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Bone cell matrix promotes the adhesion of human prostatic carcinoma cells via the $\alpha 2\beta 1$ integrin

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Prostatic carcinoma cells have a propensity to metastasize to bone, and we propose that this phenomenon may be promoted by the adhesion of metastatic cells to bone matrix. Bone matrix is produced by osteoblasts, and we have developed an *in vitro* model of bone matrix by isolating the substratum deposited by human osteoblast-like U2OS cells. The collagenous nature of this matrix was demonstrated by the incorporation of [^{3}H]proline and its subsequent release by purified collagenase. Both U2OS matrix and purified type I collagen stimulated the adhesion of human PC-3 prostatic carcinoma cells. Human laminin supported adhesion to a much lesser extent, and PC-3 cells did not adhere to fibronectin. Adhesion of PC-3 cells to U2OS matrix closely resembled adhesion to purified type I collagen with respect to (a) inhibition by a collagen-derived peptide and by antibodies raised against $\alpha 2$ or $\beta 1$ integrin collagen receptor subunits; (b) lack of inhibition by RGD (Arg–Gly–Asp) peptides; (c) stimulation by Mn^{2+} and Mg^{2+} ions but not by Ca^{2+} ion; and (d) stimulation by the phorbol ester PMA (phorbol 12-myristate 13-acetate). This adhesion was also stimulated (2.3-fold) by transforming growth factor β (TGF-β), which is a major bone-derived growth factor. We conclude that human osteoblast-like matrix is an adhesive substrate for PC-3 prostate carcinoma cells. This adhesion appears to be mediated by the interaction of $\alpha 2\beta 1$ integrins on PC-3 cells with matrix-derived collagen. The stimulation of this adhesion by TGF-β suggests that the co-expression of TGF-β and type I collagen in bone may synergistically facilitate the adhesion of metastatic cells to bone matrix proteins and thereby increase their localization in the skeleton.

Keywords: $\alpha 2\beta 1$ integrins, bone cell matrix, prostatic carcinoma cells

Introduction

Bone is one of the most frequent targets for the metastasis of several common human malignancies, including carcinomas of the breast and prostate [1]. In prostate cancer, the skeleton is usually the earliest and most extensively involved target organ [2]. Due to the modest blood supply of bone [3], it is likely that extravascular factors contribute to the frequent metastatic colonization of the skeleton. Several reports have indicated that bone matrix proteins may promote the expression of various metastatic phenotypes [4–6], including adhesiveness [7], in a variety of cancer cell lines. We hypothesize that bone matrix proteins stimulate the adhesion of metastatic prostate cancer cells, which may promote the localization of these cells in the skeleton.

Bone matrix is produced by osteoblasts, and represents an enormous reservoir of extracellular matrix proteins. Type I collagen is the major adhesion protein in bone, and purified type I collagen is an adhesive substrate for metastatic cells [8–10]. Bone-derived collagen may also be an adhesive substrate for cancer cells *in vivo*. The adhesion of multiple myeloma cells to type I collagen within bone has been proposed to contribute to the propensity of these cells to remain localized within the skeleton [11]. Embedded within collagenous bone matrix are a large number of polypeptide growth factors, including

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transforming growth factor β (TGF-β1) [12]. TGF-β and other factors are released from the matrix during bone resorption, a process which has been shown to increase the adhesion of metastatic cancer cells to bone *in vitro* [7].

Cell adhesion to matrix proteins is mediated by cell surface integrin receptors, which are heterodimeric proteins comprised of an α subunit non-covalently bound to a β subunit. The type I collagen receptors include the α1β1, α2β1, and α3β1 integrins [9, 13]. These receptors are constitutively expressed on the surface of many types of metastatic cells [10, 13], which may allow them to adhere to collagen within metastatic sites. The expression of collagen receptors on cancer cells is often enhanced in comparison with non-transformed counterparts [8, 9], a phenomenon which has been associated with aggressive behavior and enhanced metastatic potential [14, 14].

