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Report

Protein production by osteoblasts: modulation by breast cancer cell-derived factors

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Summary

Breast cancer cells (BCC) frequently metastasize to bone where they may cause tumor-induced osteolysis (TIO). While the important eroding role of the osteoclasts in TIO is well admitted, the possibility that BCC and/or osteoblasts activated by tumoral factors could also directly degrade bone matrix in this pathology has been much less investigated. We show here that the net collagen amount produced *in vitro* by normal human osteoblasts and osteoblast-like cells was significantly reduced by culture medium conditioned by several BCC lines, including three newly isolated ones. There was no evidence for a decrease in collagen synthesis, as assessed by the production of the carboxyterminal propeptide of type I collagen. In contrast, the effect of BCC-derived medium on collagen amount was attenuated by inhibitors of matrix metalloproteinases (MMPs) as well as by tranexamic acid, an inhibitor of the plasminogen conversion to plasmin, while it was abolished in presence of the two kinds of proteinase inhibitors. This osteoblastic protein degradation activity appeared to be attributable to factors secreted by the osteoblasts as well as by BCC. These factors had molecular weights lower as well as higher than 10 kD. Our data suggest that besides the eroding action of osteoclasts, BCC- and osteoblast-derived MMPs and serine proteinases might play a direct role in bone collagen degradation in TIO.

Introduction

Breast cancer cells (BCC) frequently metastasize to bone where they may cause extensive tumor-induced osteolysis (TIO). TIO is often characterized by a dramatic degradation of bone matrix, with little evidence for a normal repair, leading to the considerable morbidity of bone metastases [1]. Although its mechanism remains not yet fully understood, TIO is mainly attributed to the action of the bone-resorbing cells, the osteoclasts, whose number and activity are increased near the metastatic foci of BCC [2], presumably due to the action of one or more factor(s) secreted by cancer cells.

Several observations suggest that besides osteoclasts, osteoblasts and BCC could participate directly to the process of TIO: first, both cell types secrete various proteinases and BCC may erode bone slices *in vitro* [3]; secondly, the bone resorption in TIO is not compensated by an equal formation of a new bone matrix, as observed in normal skeletal homeostasis where osteoclastic and osteoblastic activities are balanced. This relative lack of bone matrix formation in TIO could result from a reduced anabolic activity of the osteoblasts and/or from an excessive, non-osteoclastic degradation of the bone matrix proteins; thirdly, the erosive action of the osteoclasts is thought to need a pre-digestion step of the collagen present at the resorption site. This may be performed in normal conditions by stromal or osteoblast-derived interstitial collagenase [4]. In the process of TIO, this pre-digestion step could be enhanced directly

by BCC-derived proteinases or indirectly through the stimulation of osteoblastic proteinases production by BCC-derived factors.

Since the direct participation of osteoblasts and BCC to the process of protein degradation in TIO remains poorly studied, we have determined whether culture medium conditioned by BCC lines might modulate the amount of proteins produced by osteoblastic cell lines or normal human osteoblasts. Our data indicate that secretory products of BCC reduce the amount of osteoblastic proteins, notably collagen, by a mechanism involving proteinases from the matrix metalloproteinases and plasminogen/plasmin families.

Materials and methods

Materials

Highly purified collagenase (MMP-1, Form III) was bought from Advanced Biofactures Corporation (ABC, Lynbrook, NY), ascorbic acid, phenylmethylsulfonylfluoride (PMSF), *N*-ethylmaleimide (NEM), Hepes, tranexamic acid (TrA) were from Sigma (St Louis, MO), L-[2,3,4,5-^{3}H] proline (Code TRK534) was from Amersham International, (Little Chalfont, UK), BB94 ('batimastat') and BB274 [5] were provided by British Biotechnologies Ltd (Cowley, Oxford, UK) and Ro31-9790 [6] was given by Roche Products Ltd (Welwyn Garden City, UK).

For each proteinase inhibitor (TrA, BB94, Ro31-9790), dose-response curves were obtained and the highest nontoxic concentrations were used in the experiments. Lack of toxicity on cells was assessed by Trypan Blue exclusion test and microscopic examination of cell detachment.

