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Production and regulation of interleukin-11 by breast cancer cells

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Abstract

We have studied the production of interleukin-11 (Il-11) in 13 breast cancer cell (BCC) lines. Two of these cell lines (MDA-MB-231 and Hs578T) expressed the cytokine at both the protein and mRNA levels. Il-11 did not modulate the growth of five BCC lines examined, including the two cytokine-producing BCC lines. The production of Il-11 was increased by transforming growth factor-β_1 in a dose-dependent manner with a rapid (2 h) and transient (24 h) mRNA induction, but not by epidermal growth factor, insulin-like growth factor-I and -II, basic fibroblast growth factor, platelet-derived growth factor or parathyroid hormone. The cyclic AMP inducer, forskolin, and the activator of protein kinase C, phorbol 12-myristate 13-acetate, also stimulated the production of Il-11. Besides Il-11, MDA-MB-231 and Hs578T were the only BCC lines to produce interleukin-6 (Il-6) protein and mRNA. Since Il-11 and Il-6 are potent stimulators of osteoclast development and bone is a major source of TGF-β_1, our data suggest that Il-11, together with Il-6, contributes to the high bone destructive capacity of MDA-MB-231 cells and could play a role in breast cancer-induced osteolysis. Published by Elsevier Science Ireland Ltd.

Keywords: Interleukin-11; Interleukin-6; Breast cancer cells; TGF-β; Bone metastases

1. Introduction

Interleukin-11 (Il-11) is a pleiotropic cytokine that was first identified in supernatants from a primate bone marrow stromal cell line. Like interleukin-6 (Il-6), Il-11 stimulates acute phase protein production, platelet production, B-cell activation and hematopoiesis [1]. Bone tissue is also affected by Il-11, which decreases osteoblast activity [2] and is an important stimulator of osteoclast development [3].

Il-11 is produced by cells of various origin [1], but its synthesis by breast cancer cell (BCC) lines has not been reported. BCC lines, when metastasizing, colonize bone marrow and frequently lead to osteolysis [4], mainly by increasing the number of osteoclasts [5]. This background led us to hypothesize that BCC could produce Il-11. We looked for a regulation of Il-11 production by several growth factors which are particularly abundant in bone matrix. We also examined Il-6 production by the same BCC lines since Il-6

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is another cytokine which increases osteoclast formation [6].

2. Materials and methods

2.1. Materials

Epidermal growth factor (EGF), forskolin and phorbol 12-myristate 13-acetate were from Sigma (St. Louis, MO), parathyroid hormone was from Bissendorf Biochemicals (Hanover, Germany) and basic fibroblast growth factor (bFGF), insulin-like growth factor-I and -II (IGF-I and -II), platelet-derived growth factor (PDGF), transforming growth factor-β_1 (TGF-β_1) and Il-11 were from R&D Systems (Abingdon, UK). All factors were used within 3 months following preparation and stored at −70°C.

2.2. Cell lines

All BCC lines were from American Tissue Culture Collection, except for IBEP-1, IBEP-2 and IBEP-3, which were obtained in our laboratory [7]. Cells were cultured in DMEM medium (GIBCO, Ghent, Belgium) supplemented with 2 mM L-glutamine, 2% penicillin–streptomycin (10 000 U/ml) and 5% heat-inactivated fetal calf serum (GIBCO) in a humidified atmosphere of 95% air/5% CO_2 at 37°C.

2.3. Quantification of Il-6 and Il-11

Il-6 and Il-11 levels were measured by enzyme-linked immunosorbent assays (ELISA) using Quantikine kits from R&D Systems. The lowest detectable values were 3.1 and 15.6 pg/ml for Il-6 and Il-11, respectively. Determinations were made in 1% FCS-containing culture medium incubated for 24 h with BCC and then centrifuged for 10 min at $2500 \times g$ and kept at −20°C for no more than 1 month before cytokine determination. Values are expressed as mean ± SD.