Collagen receptors can be upregulated *in vitro* by growth factors which are present in bone matrix, such as TGF-β. TGF-β is found in greater quantities in bone matrix than in any other tissue [16], and this growth factor has been shown to upregulate collagen receptor expression [9, 17] and to enhance cell adhesion to type I collagen [18]. We propose that bone-derived TGF-β may locally upregulate the expression of integrin receptors on metastatic cells, thus enhancing their ability to adhere to bone matrix ligands. In this study we describe an *in vitro* model for studying the adhesive interaction between metastatic cancer cells and bone cell matrix. We studied human PC-3 prostatic carcinoma cells, which were originally isolated from a metastatic bone lesion [19] and which are known to form experimental [20] and spontaneous [21] bone metastases in nude mice. Bone cell matrix was derived from human U2OS osteosarcoma cells, which are transformed osteoblasts which possess many of the phenotypic properties of differentiated osteoblasts [22–24]. We demonstrate the rapid adhesion of PC-3 prostatic carcinoma cells to the collagenous extracellular matrix produced by cultured U2OS cells. This adhesion is stimulated by TGF-β, and appears to be mediated by the interaction of matrix-derived collagen with α2β1 integrin receptors on PC-3 cells.

Materials and methods

Cell culture

Human PC-3 prostatic carcinoma cells were purchased from ATCC and were maintained in Ham's F-12K medium with 1% penicillin/streptomycin (P/S) (Gibco, Burlington, Ontario, Canada) and 7% fetal bovine serum (FBS). Human U2OS osteosarcoma cells were purchased from ATCC, and were maintained in α-MEM medium with 1% P/S, 10% FBS, and ascorbic acid (50 μg/ml) (Sigma). U2OS cells were cultured to confluent density for 7 days in Falcon™ 96-well tissue culture plates (Becton Dickinson, Lincoln Park, NJ, USA). The extracellular matrix produced by U2OS cells was isolated by removing the culture media, rinsing the U2OS cell monolayers with phosphate-buffered saline (PBS), and lysing the cells with 20 mM ammonium hydroxide [25]. The lysates were aspirated, and wells were rinsed with PBS and air-dried. In some experiments, monolayer cultures of PC-3 cells were pretreated with the phorbol ester PMA (phorbol 12-myristate 13-acetate, 20 nM) (Sigma) for 30 min prior to trypsinization and use in adhesion assays. In other experiments, PC-3 cells were pretreated with recombinant human (TGF-β1 10 ng/ml) (R&D Systems, Minneapolis, MN, USA) for 24 h prior to adhesion assays.

[^{3}H]Proline incorporation and release by U2OS matrix

The collagen content of U2OS matrix was analysed by adapting a [^{3}H]proline incorporation and release assay [26]. U2OS matrices were labeled by culturing confluent U2OS cell monolayers in supplemented α-MEM containing 5 μCi/ml [^{3}H]proline (New England Nuclear, Boston, MA, USA) in 96-well plates for 5 days. Matrices were isolated by lysing cells with 20 mM ammonium hydroxide, as described above. After extensive rinsing with PBS, matrices were incubated with 200 μl/well of serum-free α-MEM (control), or serum-free α-MEM plus 1% SDS, or serum-free α-MEM plus 50 U/ml of purified leech collagenase [27] (Biopharm, Charlston, SC, USA). The release of ^{3}H into the media was determined 24 h later with a scintillation counter (Beckman LS1801; Fullerton, CA, USA).

Antibodies, peptides, and proteins

The following monoclonal antibodies were obtained for specific integrin subunits: anti-α2 (clone P1E6), anti-α3 (clone P1B5), and anti-β1 (clone P4C10), from Gibco, and anti-αv (clone VNR147) from Telios (San Diego, CA, USA). Antibodies were used in attachment assays at titers of 1:250, 1:500, and 1:1000. Data are presented using a 1:500 dilution, as no additional inhibitory effects were seen using higher titers with any of the antibodies. The following peptide sequences and proteins were obtained: GRGDSP (Gly–Arg–Gly–Asp–Ser–Pro), GRGESP (Gly–Arg–Gly–Glu–Ser–Pro), and YIGSR (Tyr–Ile–Gly–Ser–Arg), from Gibco (Grand Island, NY, USA); DGEA (Asp–Gly–Glu–Ala), from Peninsula Labs (Belmont, CA, USA);

and bovine serum albumin (BSA), human type I collagen, laminin, and fibronectin, from Gibco. Peptides were tested in attachment assays at 250, 500 and 1000 μg/ml. The maximal effects of peptides were fully realized at 250 μg/ml, at which concentration the data are presented.