Cell cultures

Transformed but non-tumorigenic HBL-100 breast cells; T-47D, MDA-MB-231 breast adenocarcinoma cells; SaOS-2 and MG-63 osteosarcoma cells were all obtained from the American Type Culture Collection (Rockville, MD). IBEP-1, -2, and -3 breast adenocarcinoma cells were isolated and characterized in our laboratory [7]. Normal human osteoblasts were isolated and cultured as previously described [8]. All cell types were maintained 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 (FCS, Gibco), in a humidified atmosphere of 95% air-5% CO_2.

Preparation of conditioned medium

Cancer cells cultured up to confluency were incubated for 24 h in FCS-free DMEM. At the end of this period of time, the medium was collected, centrifuged for 10 min at $500 \times g$ and the supernatant was diluted with fresh DMEM to obtain a final volume of 1 ml medium per 500,000 conditioning cells. Control medium was obtained by incubating FCS-free DMEM on plastic. All medium were kept at -20°C until use.

Collagen measurement

We adapted the collagen assay method of Peterkofsky and Diegelmann [9]. Normal human osteoblasts and osteoblast-like cells were cultured in DMEM supplemented with 5% FCS in 24-wells plastic plates (Falcon) up to confluency. They then received 250 µl per well of FCS-free DMEM medium/conditioned medium (1:1) supplemented with 1 µCi L-[2,3,4,5-^{3}H] proline and 12.5 µg ascorbic acid. After 18 h of incubation, the medium was discarded and the cell layer was sonicated briefly (10", level 1, B-12 Sonifier, Branson, CT) in homogeneization buffer (PBS, PMSF 2 mM, NEM 6.3 mM, EDTA 25 mM, 1 mg/ml BSA). Proteins were precipitated and the pellets washed three times in 10% trichloroacetic acid (TCA). Traces of TCA were removed by two careful washes with ethanol/ether (3:1). The pellets were solubilized in 800 µl 0.12 M Hepes (pH 7.2), 0.5 mM $CaCl_2$, 2.5 mM NEM. 300 µl were incubated for 90 min at 37°C with 2U collagenase, while 300 µl were incubated without collagenase and served as control. After TCA precipitation, the supernatant (collagenase-degraded proteins or CDP) and the resolubilized pellet (non-collagenous proteins or NCP) were counted in separate scintillation vials.

Dosage of P1CP

The carboxyterminal propeptide of type I procollagen (P1CP) is released by cleavage from the biochemical precursor of type I collagen, the most abundant matrix protein in osteoblastic cells. Its measurement provides a direct information on the rate of type I collagen synthesis [10]. P1CP determinations were made using a specific radioimmunoassay from Orion Diagnostica (Espoo, Finland), according to the manufacturer's instructions.

Ultrafiltration of conditioned medium

Confitioned medium was ultrafiltrated (two successive steps) using Amicon YM10 membranes (cut-off: 10 kD, Amicon, Beverly, MA). The retentate (R) was diluted to reach initial volume with fresh culture medium.

Statistics

All collagen amounts values were corrected for cell number (expressed in thousands), measured as previously described [11] and they are given as means +/− SD. Statistical significance of the differences in mean values was assessed by two-tailed Student's *t*-test. A P-value < 0.05 was considered significant.

Results

Culture medium conditioned by T-47D BCC reduced the net protein amount in osteoblastic cells

We tested whether culture medium conditioned by the T-47D BCC line or by the 'control' HBL-100 non-tumorigenic breast cell line could modulate the net collagenous (CDP) and non-collagenous (NCP) protein amount produced by normal human osteoblasts (hOB) or by two osteoblast-like cell lines (SaOS-2 and MG-63). The upper panel of Figure 1 shows that the CDP amount was reduced in all types of osteoblastic cells only by culture medium conditioned by T-47D BCC. The observed CDP values were 77%, 74%, and 42% of controls, in hOB, MG-63, and SaOS-2, respectively (all P-values < 0.01). In SaOS-2, but not in MG-63 or hOB, the NCP amount was also significantly ($P < 0.05$) reduced to 71% of control values by the medium conditioned by T-47D BCC (Figure 1, lower panel), thus to a much lower extent than what was observed for the CDP amount.

The effects on SaOS-2 cells of culture medium conditioned by T-47D BCC were dose-dependent, since they progressively disappeared when the conditioned medium was diluted with increasing amounts of fresh RPMI (data not shown).

