Biochemical and Biophysical Research Communications **275,** 60–62 (2000)
doi:10.1006/bbrc.2000.3241, available online at http://www.idealibrary.com on IDEAL®

Cancer Cell-Derived Interleukin 1α Contributes to Autocrine and Paracrine Induction of Pro-metastatic Genes in Breast Cancer

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Received July 3, 2000

Invasion and metastasis of cancer cells is a complex process requiring the activity of proteins that promote extracellular matrix degradation, motility of cancer cells, and angiogenesis. Although exclusively the cancer cells make several of these proteins, few key proteins are derived from stromal cells in response to cancer cell–stromal cell interaction. In this report, we show that the breast cancer cell-derived interleukin-1alpha (IL-1α) plays an important role in expression of pro-metastatic genes in cancer as well as in stromal cells. Neutralizing antibody against IL-1α inhibited IL-6, and IL-8 expression in IL-1α-expressing cancer cells. In addition, this antibody also prevented induction of IL-6, IL-8, and matrix metalloproteinase 3 (MMP3) but not vascular endothelial growth factor (VEGF) in fibroblasts by conditioned medium (CM) from IL-1α-expressing breast cancer cells. These results suggest that inhibition of IL-1α activity by either neutralizing antibody against IL-1α or chemical inhibitor of IL-1α processing may prevent invasion and metastasis of breast cancer.

Key Words: **breast cancer; metastasis; interleukin-1α; nuclear factor-κB.**

Several cytokines and growth factors play an important part in cancer progression [1–4]. These cytokines and growth factors are the principal mediators of cancer cells–stromal cell interaction, which is critical for invasion of cancer cells to surrounding tissues and metastatic dissemination to distant organs. Cancer cell–stromal cell interactions trigger functional responses in endothelial and fibroblasts, local basement membrane dissociation, endothelial cell migration, and microvessel morphogenesis. Identification of growth factor/cytokines involved in this process as well as their functional targets in cancer and stromal cells is critical for developing anti-metastatic therapeutic agents.

The transcription factors that regulate the expression of pro-metastatic genes are the likely target of growth factors and cytokines. The transcription factor nuclear factor-kappaB (NF-κB) is an ideal candidate as it regulates the expression of pro-metastatic genes urokinase plasminogen activator (uPA), MMP9, integrin αV, pro-angiogenic gene IL-8, and pro-motility gene IL-6 [5–8]. In normal cells, NF-κB is maintained in the cytoplasm by protein–protein interaction with inhibitor-of-κB (IκB) [5]. Cytokines/growth factors such as IL-1, tumor necrosis factor α (TNFα), and leukemia inhibitory factor (LIF) activate kinases that phosphorylate IκBs [5]. NF-κB is released from phosphorylated IκBs, translocates to nucleus, and activates target genes. We and others have shown that the activity of NF-κB is constitutive in a subset of breast cancers [9–11]. Furthermore, we have shown that the conditioned medium (CM) from a subset of breast cancer cells induce NF-κB in fibroblasts, which is blocked by neutralizing antibody against IL-1α [12]. In this report, we show that cancer cell-derived IL-1α contributes to IL-6 and IL-8 expression in cancer cells, and IL-6, IL-8, and MMP3 expression in fibroblasts. These results suggest that inhibitors of IL-1α activity may prevent NF-κB activation and metastasis in breast cancer.

MATERIALS AND METHODS

Cell culture, collection of CM, and antibodies. All cell lines were purchased from American Type Culture Collection. The media were described previously [12]. Confluent cultures were washed in PBS and incubated with 10 ml serum-free MEM for 24 h. The medium

Abbreviations used: NF-κB, nuclear factor-kappaB; IL-1α, interleukin 1alpha; IL-6, interleukin 6; IL-8, interleukin 8; uPA, urokinase plasminogen activator; MMP3, matrix metalloproteinase 3; VEGF, vascular endothelial growth factor; LIF, leukemia inhibitory factor.

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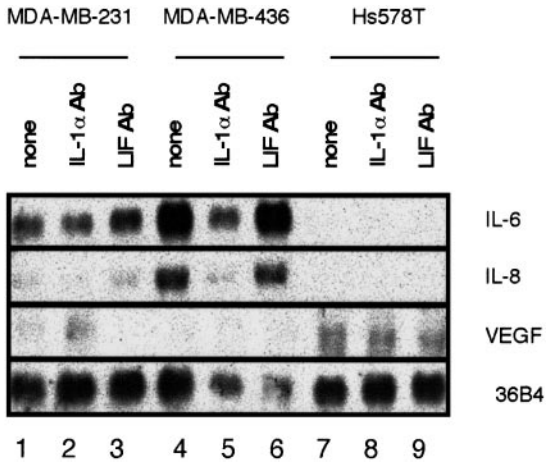

FIG. 1. IL-1α is responsible for expression of IL-6 and IL-8 in breast cancer cells. Neutralizing antibody against IL-1α prevents IL-6 and IL-8 expression in breast cancer cells. 15 μg of total RNA from breast cancer cells incubated with serum-free medium for 48 h and indicated antibodies (3 μg/ml) for 6 h was subjected to Northern blotting with indicated probes. Integrity RNA was examined by reprobing the blot with the ribosomal protein gene 36B4.

was collected after 24 h and used for treating human lung fibroblasts (HLF-1) (80% confluent) [12]. All antibodies were purchased from R & D Systems, Minneapolis, MN.

Northern blotting. Total RNA was prepared by RNeasy kit (Qiagen) or guanidinium isothiocyanate/ceseium chloride centrifugation method [12]. RNA from breast cancer cells incubated with antibodies for 6 h or fibroblasts treated with CM ± antibodies for 4 h was electrophoresed on an agarose-formaldehyde gel and subjected to Northern blot analysis as described previously [12].

