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The regulation of prostate cancer cell adhesion to human bone marrow endothelial cell monolayers by androgen dihydrotestosterone and cytokines

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Abstract

A previous study from our laboratory suggested that prostate cancer metastasis to bone may be mediated, in part, by preferential adhesion to human bone marrow endothelial (HBME) cells. Tumor cell adhesion to endothelial cells may be modulated by the effect of cytokines on cell adhesion molecules (CAMs). Tumor necrosis factor-alpha (TNF-α) regulates VCAM expression on the endothelium and this effect is enhanced by dihydrotestosterone (DHT). Transforming growth factor-beta (TGF-β) stimulates the expression of $\alpha_2\beta_1$integrin on PC-3 cells. The current study investigated the effects of the above cytokines and DHT (singularly and in various combinations) upon HBME and prostate cancer cell expression of VCAM, α_2 integrin subunit, and β_1 integrin subunit by flow cytometry. We also monitored the effects of the above treatments on PC-3 cell adhesion to HBME monolayers. The data demonstrate that none of the treatments significantly altered the expression of selected CAMs on HBME cell and neoplastic prostate cell lines. The treatment of HBME monolayers with various combinations of cytokines and DHT prior to performing adhesion assays with PC-3 demonstrates that treatments containing TGF-β reduced PC-3 cell adhesion to HBME monolayers by 32% or greater ($P < 0.05$). The reduction in PC-3 cell adhesion to TGF-β-treated HBME monolayers was dose dependent. Interestingly, LNCaP cells but not PC-3 cells treated with TGF-β had a reduced ability to adhere to untreated HBME monolayers. These results suggest that TGF-β may reduce tumor cell adhesion to bone marrow microvascular endothelium, *in vivo*. The biological significance of this observation is discussed.

Introduction

During cancer cell metastasis, tumor cells detach from the primary mass, enter blood circulation, adhere to vascular endothelial cells at a specific site, exit blood circulation at that site, induce angiogenesis, and establish a secondary mass [1]. Prostate cancer preferentially metastasizes to bone and causes significant bone pain [2]. A previous study suggested that prostate cancer metastasis to bone may be, in part, a result of preferential adhesion to human bone marrow endothelial (HBME) cells [3]. The cell adhesion molecules (CAMs) responsible for PC-3 cells' (a prostate cancer cell line derived from a bone metastasis) interaction with HBME cells, however, were not extensively examined in that study.

CAMs mediate, in part, the adhesion of tumor cells to the endothelium. The CAMs on endothelial cells are typically influenced by inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) [4, 5]. Endothelial cells stimulated with TNF-α express increased amounts of vascular cell adhesion molecule-1 (VCAM-1) and E-selectins. Both CAMs have been shown to mediate tumor cell adhesion to vascular endothelial cells [5–7]. Interestingly, the TNF-α-induced expression of VCAM-1 on human umbilical vein endothelial cells (HUVECs) is enhanced by treatment of HUVECs with dihydrotestosterone (DHT) [8].

The expression of CAMs on prostate cancer cells is also regulated by cytokines [9]. The integrin $\alpha_2\beta_1$ mediates PC-3 cell adhesion to collagen I, which is a major component of the bone matrix. Furthermore, the expression of this integrin on PC-3 cells is upregulated by transforming growth factor-beta (TGF-β), which is a prevalent cytokine in the bone marrow. TGF-β first binds TGF-β receptor type II, which presents the TGF-β ligand to TGF-β receptor type I. The TGF-β receptor type I is activated by phosphorylation and interacts with an adaptor protein SARA (Smad anchor for receptor activation). SARA then propagates the TGF-β signal to intracellular signaling mediators known as Smad2 and Smad3. Smad 2 and 3 interact with Smad 4 and the Smad complexes are translocated to the nucleus, where they activate specific genes. Interestingly, Smad 3 is an important

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coregulator for androgen-signaling in prostate cancer cells [10].

Nakashima et al. [11] reported that serum levels of TNF-α are elevated in prostate cancer patients. TNF-α facilitates metastasis by enhancing endothelial cell expression of CAMs that would in turn mediate cancer cell adhesion to the endothelium [7]. In addition, the normal physiological levels of DHT (4–40 nmol/l) can enhance surface expression of VCAM on TNF-α stimulated endothelial cells [8]. TGF-β is a prevalent cytokine in the bone matrix and has been demonstrated to enhance the expression of the collagen I receptor, $\alpha 2\beta 1$ [12]. It is therefore conceivable that TGF-β regulates CAM expression on both prostate cancer cells and HBME cells that can affect their interaction.

Based upon these findings, we examined the interplay between CAMs, cytokines and androgens such as DHT. Specifically, the present study investigated the effects that TGF-β, TNF-α, and DHT (singularly and in various combinations) have upon selected prostate cancer cell adhesion to HBME cell monolayers. We also determined the effect these soluble factors have on HBME cell expression of three CAMs: $\alpha 2$ integrin subunit, $\beta 1$ integrin subunit, and VCAM. Lastly, we investigated the effects of these soluble factors on the surface expression of $\alpha 2$ integrin and $\beta 1$ integrin subunits on normal and neoplastic prostate cell lines. TNF-α was excluded from the treatment protocol for normal and neoplastic prostate epithelial cell lines because it has a cytotoxic effect on some cancer cell lines in this study [13].