Effect of culture medium conditioned by various other breast cancer cell lines

Using the SaOS-2 cell line as the osteoblastic target, we extended our initial studies with T-47D BCC to another widely used BCC line, MDA-MB-231, and

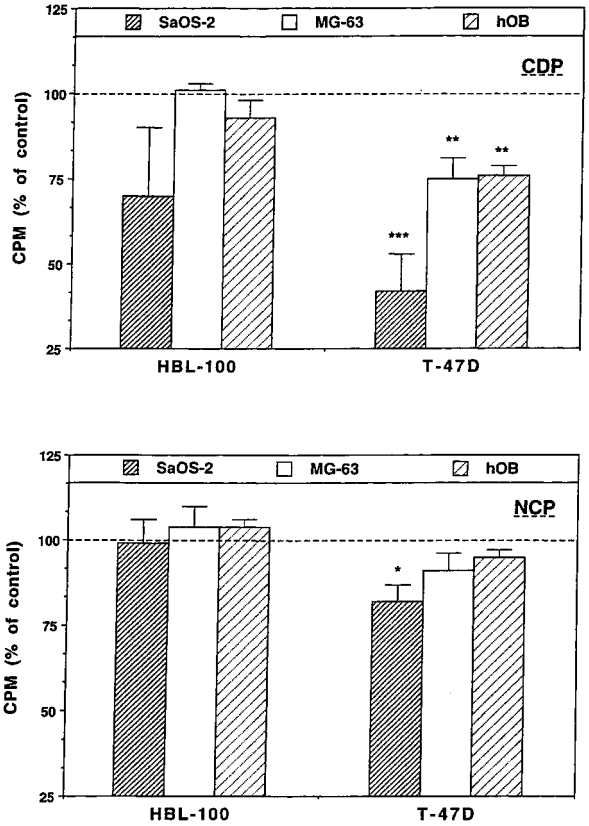

Figure 1. CDP (upper panel) and NCP (lower panel) amounts in two osteosarcoma cell lines (SaOS-2 and MG-63) and in normal human osteoblasts (hOB): modulation by culture medium conditioned by T-47D BCC or by control HBL-100 cells which are non-tumorigenic SV40-transformed mammary epithelial cells. Results are mean ± SD ($n = 3$) and are expressed as percentages of controls (osteoblastic cells treated with culture medium conditioned on plastic dish free of cancer cells). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$.

to three additional BCC lines isolated and characterized in our laboratory, IBEP-1, IBEP-2 and IBEP-3 [7]. Culture medium conditioned by all these lines decreased the net CDP amount in SaOS-2 cells to a variable extent. The medium conditioned by MDA-MB-231 cells was the most potent with a reduction of the CDP amount to 28% of the control value (Table 1). Except for the medium preincubated on IBEP-3 BCC, all conditioned medium also decreased the NCP amount in SaOS-2 cells. Contrasting with the data obtained using T-47D and MDA-MB-231 BCC, no specific degradation of collagenase-sensitive proteins was caused by the medium conditioned by IBEP-1 and IBEP-2 BCC; indeed, these cell lines led to a similar decrease in the NCP and the CDP amount produced by SaOS-2 cells.

Table 1. CDP and NCP amounts in SaOS-2 osteoblast-like cells: modulation by culture medium conditioned by various breast cancer cell lines

	CDP		NCP	
	cpm/million cells	% of control	cpm/million cells	% of control
Control	47.0 ± 1.8	100 ± 4	88.3 ± 2.5	100 ± 3
T-47D	21.8 ± 1.3	$46 \pm 3^{**}$	62.8 ± 1.7	$71 \pm 2^{**}$
MDA-231	13.0 ± 1.4	$28 \pm 3^{**}$	64.3 ± 1.0	$73 \pm 1^{**}$
IBEP-1	28.0 ± 0.8	$60 \pm 2^{**}$	53.5 ± 2.4	$61 \pm 3^{**}$
IBEP-2	38.0 ± 1.2	$81 \pm 2^{**}$	75.8 ± 5.5	$86 \pm 6^{*}$
IBEP-3	35.3 ± 1.7	$75 \pm 4^{**}$	84.0 ± 2.5	95 ± 3

Results (mean $\pm$ SD) are expressed as cpm/million cells and as percentages of control (SaOS-2 cells treated with culture medium preincubated on plastic dish free of cancer cells).
$^{*}P < 0.05$, $^{**}P < 0.005$.

