-free Hanks’ balanced salt solution. Only single-cell suspensions with greater than 95% viability, as determined by Trypan blue exclusion, were used for the injections. To produce tumors, 2 × 10⁶ SKOV3.ip1 cells (in 0.1 ml) were injected subcutaneously into the right flank of each mouse (5 mice/group). Three days after tumor cell injection, mice were treated with daily injections of norepinephrine, epinephrine, or isoproterenol, and tumors were assayed for IL-6 by ELISA (Fig. 1, A–C). In all three human ovarian carcinoma cell lines stimulated with isoproterenol or physiological stress levels of norepinephrine or epinephrine, a significant increase in IL-6 secretion resulted. Specifically, stimulation of SKOV3.ip1 cells with norepinephrine resulted in a peak >20-fold increase in IL-6 production at the 3-h time point (p = 0.004). The mean basal level of IL-6 secretion by SKOV3.ip1 cells at 3 h was 6.4 ± 2.25 pg/ml, and after treatment with 1 and 10 μM norepinephrine IL-6 levels increased to 152.38 ± 5.12 and 129.47 ± 8.70, respectively. Similarly, norepinephrine led to a 4-fold increase in IL-6 secretion by EG cells (p = 0.02) and a 120-fold increase in IL-6 secretion by Hey-A8 cells (p < 0.001). Stimulation of human ovarian cancer cell lines with epinephrine also increased IL-6 production. Specifically, Hey-A8 cells treated with 10 μM epinephrine produced 656.06 ± 151.02 pg/ml of IL-6, resulting in a 77-fold increase (p = 0.018) in comparison to controls. Epinephrine induced an ~5-fold increase in IL-6 secretion in both.
SKOV3.ip1 (p = 0.001) and EG cells (p < 0.001). Moreover, the synthetic β-adrenergic receptor agonist isoproterenol also induced a significant increase in IL-6 secretion. Following treatment of Hey-A8 and SKOV3.ip1 cells, we observed an ~200-fold increase in IL-6 expression (p < 0.004; Fig. 1). Western blot analysis of the SKOV3.ip1 cells treated with norepinephrine or epinephrine confirmed the increases in IL-6 protein observed with ELISA (Fig. 1D).

**Norepinephrine Stimulation Increases IL-6 RNA**—To more clearly define the mechanisms by which catecholamines regulate IL-6 production by ovarian cancer cells, we examined transcriptional changes in IL-6. SKOV3.ip1 cells were stimulated with 10 μM norepinephrine, and IL-6 mRNA levels were quantified using real-time RT-PCR at multiple time points ranging from 3 to 24 h. The level of IL-6 mRNA was normalized against 18 S RNA levels. As shown in Fig. 2, norepinephrine increased IL-6 mRNA by 45-fold at 6 h, and IL-6 mRNA levels returned to baseline within 12 h. Norepinephrine had a similar effect on other ovarian cancer cell lines (data not shown).

**Norepinephrine Increases IL-6 Promoter Activity**—To determine whether increased transcriptional activity of the IL6 promoter was responsible for norepinephrine enhancement of IL6 gene expression, we examined the effects of norepinephrine on a luciferase reporter construct driven by 651 bp of human DNA sequence upstream of the IL6 gene transcription start site (34). When this construct was transfected into SKOV3.ip1 cells, norepinephrine enhanced luciferase production by ~10-fold within 3 h (Fig. 3), suggesting that increased IL-6 mRNA was due to increased transcription rather than increased stability. Similar effects were observed in SKOV3 and Hey-A8 cell lines (data not shown). Pharmacologic stimulation of the cellular cAMP signaling pathway with Bt2cAMP also enhanced activity of the IL6 promoter, suggesting that the effects of norepinephrine might be mediated by the G-protein-linked receptors that activate the adenylyl-cyclase/cAMP system. The ~10-fold induction by catecholamine was similar in magnitude to that induced by the protein kinase C activator phorbol 12-myristate 13-acetate, which functioned as a positive control in these studies.

To examine the effects of norepinephrine at the post-translational level, IL-6 protein levels in SKOV3.ip1 cells were evaluated in the presence of cycloheximide, 1 μg/ml. Norepinephrine had no effect on the degradation of the IL-6 protein (Fig. 3B). These results indicate that norepinephrine increases IL-6 levels by transcriptional activation rather than by affecting protein stability.

