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Different signalling pathways regulate VEGF and IL-8 expression in breast cancer: implications for therapy

Dina Chelouche-Lev, Claudia P. Miller, Carmen Tellez, Maribelis Ruiz, Menashe Bar-Eli, Janet E. Price *

Department of Cancer Biology, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Box 173, Houston, TX 77030, USA
Cancer Biology Program, University of Texas Graduate School of Biomedical Sciences, Houston, TX 77030, USA

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Abstract

Elevated expression of pro-angiogenic cytokines is associated with aggressive tumour growth and decreased survival of patients with breast cancer. In general, the breast cancer cell lines with high vascular endothelial growth factor (VEGF) expression also express high levels of interleukin-8 (IL-8). The consequence of inhibiting mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K), both implicated in regulation of these cytokines, was examined in four cell lines. Treatment with the mitogen-activated protein kinase/extracellular signal-related kinase (MEK) inhibitor U0126 reduced expression of VEGF and IL-8 in MDA-MB-231 cells, partially inhibited expression in MDA-MB-468 and Hs578T cells, with minimal effects in GI101A cells. Treatment with LY294002 reduced cytokine expression in GI101A and MDA-MB-468 cells, with partial reduction in Hs578T and less effect in MDA-MB-231 cells. Thus, IL-8 and VEGF were regulated by different signalling pathways in different cell lines; this suggests that inhibition of the dominantly active pathway can downregulate both angiogenic cytokines. Recognising which signalling pathway is active may identify targets for anti-angiogenic therapy of breast cancer.

Keywords: Angiogenic cytokines; Breast cancer; Mitogen-activated protein kinase; Phosphatidylinositol-3-kinase

1. Introduction

With growing disappointment in the failure of conventional therapy to treat metastatic breast cancer, many investigators are seeking to focus therapy on targets that are necessary and specific for tumour progression. Targeting blood vessels in tumours seems logical as breast cancer, similar to other solid tumours, cannot grow beyond a few mm in diameter, nor metastasise, without neovascularisation [1]. Increased expression of pro-angiogenic factors has been associated with aggressive tumour growth and decreased survival of patients with breast cancer [2]. Vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) are two potent angiogenic factors secreted by breast cancer cells, which contribute to the establishment and expansion of tumour neovasculature. VEGF-A, the first member of the vascular endothelial growth factor family, was originally described as vascular permeability factor [3], and will be referred to as VEGF. While primarily known as a pro-angiogenic factor, there is emerging evidence of additional functions for VEGF, including promoting breast cancer cell survival and invasiveness [4,5]. IL-8 is a CXC chemokine originally identified as a neutrophil chemotactic factor and subsequently recognised to have many functions that promote tumour growth, motility

* Corresponding author. Tel.: +1-713-563-5484; fax: +1-713-792-8747.
E-mail address: jprice@mdanderson.org (J.E. Price).

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and metastasis [6]. While there is extensive information about VEGF and breast cancer, less has been reported about IL-8. Expression of IL-8 is higher in oestrogen receptor (ER)-negative breast cancer cell lines than ER-positive cell lines [7], and ERα has been shown to downregulate IL-8 expression [8]. This cytokine is also expressed by stromal and inflammatory cells in the tumour microenvironment, and in different contexts may have either pro- or anti-tumourigenic effects. IL-8 released by tumour-associated macrophages is considered to have pro-malignant activity [9]; in contrast, IL-8 was induced in breast cancer cells following CD40 ligation, coincident with reduced tumour cell proliferation and viability [10]. Several reports have suggested that the high expression of IL-8 is associated with the invasive potential of breast cancer cells [7,11], and with metastatic potential in immunodeficient mice [12].

While VEGF and IL-8 may be potential targets for cancer therapy, neither cytokine is unique to cancer. Both are expressed by breast epithelial cells [13,14]; however, the signalling pathways that regulate their expression in the normal breast have not been reported. Since both cytokines play a role in normal physiology (of breast and other cells and tissues), the non-selective inhibition of their functions might lead to severe side-effects. Understanding what controls elevated expression of VEGF and IL-8 in breast cancer cells may reveal ways to specifically inhibit expression in malignant cells. Both cytokines are responsive to various stimuli, including oncogene activation, growth factors and a variety of stresses, of which hypoxia is the best characterised [15]. However, less has been reported on the regulation of VEGF and IL-8 in the absence of stimulation, i.e., the basal or constitutive expression.

Two of the major signal transduction pathways implicated in the induction of VEGF and IL-8 are the mitogen-activated protein kinase (MAPK) pathway and the phospatidylinositol-3-kinase (PI3K)/Akt pathway [16–18]. In the present study, we investigated the expression of VEGF and IL-8 in a panel of breast cancer cell lines to provide insights into these signalling pathways.

2. Materials and methods

2.1. Cell lines and tissue culture

Breast cancer cell lines were obtained from the American Type Culture Collection (ATCC) (Manassas, VA) (T47D, SKBR3, Hs578T, MCF-7), or provided by the Goodwin Institute (Plantation FL) (GI101A), Dr. Stephen Ethier (University of Michigan) (SUM149), or Dr. Relda Cailleau (MDA-MB-231, MDA-MB-435, MDA-MB-468, MDA-MB-361). The cells were maintained in medium (either Minimum Essential Medium (MEM), or Dulbecco's Modified Eagle Medium (DMEM)-F12) with 5% or 10% fetal bovine serum and L-glutamine, in a humidified incubator with 5%-CO_2.