Materials and methods

Cell lines

Human bone marrow endothelial (HBME) cells were developed and described in a previous publication [3]. Normal prostate epithelial cells, 267B1, were a generous gift from Dr Jill Mascoska, University of Michigan Comprehensive Cancer Center (Ann Arbor, Michigan). WiDr (colon cancer cell line), LNCaP (prostate cancer cell line derived from a lymph node metastasis that expresses androgren receptor) and PC-3 (prostate cancer cell line derived from a bone metastasis that does not express androgren receptor) were obtained from the American Type Culture Collection (ATCC). HBME and WiDr were maintained in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin; LNCaP and PC-3 were maintained in RPMI Medium with 10% FBS and 1% penicillin/streptomycin. The immortalized normal prostate epithelial cell line, 267B1, was maintained in Defined Keratinocyte-SFM supplemented with growth factors (Gibco), 10% FBS and 1% penicillin/streptomycin.

Flow cytometry procedure and analysis

The cell lines were seeded in either T-75 or T-150 flasks and grown to confluence. Once confluent, the HBME cell monolayers, normal and neoplastic epithelial cells were treated as described in respective figure legends. The concentrations of DHT and TNF-α were used because they were demonstrated to increase the expression of VCAM on endothelial cells [8]. TGF-β concentration was selected because it increased PC-3 adhesion to bone matrix [12]. Before flow cytometric procedures, one million cells were incubated with 100 μl of antibodies to α_2 integrin subunit, β_1 integrin subunit or VCAM (for HBME cells only) at a concentration of 10 μg/ml for 45 min at room temperature. The cells were washed with 10 volumes of PBS, centrifuged and the pellets were incubated with 10 μg/ml of anti-mouse FITC conjugate IgG (Sigma Chemical Co., St. Louis, Missouri) for 45 min at room temperature. Cells incubated with secondary antibodies only served as negative controls. After incubation with secondary antibodies, cells were washed again with 10 volumes of PBS, fixed with 3% paraformaldehyde, pH 7.4, and analyzed for fluorescence with an Epics Elite Flow Cytometer (Coulter Corp., Miami, Florida). The percentage of positive cells was determined by subtracting the negative control value of the histogram from tested sample values. The average of two replications is reported.

Adhesion assays

Adhesion assays were performed as reported earlier [3]. Briefly, human bone marrow endothelial (HBME) cells were seeded onto plastic and grown to confluence in 'snap-apart' 96-well tissue culture plates (Fisher Scientific, Pittsburgh, Pennsylvania). Prostate cancer cells (PC-3 or LNCaP) or HBME cells were treated with various combinations of cytokines and DHT for approximately 24 h prior to the adhesion assay. The media containing the treatments were removed shortly before starting the adhesion assay and replaced with adhesion media (MEM, 1% bovine serum albumin). Prostate cancer cells were labeled with ^{51}Cr sodium salt and layered over monolayers of HBME cells for 30 min at 37 °C. The plates were gently washed three times with PBS and assayed for gamma particle emission using a gamma counter. Cell adhesion was reported as counts per minute (CPM) relative to the adhesion of controls, which were set to 100. Experiments were done in quadruplicates and repeated a minimum of two times.

Membrane preparation and Western blotting

Cells were dounced in a 20 mM HEPES buffer (pH 7.4) supplemented with 5 mM Na_3VO_4 and 1 mM phenylmethylsulfonylflouride (PMSF). The resulting slurry was centrifuged at 100 000 $\times$ g for 35 min at 4 °C to pellet membrane fractions. The membrane fractions were solubilized in lysis buffer (20 mM sodium phosphate (pH 7.5), 100 mM NaCL, 1% triton X-100, 0.5% Na^+ deoxycholate and 0.1% SDS) and the protein concentrations were determined. Fifty micrograms of protein were resolved on a 7.5% polyacrylamide ready gel and transferred to a nitrocellulose membrane in accordance with manufacturer's instructions (Bio-Rad Laboratories, California). The membrane was blocked overnight at 4 °C with blocking buffer (Tris-buffered saline (TBS) pH 7.7 {0.05M Tris base, 0.15M NaCL} supplemented with 0.05% tween 20 and 10% nonfat powdered milk), probed for

1 h at room temperature (RT) with rabbit polyclonal anti-TGF-β type II receptor (Santa Cruz Biotechnology) diluted 1/5000 in probing solution (TBS pH 7.7 supplemented with 0.1% tween 20 and 2.5% nonfat powdered milk) and washed four times with probing solution. Next, the membrane was subjected to goat anti-rabbit IgG secondary antibody diluted 1/1000 in probing solution for 1 h at RT, washed four times and band visualized by chemiluminesence according to manufacturer's instructions.

Statistical analysis

For flow cytometry data, equality among treatment effects were assessed by Kruskal–Wallis tests due to the small number of replications for each treatment. For adhesion assay data, natural-log transformed measurements were analyzed by analysis of variance (ANOVA) to assess treatment effects. Adjustments for experimental groups were made where applicable. The estimates represent the expected values of the experimental group relative to the control group. In the graphs, the control group is plotted at 100. The estimated value and its 95% confidence interval were obtained by taking the anti-logarithm of the results from the analysis of variance. Statistical analyses were carried out with the SAS 8 software.

Results

The effects of cytokines and DHT on prostate cancer cell adhesion to HBME monolayers

Adhesion assays were done to determine the effect of soluble factors on HBME cell monolayers' ability to bind PC-3 cells. The data demonstrates a 39% reduction (95% confidence interval (22%, 52%) in PC-3 cell adhesion to HBME cells only when the treatment included TGF-β1 (Figure 1, $P = 0.001$). Neither TNF-α nor DHT, alone or in combination, affected PC-3 cell adhesion to HBME cell monolayers. To determine if the observed decrease in PC-3 cell adhesion to TGF-β-treated HBME cells is dose dependent, HBME cells were treated with various doses of TGF-β and then used in adhesion assays. The data demonstrate that the adhesion of PC-3 cells to TGF-β-treated-HBME cell monolayers is dose-dependent beyond a concentration of 0.1 ng/ml (Figure 2, $P = 0.001$).