Collagen synthesis was not reduced by culture medium conditioned by BCC

A reduction of the net protein amounts produced by cells may result from a decreased production as well as from an increased degradation of these proteins. As carboxyterminal propeptide of type I collagen (P1CP) release by cells is directly related to their production of type I collagen (the most abundant bone matrix protein), we assayed the P1CP amount produced by osteoblastic cells treated with medium conditioned by BCC. We did not observe any significant reduction of P1CP amount whether in hOB, MG-63 or SaOS-2 cells. Instead, in some cases, the medium conditioned by BCC caused an increase in the level of P1CP released by osteoblastic cells. For instance, while a P1CP value of 1.94 ± 0.48 ng/μg DNA was found in SaOS-2 cells treated with control medium, the values were 1.75 ± 0.49; 1.85 ± 0.35; 6.5 ± 0.14; 2.05 ± 0.07; 3.2 ± 0.71 ng/μg in SaOS-2 cells incubated with medium conditioned by T-47D; MDA-MB-231; IBEP-1; IBEP-2; IBEP-3 cells, respectively. Similar results were obtained using MG-63 cells and hOB as target cells instead of SaOS-2 cells (data not shown).

Proteinase inhibitors reduced the effects of medium conditioned by BCC on protein amounts in osteoblastic cells

Collagenous proteins may be specifically degraded by members of the matrix-metalloproteinase (MMP) family of enzymes. We tested whether the reduction of CDP amount in osteoblasts treated with culture medium conditioned by BCC could be attributable to the action of MMPs. As shown in Table 2, the addition of the MMP-specific [5] proteinase inhibitor BB94 ('batimastat', 10^{-3} M) to the culture medium conditioned by T-47D or MDA-MB-231 partly reduced their effects on the CDP, but not at all on the NCP produced by SaOS-2 cells. BB274 (10^{-3} M), an analogue of batimastat unable to inactivate MMPs, had no effect on the amount of proteins found in osteoblastic cells treated with conditioned medium. Similar results were obtained with another MMP inhibitor, Ro31-9790. Again, the effects of medium conditioned by BCC on osteoblastic CDP amounts were significantly reduced, while NCP amounts were not significantly affected in presence of 10^{-5} M Ro31-9790 (Table 2). Significant effects of batimastat and Ro31-9790 were already observed at concentrations 10 times lower (data not shown).

Plasmin is a serine proteinase whose involvement in cancer invasion and metastasis has been widely documented (for review, see [12]). The generation of plasmin by cleavage of plasminogen may be blocked by tranexamic acid (TrA) [13]. Table 2 shows that addition of TrA (10^{-4} M) to the medium conditioned by BCC reduced their effects on the CDP amounts and abolished their effects on NCP amounts in SaOS-2 cells.

Co-addition of BB94/batimastat and TrA to the medium conditioned by BCC abolished their modulation of CDP concentrations in osteoblastic cells (Table 2).

Since cathepsin D is found in several BCC lines including MDA-MB-231 cells [14], the aspartic proteinase inhibitor pepstatin was also tested, but revealed no significant inhibitory activity on the effects of medium conditioned by T-47D or MDA-MB-231 BCC (data not shown).

Table 2. Modulation of CDP and NCP amounts in SaOS-2 cells by conditioned medium: effect of proteinase inhibitors

