Endothelin-1 Induces CXCL1 and CXCL8 Secretion in Human Melanoma Cells

Catherine R. Mangahas,*[1] Gelo V. dela Cruz,*[1] George Friedman-Jiménez,† and Sumayah Jamal*
*The Ronald O. Perelman Department of Dermatology, New York University School of Medicine, New York, New York, USA; †Division of Epidemiology and Biostatistics, New York University School of Medicine, New York, New York, USA

The endothelin pathway plays a critical role in melanoma tumor progression by a variety of mechanisms that enhance tumor cell growth, invasion, and metastasis. Here, we investigate the effect of this pathway on CXC chemokine expression in human melanoma cells and melanocytes. As determined by ELISA, endothelin-1 (ET-1) induces CXCL1 and CXCL8 secretion in three human melanoma cell lines in a concentration-dependent fashion. These responses are mediated by the endothelin-B receptor and are sustained over a 40 h time course. ET-1 does not induce CXCL1 secretion in primary human melanocytes but ET-3, an endothelin isoform, induces a low level of CXCL1 secretion in certain cultures. Neither ET-1 nor ET-3 induces secretion of CXCL8 in primary human melanocytes; thus, this response may be specific for melanocytic cells that have undergone malignant transformation. We have previously demonstrated that ET-1 induces changes in the expression of adhesion molecules in melanoma cells such that invasion and metastasis are favored. This study demonstrates that ET-1 additionally induces secretion of CXC chemokines critical for melanoma metastasis and tumor progression.

Key words: BQ788/endothelin-A receptor/endothelin-B receptor/skin/tumor progression
J Invest Dermatol 125:307–311, 2005

CXCL1 and CXCL8 are members of the CXC chemokine family that play an important role in melanoma progression (Payne and Cornelius, 2002). CXCL1 is also known as melanoma growth-stimulatory activity and, as its name suggests, it stimulates melanoma cell proliferation in an autocrine fashion (Richmond *et al*, 1985, 1988; Richmond and Thomas, 1986). CXCL1 is the gene product of the oncogene Gro and in addition to its ability to stimulate proliferation, it is also a potent stimulator of angiogenesis (Strieter *et al*, 1995). Antibodies directed against CXCL1 are inhibitory for melanoma cell growth (Lawson *et al*, 1987), and overexpression of CXCL1 in immortalized melanocytes confers the ability to form tumors on nude mice (Balentien *et al*, 1991). CXCL8 expression is correlated with melanoma cell growth, angiogenesis, and metastatic potential in nude mice (Singh *et al*, 1994; Norgauer *et al*, 1996; Belperio *et al*, 2000). The regulation of CXCL1 and CXCL8 in melanocytic cell lineages is complex. In the case of CXCL1, regulation occurs at both the levels of transcription and translation and involves activation of nuclear factor-κB (NF-κB) (Bordoni *et al*, 1990; Shattuck-Brandt *et al*, 1997). In the case of CXCL8, regulation of expression is linked to factors present in the epidermal microenvironment such as keratinocyte-derived interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), which activate the transcription factors AP-1 and NF-κB (Gutman *et al*, 1995; Singh *et al*, 1995; Mohler *et al*, 1996). Here, we investigate the regulatory role of another factor present in the epidermal microenvironment, endothelin-1 (ET-1), in CXCL1 and CXCL8 expression.

ET-1 is a 21-amino acid peptide secreted by epidermal keratinocytes in response to ultraviolet (UV) irradiation, which plays an important role in the tanning response of the skin (Imokawa *et al*, 1995; Tada *et al*, 1998). Melanocytes and melanoma cells express two high-affinity endothelin receptors, endothelin-A receptor (ET_A) and endothelin-B receptor (ET_B), that mediate physiologic functions such as chemotaxis, mitogenesis, and pigment production (Yohn *et al*, 1994; Imokawa *et al*, 1996). The ET-1/ET_B receptor pathway has been linked to melanoma tumor progression and is important for melanoma cell viability *in vivo* and *in vitro* (Lahav *et al*, 1999; Jamal, 2000; Jamal and Schneider, 2002). We have previously demonstrated that the ET-1/ET_B pathway alters adhesion molecule expression in a manner favoring invasive behavior. Specifically, ET-1 downregulates the tumor invasion suppressor E-cadherin (Jamal, 2000; Jamal and Schneider, 2002) and upregulates the tumor invasion promoter melanoma cell adhesion molecule (MCAM) in melanocytic cells *in vitro* (Mangahas *et al*, 2004). Other studies have demonstrated that this same pathway activates metalloproteinases in melanoma cells, with a concomitant increase in migration and invasive behavior as determined by *in vitro* chemotaxis and invasion assays (Bagnato *et al*, 2004). Since the CXC family of chemokines also plays an important role in melanoma migration and metastasis (Payne and Cornelius, 2002), this study

