

Bradykinin Receptor Subtype 1 Expression and Function in Prostate Cancer¹

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ABSTRACT

Kinins exert multiple pathophysiological functions, including vascular permeability and mitogenesis, by activating their cognate receptors, bradykinin subtype 1 receptor (B1R) and bradykinin subtype 2 receptor (B2R), which belong to the superfamily of G protein-coupled receptors. Tissue-specific expression pattern or contribution of the individual kinin receptors to pathological prostate cell growth is not known. We report here the differential expression of B1R and B2R in human benign and malignant prostate specimens. Whereas B2R is ubiquitously expressed, the B1R is detected only in prostatic intraepithelial neoplasia and malignant lesions and not in benign prostate tissues. Using androgen-insensitive prostate cancer PC3 cells, we show that specific stimulation of endogenous B1R promotes cell growth, migration, and invasion. These findings identify B1R as an early marker for pathological growth of the prostate and suggest its potential utility as a drug target effective for the treatment of prostate cancer.

INTRODUCTION

Prostate cancer is the most common noncutaneous malignancy affecting males in the United States (1). Growth of normal and malignant prostate is tightly regulated by action of the androgenic hormones, testosterone and dihydrotestosterone. This observation led to the development of effective endocrine-based, androgen-ablative therapy for prostate carcinoma (2). However, the observations that (a) prostate cancer afflicts mostly older men, who have decreased serum testosterone, and (b) androgen-ablative therapy fails in a substantial portion of patients who develop androgen-insensitive prostate cancer (3, 4) strongly suggest the existence of additional growth factors that contribute to prostate pathology.

hK2³ and hK3, also called prostate-specific antigen, are the best available cancer markers, and they are used for screening, diagnosis, and management of prostate cancer (5, 6). Importantly, hK2 is a glandular kallikrein with demonstrable kininogenase activity that is able to produce kinins, including BK and Lys-BK (7, 8). These kinins are rapidly metabolized to produce biologically active peptides, including des-Arg⁹-BK (9). Available evidence demonstrates that kinin expression is dramatically up-regulated in patients with ovarian, gastric, and hepatic cancers (10). These data suggest that the kinin system may be activated in prostate cancer and could contribute to the initiation and/or progression of the disease.

Kinins exert their effects on target cells by activating their cognate receptors, B1R and B2R (11), which are members of the superfamily of G protein-coupled receptors (12). B1R and B2R share little sequence homology and differ in their rank order of potency toward

specific agonists and antagonists. For example, des-Arg⁹-BK is a specific stimulant of B1R, and BK serves as a selective agonist for the B2R (13). In addition, whereas the B2R is constitutively expressed and is thought to mediate most of the physiological actions of kinins, the B1R is generally not expressed under nonpathological conditions but undergoes marked up-regulation after cell injury and stress (14), suggesting that B1R may become the dominant kinin receptor subtype in diseased tissue. Little is known about expression pattern and contribution of the individual kinin receptors to pathological growth of the prostate. We report here the specific expression of B1R in prostatic intraepithelial neoplasia (PIN) and malignant (but not benign) human prostate tissues. Furthermore, using prostate cancer PC3 cells as a model for the study of androgen-insensitive prostate cancer, we show that these cells express B1R and demonstrate that specific inhibition of B1R signaling attenuates cell growth, migration, and invasion.

MATERIALS AND METHODS

Materials. Prostate tissue samples were obtained from patients undergoing radical prostatectomy for biopsy-proven adenocarcinoma at Duke University Medical Center in accordance with guidelines of the Institutional Review Board for handling human materials. Specimens were obtained from the operating room immediately after resection. Tissue suspicious for cancer and tissue with normal appearance contralateral to the biopsy-proven site were obtained, fixed in 10% buffered neutral formalin, and embedded in paraffin. Sectioning of both benign- and malignant-appearing tissues was performed and interpreted by a single pathologist.

The B1R-specific agonist des-Arg⁹-BK and antagonist des-Arg⁹-[Leu⁸]BK were purchased from Sigma. Concentrations of the agonist and antagonist were empirically determined for each assay so that maximal but specific responses were obtained. Fibronectin was purchased from Sigma, and cell proliferation ELISA BrdU kit was from Roche Molecular. Peptides containing human B1R residues 317–353 and B2R residues 310–364 were synthesized and used to immunize New Zealand White rabbits. IgGs were purified using protein A resin, as described by the manufacturer (Pierce). Anti-phospho-ERK and anti-phospho-Akt antibodies were purchased from Cell Signaling, anti-ERK2 was purchased from Upstate Biotechnology, and horseradish peroxidase-conjugated secondary antibodies were purchased from Jackson ImmunoResearch. Peroxidase ABC immunostaining kit was purchased from Vector Laboratories. Cell culture media and supplements were purchased from Life Technologies, Inc. All other reagents were standard laboratory grade.