To determine the effect TGF-β had on PC-3 cells' and LNCaP cells' ability to adhere to HBME monolayers, PC-3 and LNCaP cells were treated with 10 ng/ml of TGF-β. This concentration was demonstrated to increase PC-3 cell adhesion to collagen type I enriched matrix [9]. Our result demonstrates that treatment of LNCaP cells ($P = 0.02$), but not PC-3 cells ($P = 0.14$), with TGF-β significantly reduces their ability to adhere to HBME cell monolayers (Table 1). Western analysis was done on membrane preparations from cell lines used in this study to evaluate the expression of TGF-β receptor type II, which binds TGF-β ligand from the culture fluid [14]. Beta-1 integrin subunit was probed as control for membrane preparations. The result demonstrates that TGF-β receptor type II is only expressed in the WiDr and LNCaP cell lines (Figure 3). As expected, all cell lines express beta-1 integrin subunit, thus demonstrating the success of our procedure for isolating membrane-associated proteins.

Table 1. The effect of TGF-β treatment on PC-3 and LNCaP adhesion to HBME cell monolayers.

Cell lines/treatment	Percentage relative to control, 95% confidence interval
LNCaP /Control	100
LNCaP/Treated	56 (36, 87)
PC-3/Control	100
PC-3/Treated	88 (74, 104)

Percent relative to controls of LNCaP and PC-3 cell adhesion to HBME monolayers.
Both LNCaP and PC-3 cells were treated for 24 h with 10 ng/ml of TGF-β prior to being used in adhesion assays.

Table 2. The effect of TNFα on endothelial CAM expression.

Antibodies	Non-stimulated	TNFα-stimulated
E-selectin	2	45
VCAM	3	65
PeCAM	99	–

HUVEC were probed for the above cell adhesion molecules under non-stimulated and cytokine stimulated conditions. Maximal expression of E-selectin was detected at 4 h of cytokine stimulation and VCAM maximal expression was detected at 16–24 h of cytokine stimulation. PeCAM does not require cytokine activation and normally found in high amount on endothelial cells. The numbers are percentage above background. Background was determined by the amount of fluorescence associated with cells untreated with antibodies.

The role of DHT in prostate cancer cell adhesion to HBME was next determined. Unlike PC-3 cells, LNCaP cells are androgen responsive. The effect of DHT on the proliferation of LNCaP cell is known [15]. To the authors' knowledge, however, no information exists regarding the effect DHT has on LNCaP cells' ability to adhere to an endothelial monolayer. Using a broad range of DHT concentrations in standard medium supplemented with charcoal-stripped fetal bovine serum, we demonstrate that DHT does not reduce LNCaP cell adhesion to HBME monolayers (Figure 4a). DHT only marginally decreases PC-3 cell adhesion at the highest dose (200 nmolar), which is above the normal physiological range [9] (Figure 4b). Because PC-3 cells do not express androgen receptors, this outcome was not expected and cannot be explained.

The effects of cytokines and DHT on CAM expression

HBME cells were treated with cytokines and DHT as described in Figure 1 for the expression of VCAM-1, α_2 and β_1. The concentration of DHT used in this study is within the normal range of testosterone in human male serum. The data