Abbreviations: ET-1, endothelin-1; ET_A, endothelin-A receptor; ET_B, endothelin-B receptor
[1]Co-first authors.

investigates the role of the endothelin pathway in the regulation of CXC chemokine expression in human melanoma cells.

Results

ET-1 induces secretion of CXCL1 and CXCL8 in a concentration-dependent fashion Microarray analysis was performed using total RNA samples prepared from SKMEL28 human melanoma cells stimulated with 10 nM ET-1 over a 40 h time course. Analysis of the data revealed an induction of signals corresponding to CXCL1 and CXCL8 mRNA at all time points tested (data not shown). To corroborate these preliminary data, ELISA was performed to determine the effect of ET-1 stimulation upon CXCL1 and CXCL8 protein secretion in SKMEL28 human melanoma cells. In Fig 1*A*, SKMEL28 human melanoma cells were stimulated with 10 nM ET-1 over a 40 h time course. As shown, there was an ET-1-dependent induction of CXCL1 secretion ($p < 0.001$; *left panel*) observed at all time points with a peak induction observed at the 24-h time point. Fold inductions of CXCL1 ranged from approximately 1.8 × to 3 × . An ET-1-dependent induction of CXCL8 secretion was also observed at all time points tested ($p < 0.001$; see Fig 1*A*, *right panel*), with a peak of induction observed at 40 h. Fold inductions of CXCL8 ranged from approximately 2 × to 5 × . To determine whether these responses occurred in a concentration-dependent fashion, we stimulated SKMEL28 cells with ET-1 at concentrations ranging from 0.1 to 10 nM. As shown in Fig 1*B*, increasing concentrations of ET-1 resulted in progressively higher levels of both CXCL1 and CXCL8 secretion ($p < 0.001$ for each, left and right panels, respectively). ET-3, an endothelin isoform that is selective for the ET_B, also induced a 2.6-fold increase in CXCL1 secretion and a 5.9-fold increase in CXCL8 secretion, suggesting that these responses are mediated by the ET_B receptor. ET-1 also induced CXCL1 and CXCL8 secretion in M20 and WM266-4 human melanoma cells (data not shown), indicating that these responses are not specific to SKMEL28 cells alone. Overall, these data demonstrate that ET-1 induces secretion of CXCL1 and CXCL8 proteins in human melanoma cells in a concentration-dependent fashion and with kinetics that are sustained and prolonged.

ET-1-dependent secretion of CXCL1 and CXCL8 is mediated by the ET_B receptor There are two different