2.4. mRNA isolation and analysis

Total RNA was extracted with TriZol (BIOTECX Laboratories, Houston, TX) and 15 μg was separated on a 0.9% agarose gel in 2.2% formaldehyde, 0.02 M 3-(*N*-morpholino) propane sulfonic acid (MOPS), 5 mM sodium acetate and 1 mM EDTA before transfer onto a nylon membrane (Hybond-N, Amersham). Prehybridization, hybridization and autoradiography were performed as previously described [8]. The Il-11 probe was a 1250 bp cDNA obtained from Genetics Institute and the Il-6 probe was a 500 bp cDNA provided by Dr Droogmans (Rhode St. Genèse, Belgium). Control DNA was a 29-mer oligonucleotide specific for the 28S ribosomal RNA (Clontech, Palo Alto, CA). All probes were labeled by random-priming to specific activities higher than 10^9 cpm/μg DNA.

2.5. Cell proliferation

BCC were seeded at 25 000 cells/well in 24-well plates. After 72 h, the FCS concentration was lowered to 1% and Il-11 (1 nM) was added for 48 or 96 h. Cells were labeled with 0.5 μCi of 6-[^{3}H]thymidine/well during the last 18 h of culture and DNA synthesis was measured as previously described [9]. Values are expressed as mean ± SD.

3. Results

3.1. Il-11 production by BCC lines

Thirteen BCC lines were tested for Il-11 production. Ten of these (BT-20, BT-483, CAMA-1, Hs578T, MCF-7, MDA-MB-231, MDA-MB-361, SK-Br-3, T-47D and ZR-75-B) are widely used BCC lines; IBEP-1, -2 and -3 have been isolated from pleural effusions and characterized in our laboratory as mammary adenocarcinoma cells [7]. The BCC lines were distributed along a spectrum of differentiation from epithelial to fibroblastic phenotypes [10]. We found that Il-11 was produced by MDA-MB-231 (0.81 ± 0.10 ng/10^6 cells in 24 h, $n = 17$) and Hs578T (0.64 ± 0.06 ng/10^6 cells in 24 h, $n = 8$). The cytokine could not be detected in the culture medium of any other BCC line.

Similarly, as shown in Fig. 1, Il-11 mRNA was expressed by MDA-MB-231 and Hs578T cells but not by other BCC lines. The sizes of the transcripts (2.5 and ~1.5 kb) were similar to those observed in human fibroblasts and osteoblasts [11,12]. Although the shorter transcript was more abundant than the lar-

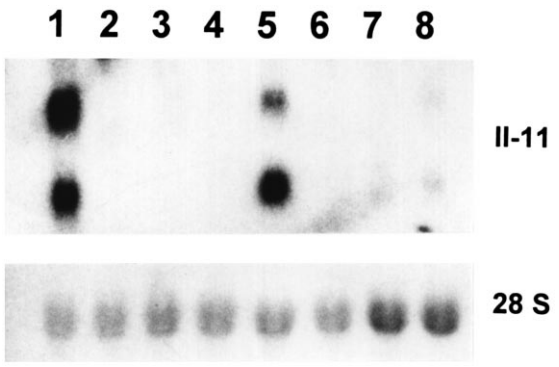

Fig. 1. Il-11 mRNA expression in BCC lines. Total RNA was successively hybridized with an Il-11 cDNA and a control 28S rRNA oligonucleotide. Lane 1, Hs578T; lane 2, IBEP-1; lane 3, IBEP-2; lane 4, IBEP-3; lane 5, MDA-MB-231; lane 6, MCF-7; lane 7, T-47D; lane 8, SaOS-2 osteosarcoma cell line, known to express Il-11 mRNA [12]. Despite long (>1 week) exposure, Il-11 mRNA signals were undetectable in BCC other than MDA-MB-231 and Hs578T cells. A weak signal was observed in SaOS-2 cells.

ger one in MDA-MB-231 cells, this was not the case for Hs578T cells.