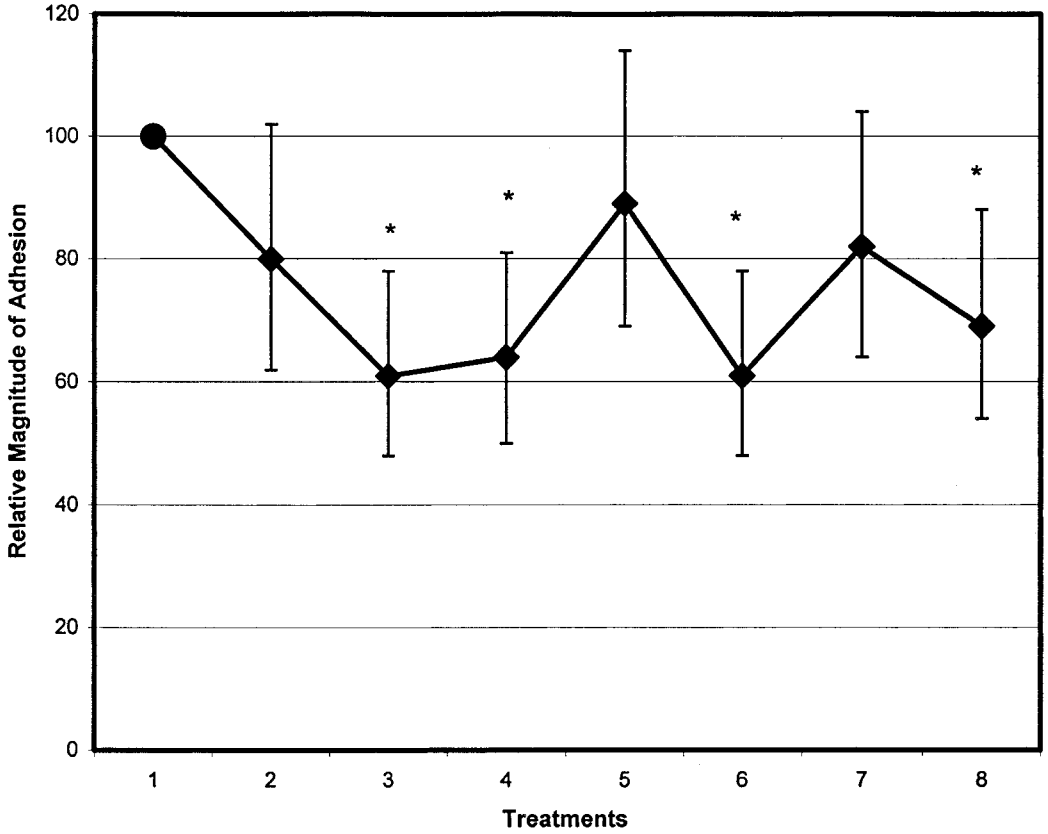

Figure 1. PC-3 cell adhesion to HBME cell monolayers treated with cytokines and DHT. The control (solid circle) is the magnitude of PC-3 cell adhesion to untreated HBME cell monolayer, which is defined as treatment 1. Treatments are as follows: (1) HBME culture media (Control), (2) 500 U/ml of TNF-α, (3) 10 ng/ml of TGF-β, (4) 500 U/ml of TNF-α and 10 ng/ml of TGF-β, (5) 40 nmol/l of DHT and 500 U/ml of TNF-α, (6) 40 nmol/l of DHT and 10 ng/ml, (7) 40 nmol/l of DHT, and (8) 40 nmol/L of DHT, 10 ng/ml of TGF-β, and 500 U/ml of TNF-α. The original culture medium was used as a solvent for each treatment. HBME cells were treated for approximately 24 h. Statistically significant values relative to the control are indicated by $^*(P < 0.05)$. Bars represent 95% confidence interval based on the ANOVA.

demonstrate no statistically significant difference between the 8 experimental conditions for surface expression of VCAM, alpha-2 integrin subunit, and beta-1 intregin subunit (all P-values are > 0.17) (Figure 5). HUVEC monolayers were treated as a control for TNF-α effect on endothelial cells (Table 2). HUVEC are sensitive to TNF-α stimulation and respond by expressing VCAM and E-selectin. Our data demonstrate that VCAM and E-selectin expression on HUVECs are upregulated by TNF-α treatment, thus indicating that our TNF-α treatment is effective.

The effect of TGF-β and DHT on α_2 and β_1 integrin subunit expression in normal and neoplastic cell lines was investigated. TNF-α was excluded from treatment regimens because of its reported cytotoxity on the LNCaP cell line [13, 16]. The data demonstrate that none of the four treatments significantly affected the expression of α_2 and β_1 integrin subunits in any cell line evaluated (all P-values > 0.14) (Figures 6A and B).

Discussion

The role that important cytokines (i.e., TNF-α and TGF-β) and androgen may play in prostate cancer cell adhesion to HBME monolayers has not been studied well. Cytokines have been demonstrated to regulate the expression of CAMs on both endothelial cells and prostate cancer cells [12, 17]. The cytokine activation of some endothelial cell types is enhanced in the presence of DHT [8]. The current study reports the effect of DHT and selected cytokines on endothelial cells and prostate cancer cells, and gives a rationale for the cytokines that were evaluated.

Serum levels of TNF-α have been demonstrated to be elevated in patients with advanced prostate cancer [11]. TNF-α is a potent activator of CAM expression on endothelial cells [17]. CAMs typically expressed on endothelial cell surfaces in response to TNF-α stimulation are VCAM, E-selectin and intracellular cell adhesion molecule (ICAM) [17]. These CAMs are known to mediate adhesion of a variety of tumor cells to HUVEC [7, 18, 19]. TNF-α induced VCAM expression on HUVEC is enhanced in the presence of physiologic concentrations of DHT [8]. DHT alone does not induce VCAM expression. The current study demonstrates that our immortalized HBME cells do not express VCAM in response to TNF-α stimulation. This is not the case for HBME cells immortalized in other laboratories [17, 20]. Interestingly, prostate cancer cell lines used in this study do not express receptors for VCAM, ICAM and E-selectin [21, 22]. Galectin-3, which is carbohydrate-binding protein, is expressed in PC-3 and LNCaP cells and it has been demonstrated to mediate PC-3 cell adhesion to HBME monolayers [4, 23]. Although the receptor for