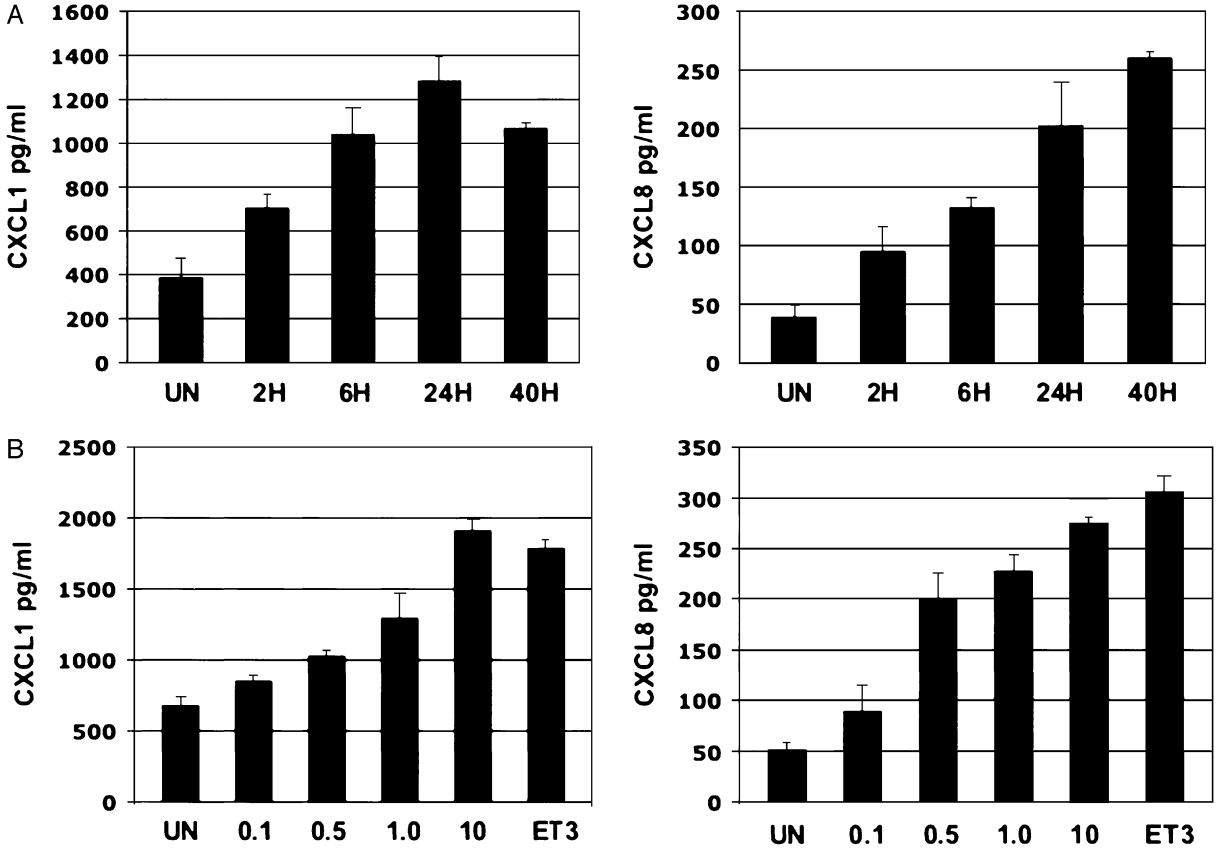

Figure 1
Time course and concentration dependence of CXCL1 and CXCL8 induction of secretion by endothelin-1 (ET-1) using ELISA. (*A*) *Left panel*: SKMEL28 cells were stimulated with 10 nm ET-1 for the indicated times. Culture medium was collected and used for CXCL1 ELISA. *Right panel*: SKMEL28 cells were stimulated with 10 nm ET-1 for the indicated times. Culture medium was collected and used for CXCL8 ELISA. (*B*) *Left panel*: SKMEL28 cells were stimulated for 40 h with ET-1 at concentrations ranging from 0.1 to 10 nM. Cells were also stimulated with 10 nM of ET-3. Culture medium was collected and used for CXCL1 ELISA. *Right panel*: SKMEL28 cells were stimulated for 40 h with ET-1 at concentrations ranging from 0.1 to 10 nM. Cells were also stimulated with 10 nM of ET-3. Culture medium was collected and used for CXCL8 ELISA. Bars represent levels of secreted CXCL1 and CXCL8 proteins, respectively, in pg per mL. Values were averaged over three independent trials, and error bars represent standard deviation. All p-values are two sided. (*A*, *left panel*, time graph), "from linear regression, $p < 0.001$"; (*A*, *right panel*, time graph), "from linear regression, $p < 0.001$"; (*B*, *left panel*, dose graph), "from linear regression, $p < 0.001$"; (*B*, *right panel*, dose graph), "from linear regression, $p < 0.001$".