Adhesion assays

Purified extracellular matrix proteins were diluted in distilled, deionized water, and tissue culture microwells were coated with varying concentrations of these proteins by overnight incubation. Coated and uncoated (control) wells were then incubated with BSA (1 mg/ml) for 4 h to prevent the non-specific adsorption of serum-derived adhesion proteins, and were then rinsed with distilled, deionized water and air dried. PC-3 cells were released from culture by trypsin/EDTA (Gibco), pelleted by centrifugation, and resuspended in FBS-supplemented Ham's F-12K media. Cells were added to purified protein-coated wells (2×10^4 cells/well) for 30 min. For wells coated with U2OS matrix, PC-3 cells were added for varing durations in order to assess the kinetics of adhesion. After defined durations, media and non-adherent cells were aspirated and wells were rinsed twice with PBS to remove loosely adherent cells. To examine divalent cation-dependent adhesion, PC-3 cells were released from tissue culture flasks by trypsin/EDTA, and were then washed three times with an attachment buffer (145 mM NaCl, 5 mM KCl, 20 mM HEPES, pH 7.4, 0.5% BSA, 25 μM EDTA) [28]. Cells were reconstituted at 10^5/ml in attachment buffer in the presence or absence of different concentrations of $MnCl_2$, $MgCl_2$, or $CaCl_2$. For all experiments, cell adhesion was quantified as described previously [29]. Briefly, adherent cells were stained for 10 min with a solution of 0.1% crystal violet in 2% ethanol. After gentle aspiration of the staining solution, wells were rinsed carefully with tap water, and the stained adherent cells were solubilized with 100 μl of 1% sodium dodecyl sulfate (SDS). Background staining of U2OS matrix or of purified matrix proteins was negligible, as determined by crystal violet staining of these surfaces without added cells. Absorbance was determined at 570 nm with a Bio-Tek EL340 microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

Results

[^{3}H]Proline incorporation into U2OS matrix

We monitored the incorporation of [^{3}H]proline by U2OS cell matrices in order to evaluate their collagen content. After 5 days of radiolabeling, isolated matrices contained significant amounts of [^{3}H]proline which could be released by solubilizing the matrix with 1% SDS. A significant proportion of the radiolabeled matrix protein was also digestible by treatment with purified leech collagenase, as determined by [^{3}H]proline release from the matrix (Table 1).

PC-3 cell adhesion to U2OS matrix

We examined the ability of isolated U2OS matrix to promote the adhesion of an osteotropic prostatic carcinoma cell line (PC-3). U2OS matrix stimulated the rapid attachment and spreading of human PC-3 prostatic carcinoma cells. The rate of PC-3 cell adhesion to U2OS matrix was approximately 5-fold more rapid than adhesion to tissue culture wells coated with poly-L-lysine (Figure 1). PC-3 cell adhesion to U2OS matrix was partially inhibited (22%) by the addition of a type I collagen derived recognition sequence peptide, DGEA (Asp–Gly–Glu–Ala) (250 μg/ml) (Table 2). Adhesion to U2OS matrix was not inhibited by similar concentrations of the vitronectin/osteopontin recognition sequence peptide GRGDSP (Gly–Arg– Gly–Asp–Ser–Pro), nor by a negative control peptide, GRGESP (Gly–Arg–Gly–Glu–Ser–Pro) (Table 2). GRGDSP peptide concentrations as high as 1000 μg/ml also failed to inhibit PC-3 cell adhesion.