**Induction of IL-6 Expression by Stress Hormones Requires Activation of β-Adrenergic Receptors**—Human ovarian carcinoma cell lines express β-1 and β-2 adrenergic receptors that can activate the cAMP signaling pathway through G-protein-
mediated stimulation of adenylyl cyclase (14). To determine whether IL-6 expression could be regulated through \( \beta \)-adrenergic receptors, SKOV3.ip1 and Hey-A8 cells were treated with 1 \( \mu \)M propranolol, a nonspecific \( \beta \)-adrenergic receptor antagonist, for 1 h prior to stimulation with 10 \( \mu \)M norepinephrine or isoproterenol. As shown in Fig. 4A, induction of IL-6 by norepinephrine and isoproterenol was blocked by propranolol. These effects were specific to \( \beta \)-adrenergic receptors, as the adrenergic antagonist prazosin (5 \( \mu \)M) had no effect on the ability of stress hormones to induce IL-6 production (data not shown). Because \( \beta \)-adrenergic receptors are known to signal via cAMP, we stimulated SKOV3.ip1 and Hey-A8 cells with the adenylyl cyclase activator forskolin and found an increase in IL-6 production similar to that induced by norepinephrine (Fig. 4B). Stimulation of SKOV3.ip1 cells with forskolin resulted in a 4-fold increase in IL-6 expression \( (p < 0.001) \), and forskolin induced an ~8-fold increase in Hey-A8 IL-6 production \( (p = 0.034) \). Because stimulation of the \( \beta \)-adrenergic receptors has been shown previously to also lead to activation of Epac (exchange protein directly activated by cAMP) (35, 36), we sought to determine whether activation of Epac could contribute to IL-6 expression in human ovarian carcinoma cells. The SKOV3.ip1 and Hey-A8 cells were treated with 100 \( \mu \)M 8-CPT-2Me-cAMP, a cAMP analog that specifically targets Epac. In both cell lines, 8-CPT-2Me-cAMP did not increase secretion of IL-6 (Fig. 4B).

Induction of IL-6 Expression by Norepinephrine Is Src-dependent—Recent reports indicate a role for the protein tyrosine kinase Src in \( \beta \)-adrenergic receptor signal transduction (37). Based on suggested IL-6 regulation by Src (38, 39), we asked whether the catecholamine-mediated IL-6 production was Src-dependent. Following serum starvation for 18 h, the SKOV3.ip1 cells were incubated with 10 \( \mu \)M norepinephrine in serum-free medium for 1–45 min. Western blot analysis revealed that by 10 min after NE stimulation, Src phosphorylation increased, and the maximum increase in Src phosphorylation at Tyr-418, indicative of increased Src activity, occurred after 45 min (Fig. 5A). Additionally, the Src-selective inhibitor AP23846 (40) completely inhibited norepinephrine-induced Src phosphorylation. To determine whether Src phosphorylation is required for the stimulation of IL-6 production by norepinephrine, SKOV3.ip1 cells were pretreated in control medium or medium containing AP23846 for 18 h and then stimulated for 3 h with medium containing 10 \( \mu \)M norepinephrine in the presence or absence of AP23846. ELISA analysis of IL-6 in conditioned medium was then performed. The results demonstrated that inhibition of Src activity by AP23846 blocked the stimulatory effect of norepinephrine on IL-6 secretion (Fig. 5B). Furthermore, AP23846 inhibited forskolin-induced IL-6 expression in SKOV3.ip1 cells. Similar results were obtained using another Src-selective inhibitor, PP2 (Fig. 5C), and Src-targeted siRNA (Fig. 5D).

Role of Src in NE-mediated Activation of the IL6 Promoter—To determine whether norepinephrine-mediated activation of the IL6 promoter required Src activity, SKOV3 or Hey-A8 cells were treated with AP23846 or vehicle for 30 min prior to luciferase reporter assay. As shown in Fig. 6, pharmacologic inhibition of Src completely abrogated norepinephrine-mediated induction of IL6 promoter activity.

Analysis of IL6 promoter constructs bearing point mutations in transcription factor binding sites showed that NE activation did not require a functional CRE (Fig. 6B). However, mutation of the C/EBP-\( \beta \) (NFIL6) motif completely abrogated NE-mediated induction of the IL6 promoter. This effect was specific to NE-mediated signaling, as the C/EBP-\( \beta \) mutation did not impair IL6 promoter response to the positive control inducer phorbol 12-myristate 13-acetate. These data suggest that \( \beta \)-adrenergic activation of the IL6 promoter via the Src signaling pathway is not mediated by classical CREB response to cAMP/protein kinase A signaling but instead involves Src-dependent activation of C/EBP-\( \beta \).