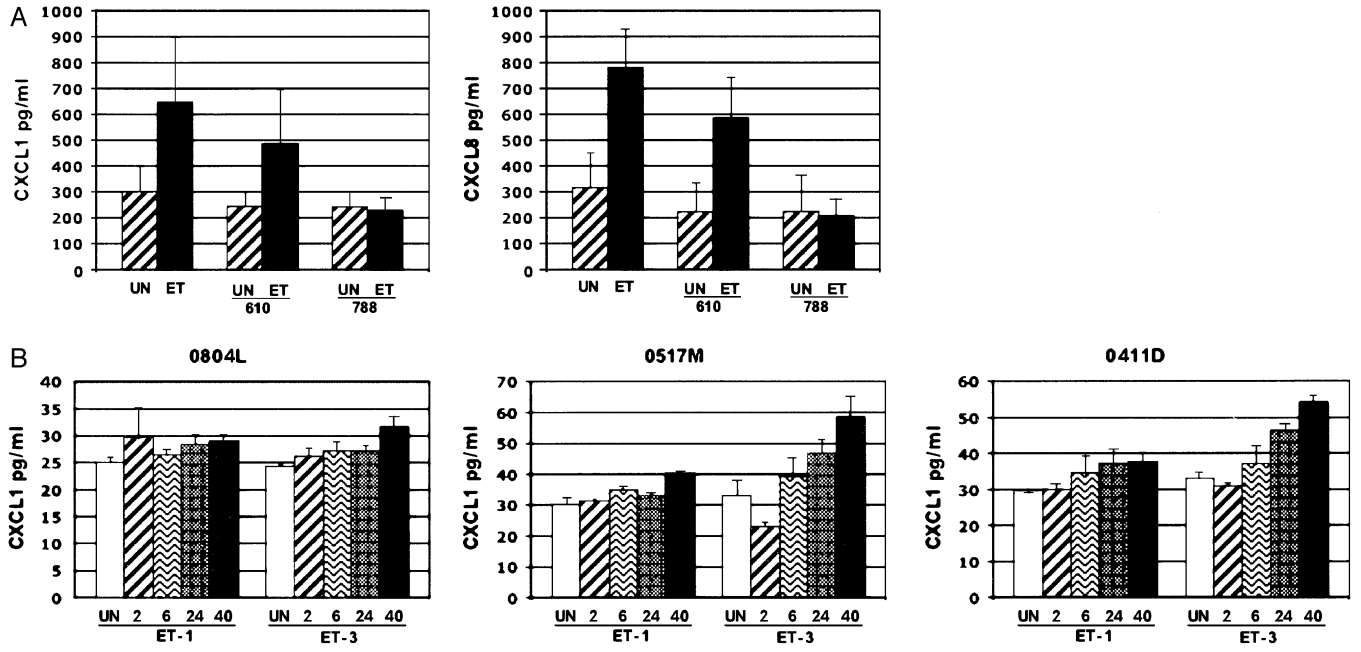

Figure 2
Endothelin-1 (ET-1)-dependent CXCL1 and CXCL8 secretion are inhibited by endothelin-B (ET_B) receptor antagonist BQ788. (*A*) *Left panel*: SKMEL28 cells were stimulated with 10 nM ET-1 for 40 h. Where indicated, cells were pre-treated with either endothelin-A receptor (ET_A) receptor antagonist BQ610 or ET_B receptor antagonist BQ788. Culture medium was collected and used for CXCL1 ELISA. *Right panel*: SKMEL28 cells were stimulated with 10 nM ET-1 for 40 h. Where indicated, cells were pre-treated with either ET_A receptor antagonist BQ610 or ET_B receptor antagonist BQ788. Culture medium was collected and used for CXCL8 ELISA. (*B*) Primary melanocytes were stimulated with 10 nM ET-1 and 10 nM ET-3 for 2–40 h. Culture medium was collected and CXCL1 ELISA was performed. *Left panel*: lightly pigmented melanocytes. *Middle panel*: medium pigmented melanocytes. *Right panel*: darkly pigmented melanocytes. Bars represent levels of secreted CXCL1 protein in pg per mL. Values were averaged over three independent trials, and error bars represent standard deviation. Melanocytes were between the fifth and tenth passage.