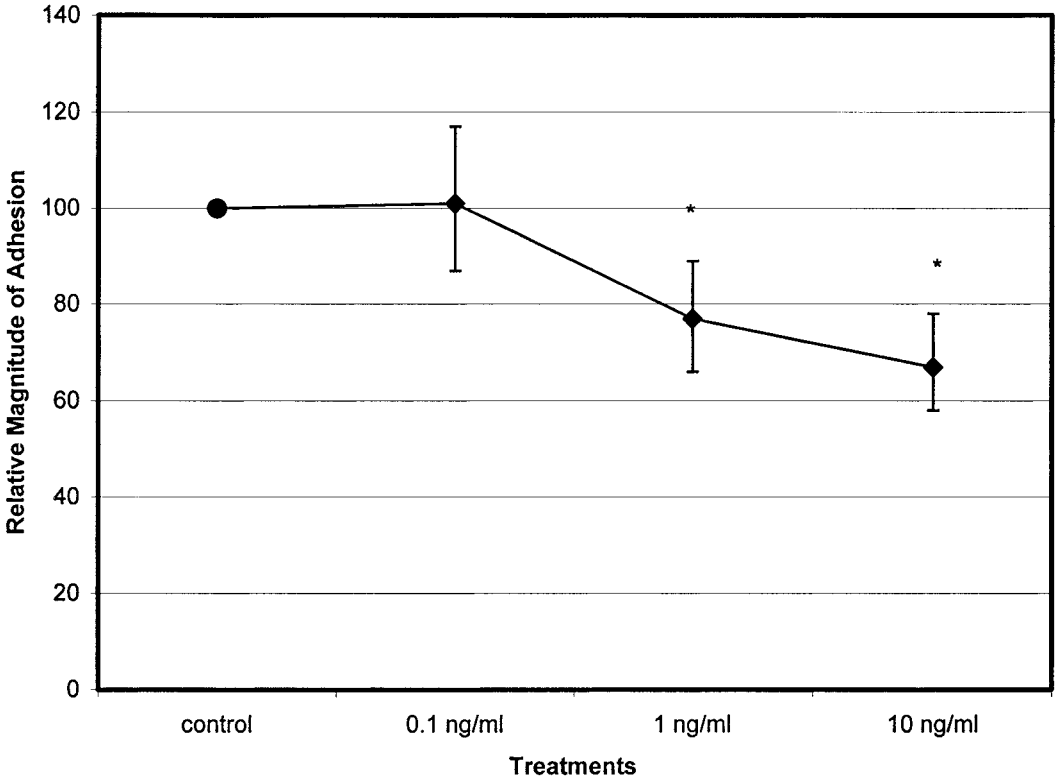

Figure 2. The effect of ascending concentrations of TGF-β on PC-3 cell adhesion to HBME cell monolayers. The control (solid circle) is the magnitude of PC-3 cell adhesion to untreated HBME cell monolayers. Statistically significant values relative to the control are indicated by $*(P < 0.05)$. Bars represent 95% confidence interval based on the ANOVA.

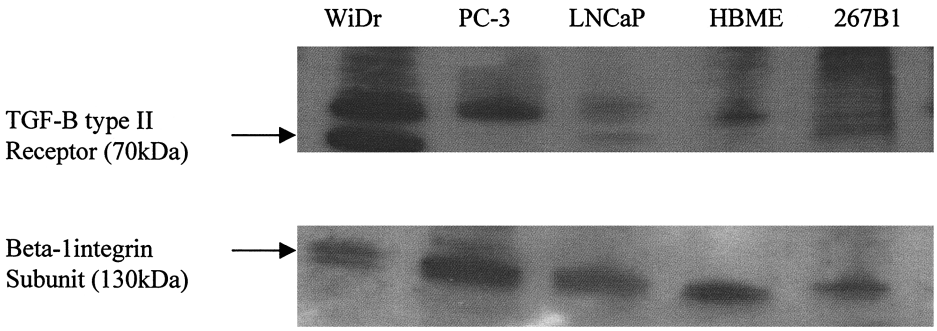

Figure 3. Western blot analysis of TGF-β receptor type II in membrane preparations of selected human cell lines. Beta-1 integrin subunit was evaluated as a control for the membrane associated proteins.

galectin-3 has not been identified, the data demonstrate that TNF-α stimulation does not alter its expression. The data generated during this investigation, therefore, suggest that TNF-α stimulation does not mediate prostate cancer cell adhesion to HBME cell monolayers via well-known inducible endothelial CAMs or unknown CAMs.

Although concentrations of plasma TGF-β do not correlate with progression of prostate cancer [23], this cytokine is prevalent in the bone, the preferred metastatic site for prostate cancer [24]. Kostenuik et al. [12] demonstrated that TGF-β upregulated PC-3 expression of $\alpha_2\beta_1$ and subsequently upregulated PC-3 cell adhesion to collagen type I. Data presented here does not support this phenomenon. Flow cytomeric analysis demonstrated that TGF-β did not alter $\alpha_2\beta_1$ expression on PC-3 cells and several cell lines including a colon cancer cell line (WiDr), a prostate cancer cell line derived from a lymph node metastasis (LNCaP), a normal prostate epithelial cell line immortalized with SV40 (NP,267B-1) [25] and our immortalized HBME cells. The difference between Kostenuik et al.'s study and the current study is that cell lysates were probed for $\alpha_2\beta_1$ in the former study. In the latter study, flow cytometry was used to evaluate $\alpha_2\beta_1$ expression at the cell surface. The inability of TGF-β to induce any response in selected cell lines was not due to the presence or absence of TGF-β receptor type II. We focused solely on receptor type II because it is responsible for binding TGF-β ligand from the medium and activating the signaling pathway [10]. TGF-β type II receptors were detected in WiDr and LNCaP cell membrane preparations by Western blotting. A previous study demonstrated that LNCaP and PC-3 cells expressed TGF-β receptor type II [15]. A more recent study [26] demonstrated that LNCaP cells do not express TGF-β receptor type II under conventional cell culture conditions. The objectives of these