We also characterized PC-3 cell adhesion to U2OS matrix by examining the effects of a number of monoclonal antibodies raised against various integrin subunits, including those which form collagen receptors. An anti-β1 antibody (P4C10) which effectively inhibits cellular adhesion to type I collagen [30], inhibited PC-3 cell adhesion to U2OS matrix by

Table 1. [^{3}H]Proline release from U2OS matrix following SDS-denaturation or purified leech collagenase treatment

	Release of [^{3}H]proline from U2OS matrix (cpm ± SEM)[a]		
	Control[b]	1% SDS	Collagenase[c]
Experiment 1	375 ± 17	—	573 ± 33[d]
Experiment 2	251 ± 24	841 ± 36[d]	357 ± 19[d]
Experiment 3	239 ± 17	1771 ± 378[d]	415 ± 81

[a]Each experiment included eight replicates for each condition.

[b]Control represents the amount of [^{3}H]proline release into serum-free α-MEM media.

[c]Purified leech collagenase (50 U/ml) was added to serum-free α-MEM.

[d]Significantly greater than corresponding control ($P < 0.005$, Student's t-test).

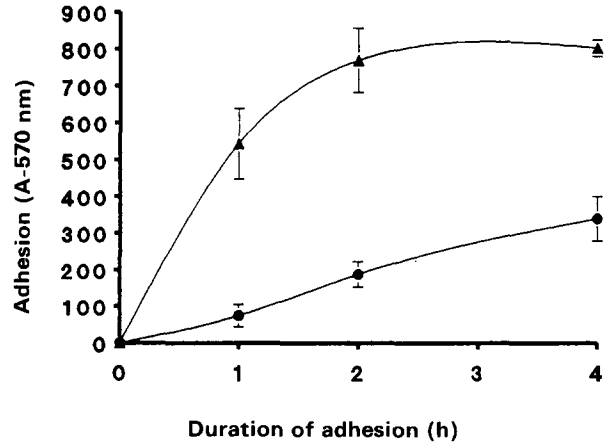

Figure 1. Kinetic analysis of PC-3 cell adhesion to U2OS matrix. PC-3 cells were suspended in Ham's F-12K medium and then added to 96-well plates coated with either U2OS matrix (—▲—) or with poly-L-lysine (—●—), as described in Materials and methods. Adhesion assays were terminated after 1, 2 or 4 h, with six replicates per experiment. This figure is representative of three independent experiments, all of which showed similar differences in adhesion rates. Data represent mean ± SEM, and all differences are significant ($P < 0.001$).

Table 2. Properties of PC-3 cell adhesion to U2OS extracellular matrix

Adhesion (% control)[a]			
Peptides[b]		Antibodies[c]	
DGEA	78 ± 6[d]	P4C10 (anti-β1)	18 ± 7[d]
GRGDSP	90 ± 8	P1E6 (anti-α2)	68 ± 3[d]
GRGESP	105 ± 17	P1B5 (anti-α3)	113 ± 8
YIGSR	98 ± 3	P1E6 + P1B5	65 ± 9[d]
		VNR147 (anti-αv)	97 ± 4
		NMAF (control)	104 ± 6

[a]Data represent the mean of three or four independent experiments with six replicates per experiment (± SEM).
[b]Peptides (250 μg/ml) were added to cell suspensions for 20 min prior to adhesion assays.
[c]Antibodies (1:500) were added to cell suspension for 20 min prior to adhesion assays.
[d]Significantly less than control (100%) adhesion ($P < 0.01$, Student's *t*-test).

over 80% (Table 2). An antibody raised against the α2 subunit (P1E6) inhibited PC-3 cell adhesion to U2OS matrix by 32%, while an anti-α3 antibody (P1B5) had no effect. We had determined previously by radioimmunoprecipitation and by flow cytometry that the collagen receptor α1β1 is not expressed by PC-3 cells [31] (manuscript in preparation), and thus anti-α1 monoclonal antibodies were not tested for effects on adhesion. An antibody (VNR147) directed against the αv subunit of the αvβ3 osteopontin receptor had no effect on PC-3 cell adhesion to U2OS matrix, nor did normal mouse ascites fluid (NMAF, negative control, Table 2). Increasing the titre of all antibodies from 1:500 to 1:100 had no additional affect on adhesion. Because many cell types express multiple collagen receptor types, we also examined the combined effects of anti-α2 and anti-α3 antibodies on adhesion of PC-3 cells to U2OS matrix. The addition of anti-α3 antibody did not enhance the inhibitory effect of the anti-α2 antibody (Table 2).