endothelin receptor subtypes expressed by melanocytic cells: the ET_A and the ET_B (Baynash *et al*, 1994). In order to determine which of these subtypes mediates ET-1-dependent secretion of CXCL1 and CXCL8, we repeated our experiments using a selective ET_A receptor antagonist (BQ610) and a selective ET_B receptor antagonist (BQ788). As shown in Fig 2*A* (*left panel*) BQ610 failed to inhibit ET-1-dependent secretion of CXCL1. Although BQ610 slightly reduced CXCL1 secretion at baseline, the average fold induction of CXCL1 secretion by ET-1 was not significantly altered, being approximately 2 $\times$ whether cells were stimulated in the presence or absence of BQ610. We thus conclude that the ET_A receptor is not responsible for mediating this response. In sharp contrast, however, BQ788 completely blocked induction of CXCL1 secretion by ET-1 (Fig 2*A*, *left panel*). Therefore, ET-1-dependent secretion of CXCL1 is likely mediated by the ET_B receptor. This conclusion is strongly supported by our finding that the selective ET_B receptor agonist ET-3 is a potent inducer of CXCL1 and CXCL8 secretion (see Fig 1). Similar results were obtained for ET-1-dependent secretion of CXCL8. As shown in Fig 2*A* (*right panel*) ET_A receptor antagonist BQ610 failed to inhibit ET-1-dependent secretion of CXCL8. As with CXCL1, whereas there was a slight suppression of CXCL8 secretion at baseline, BQ610 did not significantly alter the average fold induction of CXCL8 secretion by ET-1 (approximately 2.6 $\times$ either with or without BQ610). Once again, as shown in Fig 2*A* (*right panel*) BQ788 completely blocked the ability of ET-1 to induce CXCL8 secretion. We therefore conclude that ET-1-dependent secretion of CXCL8 is also mediated by the ET_B receptor.

ET-3 induces CXCL1 secretion in primary human melanocytes We next determined whether ET-1 and ET-3 induced CXCL1 and CXCL8 secretion in primary human melanocytes. Three different melanocyte cultures derived from lightly pigmented, medium pigmented, and darkly pigmented individuals were stimulated with 10 nM ET-1 and 10 nM ET-3 over a 40 h time course. Conditioned medium was collected and used for CXCL1 and CXCL8 ELISA. As shown in Fig 2*B*, ET-1 did not induce secretion of CXCL1 to any significant degree in the three cultures tested. ET-3, however, did induce modest levels of CXCL1 secretion in all three cultures. This effect was more evident in the cultures derived from medium and darkly pigmented individuals (Fig 2*B*, *middle and right panels*, respectively). The average fold induction of CXCL1 secretion by ET-3 in these cultures was approximately 1.8 $\times$ as compared with approximately 2.5 $\times$ in SKMEL28 human melanoma cells (Fig 1*A*). Neither ET-1 nor ET-3 induced CXCL8 secretion in melanocytes (data not shown), suggesting that this response may be specific for cells that have undergone malignant transformation. In addition, baseline levels of both CXCL1 and CXCL8 secretion in human primary melanocytes were much lower than those observed in melanoma cells (see Figs 1 and 2).

Discussion

We have demonstrated that both ET-1 and ET-3 induce the secretion of CXCL1 and CXCL8 proteins in human melanoma cells. This induction occurs within hours of ET-1

exposure and remains elevated over an extended time course. CXCL1 and CXCL8 secretion can be blocked by the selective ET_B antagonist BQ788 but not by the selective ET_A antagonist BQ610. BQ610 and BQ788 are cyclic peptides that function as competitive inhibitors of the ET_A and ET_B receptors, respectively (Huggins and Pelton, 1997). They are routinely used in the endothelin literature to aid in the dissection of endothelin-dependent pathways (Huggins and Pelton, 1997; Lahav *et al*, 1999; Bohm *et al*, 2002). Although the use of these inhibitors does not allow for elucidation of the exact mechanism for ET-1-dependent chemokine secretion, our results clearly demonstrate that activation of the ET_B receptor is critical for ET-1-dependent induction of CXCL1 and CXCL8 secretion by melanoma cells. The fact that the selective ET_B agonist ET-3 also induces CXCL1 and CXCL8 secretion reinforces these data. ET-1-dependent induction of CXCL1 and CXCL8 secretion is concentration dependent and can occur with doses as low as 0.1 nM. This suggests that the response is because of ET-1 addition to the cells and not because of an *in vitro* artifact. The fact that the response can be specifically inhibited by ET_B receptor blockade and not by ET_A receptor blockade also indicates that the response is specific for ET-1 stimulation and activation of the ET_B receptor.