2.2. ELISA

Tumour cells were plated in 35-mm dishes, 2×10^5 cells per dish. When the cultures reached 70–80% confluence, fresh medium was applied and collected after 24 h incubation. The cells were harvested with trypsin-ethylene diamine tetra acetic acid (EDTA) and counted. VEGF and IL-8 levels were measured using quantitative immunometric sandwich enzyme immunoassays (ELISA), following the manufacturer's recommended procedure (R&D Systems, Minneapolis, MN). Triplicate cultures of cells were tested for each experimental condition.

2.3. Antibodies and immunoblotting

Cells were exposed to either the PI3K inhibitor LY294002, or the MEK inhibitor U0126 (Cell Signaling Technology, Inc., Beverly, MA), prepared in dimethyl sulphoxide (DMSO), or equivalent volumes of DMSO (control). After 1 h incubation, the cultures were washed with phosphate-buffered saline (PBS) and lysates prepared as described previously [19]. The antibodies used for immunoblotting were against phospho-Akt (Ser473), Akt, phospho-p44/p42 MAP kinase (Thr202/Tyr204) and ERK1/2 (Cell Signaling Technology, Inc.), detected using a horseradish peroxidase-conjugated anti-rabbit IgG, and the Amersham enhanced chemiluminescence (ECL) system (Amersham, Arlington Heights, IL).

2.4. Quantitative real-time PCR

Total RNA was isolated using TriReagent (Sigma Chemical Co.) and reverse-transcribed with random primers from the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). cDNA was amplified in duplicate samples using the ABI 7000 Sequence Detection System for the expression of VEGF, IL-8 and 18S using Predeveloped TaqMan® Assay Reagents (Applied Biosystems) following the manufacturer's recommended amplification procedure. Results were recorded as mean threshold cycle (Ct), and relative expression was determined using the comparative Ct method. The ΔCt was calculated as the difference between the average Ct value of the endogenous control, 18S, from the average Ct value of test gene. To compare the relative amount of target gene expression in different samples, human placenta RNA (Promega, Madison, WI) was used as a calibrator. The ΔΔCt was determined by