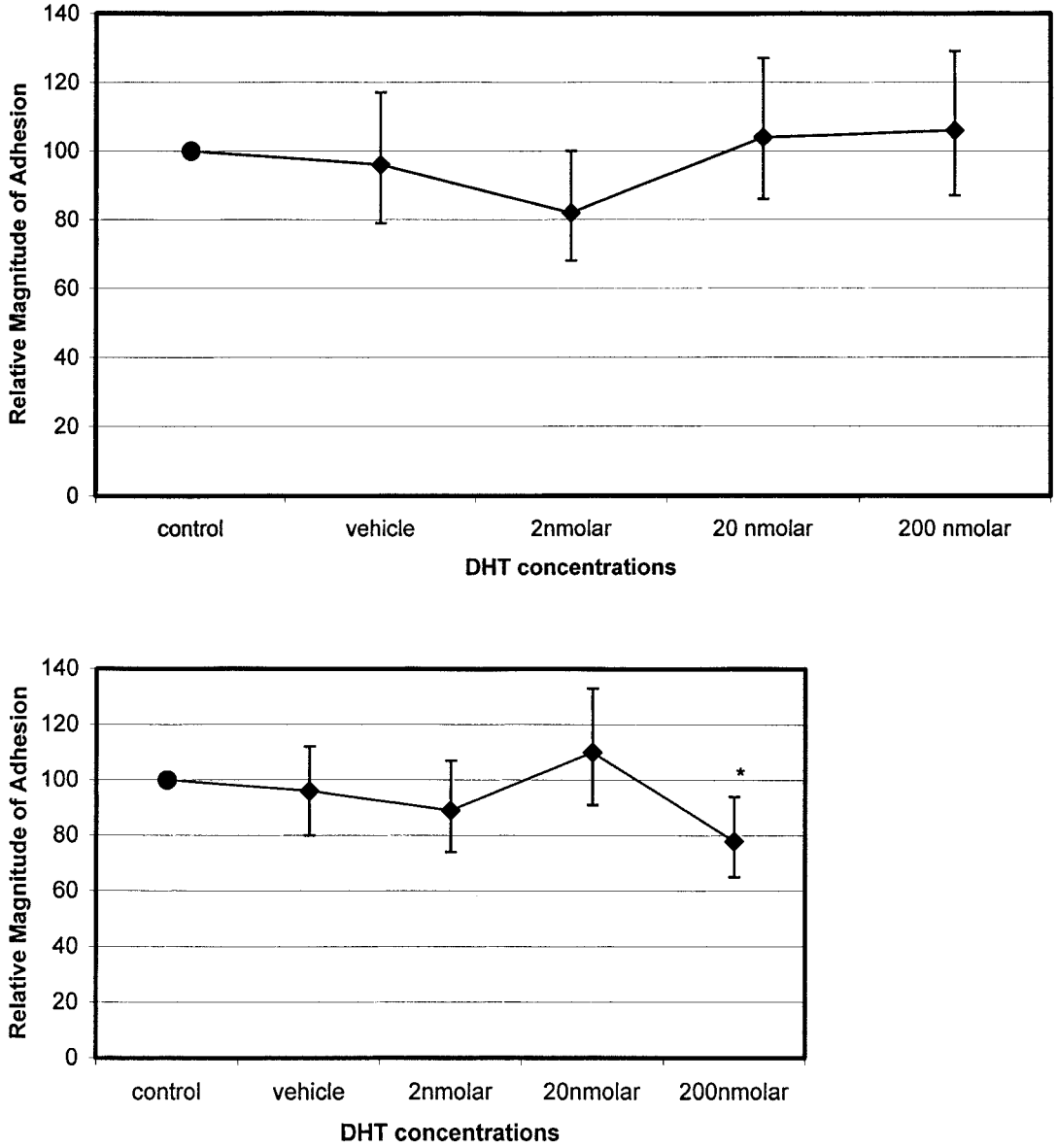

Figure 4. The effect of DHT on prostate cancer cell adhesion to HBME cell monolayers. A. LNCaP cells (A) and PC-3 cells (B) were treated for 24 h with increasing nanomolar concentrations of DHT dissolved in standard culture medium supplemented with charcoal-stripped fetal bovine serum. The control (solid circle) is the magnitude of untreated LNCaP cell adhesion to HBME cell monolayers. Statistically significant values relative to the control are indicated by $^{*}(P < 0.05)$.

particular experiments were (1) to determine if TGF-β up-regulated $\alpha_2\beta_1$ integrin expression in PC-3 cells only and (2) to determine if $\alpha_2\beta_1$ integrin plays a role in prostate cancer cell preferential adhesion to HBME *in vitro*. Alpha-2, beta-1 integrin mediates cell adhesion to extra-cellular matrix (ECM) components collagen type I, II, III and IV [22]. However, it is known that CAMs that mediate cell-to-ECM interactions can also mediate cell-to-cell interactions such as $\alpha_5\beta_1$ mediating the adhesion of human osteosarcoma cells, human melanoma cells and human kidney carcinoma cells to HUVEC [27]. The ligand for $\alpha_5\beta_1$ integrin is ECM component fibronectin [22]. Our data demonstrate that $\alpha_2\beta_1$ integrin is equally expressed on LNCaP, normal prostate epithelial cells and PC-3 cells, and slightly more expressed on the WiDr cells. WiDr cells adhere poorly to HBME cell monolayers and are used as a negative control in our adhesion assays [3]. Based on these observations, we conclude that $\alpha_2\beta_1$ integrin does not mediate preferential adhesion of prostate cancer cells to HBME cell monolayer.

The current study demonstrates that treatment of HBME monolayers with TGF-β significantly reduces their ability to bind PC-3 cells. The TGF-β treatment of LNCaP and not PC-3 cells also reduces their ability to bind HBME monolayers. This occurred despite our inability to detect TGF-β type II receptors on HBME cells. The TGF-β mediated inhibition was first demonstrated in a murine model consisting of P815 mastocytoma and microvascular endothelial cells [28]. The CAMs regulated by TGF-β were not identified in that study or the current study. The amount of TGF-β in the bone is greater than in any other organ [12]. Based on Bereta et al.' [28] and our observations, its presence in the bone should reduce the incidence of bone metastasis. This suggests that