Although ET-3 induced a mild induction of CXCL1 secretion in two of three melanocyte cultures tested, ET-1 did not induce either CXCL1 or CXCL8 secretion in primary human melanocytes. The same result was previously demonstrated by Mockenhaupt *et al* (2003). In this study, ET-1-dependent secretion of CXCL1 and CXCL8 in melanocytes required the additional presence of basic fibroblast growth factor (bFGF) and/or α-melanocyte stimulating hormone (Mockenhaupt *et al*, 2003). Our results suggest that in melanoma cells, this requirement for other factors has been abrogated and that ET-1 stimulation alone is sufficient for CXCL1 and CXCL8 induction of secretion. It is well known that unlike melanocytes, nevus cells and melanoma cells produce endogenous bFGF, and this may account for the ability of ET-1 to induce secretion of CXCL1 and CXCL8 in melanoma cells without the need for bFGF addition (Mancianti *et al*, 1993). Most melanocytes express both the ET_A and ET_B receptors (Eberle, 1999). Stimulation of melanocytes with ET-1, which binds with equal affinity to both receptors (Huggins and Pelton, 1997), activates both receptors simultaneously. In contrast, stimulation with ET-3, a selective ET_B agonist, would only activate the ET_B receptor in these cells. The fact that ET-3, and not ET-1, induced CXCL1 secretion in melanocytes may be because of cross-talk from the ET_A receptor that is inhibitory to ET_B signaling. Most melanoma cells, however, including SKMEL28 cells, do not express the ET_A receptor (Eberle, 1999); thus, there should be no difference between responses elicited by ET-1 and ET-3, and this is what our data reflect. We have demonstrated previously that ET_A receptor activation likely inhibits ET-1-dependent upregulation of the pro-metastatic adhesion molecule MCAM, whereas ET-3 circumvents this inhibition and induces MCAM with a much greater relative efficacy (Mangahas *et al*, 2004). Our future studies will investigate the mechanisms by which ET-1 induces CXCL1 and CXCL8.

Since CXCL1 and CXCL8 induce migration, metastasis, and invasion of melanoma cells (Payne and Cornelius, 2002), any factor capable of upregulating these chemokines should have a similar effect upon cells. Indeed, ET-1 is known to have a pro-migratory effect on melanoma cells and also induces melanoma cell invasion *in vitro* (Bagnato *et al*, 2004). These pro-migratory and pro-invasive responses require activation of the ET_B receptor (Bagnato *et al*, 2004). Since ET-1-dependent secretion of CXCL1 and CXCL8 also requires ET_B receptor activation, it is possible that CXCL1 and CXCL8 play a critical role in ET-1-dependent melanoma cell migration and invasion. Under normal conditions, melanocytes are transiently stimulated by ET-1 of keratinocyte origin when the epidermis is exposed to UV irradiation (Hara *et al*, 1995). Unlike melanocytes, however, melanoma cells secrete IL-1 and TNF-α, both of which induce ET-1 secretion by epidermal keratinocytes (Moretti *et al*, 1999). This may initiate an abnormal and persistent signaling loop within the epidermal microenvironment that results in the constitutive stimulation of melanoma cells with ET-1. Given our data, we hypothesize that such a stimulation may result in CXCL1 and CXCL8 secretion by melanoma cells, with a concomitant increase in melanoma invasion and metastasis via an autocrine signaling loop. ET-1 plays an important role in a variety of different types of cancers including prostate, lung, and colon cancer (Jamal, 2000; Jamal and Schneider, 2002; Bagnato *et al*, 2004). Overall, this study provides evidence that the ET-1/ET_B pathway may also play an important role in melanoma progression through the induction of key CXC chemokines, and identifies this pathway as a potential target for therapeutic intervention.