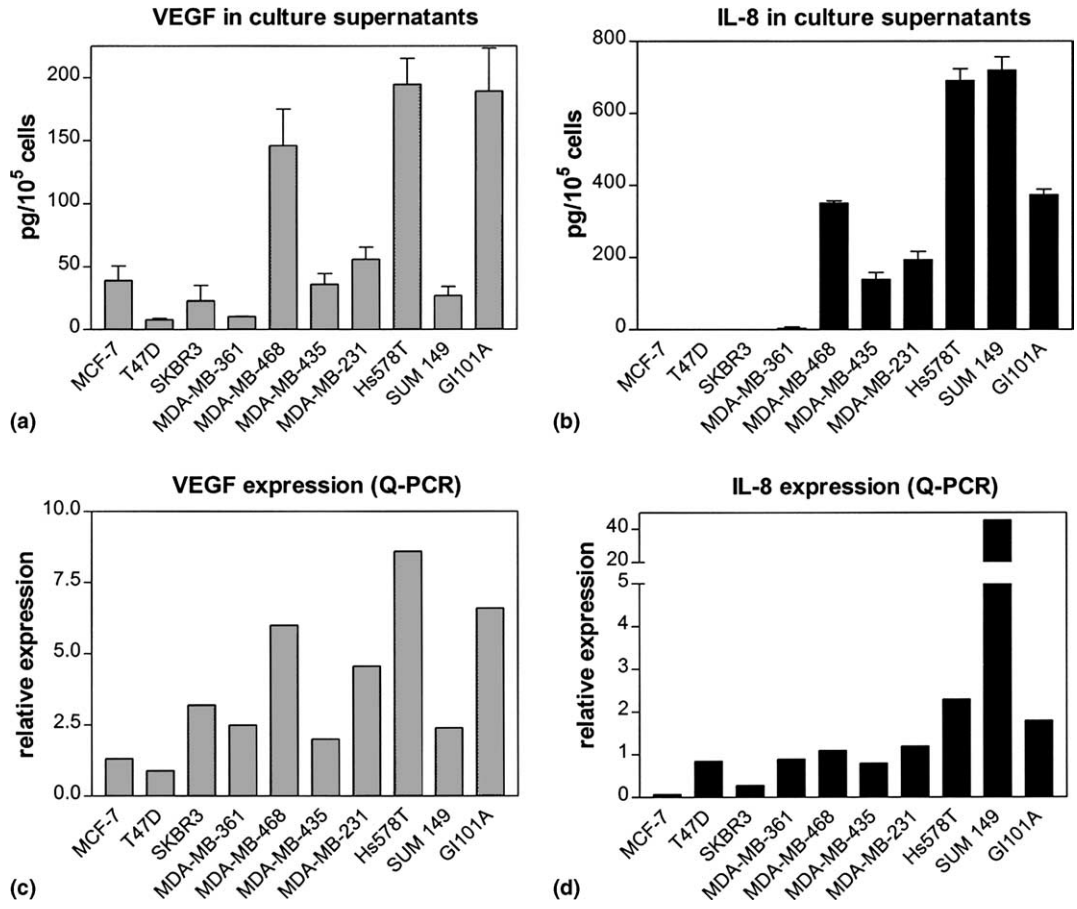

Fig. 1. Expression of vascular endothelial growth factor (VEGF) and IL-8 by human breast cancer cell lines. The concentrations of VEGF (a) and IL-8 (b) were measured by enzyme-linked immunosorbent assays (ELISA) in supernatants from breast cancer cells cultured for 24 h, and expressed as pg/10^5 cells/ml. Results shown are means and standard deviation (SD) from replicate samples and are representative of 3 assays. Relative expression levels of RNA for VEGF (c) and IL-8 (d) assayed by quantitative RT-PCR (QPCR), as described in Section 2. Values shown are from duplicate samples and are representative of repeat assays using different samples of RNA.

subtracting the ΔCt of the calibrator from the ΔCt of the test sample. Relative expression of the target gene is calculated by the formula, $2^{-\Delta\Delta Ct}$, which is the amount of gene product, normalised to the endogenous control and relative to the calibrator sample.

2.5. Transient transfection and reporter assays

Promoter activity was measured by transient transfection with the pGL-IL-8 construct, or the pGL3VEGF 3.317 construct, as described previously [19]. As a control for transfection efficiency, the cells were cotransfected with a β-actin Renilla construct, in which the β-actin promoter regulates Renilla luciferase expression. Transient transfections of the reporter constructs were done using Lipofectin Reagent (BRL-GIBCO Life Technologies, Rockville, MD). Cells were incubated with the Lipofectin-DNA complexes in OPTI-MEM (BRL-GIBCO Life Technologies) medium for 12–18 h, and then with culture medium with or without 10 μM U0126 or 20 μM LY294002. After 24–30 h, the cells were lysed in 1× passive lysis buffer and luciferase activity measured using the Dual-Luciferase Reporter Assay System (Promega, Madison WI). Luciferase activity was calculated using the following formula: (firefly luciferase units/Renilla luciferase units). Fold-induction was calculated using the following formula: (luciferase activity in treated cells/control luciferase activity) – 1; Fold-reduction was calculated: 1 – (luciferase activity in control/treated cells luciferase activity). Experiments to determine the effects of U0126 and LY294002 on transactivation used reporter constructs with either three activator protein-1 (AP-1) or three nuclear factor (NF)-κB consensus elements subcloned in front of a luciferase reporter [19]. After incubating cells with the Lipofectin-DNA complexes for 10 h, the transfection medium was replaced with culture medium for 16 h. The cells were incubated with the inhibitors for 4, 8 or 24 h, then lysed, and the luciferase activity measured.

2.6. Electrophoretic mobility shift assay

Nuclear extracts of MDA-MB-231 and GI101A cells were prepared, and binding reactions performed as described previously [19]. AP-1 or NF-κB consensus oligos were end-labelled with [γ-P^{32}]adenosine triphosphate (ATP) (Promega) and incubated with 5 μg of nuclear extract. For competition, nuclear extracts were incubated for 30 min with unlabelled oligonucleotide, followed by incubation with labelled consensus sequence for 30 min. For supershifting, nuclear extracts were incubated with the labelled consensus sequence for 30 min followed by incubation with antibodies against c-fos, c-jun, p50, p52, or p65 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for 30 min.

3. Results

3.1. VEGF-A and IL-8 expression in breast cancer cell lines

VEGF and IL-8 was measured in supernatants from 10 breast cancer cell lines using ELISA (Fig. 1(a) and (b)). There was a general correlation between the levels of expression of the 2 cytokines in the same cell line, i.e., if the cells expressed a high level of VEGF, the level of IL-8 was also high. The exception was the SUM 149 cell line, which expressed the highest amount of IL-8, yet ranked 7th for the expression of VEGF protein and RNA. RNA measurements by quantitative RT-PCR (QPCR) (Fig. 1(c) and (d)) were consistent with the ELISA results. The value of cytokine RNA expression was relative to that of the calibrator sample (placenta), which was assigned the value of one. Four cell lines, MDA-MB-231, MDA-MB-468, GI101A and Hs578T, expressing relatively high levels of both cytokines, were studied further.

3.2. MEK inhibition reduces VEGF and IL-8 protein expression in MDA-MB-231, but not GI101A cells

Since the MAPK/ERK1/2 pathway is involved in the inducible regulation of VEGF and IL-8, we investigated its contribution to constitutive expression. MEK inhibition resulted in a dose-dependent reduction of both VEGF and IL-8 protein (86.6% and 95% inhibition with 10 μM U0126) in the MDA-MB-231 cells treated for 24 h (Fig. 2). Similar results were found after 72 h treatment (data not shown). In contrast, the GI101A cells showed little response to U0126.