Role of IL-6 in Isoproterenol-induced In Vivo Tumor Growth—To determine the role of IL-6 in mediating catecholamine-induced tumor growth, we used a neutral liposomal approach (DOPC) for \textit{in vivo} siRNA delivery that we have previously optimized for \textit{in vivo} applications (32, 33). We first identified an IL-6-targeted siRNA sequence that decreased IL-6 levels in the SKOV3.ip1 cells (data not shown). Next, female SCID mice were injected subcutaneously with SKOV3.ip1 cells and treated with 10 \( \mu \)M isoproterenol in serum-free medium for 3 h. As shown in Fig. 7, tumors from mice injected with SKOV3.ip1 cells treated with isoproterenol in the presence of 8-CPT-2Me-cAMP were significantly smaller than tumors from mice injected with SKOV3.ip1 cells treated with isoproterenol alone.
according to the following groups (n = 5/group): (a) control siRNA-DOPC twice weekly + PBS intraperitoneal daily; (b) IL-6 siRNA-DOPC twice weekly + PBS intraperitoneal daily; (c) control siRNA-DOPC twice weekly + isoproterenol intraperitoneal daily; (d) IL-6 siRNA-DOPC twice weekly + isoproterenol intraperitoneal daily. Isoproterenol treatment with control siRNA increased tumor volume by >500% (p < 0.01; Fig. 7). IL-6 siRNA-DOPC reduced tumor growth by 47% in the PBS control group (p = 0.14) and blocked the isoproterenol-induced tumor growth (Fig. 7).

**DISCUSSION**

This study provides direct evidence that chronic stress hormones affect expression of IL-6, a pro-angiogenic cytokine in ovarian carcinoma cells, and do so through a β-adrenergic receptor/Src tyrosine kinase signaling axis. For these studies,
we stimulated cultured ovarian cancer cells with physiologic catecholamine concentrations and found that for all three of the cell lines tested (SKOV3.ip1, Hey-A8, and EG) norepinephrine and epinephrine significantly increased IL-6 production. Moreover, we have demonstrated that incubation of SKOV3.ip1 cells with norepinephrine results in substantial increases in IL-6 RNA and promoter activity, indicating that stress mediators regulate expression of the \textit{IL6} gene at the transcriptional level.

The biological effects of catecholamines are mediated through binding to \( \beta \)-adrenergic receptors on the surface of target cells. This results in activation of adenylyl cyclase, which catalyzes the formation of the second messenger cAMP from ATP. Cyclic AMP binds to and activates protein kinase A, leading to the phosphorylation of downstream molecules (41). In our studies the effect of catecholamines on IL-6 expression was blocked by the addition of \( \beta \)-adrenergic receptor antagonist propranolol but not by the \( \alpha \)-adrenergic receptor inhibitor prazosin. Additionally, by utilizing forskolin as a direct activator of adenylyl cyclase, Br2cAMP as a generalized activator of cAMP effector pathways, and 8CPT-2Me-cAMP as a specific activator of the EPAC pathway, we determined that cAMP induced IL-6 expression through an EPAC-independent pathway. Recent reports have demonstrated that activation of \( \beta \)-adrenergic receptors results in enhanced Src activity (42, 43). Therefore, we evaluated the role of Src in norepinephrine-induced IL-6 expression and observed that norepinephrine stimulation led to an increase in Src phosphorylation. Moreover, the Src inhibitors AP23846 and PP2 blocked the induction of IL-6 expression and activation of the human \textit{IL6} promoter by norepinephrine. These data indicate that stress-related hormones activate \( \beta \)-adrenergic receptors on ovarian cancer cells by increasing the kinase activity of Src, which results in enhanced transcription of the \textit{IL6} promoter and subsequent production of IL-6 protein. Studies evaluating human ovarian cancer clinical specimens by immunohistochemistry have demonstrated that activated Src is overexpressed in the majority of ovarian carcinomas (44) and contributes to angiogenesis in these tumors; however, the mechanism(s) by which Src is activated are unclear. The current study provides a mechanism by which Src may be activated in these tumors, \textit{i.e.} by response to stress hormones through \( \beta \)-adrenergic receptors on tumor cells and enhance expression of IL-6 and vascular endothelial growth factor.

Studies showing that norepinephrine levels increase in preovulatory follicles indicate that catecholamines play a role in the physiology of the normal ovary (28). Tumor cells may usurp these mechanisms to promote disease progression. Epinephrine and norepinephrine have been demonstrated to regulate IL-6 expression in adipose cells and myocytes (24, 25). Our
findings are consistent with these reports and reveal a novel pathway by which stress hormones enhance tumor production of IL-6. In other studies, catecholamines have been shown to augment ovarian cancer cell expression of vascular endothelial growth factor (14). Collectively, these data indicate that stress-induced activation of β-adrenergic signal transduction pathways on ovarian tumor cells can enhance expression of multiple proangiogenic molecules critical to ovarian cancer progression (Fig. 8).

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