RESULTS

To study the role of IL-1α in autocrine induction of IL-6 and IL-8 in breast cancer cells, we studied the effect of neutralizing antibody against IL-1α on two cell lines that express IL-1α (MDA-MB-231 and MDA-MB-436) and a cell line that do not express IL-1α (Hs578T) [12]. Cells were maintained in serum-free medium for 48 h and incubated with neutralizing antibody against either IL-1α or LIF for 6 h. Antibody against IL-1α but not LIF inhibited IL-6 expression in MDA-MB-231 and MDA-MB-436 cells (Fig. 1). Antibody against IL-1α also inhibited IL-8 expression in MDA-MB-436 cells. Neither antibodies inhibited vascular endothelial growth factor (VEGF) expression in Hs578T cells. These results suggest that autocrine activity of IL-1α is responsible for IL-6 and IL-8 expression in breast cancer cells.

To determine the paracrine activity of breast cancer cell-derived IL-1α, we incubated fibroblasts with CM from MDA-MB-231 cells that have been pretreated with either IL-1α antibody or LIF antibody. CM induced IL-6, IL-8, MMP3, and VEGF in fibroblasts (Fig. 2, lane 2). Induction of IL-6, IL-8, and MMP-3 but not VEGF was inhibited by IL-1α antibody (lanes 3 and 4). In contrast, LIF antibody had no effect on IL-6, IL-8, MMP3, and VEGF expression. These results suggest that breast cancer cell-derived IL-1α contributes to IL-6, IL-8, and MMP3 expression in fibroblasts. Furthermore, an unknown factor(s) from cancer cells induces VEGF in fibroblasts.

DISCUSSION

In this report, we show that breast cancer cell-derived IL-1α promotes cancer cell growth and metastasis by altering gene expression in cancer cells as well as stromal cells by autocrine and paracrine mechanism. IL-1α specifically targets the pro-metastatic genes IL-6, IL-8, and MMP3. We propose that IL-1α induces NF-κB activity in cancer cells as well as fibroblasts, which leads to activation of IL-6 and IL-8. At present, it is not known whether NF-κB is involved in induction of MMP3. However, MMP3 promoter contains AP-1 binding site, which can be activated by IL-1α [13]

There is considerable evidence for overexpression of IL-1α, IL-6, IL-8, and MMP3 in cancers including breast cancers. Also, in most cases, overexpression correlates with poor prognosis. For example, increased serum IL-6 correlates with tumor progression, resistance to chemo-endocrine therapy and reduced survival rate due to extensive metastasis and pleural ef-

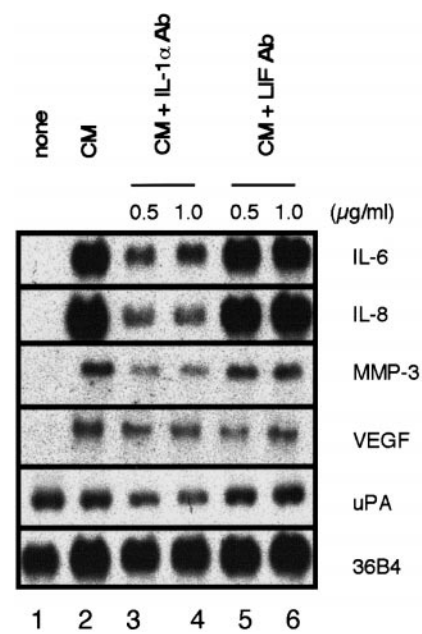

FIG. 2. IL-1α is responsible for induction of IL-6, IL-8, and MMP3 in fibroblasts by CM from MDA-MB-231. MDA-MB-231 CM was incubated with neutralizing antibodies against IL-1α or LIF at indicated concentration (μg/ml) for 1 h at room temperature. Fibroblasts were incubated with CM for 4 h and RNA was analyzed as in FIG. 1.

fusion in breast cancer [14]. IL-6 may also increase the motility of cancer cells and cause cancer-associated cachexia [14, 15]. Approximately 48% of breast cancer patients have elevated IL-6 [14]. IL-8, whose overexpression correlates with poor prognosis in melanomas, increases angiogenesis, thereby allowing metastatic cells to colonize [16, 17]. The stromal expression of MMP3 is essential for the establishment of invasive mesenchymal-like mammary tumors [18]. Inhibition of IL-6, IL-8, and MMP3 activity, therefore, may be an ideal approach to reduce breast cancer metastasis. Our studies raise the possibility that tumor cell-derived IL-1α is responsible for elevated IL-6, IL-8, and MMP3 in the tumor microenvironment. Consistent with this possibility, IL-1α is overexpressed in invasive-ductal but not benign primary breast cancers. Inhibition of IL-1α activity may be an efficient way of preventing breast cancer metastasis. IL-1α activity can be inhibited by neutralizing antibodies, as shown in this study, or by chemical inhibitor that prevent processing of proIL-1α to mature IL-1α [19]. Because IL-1α is released either after cell death or by transformed cells, specific inhibition of this cytokine is less likely to be harmful [20]. Moreover, unlike IL-1β knockout animals, IL-1α knockout mice are healthy and have normal febrile-neuro-endocrine response [21]. Therefore, we propose that IL-1α should be considered as a therapeutic target in breast cancer.

ACKNOWLEDGMENTS

We thank J. R. Dunn and P. Bhat-Nakshatri for technical assistance. This work is supported by the grants from Catherine Peachy Fund, Inc. (G.S.W. and H.N.), Breast Cancer Research Foundation of America (to G. W. S), and National Institutes of Health Grant CA82208-01A1 (to H.N.).

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