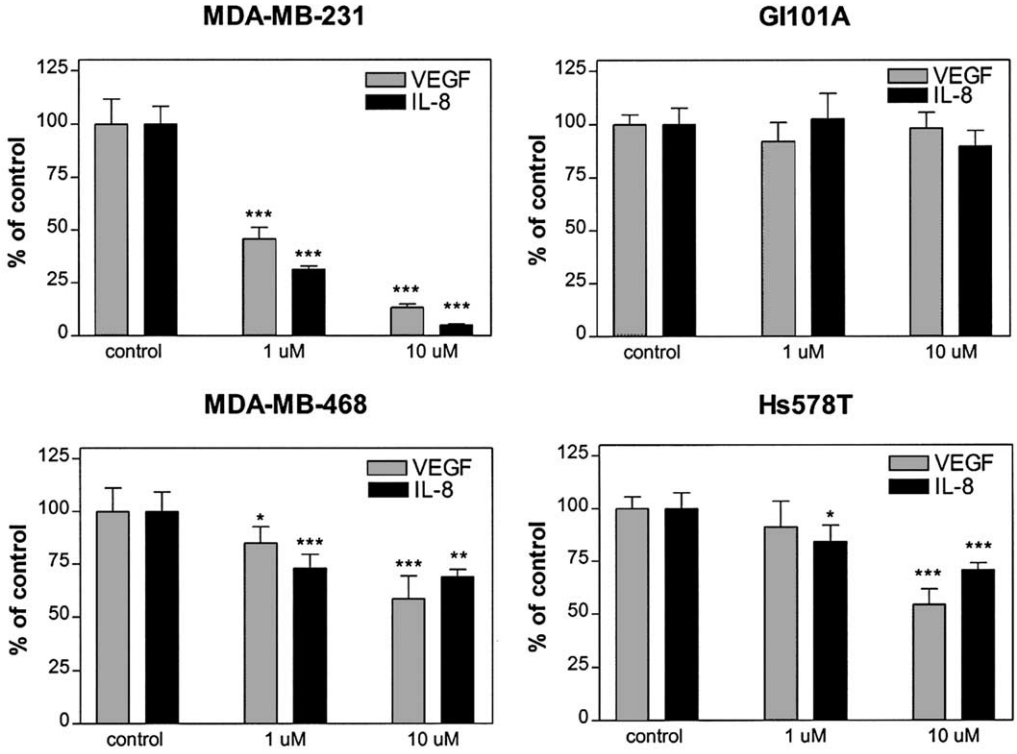

Fig. 2. Effect of MEK inhibition on VEGF and IL-8 secretion. Breast cancer cells were treated with 1 or 10 μM U0126 for 24 h, and the VEGF and IL-8 in culture supernatants measured by ELISA. Results shown are means and SD of triplicate samples, expressed as a percent of control values, and are representative of repeated experiments. The P values (*, $P = 0.01-0.05$; **, $P = 0.001-0.01$; ***, $P < 0.001$) were calculated using Student's t-tests.

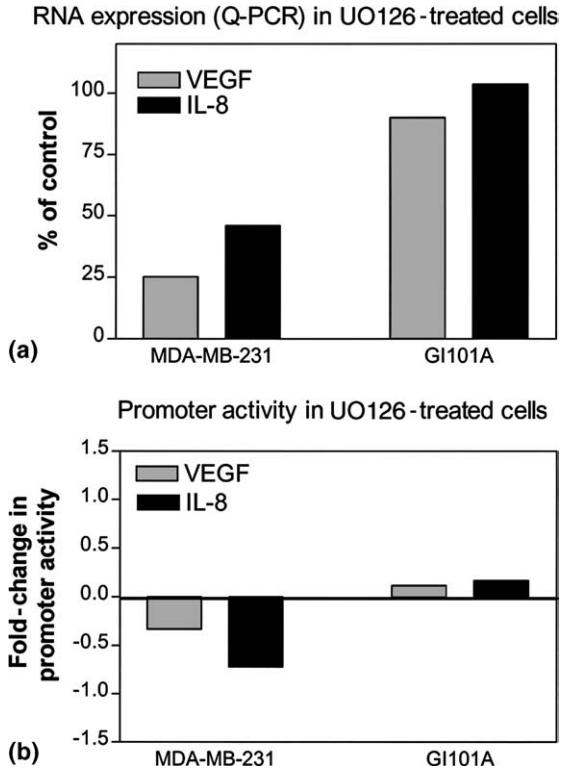

Fig. 3. Effect of MEK inhibition on cytokine expression and promoter activity. (a) Relative levels of VEGF and IL-8 RNA were assessed from samples of GI101A and MDA-MB-231 cells treated with 10 μM U0126, and results expressed as percent of control samples. (b) Luciferase activity driven by VEGF and IL-8 promoters was assayed in GI101A and MDA-MB-231 cells treated with 10 μM U0126. The fold-change in promoter activity was relative to the values from untreated cells, assigned the value of 1.

There was a partial reduction of VEGF and IL-8 in the supernatants of MDA-MB-468 (41.5% and 31%, respectively) and Hs578T cells (45% and 29.3%, respectively). ELISA results were calculated as pg/10^5 viable cells; exposure to 10 μM U0126 for 24 h produced 2.9%, 3.1% and 31% growth inhibition in MDA-MB-231, GI101A and MDA-MB-468 cultures, respectively, with no decrease in the Hs578T cultures. Thus, the reduction in cytokines was not due to a loss of cell viability. These results suggest differences in the contribution of ERK1/2 to cytokine regulation in the different cell lines.