The inhibition of adhesion by the P1E6 (anti-α2) and P4C10 (anti-β1) antibodies, and by the DGEA peptide, suggest that the α2β1 integrin receptor contributes to the adhesion of PC-3 cells to U2OS matrix-derived collagen. On some cell types, the α2β1 integrin is also a potential receptor for laminin and fibronectin as well as for collagen [31]. We thus examined the ability of PC-3 cells to adhere to these purified proteins. PC-3 cells adhered most rapidly to type I collagen, and also adhered to laminin (Figure 2). Fibronectin did not support PC-3 cell adhesion even

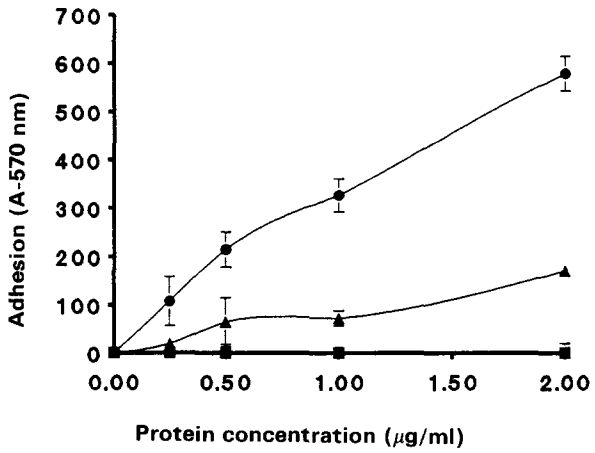

Figure 2. PC-3 cell adhesion to purified matrix proteins. Tissue culture microwells were coated with different concentrations of purified human type I collagen (—●—), laminin (—▲—), or fibronectin (—■—), as described in Materials and methods. PC-3 cells were suspended in Ham's F-12K media, and added to the wells for 30 min (2×10^4 cells/well). This figure is representative of three independent experiments, all of which demonstrated similar relative differences in PC-3 cell adhesion to each substrate. PC-3 cell adhesion to type I collagen is significant at all concentrations, and adhesion to laminin is significant from 5 to 10 μg/ml ($P < 0.01$). PC-3 cells did not adhere to fibronectin in significant numbers at any concentration.

at high concentrations (10 μg/ml). Although PC-3 cells were capable of adhering to purified laminin, the laminin recognition sequence peptide YIGSR (Tyr–Ile–Gly–Ser–Arg) did not inhibit PC-3 cell adhesion to U2OS matrix (Table 2). These data suggest that neither laminin nor fibronectin contribute to the adhesion of PC-3 cells to U2OS matrix.

Collagen receptors have a distinct profile of divalent cation dependence, which was mimicked by PC-3 cells adhering to U2OS matrix. PC-3 cells were suspended in a cation-free attachment buffer to which varying concentrations of divalent cations were added prior to adhesion assays. PC-3 cell adhesion to U2OS matrix was significantly stimulated by Mn^{2+} and Mg^{2+} ions, while Ca^{2+} ions caused a small but insignificant amount of adhesion (Figure 3). This profile of cation dependence is virtually identical to that observed for PC-3 cell adhesion to purified type I collagen [10].

Regulation of PC-3 cell adhesion to U2OS matrix

The type I collagen-binding activity of the α2β1 integrin receptor has been reported to be inducible by the phorbol ester PMA [32] and by TGF-β1 [33]. We thus pretreated PC-3 cells with PMA (20 nM) for 30 min prior to their addition to wells coated with either purified type I collagen or with U2OS matrix. The adhesion of PMA-treated PC-3 cells to both substrates was increased by approximately 35% compared to untreated cultures ($P<0.05$) (Figure 4). We previously reported that TGF-β1 also stimulates PC-3 cell adhesion to purified type I collagen [10]. Pretreatment of PC-3 cells with TGF-β1 for 24 h (1