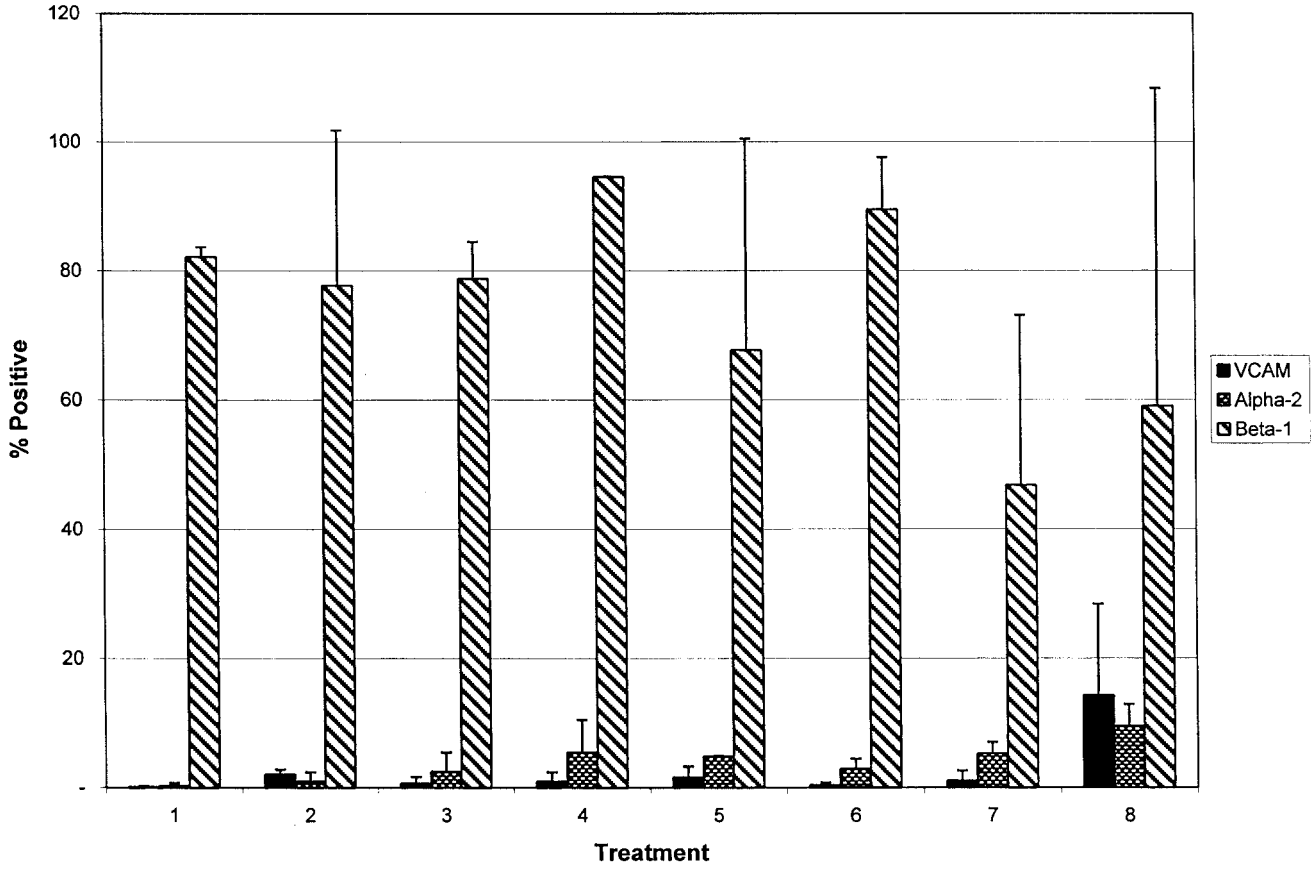

Figure 5. The effect of selected cytokine and DHT treatments on $\alpha_2\beta_1$ and VCAM expression on HBME cells as determined by flow cytometry. Treatments are as follows: (1) HBME culture media (Control), (2) 500 U/ml of TNF-α, (3) 10 ng/ml of TGF-β, (4) 500 U/ml of TNF-α and 10 ng/ml of TGF-β, (5) 40 nmol/l of DHT and 500 U/ml of TNF-α, (6) 40 nmol/l of DHT and 10 ng/ml, (7) 40 nmol/l of DHT, and (8) 40 nmol/l of DHT, 10 ng/ml of TGF-β, and 500 U/ml of TNF-α. The original culture medium was used as a solvent for each treatment. HBME cells were treated for approximately 24 h, the medium was removed and replaced with adhesion assay medium.

other factors in the bone may be negating the TGF-β effect on bone marrow endothelial cells and/or the concentration of TGF-β in the bone is not sufficient to alter CAM expression on the endothelium or metastasizing prostate cancer cells. Our data demonstrate a reduction in PC-3 cell adhesion to HBME cell monolayers at TGF-β concentrations exceeding 0.1 ng/ml. Using a murine model, Bereta and colleagues [28] demonstrated a reduction at TGF-β concentrations exceeding 0.5 ng/ml. Another explanation may be that TGF-β reduces bone metastasis in cancer cells that are TGF-β responsive, like LNCaP. The ability of TGF-β to reduce LNCaP but not PC-3 cell adhesion to HBME monolayers may explain the inability of LNCaP cells to metastasize to bone. Because TGF-β reduces HBME monolayers' ability to bind PC-3 but enhances PC-3's ability to bind collagen I, this cytokine may have mediated PC-3's ability to detach from the bone marrow endothelium and attach to the underlying bone matrix, which is composed mainly of collagen I [12].