The investigation was continued using the MDA-MB-231 and GI101A cell lines. QPCR (Fig. 3(a)) demonstrated a major reduction in the VEGF (75%) and IL-8 (54%) RNA levels in the MDA-MB-231 cells treated with 10 μM U0126, and no effect in the GI101A cells. To test the effect of U0126 on transcription, VEGF and IL-8 reporter constructs were transiently transfected into cells exposed to the inhibitor (Fig. 3(b)). VEGF and IL-8 promoter activities were reduced in MDA-MB-231 cells, (−0.33 and −0.71, respectively) with minimal change in the GI101A cells. The results suggest that VEGF and IL-8 expression in MDA-MB-231 cells is dependent on the MAPK/ERK pathway, which has relatively little impact on the expression of these cytokines in GI101A cells.

3.3. PI3K inhibition reduces VEGF and IL-8 expression in GI101A, but not MDA-MB-231 cells

The second signalling pathway important for the inducible regulation of VEGF and IL-8 is the PI3K/Akt pathway. A similar set of experiments was performed using the PI3K inhibitor, LY294002. Exposure to 20 μM LY294002 produced an 80% and 87% reduction in the VEGF and IL-8 protein levels, respectively, in supernatants of GI101A cells, after 24 h (Fig. 4), with no loss in cell number, compared with DMSO-treated cells. LY294002 treatment significantly reduced the cytokines released by MDA-MB-468 cells (66% and 60%, respectively). A less pronounced, but still significant ($P < 0.001$), reduction was noted in Hs578T supernatants (50% reduction of VEGF and 35% reduction of IL-8). However, MDA-MB-231 cells showed a more modest response (<20% reduction). QPCR and promoter studies using MDA-MB-231 and GI101A cells produced results consistent with the ELISA results (Fig. 5). VEGF and IL-8 promoter activity was reduced 1.7- and 1.1-fold, respectively, in GI101A, with little change in the MDA-MB-231 cells (Fig. 5(b)).

Thus, the PI3K pathway is essential for the constitutive expression of VEGF and IL-8 in GI101A cells, contributes to the regulation in MDA-MB-468 and Hs578T cells, but its inhibition has little impact in MDA-MB-231 cells.

3.4. Phosphorylation status of ERK and Akt in the breast cancer cell lines

The phosphorylation of ERK1/2 and Akt was assessed in the four cell lines (Fig. 6). MDA-MB-231 cells expressed a substantial amount of pERK that was inhibited by U0126; however pAkt was not detected. In GI101A cells, which appear dependent on PI3K signalling for cytokine regulation, both pathways showed constitutive activation, with high levels of pERK and pAkt. MDA-MB-468 and Hs578T cells also expressed pERK and pAkt.

3.5. DNA binding activity of AP-1 and NF-κB in MDA-MB-231 and GI101A cells

AP-1 and NF-κB are reported to regulate VEGF and IL-8, and GI101A and MDA-MB-231 cells express high levels of these transcription factors. Treatment with LY294002 or U0126 did not affect their abundance. Incubation of nuclear extracts with AP-1 or NF-κB probes resulted in robust DNA-protein complexes