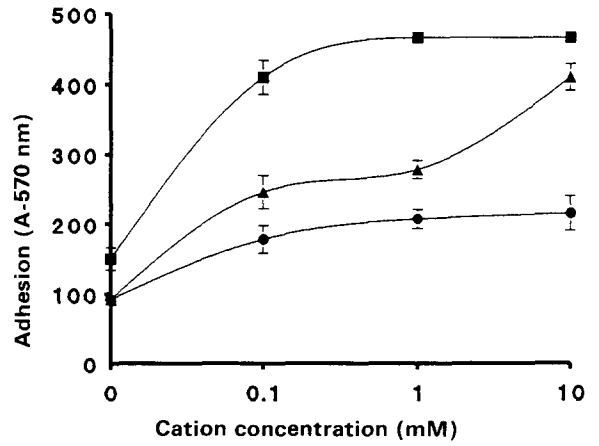

Figure 3. Cation dependence of PC-3 cell adhesion to U2OS matrix. PC-3 cells were suspended in a cation-free attachment buffer, to which were added different concentrations of $MnCl_2$ (–■–), $MgCl_2$ (–▲–), or $CaCl_2$ (–●–) prior to their use in adhesion assays (2×10^4 cells/well). Adhesion assays were terminated after 30 min. This figure represents data obtained from three independent experiments, each of which contained six replicates per condition. Adhesion was stimulated in the presence of all concentrations of $MnCl_2$, and in the presence of 0.1–10 mM $MgCl_2$, compared to cation-free adhesion ($P<0.01$). $CaCl_2$ did not significantly stimulate adhesion at any concentration.

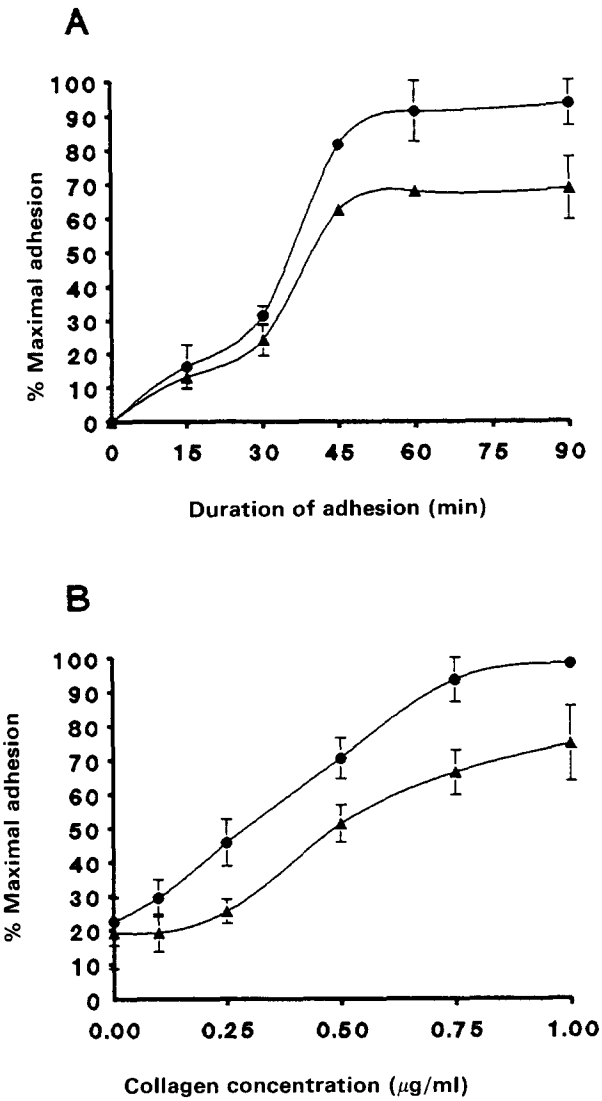

Figure 4. Effect of the phorbol ester PMA on PC-3 cell adhesion to U2OS matrix and to type I collagen. PC-3 cells were suspended in Ham's F-12K media in the presence (–●–) or absence (–▲–) of 20 nM PMA. After 30 min, cells were added to tissue culture microwells coated with either U2OS matrix (**A**) or with different concentrations of purified human type I collagen (**B**). Adhesion to U2OS matrix was permitted for the durations indicated in (**A**), and adhesion to type I collagen was permitted for 30 min. This figure is representative of three independent experiments. PMA treatment significantly enhanced PC-3 cell adhesion to U2OS matrix from 45 to 90 min, and significantly increased adhesion to type I collagen at concentrations of 0.25–1 μg/ml ($P<0.01$).