In summary, this investigation demonstrates that DHT and TNF-α do not alter prostate cancer cell adhesion to bone marrow endothelial cells *in vitro*. Although TNF-α induces the expression of VCAM and E-selectin on HUVEC, it does not induce the expression of these CAMs on our established HBME cell line. HBME cell monolayers treated with TGF-β have a reduced affinity for prostate cancer cells *in vitro*. The TGF-β mediated effect is dose dependent. Because TGF-β is a common cytokine in the bone microenvironment, which is a common site for prostate cancer metastasis [12], it is unlikely that the effect of TGF-β on bone marrow endothelial cells prevents metastasis. We therefore speculate that TGF-β may play a role in releasing the attached cancer cells from the endothelium and upregulating their adhesion to the collagen I enriched bone matrix via enhanced $\alpha_2\beta_1$ expression [9, 12]. The prevalence of TGF-β in the bone and its ability to reduce endothelial cell binding to prostate cancer cells may be exploited to prevent skeletal complications associated with metastatic prostate cancer. Studies are currently underway to identify the CAMs on HBME cells that are regulated by TGF-β.

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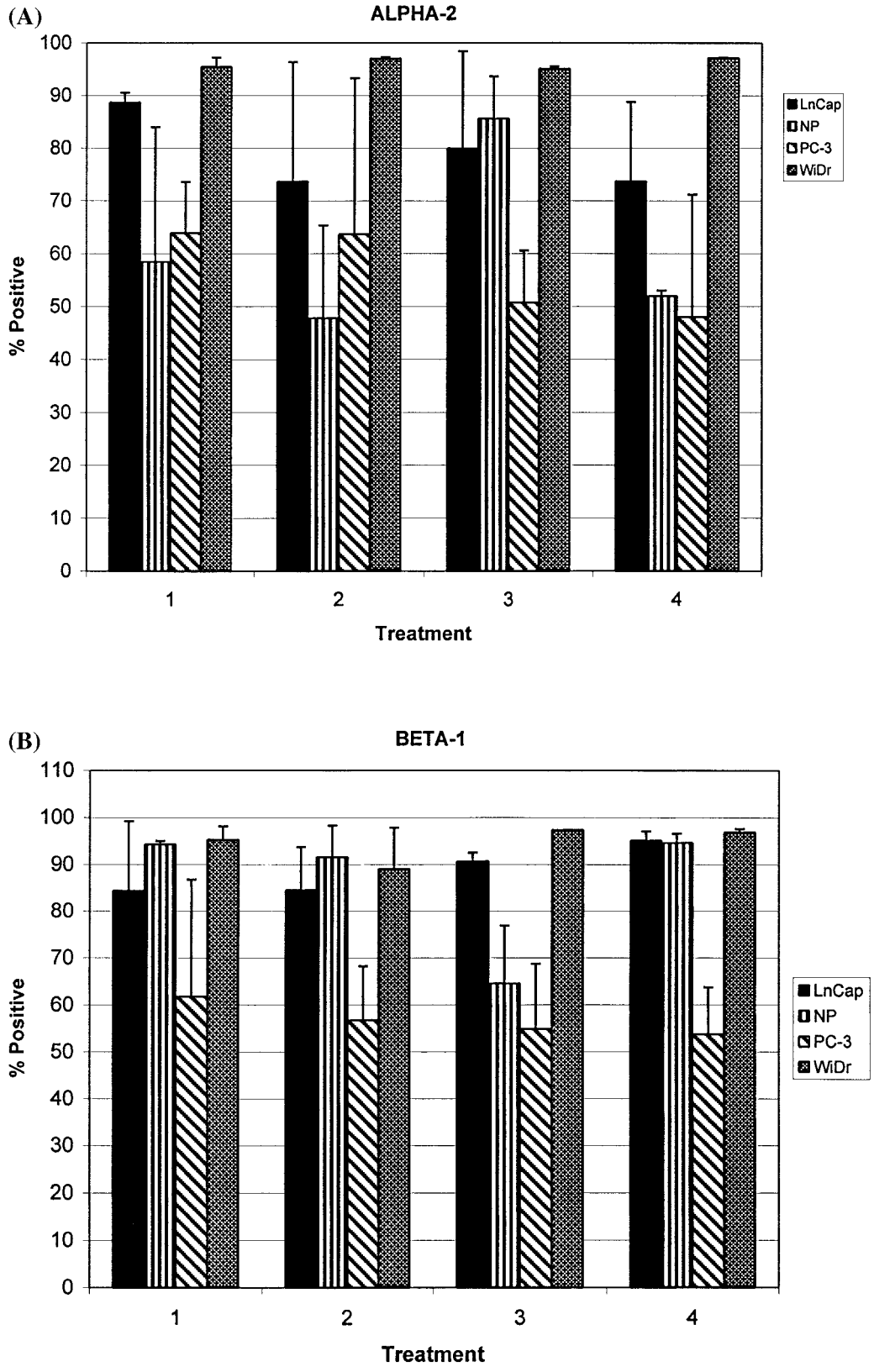

Figure 6. The effect of TGF-β and DHT treatments on $\alpha_2\beta_1$ expression in selected cell lines. A) Flow cytometric analysis of the $\alpha 2$ intregin subunit. B) Flow cytometric analysis of the $\beta 1$ intregin subunit. NP: normal prostate epithelial cell, 267B1. Treatments are as follows: (1) respective culture media (Control) for each cell lines, (2) 10 ng/ml of TGF-β, (3) 40 nmol/l of DHT, and (4) 40 nmol/l of DHT and 10 ng/ml. The original culture medium was used as a solvent for each treatment. HBME cells were treated for approximately 24 h, the medium was removed and replaced with adhesion assay medium.

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