Materials and Methods

Cells and cell culture SKMEL28 cells, M20 cells, and WM2664 cells were cultured as described previously (Jamal and Schneider, 2002). Endothelin stimulations were carried out in melanoma growth medium (MCDB153, insulin 5 μg per mL, 1% penicillin–streptomycin solution; Invitrogen Life Technologies, Carlsbad, California) supplemented with 2% heat-inactivated fetal bovine serum (Cellgro Mediatech, Herndon, California). ET-1 and ET-3 were purchased from Bachem Bioscience (catalog # H-7625 and H-9025, respectively, King of Prussia, Pennsylvania). BQ610 and BQ788 (Bachem Bioscience) were added to cells at a final concentration of 1 μM 1 h prior to ET-1 addition. All other cell culture reagents were urchased from Sigma-Aldrich (St Louis, Missouri).

ELISA Cells were stimulated with ET-1 with or without BQ610 and BQ788 for 48 h and then conditioned medium was collected and used for ELISA. CXCL1 ELISA were performed using the Gro-1, ELISA kit (RND systems). CXCL8 ELISA were performed using the IL-8 ELISA kit (RND systems, Minneapolis, Minnesota).

Statistical analyses Hypotheses of association of CXCL1 and CXCL8 with time and with concentration of ET-1 were tested using linear regression. All p-values are two sided.

DOI: 10.1111/j.0022-202X.2005.23820.x

Manuscript received June 21, 2004; revised March 21, 2005; accepted for publication March 22, 2005

Address correspondence to: Sumayah Jamal, Department of Dermatology, New York University School of Medicine, 550 First Ave, Rm. TH100, New York, New York, USA. Email: sumayah.jamal@med.nyu.edu

References

Bagnato A, Rosano L, Spinella F, Di Castro V, Tecce R, Natali PG: Endothelin B receptor blockade inhibits dynamics of cell interactions and communications in melanoma cell progression. Cancer Res 64:1436–1443, 2004

Balentien E, Mufson BE, Shattuck RL, Derynck R, Richmond A: Effects of MGSA/GRO alpha on melanocyte transformation. Oncogene 6: 1115–1124, 1991

Baynash AG, Hosoda K, Giaid A, Richardson JA, Emoto N, Hammer RE, Yanagisawa M: Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. Cell 79:1277–1285, 1994

Belperio JA, Keane MP, Arenberg DA, Addison CL, Ehlert JE, Burdick MD, Strieter RM: CXC chemokines in angiogenesis. J Leukoc Biol 68:1–8, 2000

Bohm F, Ahlborg G, Johansson BL, Hansson LO, Pernow J: Combined endothelin receptor blockade evokes enhanced vasodilatation in patients with atherosclerosis. Arterioscler Thromb Vasc Biol 22:674–679, 2002

Bordoni R, Fine R, Murray D, Richmond A: Characterization of the role of melanoma growth stimulatory activity (MGSA) in the growth of normal melanocytes, nevocytes, and malignant melanocytes. J Cell Biochem 44:207–219, 1990

Eberle J: Downregulation of endothelin B receptor in human melanoma cell lines parallel to differentiation genes. J Invest Dermatol 112:925–933, 1999

Gutman M, Singh RK, Xie K, Bucana CD, Fidler IJ: Regulation of interleukin-8 expression in human melanoma cells by the organ environment. Cancer Res 55:2470–2475, 1995

Hara M, Yaar M, Gilchrest BA: Endothelin-1 of keratinocyte origin is a mediator of melanocyte dendricity. J Invest Dermatol 105:744–748, 1995

Huggins JP, Pelton JT: Endothelins in Biology and Medicine. Boca Raton, FL: CRC Press Inc., 1997

Imokawa G, Miyagishi M, Yada Y: Endothelin-1 as a new melanogen: Coordinated expression of its gene and the tyrosinase gene in UVB-exposed human epidermis. J Invest Dermatol 105:32–37, 1995