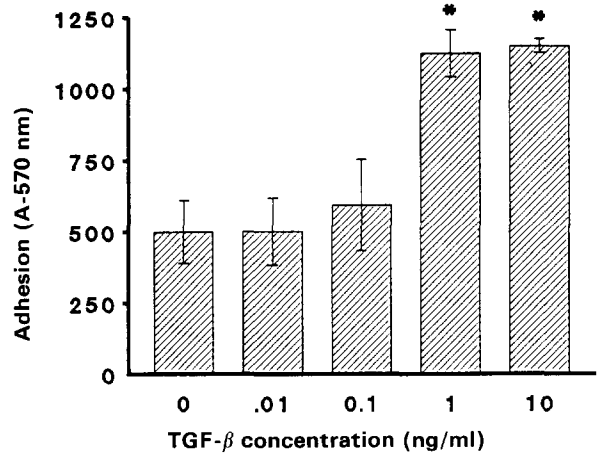

Figure 5. Effect of transforming growth factor β (TGF-β) on PC-3 cell adhesion to U2OS matrix. PC-3 cells were cultured for 24 h in 25 cm^2 flasks with Ham's F-12K media (with 7% FBS) containing various concentrations of human recombinant TGF-β1 (0–10 ng/ml). PC-3 cell monolayers were then rinsed extensively with PBS, trypsinized, resuspended in TGF-β-free Ham's F-12K media (with 7% FBS), and then assayed for adhesion to U2OS matrix. This figure is the mean of two independent experiments, each of which included six replicates per condition. TGF-β treatment at 1 and 10 ng/ml caused significantly increased adhesion ($P < 0.001$).

or 10 ng/ml) also stimulated their attachment to U2OS matrix by over 2-fold ($P < 0.001$) (Figure 5).

Discussion

The skeleton is one of the earliest, most common, and most extensively involved target organs for the dissemination of many common human malignancies, including carcinoma of the prostate [2]. We have proposed that the metastasis of prostate cancer cells to bone may be regulated by the adhesion of metastatic cells to bone matrix proteins. Bone matrix is 90% type I collagen, and type I collagen can promote the adhesion of metastatic cancer cells [7, 8–11]. Bone matrix also contains a number of polypeptide growth factors which upregulate the expression and function of collagen receptors *in vitro*. These factors include TGF-β [17, 18, 3], insulin-like growth factor-I (IGF-I) [35], and basic fibroblast growth factor (bFGF) [35]. Bone-derived growth factors might further enhance metastatic osteotropism by potentiating the adhesion of metastatic cells to bone matrix proteins in a spatially regulated manner.

We have developed an *in vitro* model to examine the adhesion of metastatic PC-3 prostatic carcinoma cells to the extracellular matrix produced by human bone cells. This matrix was obtained from U2OS osteosarcoma cells, which exhibit many of the phenotypic properties of differentiated osteoblasts [22–24]. The U2OS matrix was collagenous, as determined by the release of previously incorporated [^{3}H]proline through exposure to purified leech collagenase [36]. This collagenase specifically cleaves type I collagen in a manner similar to that of mammalian collagenase [27]. We were unable to obtain sufficient quantities of matrix-derived collagen to demonstrate on Coomassie-stained SDS polyacrylamide gels (not shown), a problem which others have reported with human osteosarcoma cell lines due to their characteristically low levels of collagen production [37]. Nonetheless, PC-3 cells are capable of rapidly adhering to low microgram quantities of type I collagen, and the rate of PC-3 cell adhesion to U2OS matrix is similar to the rate of adhesion to tissue culture wells coated with saturating quantities of type I collagen. The low level of collagen in U2OS matrix therefore appears to be sufficient to maximally support PC-3 cell adhesion.