		CDP (% of control)	NCP (% of control)
Control		100 ± 5	100 ± 6
T-47D		46 ± 4	78 ± 5
MDA-231		31 ± 2	83 ± 6
Control	+ BB94 (1 mM)	113 ± 5	98 ± 3
T-47D	+ BB94 (1 mM)	86 ± 4*	76 ± 2
MDA-231	+ BB94 (1 mM)	76 ± 8*	77 ± 3
Control	+ BB274 (1 mM)	94 ± 4	98 ± 2
T-47D	+ BB274 (1 mM)	46 ± 4	65 ± 3
MDA-231	+ BB274 (1 mM)	25 ± 3	77 ± 2
Control	+ Ro31-9790 (0.01 mM)	104 ± 7	102 ± 6
T-47D	+ Ro31-9790 (0.01 mM)	72 ± 2*	78 ± 4
MDA-231	+ Ro31-9790 (0.01 mM)	76 ± 5*	77 ± 6
Control	+ TrA (0.1 mM)	108 ± 7	108 ± 10
T-47D	+ TrA (0.1 mM)	90 ± 8*	105 ± 8*
MDA-231	+ TrA (0.1 mM)	52 ± 5*	108 ± 8*
Control	+ BB94 (1 mM) + TrA (0.1 mM)	126 ± 15	110 ± 8
T-47D	+ BB94 (1 mM) + TrA (0.1 mM)	115 ± 15*	108 ± 8*
MDA-231	+ BB94 (1 mM) + TrA (0.1 mM)	124 ± 16*	115 ± 5*

Results are mean ± SD ($n = 3$) and are expressed as percentages of control (SaOS-2 cells treated with medium conditioned on plastic alone and not added with proteinase inhibitors).
BB94: batimastat; TrA: tranexamic acid; $^*P < 0.005$ vs SaOS-2 incubated with the same conditioned medium but without inhibitor.

Both small (MW < 10 kD) and large (MW > 10 kD) BCC-derived molecules are involved in osteoblastic protein degradation

We partitioned culture medium conditioned by T-47D and MDA-MB-231 BCC by centrifugation on a porous membrane with a cut-off value of 10 kD, since both MMPs and plasmin have a MW higher than 10 kD. We then tested the effect of the ultrafiltrate (U) and the retentate (R) on the proteins amount in SaOS-2 cells. Figure 2 (upper panel) shows that like the whole (W) medium conditioned by T-47D and MDA-MB-231 BCC, both U and R fractions reduced the CDP amount in osteoblastic cells. Except for the R fraction from medium conditioned by T-47D cells, a similar inhibitory effect was seen on the NCP amount (Figure 2, lower panel).

Discussion

Culture medium conditioned by various BCC lines decreased the net protein amounts produced by normal human osteoblasts and osteoblast-like cells, with a partial specificity for the collagenase-digestible proteins (CDP). However, no reduction in absolute collagen production by osteoblastic cells was seen, as shown by P1CP measurement. In contrast, the effects of conditioned medium were reduced by proteinase inhibitors, indicating that BCC may degrade, or induce osteoblastic cells to degrade, the bone matrix proteins. Our results are in agreement with our hypothesis that BCC metastasizing to bone may degrade the matrix proteins, notably collagen, in their vicinity independently of stimulating the eroding action of osteoclasts, and that osteoblasts are also important target cells for the action of BCC.

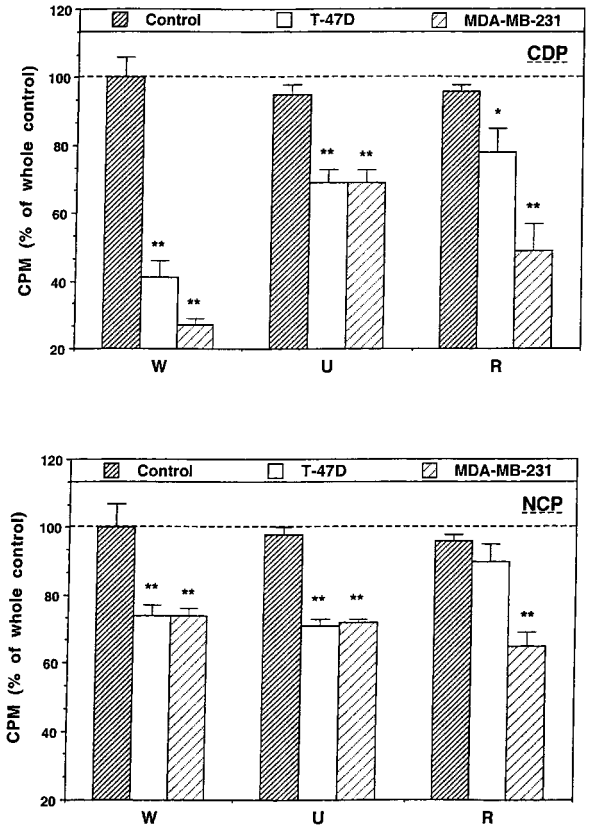

Figure 2. CDP (upper panel) and NCP (lower panel) amounts in SaOS-2 cells: effect of fractions from medium conditioned by T-47D and MDA-MB-231 BCC. U: ultrafiltrate (MW < 10 kD); R: retentate (MW > 10 kD). Results are mean ± SD ($n = 3$) and are expressed as percentages of control (whole medium – W). $^{*}P < 0.05$, $^{**}P < 0.005$.