3.2. Effects of Il-11 on BCC proliferation

Since interleukin-1 (Il-1) and Il-6 have been reported to inhibit BCC growth [13], we tested whether Il-11 altered the proliferation of our cell lines. Il-11 (1 nM) was incubated with MCF-7, T-47D, BT-20, MDA-MB-231 and Hs578T cells for 2 or 4 days. As shown in Table 1, the cytokine was, however, devoid of any significant effect on DNA synthesis in these cells.

3.3. Modulation of Il-11 production by BCC lines

We then tested whether Il-11 production by MDA-MB-231 and Hs578T cells was modulated by growth factors present in the bone matrix. As shown in Fig. 2, TGF-β_1 increased Il-11 production by these cells in a dose-dependent manner, with a much greater sensitivity for the MDA-MB-231 cell line. In contrast to TGF-β_1, EGF (10 ng/ml), bFGF (10 nM), IGF-I and -II (10 nM each) and PDGF (10 nM) were unable to significantly increase Il-11 production by the two cell lines, despite the use of concentrations higher than those known to produce most of their effects. Parathyroid hormone (10^{-8} M) had no effect on Il-11 production by the BCC lines (data not shown). Fig. 3 shows that Il-11 mRNA induction by TGF-β_1 in MDA-MB-231 cells was rapid and transient, with a maximum effect after 2 h. At 24 h, Il-11 mRNA signals had returned to basal levels (data not shown). No mRNA level variation was seen with other growth factors.

The cAMP-dependent protein kinase (PKA) and the protein kinase C (PKC) are important intracellular mediators of signals received by the cells. We have studied the response of MDA-MB-231 cells to the cAMP agonist forskolin (10^{-5} M) and the PKC activator phorbol 12-myristate 13-acetate (PMA, 100 ng/ml). Both agents induced a marked increase in Il-11 production (unstimulated cells 0.48 ± 0.05 ng Il-11/10^6 cells in 24 h, +forskolin 9.18 ± 0.46 ng Il-11/10^6 cells in 24 h, +PMA 12.55 ± 1.67 ng Il-11/10^6 cells in 24 h, $n = 3$, $P < 0.001$). In addition, these agents increased Il-11 mRNA levels for more than 24 h (data not shown).

Table 1
Lack of effect of Il-11 on BCC proliferation

	MCF-7	T-47D	MDA-231	Hs578T	BT-20
Day 2					
Control	948 ± 42	1722 ± 51	566 ± 21	602 ± 10	ND
Il-11 (1 nM)	972 ± 39	1704 ± 44	599 ± 39	587 ± 23	ND
Day 4					
Control	1215 ± 45	2013 ± 59	761 ± 19	736 ± 38	846 ± 29
Il-11 (1 nM)	1187 ± 53	1917 ± 49	782 ± 27	756 ± 26	881 ± 33

ND, not determined.
BCC were cultured for 48 or 96 h with 1 nM Il-11 and labeled with 6-[^{3}H]thymidine for 24 h before the determination of DNA synthesis. Values given are disintegration per min/well ± SD.

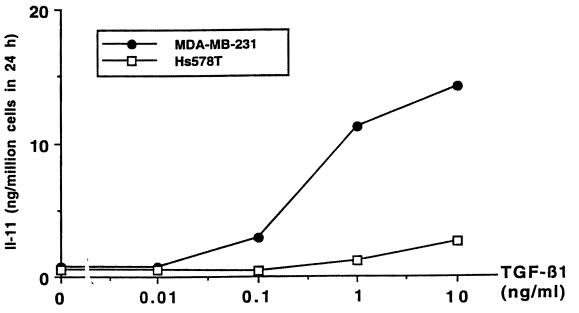

Fig. 2. TGF-β_1 increases Il-11 production in Hs578T and MDA-MB-231 cells. Standard deviations ($n = 3$) are included in the representation of the mean values and cannot be shown on the graph.