Cell Culture and Growth. Bone and lymph node metastasis human prostate cancer PC3 and LNCaP, respectively, cells were obtained from the American Type Culture Collection. The PC3 cells were used as a model for the study of androgen-independent, tumorigenic prostate cancer. Cells were maintained in RPMI 1640 containing 10% FCS and antibiotics (100 units/ml penicillin and 10 µg/ml streptomycin). For growth, high passage (passage 130–150) PC3 cells were seeded in 96-well plates at a density of 5000 cells/well in standard culture medium for 24 h to allow attachment. The culture medium was replaced with serum-free RPMI 1640 containing des-Arg⁹-BK (50 nM) or EGF (10 ng/ml). For antagonist experiments, des-Arg⁹-[Leu⁸]BK (1 µM) was added to cells 30 min before stimulation with agonist. Cell proliferation was determined after exposure to agonist for 48 h by colorimetric measurement of BrdU incorporation into nascent DNA according to the manufacturer's instructions (15). Absorbance of samples (A₄₅₀ nm) was determined using a scanning multiwell spectrophotometer, and absolute absorbance was normalized to the absorbance of unstimulated cells in each plate and expressed as a percentage of the control value.

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³ The abbreviations used are: hK, human kallikrein; B1R, bradykinin subtype 1 receptor; B2R, bradykinin subtype 2 receptor; PIN, prostatic intraepithelial neoplasia; ERK, extracellular signal-regulated kinase; BK, bradykinin; BrdUrd, bromodeoxyuridine; EGF, epidermal growth factor; ABC, avidin-biotin peroxidase complex; EGFR, epidermal growth factor receptor; HB-EGF, heparin-bound epidermal growth factor.

Immunohistochemical Staining. Sections of human prostate cancer biopsies were stained by the ABC method. Briefly, endogenous peroxidase activity of xylene-deparaffinized and rehydrated sections was inhibited by treatment with 0.3% H₂O₂/methanol. Antigen retrieval was accomplished by incubation in 10 mM citrate buffer (pH 6.0) at 94°C for 15 min. Nonspecific binding was blocked by incubating sections with 10% normal goat serum in a humidified chamber for 30 min at ambient temperature followed by overnight incubation at 4°C with a 1:200 dilution of rabbit polyclonal anti-B1R (or B2R) antibodies. The sections were washed twice with PBS, incubated with 5 µg/ml goat antirabbit biotinylated IgG in PBS containing 1% BSA for 30 min at ambient temperature, and then incubated with ABC reagent for 30 min. Specific immunostaining was visualized by 3,3'-diaminobenzidine. Slides were dehydrated through graded alcohol to xylene washing and mounted on cover slips. Hematoxylin was used for nuclear counterstaining. To determine B1R expression in PC3 and LNCaP cells, cells were grown on glass coverslips and fixed and stained with anti-B1R antibody, exactly as described above. For visualization, cells were incubated with FITC-conjugated donkey antirabbit IgG at a dilution of 1:200, and specific staining was determined using a Zeiss LSM 510 laser scanning confocal microscope equipped with a Zeiss 63 × 1.4 numerical aperture water immersion lens.

ERK and Akt Activation. PC3 cells were serum-starved for 24 h before exposure to agonist at 37°C. Antagonist was added 30 min before agonist stimulation. Reactions were terminated by direct addition of lysis buffer [150 mM NaCl, 50 mM Tris-HCl (pH 8.0), 5 mM EDTA, 10 mM NaF, 10 mM Na PP₃, 1% NP40, 0.5% deoxycholate, protease inhibitor tablet (Boehringer Mannheim), 1 mM phenylmethylsulfonyl fluoride, and 100 µM Na₃VO₄] to cell monolayers, and protein concentrations were determined using the Bradford assay. Equal amounts of protein from each sample were separated on 4–20% SDS-polyacrylamide electrophoresis gels and transferred to nitrocellulose filters. ERK and Akt phosphorylation was detected by immunoblot using phospho-specific ERK and Akt antibodies exactly as described previously (15). Phosphorylated ERK and Akt bands were visualized with enzyme-linked chemiluminescence and quantitated using Fluor-S MultiImager (Bio-Rad). Nitrocellulose filters were stripped of immunoglobulins and reprobed with ERK2 and Akt antibodies to confirm equal loading of protein.

Wound Healing Assay. PC3 cells were seeded in 6-well plates and incubated overnight in starvation medium (RPMI 1640 containing 0.1% BSA and 10 mM HEPES). Cell monolayers were wounded with a sterile 200-µl pipette tip, washed with starvation medium to remove detached cells, and stimulated with the indicated agonist for 36–48 h. Where indicated, the antagonist was

added to cells 30 min before stimulation. Next, cells were fixed in methanol, stained with 0.5% (w/v) crystal violet, washed with water, air dried, and inspected with a light microscope. Cells were photographed using a Coolpix 995 digital camera (Nikon) attached to dissection microscope (Bio-Rad), and images were imported into Adobe Photoshop 5.0 (Adobe Systems, Inc.). The number of cells in equal wounded fields was counted with a ×400 objective in an Axioskop microscope (Zeiss) to quantitate cell migration.