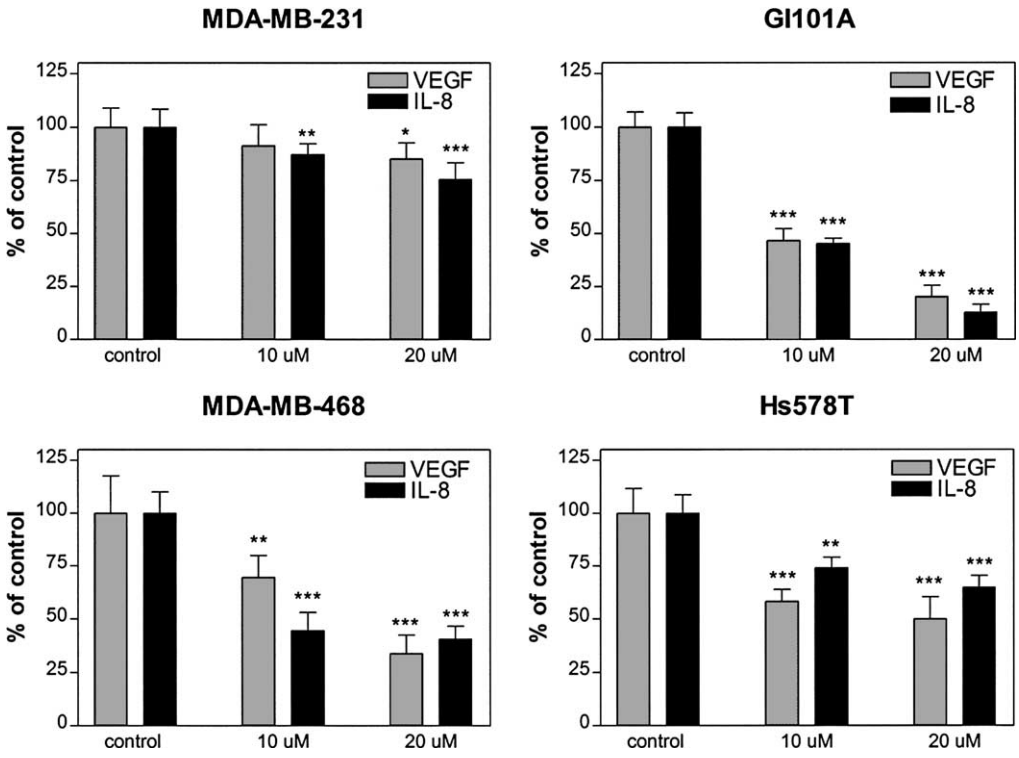

Fig. 4. Effect of PI3K inhibition on VEGF and IL-8 secretion. Breast cancer cells were treated with 10 or 20 μM LY294002 for 24 h and VEGF and IL-8 in culture supernatants measured by ELISA. Results shown are means and SD of triplicate samples, expressed as a percent of control values and are representative of repeated experiments. The P values (*, $P = 0.01-0.05$; **, $P = 0.001-0.01$; ***, $P < 0.001$) were calculated using Student's t-tests.

(Fig. 7), and treatment with the inhibitors did not substantially alter binding activity. Supershifting with antibodies showed that c-jun was the major component bound to AP-1 oligonucleotides in MDA-MB-231 extracts, while c-fos was the more prominent component in GI101A extracts (Fig. 7). Reporter assays measured the effect of U0126 and LY294002 on AP-1 and NF-κB transcriptional activity. In MDA-MB-231 cells, 8 h of treatment with U0126 led to an approximately 25% reduction in transcriptional activity of both reporter constructs, while LY294002 reduced the activity of AP-1 by 46% and NF-κB by 36%. Exposure to the inhibitors for 4 h had less effect (15–25%), while after 24 h the treated and control values were not substantially different. The results from GI101A cells were similar. However, while the inhibitors transiently reduced the transcriptional activity of AP-1 and NF-κB in both cell lines, this did not correspond with the differences in IL-8 and VEGF expression. This suggests that while AP-1 and NF-κB may contribute to the constitutive expression of the cytokines in the breast cancer cells, additional factors are involved.

4. Discussion

Angiogenesis is crucial for tumour progression, and pro-angiogenic molecules such as VEGF and IL-8 have been considered as potential targets for cancer therapy. In the present study, we found heterogeneity in the basal levels of expression of these cytokines in breast cancer cell lines. Consistent with previous reports, higher levels of IL-8 expression were seen in the ER-negative breast cancer cell lines [7,8]. Our data show that when a cell line expressed a high level of VEGF, it generally also expressed a high amount of IL-8, suggesting common regulatory pathways. There is currently no evidence of a direct relationship between the two cytokines, i.e., that one regulates the expression of the other. One cell line was an exception to the general finding, the SUM 149 cell line, which expressed a high level of IL-8 and only a low-to-moderate amount of VEGF. This cell line was established from an inflammatory breast cancer [20], an aggressive form of breast cancer reported to be highly angiogenic, and which may differ from non-inflammatory breast cancers in terms of expression and regulation of different angiogenic factors [21].

Consistent with what is known about the induced expression of VEGF and IL-8, MAPK–ERK and PI3K signalling pathways contributed to the basal expression. This was mediated, at least in part, by regulation of transcriptional activity; mRNA stabilisation and/or post-translational events may also be involved. However, differences in the contribution of each pathway to cytokine regulation in different cell lines were

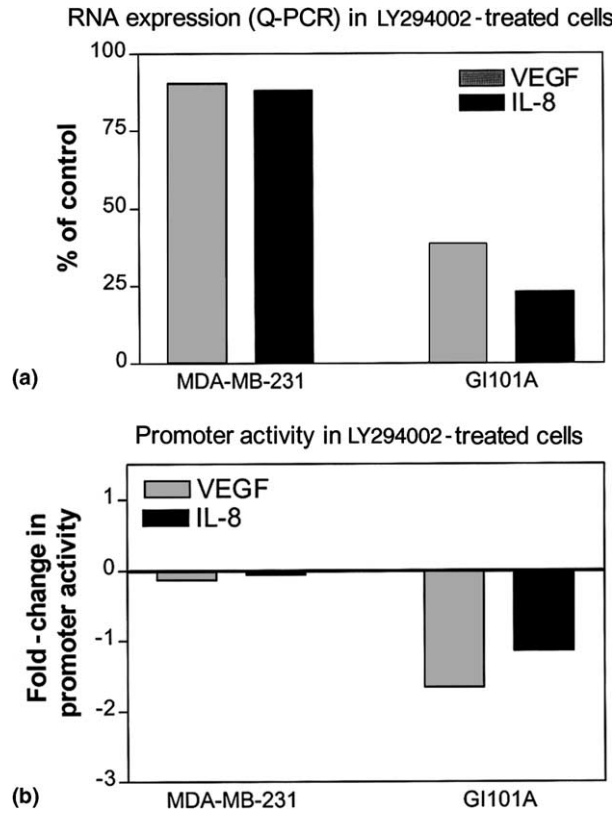

Fig. 5. Effect of PI3K inhibition on cytokine expression and promoter activity. (a) Relative levels of VEGF and IL-8 RNA were assessed from samples of GI101A and MDA-MB-231 cells treated with 20 μM LY294002, and results expressed as percent of control samples. (b) Luciferase activity driven by VEGF and IL-8 promoters was assayed in GI101A and MDA-MB-231 cells treated with 20 μM LY294002. The fold-change in promoter activity was relative to the values from untreated cells, assigned the value of 1.