3.4. Il-6 production by BCC lines

Besides Il-11, we found that MDA-MB-231 and Hs578T cells produced Il-6 (between 2 and 2.5 ng/10^6 cells in 24 h), whereas Il-6 was undetectable in other BCC lines. A similar result was obtained at the mRNA level, since a ~1.3 kb signal was expressed in MDA-MB-231 and Hs578T. No signal was observed in other BCC lines (Fig. 4) despite the use of cycloheximide (CHX), which is known to upregulate Il-6 mRNA in several cell types [14]. Indeed, the Il-6 mRNA level was increased by CHX in MDA-MB-231 but not in Hs578T cells. Thus, within the 13 BCC lines examined, Il-6 and Il-11 were found to be coexpressed in MDA-MB-231 and Hs578T cells and they were not expressed in other cell lines. On the other hand, Il-6 production was increased by TGF-β_1 in Hs578T but not in MDA-MB-231 cells (data not shown).

4. Discussion

We report here that some BCC lines express both Il-11 and Il-6, which are potent activators of bone resorption. The BCC lines that we studied were heterogeneous regarding the tissue of origin (primary tumor, pleural effusion and ascites) and the histological type of the primary tumor (carcinosarcoma, adenocarcinoma and papillary infiltrating ductal carcinoma) [15]. Hs578T cells were obtained from a primary mammary carcinosarcoma, a rare form of breast cancer [16], while MDA-MB-231 cells, isolated from a pleural effusion, are classical adenocarcinoma cells [17]. However, MDA-MB-231 cells are closer to Hs578T cells than to the other cell lines used in the present work in a number of features, i.e. spindle-shape (fibroblastic) morphology on plastic [16,17], stellate morphology on matrigel and high invasiveness in vitro and lack of several desmosomal, adherens- and tight-junction markers contrasting with a high expression of vimentin [10]. Moreover, both BCC lines show a high production of TGF-β, prostaglandin E_2 and parathyroid hormone-related peptide (PTHrP) [18–20]. Our findings that only these two cell lines produced both Il-11 and Il-6 suggest that the production of the two cytokines by the Hs578T and MDA-MB-231 cells is associated with the acquisition of an aggressive dedifferentiated phenotype [10]. It has been proposed that a link might exist between poor differentiation of breast cancers and the constitutive activation of NF-κB, a nuclear factor involved in Il-6 expression [21]. Two nucleotide sequences resembling the NF-κB consensus motif are present in the Il-11 gene promoter; they are required for transcriptional induction of Il-11 by the respiratory syncytial virus in lung epithelial cells [22]. Further studies should evaluate the role of NF-κB in Il-11 expression by BCC.

A possible explanation for the inability of Il-11 to modulate BCC growth is that these cells lack functional Il-11 receptors. No extensive study of this receptor has been performed to date, except that RT-PCR analyses revealed the presence of a transcript for its α chain [23]. However, the presence of mRNA,

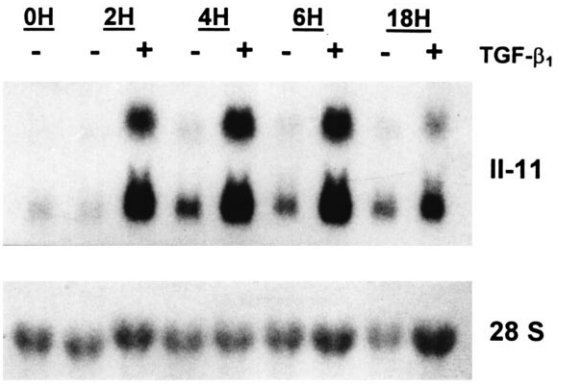

Fig. 3. Kinetics of Il-11 mRNA induction by TGF-β_1 in MDA-MB-231 cells. TGF-β_1 (10 ng/ml) induced a rapid and transient increase of both 1.5 and 2.5 kb Il-11 mRNA transcripts.