Tumor-induced osteolysis (TIO) is characterized by an uncoupling between bone formation and resorption, in favor of the latter, contrasting with the equilibrium observed in normal bone physiology. Our data indicate that this imbalance between bone formation and bone resorption in TIO could be due, at least in part, to the proteolytic activity of BCC and/or to an enhanced proteolytic activity of osteoblasts influenced by BCC-derived factors. That osteoblasts could mediate some of the effects of BCC in TIO was already suggested by precedent work in our laboratories showing that BCC-derived factors inhibit the growth rate of osteoblasts and enhance their response to osteolytic agents such as parathyroid hormone (PTH), PTH-related peptide (PTHrP) and prostaglandin E_2 (PGE_2) [15, 16].

Three of the five BCC lines tested led to a higher decrease in CDP than in NCP amount in osteoblastic cells. Our data suggest that this effect may be explained by the activity of one or more MMPs. In contrast, two of the three newly established IBEP cell lines degraded NCP and CDP to the same extent, being thus devoided of any destructive specificity for collagenous proteins. These cell lines (IBEP-1 and -2) have been obtained from pleural effusions and have been passaged only a few times *in vitro*, so that they could be much closer to and more representative of the *in vivo* tumor cell phenotype than the long-time established T-47D and MDA-MB-231 BCC lines. Our data suggest that these new lines could either not produce MMPs or produce inactive MMPs. Further studies should compare the qualitative and quantitative proteinase status in these recently isolated and few passaged cells and in the well established cell lines T-47D and MDA-MB-231.

HBL-100 cells were apparently unable to modulate the net CDP and NCP production by osteoblastic cells. HBL-100 cells have been immortalized by integration of SV40 large T-antigen. They are usually considered as 'transformed but non-tumorigenic'. Our data suggest that transformation of normal breast epithelial cells is not sufficient to give them a direct or indirect significant proteolytic activity against bone proteins. Moreover, since HBL-100 cells have been reported to be non-tumorigenic when injected to mice, we propose that the BCC-derived molecules causing the degradation of bone proteins, and apparently absent in HBL-100 cells, could also be needed to allow the growth of BCC in tissues other than bone, may be by stimulating proteinase production and/or activation by the normal host cells surrounding these BCC.

Tranexamic acid inhibits plasminogen activation to plasmin by binding to the heavy chain of the molecule. Tranexamic acid, as well as other inhibitors of the uPA/plasmin system, can reduce the *in vitro* invasion of cancer cells [13]. Our results suggest that such inhibitors, along with inhibitors of MMPs, might also be useful against bone degradation by BCC. It is of interest that, in our experimental conditions, co-addition of batimastat and TrA was sufficient to abolish the effects of BCC-derived medium on NCP and CDP production by bone cells. We conclude that cumulative or cooperative action of MMPs and members of the uPA/plasmin system may account for the total proteolytic effect of BCC-derived factors on proteins secreted by osteoblastic cells. The hypothesis of a cooperative action of MMPs and plasmin is supported by previous work from other laboratories. For instance, it

has been shown that activation of MMP-9 via a converging plasmin/stromelysin-1 cascade may enhance tumor cell invasion [17].

In contrast with batimastat and TrA, the cathepsin D inhibitor pepstatin was unable to reduce the proteolytic activity of medium conditioned by BCC. This result and data from other laboratories using the Matrigel invasion assay to study a variety of MCF-7 BCC clones [14] suggest that cathepsin D and, more widely, aspartic proteinases could be of minor importance in BCC invasion and in breast cancer-induced osteolysis. However, cathepsin D is mainly active at an acid pH. Such a condition was not realized in our experiments, but could exist *in vivo* in the vicinity of osteoclasts. These cells are known to acidify the environment between their ruffled border and the bone surface [18], dissolving thus the bony hydroxyapatite.