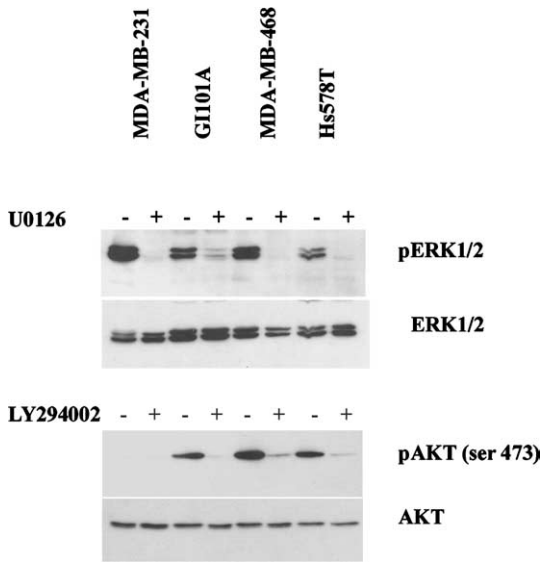

Fig. 6. Phosphorylation of ERK1/2 and Akt in breast cancer cells. Cells were exposed to 10 μM U0126 or 20 μM LY294002 for 1 h, and phosphorylation of ERK1/2 and Akt (ser 473) detected by immunoblotting using phospho-specific antibodies. The filter was re-probed with antibodies that recognise all forms of the proteins (total Akt or ERK1/2).

revealed in experiments using inhibitors of the signalling pathways. The MAPK–ERK pathway was predominantly active in MDA-MB-231 cells, while the PI3K/Akt pathway was more important in GI101A cells. In the other two cell lines studied in detail, MDA-MB-468 and Hs578T, both signalling pathways were active in regulating expression of the two cytokines.

MAPK pathways play important roles in cancer pathogenesis, controlling processes central to malignant progression such as cell growth, apoptosis and migration [22,23]. Sivaraman and collegues [24] reported MAPK activation in human breast cancer tissues, comparing primary breast cancer with benign lesions using substrate-based MAPK enzyme assays and immunoblotting. MAPK activity was significantly less in benign breast tissues compared with invasive breast cancers. In a recent study of breast cancer specimens, activated MAPK detected by immunohistochemistry was associated with lymph node metastasis, thus implicating a role in metastatic progression [25]. However, none of these reports have described a correlation between MAPK activity and VEGF and/or IL-8 expression in breast cancer.

PI3K and other components of this signalling pathway, notably Akt, have been implicated in the development of many human cancers, including breast cancer [26,27]. This study reports that inhibition of PI3K with LY294002 inhibited the basal expression of IL-8 and VEGF in breast cancer cells with constitutively activated Akt. The effect of LY294002 on cytokine expression was more robust than the effect of U0126 in cells with constitutive activation of both signalling pathways. In addition to inhibiting PI3K, LY294002 inhibits the protein kinase casein kinase 2 (CK2) with similar potency [28]. CK2 is reported to enhance activation of NF-κB, by promoting the phosphorylation and degradation of inhibitor κB-α (IκB-α) [29], and contribute to the elevated NF-κB activity seen in some human breast cancers [30]. As discussed below, NF-κB is involved in regulating IL-8 and VEGF, thus the reduced cytokine expression by some LY294002-treated cells may be due to combined inhibition of PI3K and CK2. How CK2 inhibition alone impacts the regulation of angiogenic cytokines has not been described. While U0126 is considered a relatively specific inhibitor [28], a recent report described the reduction of activity of ERK and $p70^{S6K}$ in MDA-MB-231 cells exposed to U0126 [31]. In addition to actions on other kinases, the inhibitors in this report could also potentially impact other signalling pathways, through the cross-talk reported between Akt and Raf-1 and NF-κB pathways [32].