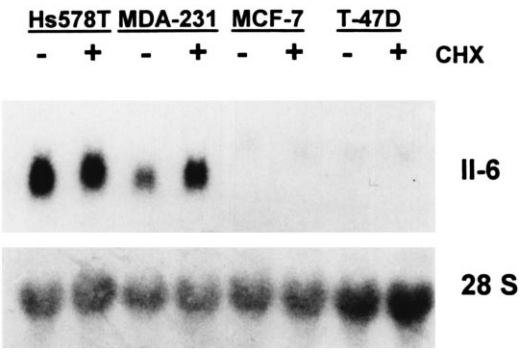

Fig. 4. Il-6 mRNA expression in BCC lines in the presence or absence of cycloheximide (CHX, 10 μg/ml given for 15 min before mRNA extraction [14]). Total RNA was hybridized with an Il-6 cDNA and a control 28S rRNA oligonucleotide.

especially in a low amount, does not guaranty the expression of enough receptor to significantly respond to Il-11. In any case, the lack of response of BCC to Il-11 does not appear to be due to the absence of the gp130 signal transduction critical component of the Il-11 receptor [24], since this protein is also a component of the Il-6 receptor and BCC responds to Il-6 [13].

The skeleton is the most common distant metastatic site for BCC [4]. MDA-MB-231 cells have been extensively used to study the pathogenesis of breast cancer-induced osteolysis in murine models [25,26]. Injection of MDA-MB-231 cells rapidly leads to dramatic bone destruction and it has been proposed that PTHrP is the main factor responsible for this process [20]. Our data suggest that Il-6 and Il-11, which are potent stimulators of bone resorption in vitro [6], could also contribute to the MDA-MB-231-induced osteolysis. In our model, the basal production of Il-11 was between 0.5 and 1 ng Il-11/10^6 cells in 24 h, corresponding to a concentration of about 25–50 pM. These Il-11 concentrations are definitely able to promote osteoclast development in vitro [3]. To our knowledge, the effects of cytokines released by Hs578T cells on bone have not yet been studied.

Skeletal colonization by breast tumor cells leads to increased bone resorption by osteoclasts, which frees space and allows the expansion of the metastatic foci. Moreover, the resorbed bone matrix releases factors attracting the BCC, such as TGF-β [27]. Our data clearly indicate that TGF-β_1, which is quite abundant in the bone matrix [28], markedly increased Il-11 production by MDA-MB-231 cells and, to a lesser extent, by Hs578T cells. Such a stimulatory effect of TGF-β on Il-11 production has been reported in fibroblasts and osteoblasts [11,12,29]. The stimulatory activity of Il-11 on osteoclast development, further enhanced by TGF-β release, might certainly contribute to increased osteoclast formation [3], therefore providing an important mechanism involved in accelerated bone destruction and invasion by breast tumor cells expressing the cytokine. Thus, Il-11 production could promote the development of the metastases through osteoclast formation and stimulation of bone resorption, rather than by a direct autocrine effect on BCC proliferation. This hypothesis deserves further investigation on the mechanisms of Il-11 expression and modulation by TGF-β in BCC. Moreover, future studies could include an analysis of TGF-β and Il-11 expression in surgical specimens from patients operated on for pathological fractures. Whether Il-11 expression in primary breast tumors could be a predictive factor for the development of skeletal metastases is another important issue.

It was previously reported that Il-6, which is expressed by normal mammary epithelial cells, is no longer detectable after cell transformation by various oncogenes (int-2, c-Ha-ras, c-erb-B2 and SV40 T-antigen) and is not produced by some BCC lines [30]. Such studies, however, did not include Hs578T and MDA-MB-231 cell lines. Since we found that these cells produce both Il-6 and Il-11, we hypothesize that some common molecular mechanism could induce the basal expression of the two corresponding genes. On the other hand, as shown by the data obtained with TGF-β_1, the modulation of this basic expression could be more specific to Il-6 or Il-11.

In conclusion, we have shown that Il-11 is abundantly produced by some BCC lines. Moreover, the strong stimulatory effect of TGF-β_1 on Il-11 production by these cell lines suggests a possible involvement of this cytokine in breast cancer-induced osteolysis.

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