Imokawa G, Yada Y, Kimura M: Signalling mechanisms of endothelin-induced mitogenesis and melanogenesis in human melanocytes. Biochem J 314:305–312, 1996

Jamal S: Endothelin-1 down-regulates E-cadherin in melanocytic cells by apoptosis-independent activation of caspase-8. J Am Acad Dermatol 43:703–704, 2000

Jamal S, Schneider RJ: UV-induction of keratinocyte endothelin-1 downregulates E-cadherin in melanocytes and melanoma cells. J Clin Invest 110:443–452, 2002

Lahav R, Heffner G, Patterson PH: An endothelin receptor B antagonist inhibits growth and induces cell death in human melanoma cells *in vitro* and *in vivo*. Proc Natl Acad Sci USA 96:11496–11500, 1999

Lawson DH, Thomas HG, Roy RG, Gordon DS, Chawla RK, Nixon DW, Richmond A: Preparation of a monoclonal antibody to a melanoma growth-stimulatory activity released into serum-free culture medium by Hs0294 malignant melanoma cells. J Cell Biochem 34:169–185, 1987

Mancianti ML, Gyorfi T, Shih IM, *et al*: Growth regulation of cultured human nevus cells. J Invest Dermatol 100:281S–287S, 1993

Mangahas CR, de la Cruz G, Jamal S: Endothelin-1 upregulates MCAM in melanocytes. J Invest Dermatol 123:1135–1139, 2004

Mockenhaupt M, Peters F, Schwenk-Davoine I, Herouy Y, Schraufstatter I, Elsner P, Norgauer J: Evidence of involvement of CXC-chemokines in proliferation of cultivated human melanocytes. Int J Mol Med 12:597–601, 2003

Mohler T, Scheibenbogen C, Hafele J, Willhauck M, Keilholz U: Regulation of interleukin-8 mRNA expression and protein secretion in a melanoma cell line by tumour necrosis factor-alpha and interferon-gamma. Melanoma Res 6:307–311, 1996

Moretti S, Pinzi C, Spallanzani A, *et al*: Immunohistochemical evidence of cytokine networks during progression of human melanocytic lesions. Int J Cancer 84:160–168, 1999

Norgauer J, Metzner B, Schraufstatter I: Expression and growth-promoting function of the IL-8 receptor beta in human melanoma cells. J Immunol 156:1132–1137, 1996

Payne AS, Cornelius LA: The role of chemokines in melanoma tumor growth and metastasis. J Invest Dermatol 118:915–922, 2002

Richmond A, Balentien E, Thomas HG, *et al*: Molecular characterization and chromosomal mapping of melanoma growth stimulatory activity, a growth factor structurally related to beta-thromboglobulin. EMBO J 7:2025–2033, 1988

Richmond A, Lawson DH, Nixon DW, Chawla RK: Characterization of autostimulatory and transforming growth factors from human melanoma cells. Cancer Res 45:6390–6394, 1985

Richmond A, Thomas HG: Purification of melanoma growth stimulatory activity. J Cell Physiol 129:375–384, 1986

Shattuck-Brandt RL, Wood LD, Richmond A: Identification and characterization of an MGSA/GRO pseudogene. DNA Seq 7:379–386, 1997

Singh RK, Gutman M, Radinsky R, Bucana CD, Fidler IJ: Expression of interleukin 8 correlates with the metastatic potential of human melanoma cells in nude mice. Cancer Res 54:3242–3247, 1994

Singh RK, Gutman M, Reich R, Bar-Eli M: Ultraviolet B irradiation promotes tumorigenic and metastatic properties in primary cutaneous melanoma via induction of interleukin 8. Cancer Res 55:3669–3674, 1995

Strieter RM, Polverini PJ, Kunkel SL, *et al*: The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem 270:27348–27357, 1995

Tada A, Suzuki I, Im S, *et al*: Endothelin-1 is a paracrine growth factor that modulates melanogenesis of human melanocytes and participates in their responses to ultraviolet radiation. Cell Growth Differ 9:575–584, 1998

Yohn JJ, Smith C, Stevens T, *et al*: Human melanoma cells express functional endothelin-1 receptors. Biochem Biophys Res Commun 201:449–457, 1994