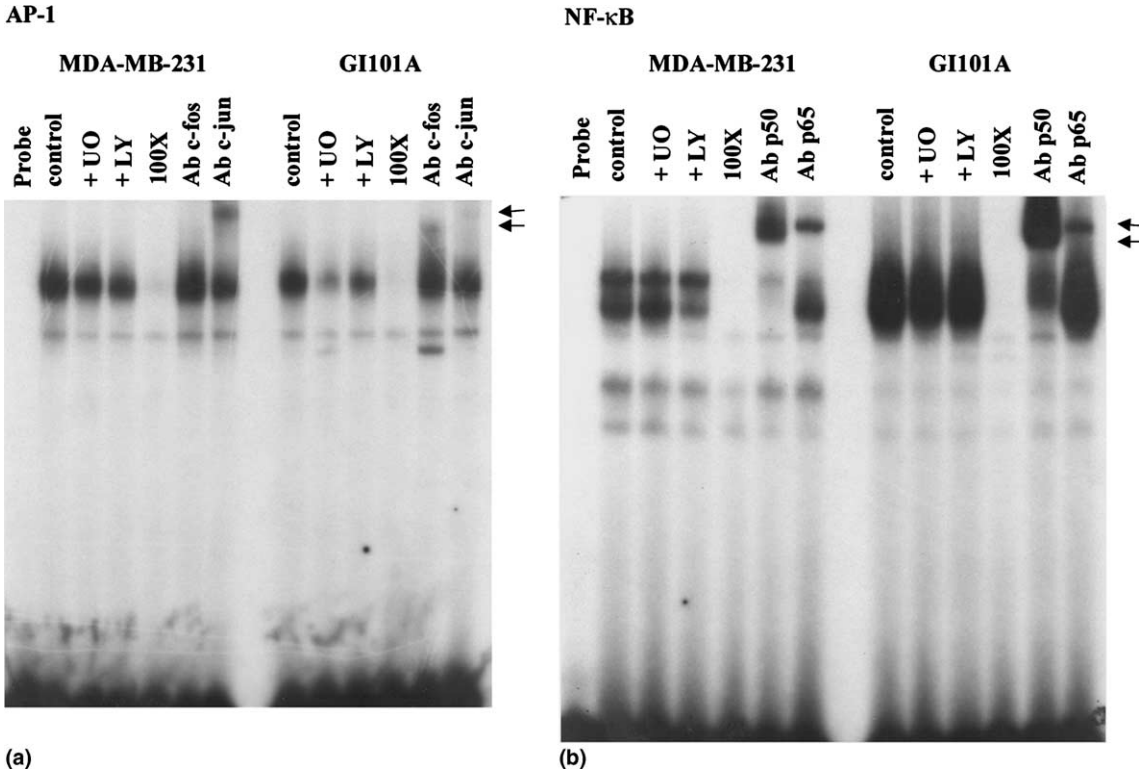

Fig. 7. AP-1 and NF-κB binding activity in MDA-MB-231 and GI101A cells. Nuclear extracts from cells treated with 10 μM U0126 or 20 μM LY294002 for 24 h were incubated with AP-1 or NF-κB consensus sequences. Specificity was confirmed by supershifting with antibodies or by competition with 100-fold excess unlabelled oligonucleotides (arrows indicate supershifted bands).

Various transcription factors are involved in regulating the expression of VEGF and IL-8 [16–18]. The regulatory regions of the promoters of both genes share binding sites for the same transcription factors, which may account for similar responses to various stimuli. Two of the transcription factors reported to regulate both IL-8 and VEGF are AP-1 and NF-κB [17,18,33,34]. Both cytokine promoters have binding sites for AP-1, while the consensus binding site for NF-κB is reported only in the IL-8 promoter. However, the inhibition of NF-κB activity in human ovarian cancer cells can suppress expression of VEGF *in vitro* and *in vivo* [35]. AP-1 is activated by MAP kinases including pERK, while NF-κB is activated by many signalling pathways, including PI3K/Akt [36]. Constitutive binding activity of both NF-κB and AP-1 was found in nuclear extracts of MDA-MB-231 and GI101A cells, and the activation of AP-1 and NF-κB reporter constructs was transiently inhibited in both cell lines by U0126 and LY294002. Similar to the regulation of IL-8 and VEGF in head and neck cancer cell lines [16], our results suggest that at least two signal pathways upstream of AP-1 and NF-κB transcription factors contribute to the expression of these angiogenic factors in the breast cancer cells studied. However, we found no apparent differences in AP-1 or NF-κB activation to account for the differences in regulation of IL-8 and VEGF in GI101A and MDA-MB-231 cells, suggesting that additional factors control their basal expression in a cell line-specific manner. Potential candidate transcription factors include Sp1 and AP-2 [17]. Levels of Sp1 in pancreatic cancer cell lines and tissue specimens were shown to correlate with constitutive VEGF expression [37]. The introduction of AP-2α into prostate cancer cells reduced the expression of VEGF, with AP-2α acting as a transcriptional repressor of the VEGF promoter [38]. While there are no reports comparing AP-2 and VEGF expression in breast cancer, immunohistochemical studies have shown that reduced expression of AP-2 is associated with more aggressive disease [39,40].

Our data suggest that the phosphorylation status of Akt and ERK combined with detection of VEGF and IL-8 expression might provide important information about the angiogenic potential of human breast cancers. Preclinical trials utilising anti-VEGF and anti-IL-8 therapies as single agents for the treatment of breast cancer did not show dramatic effects [41,42]. One explanation is that cancer cells secrete many different angiogenic factors, such that inhibition of one factor is compensated by unrestricted expression of others. Targeting common signalling pathways leading to the expression of these cytokines might result in improved tumour response. LY294002 inhibited the growth and ascites formation of ovarian cancer in nude mice [43]. Blockade of the

MAPK pathway suppressed growth of human tumour xenografts [44], and clinical trials of MEK inhibitors in patients with advanced cancer show encouraging preliminary results and tolerable side-effects [45]. Our data suggest that inhibition of MAPK signalling would inhibit IL-8 and VEGF expression in cells with activated MAPK. If the tumour cells have constitutively phosphorylated Akt, inhibition of PI3K alone or in combination with MAPK inhibitors would inhibit expression of these cytokines.

Our findings suggest that inhibition of the dominantly active signal transduction pathway can lead to downregulation of more than one angiogenic factor. This pharmacological intervention might be more tumour-specific than the direct inhibition of the cytokine and thus might reduce the likelihood of major side-effects. Recognising which signal transduction pathway is active in a tumour may have prognostic information, and may also identify a potential target for anti-angiogenic therapy of breast cancer.

Conflict of interest

None.

Acknowledgements

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