The association between MMP activity and cancer progression and invasion has been shown in numerous studies, notably pointing to the successful effect of MMP inhibitors on lymphatic and hematogenous metastasis [19], the formation of lung metastases [20] and the degradation of basal membrane by BCC [13]. A number of MMPs are expressed in breast tumors [21]. It seems, however, that only a few of these molecules are frequently produced by BCC themselves, namely, collagenase-3 (MMP-13) and matrylisin (MMP-7). The other MMPs might be overproduced by stromal cells influenced by BCC-derived factors, pointing to the importance of cell cooperation in invasive and metastatic events.

It is generally assumed that the first step in MMPs-mediated collagen degradation, that is the cleavage of native collagen, needs the intervention of a collagenase. Collagenase-1 (MMP-1), rarely found in BCC *in vivo* [21], is expressed by MDA-MB-231 but not by T-47D cells [22]; it is produced only at very low levels by MG-63 and hOB, while being apparently absent in SaOS-2 cells [23]. A second collagenase, of neutrophil origin (MMP-8), has not been found in BCC [21] and, to our knowledge, never looked for in bone cells. More recently, a third collagenase (MMP-13) has been isolated from a breast tumour, but not found in T-47D or MDA-MB-231 cells [24]. MMP-13 is found in rat bone and in human chondrocytes [25] but we failed to detect its mRNA in hOB and in SaOS-2 cells (data not shown). It was recently shown that MMP-2 (72 kD gelatinase) may act as a collagenase [26], suggesting that the enzyme might completely remove collagen molecules without the help of any other collagenase. Interestingly, MMP-2 is produced by both osteoblastic [23] and breast cancer [21] cells. This enzyme could thus play a key role in our system. More generally, MMP-2 could be the most important MMP for the metastatic dissemination of BCC, as showed by data indicating that BCC transfected by MMP-2 become more invasive and metastatic, a feature not observed with MMP-1 and MMP-3 [27].

Membrane-type MMPs (MT-MMPs) could be additional molecules involved in the process of CDP degradation induced by BCC-derived factors. They have been shown to participate to the process of MMP activation. For instance, MT1-MMP is able to generate active MMP-13 [28]. However, to our best knowledge, MT-MMP expression by osteoblasts has not been demonstrated to date.

What are the factors released by BCC able to reduce the protein amounts in osteoblastic cells? Our data indicate that some of these factors have a $MW > 10$ kD. These factors could certainly be proteinases, but other molecules could also be involved, such as interleukin-11 (IL-11). We have recently shown [29] that IL-11 is produced in high amounts by some BCC lines (i.e. MDA-MB-231 cells) and recent data revealed that IL-11 induction of type I collagen degradation in mouse calvaria is prevented by an inhibitor of MMPs [30].

We show that at least one small ($MW < 10$ kD) factor produced by BCC induces a reduction of protein amounts released by osteoblastic cells. We suggest that PGE_2 is a possible candidate. PGE_2 (MW: 352.5) is largely produced by the MDA-MB-231 cells [31, 32] and markedly stimulates MMP-2 and MMP-13 production by osteoblasts [33]. PGE_2 may also increase the production of IL-11 by MDA-MB-231 cells [32]. However, neither Il-11 nor PGE_2 are produced in high amounts by T-47D cells. Thus, if these two molecules could contribute to the higher osteolytic activity of MDA-MB-231 cells as observed in nude mice, the classical animal model of TIO, additional BCC-derived factors yet to be identified participate also to the reduction of protein amounts in osteoblastic cells.

There is an increasing interest in using specific and efficient proteinase inhibitors to prevent and control cancer metastasis. To be successful, this approach needs first to identify the cell types and the major molecules involved in the degradation of tissues accompanying cancer cell migration and invasion. Tumor-induced osteolysis (TIO) is attributed to the increased activity of osteoclasts stimulated by BCC. Our data suggest that BCC could also degrade the bone mat-

rix proteins directly and by modulating the proteolytic activity of osteoblasts. The proteinases involved in that process appears to be MMPs and members of the plasmin/plasminogen family.

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