The properties of PC-3 cell adhesion to U2OS matrix are consistent with integrin-mediated adhesion to type I collagen. PC-3 cell adhesion to U2OS matrix is stimulated by Mn^{2+} or Mg^{2+} ions, but not by Ca^{2+} ions. This profile of cation dependence is consistent with the adhesion of human chondrocytes [28] and PC-3 cells [10] to purified type I collagen. PC-3 cell adhesion to U2OS matrix was partially inhibited by a type I collagen-derived peptide (DGEA), which is a putative recognition sequence for the $\alpha 2\beta 1$ integrin-type collagen receptor [38]. Inhibitory monoclonal antibodies directed against the $\alpha 2$ or the $\beta 1$ integrin subunits could also inhibit PC-3 cell adhesion to U2OS matrix, which further suggests the interaction of $\alpha 2\beta 1$ integrin receptors with matrix-derived collagen.

Osteopontin is another adhesive protein which is synthesized by osteoblasts [39] and osteosarcoma cells [40], and which is also present in bone matrix [41]. Osteopontin is recognized by the $\alpha v\beta 3$ integrin receptor [42], and PC-3 cells have been reported to express $\alpha v\beta 3$ [43]. However, osteopontin might not play an important role in PC-3 cell adhesion to U2OS matrix, as adhesion was not inhibited by high concentrations of anti-αv antibody or GRGDS peptide, both of which inhibit the interaction of osteopontin with its $\alpha v\beta 3$ integrin receptor [42]. RGD-independent cell adhesion is consistent with integrin-mediated adhesion to type I collagen [44]. Based on their constitutive expression of the $\alpha v\beta 3$ receptor [43], it is likely that PC-3 cells are able to

adhere to osteopontin, whether it is matrix-bound or in purified form. Our inability to inhibit PC-3 cell adhesion to U2OS matrix through $\alpha v\beta 3$ inhibition suggests that the influence of type I collagen in this matrix makes the potential contribution of osteopontin difficult to unmask. The relative role of $\alpha v\beta 3$/osteopontin interactions is probably small, as indicated by the ability of the anti-$\beta 1$ antibody to inhibit adhesion to U2OS matrix by over 80%. It is also possible that some metastatic cells may not recognize matrix-derived osteopontin as an adhesive ligand. Metastatic cells have been reported to produce osteopontin themselves [45], which may function as an autocrine adhesion factor [46]. The autocrine expression of osteopontin by metastatic cancer cells may obviate their sensitivity to matrix-derived osteopontin.

The constitutive collagen-binding potential of some metastatic cells may be upregulated by growth factors which are abundant in bone matrix. Bone matrix represents the largest storage site for TGF-β, and TGF-β upregulates the expression collagen receptor subunits for a variety of cell types [9, 17]. TGF-β also increases cell adhesion to type I collagen [18, 34], and we have demonstrated that the treatment of PC-3 cells with TGF-β caused a 2.3-fold increase in their adhesion to U2OS matrix. We have reported previously that TGF-β treatment of PC-3 cells leads to the specific upregulation of the $\alpha 2\beta 1$ integrin, as well as a 2-fold increase in adhesion to purified type I collagen [10]. The phorbol ester PMA also upregulates $\alpha 2\beta 1$ expression and stimulates cancer cell adhesion to type I collagen [33]. The treatment of PC-3 cells with PMA caused a similar potentiation of their adhesion to U2OS matrix and to purified type I collagen, which further suggests that the regulation of PC-3 cell adhesion to U2OS matrix is effected through $\alpha 2\beta 1$ integrin-mediated interaction with matrix collagen.

In summary, we have demonstrated that a human bone cell line produces a collagenous matrix to which metastatic prostate cancer cells readily adhere. This adhesion appears to be mediated by the interaction of $\alpha 2\beta 1$ integrins on the cancer cell surface with matrix-derived collagen. TGF-β, a major bone-derived growth factor, also stimulated cancer cell adhesion to bone cell matrix. The abundant co-expression of type I collagen and TGF-β in bone may have implications in the pathogenesis of metastatic bone disease. We propose that bone matrix-derived type I collagen supports the adhesion of metastatic cells and consequently promotes their localization within the skeleton. Bone-derived TGF-β may provide an additional spatially-regulated stimulus for collagen binding by locally upregulating the expression of collagen receptors on metastatic cells.

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