Cell Invasion. Cell invasion assays were performed in 8-µm-pore Transwell chambers (Costar). Briefly, PC3 cells were resuspended in starvation medium and added to the upper chambers (3 × 10⁴ cells/well) of fibronectin-coated (1 µg/ml) plates in the presence or absence of growth factors. Starvation medium containing the indicated agonist was added to the lower chambers, and cells were incubated at 37°C for 4 h. Cells that invaded the fibronectin layer and migrated to the lower surface of the filter were fixed with 3.7% (w/v) paraformaldehyde, stained with 0.5% (w/v) crystal violet, and counted with a ×400 objective in an Axioskop microscope (Zeiss).

RESULTS

The presence of individual kinin receptors was determined in prostate cancer biopsies obtained from patients undergoing radical prostatectomy at Duke University Medical Center. Fig. 1 shows the differential expression of B1R and B2R in benign and malignant human prostate tissues. Immunostaining with anti-B1R antibody showed clear and specific expression of the receptor in invasive carcinoma (Fig. 1A) and PIN (Fig. 1B), but not in benign glands. Furthermore, prostate stroma, inflammatory cells, endothelial cells, and nerves were uniformly negative (Fig. 1, A–C). Specificity of the antibody used was demonstrated by inclusion of a B1R antibody-neutralizing peptide before addition of the anti-B1R antibody, which eliminated the specific detection of the receptor (16). High-magnification images demonstrated the plasma membrane localization of the B1R in the epithelial cancer cells (Fig. 1C). Immunostaining with anti-B2R antibody showed similar expression of the receptor in both benign and malignant tissues, as well as in the prostate stroma (Fig. 1D). Thus, whereas expression of the B1R appears to be restricted to PIN and malignant tissue, expression of the B2R is ubiquitous in both the normal and diseased glandular tissue, as well as the stromal

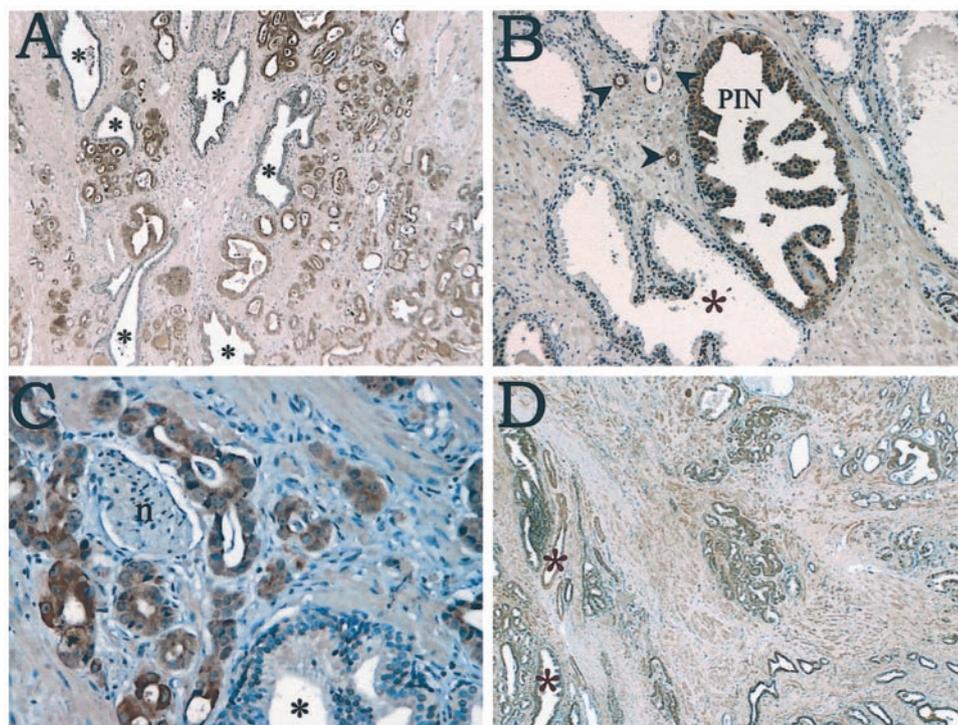


Fig. 1. Immunostaining of B1R and B2R in human prostate tissue. Paraffin-embedded sections of human prostate stained with anti-B1R (A–C) and anti-B2R (D) antibodies are shown. Specific staining was developed by the ABC method. A, low-power image of carcinoma infiltrating between benign and normal-appearing glands showing homogenous strong staining (×40). B, another case showing high intensity staining in invasive carcinoma (arrowheads) and PIN, and negative staining in normal-appearing glands (×100). C, higher-power view showing strong staining in carcinoma and negative staining in stroma, normal-appearing gland, and nerve (×400). D, low-power image of carcinoma infiltrating benign and normal-appearing glands showing homogenous staining for B2R (×40). Note positive staining of both glandular and stromal compartments. Asterisk, normal-appearing gland; arrowhead, invasive adenocarcinoma; n, nerve.

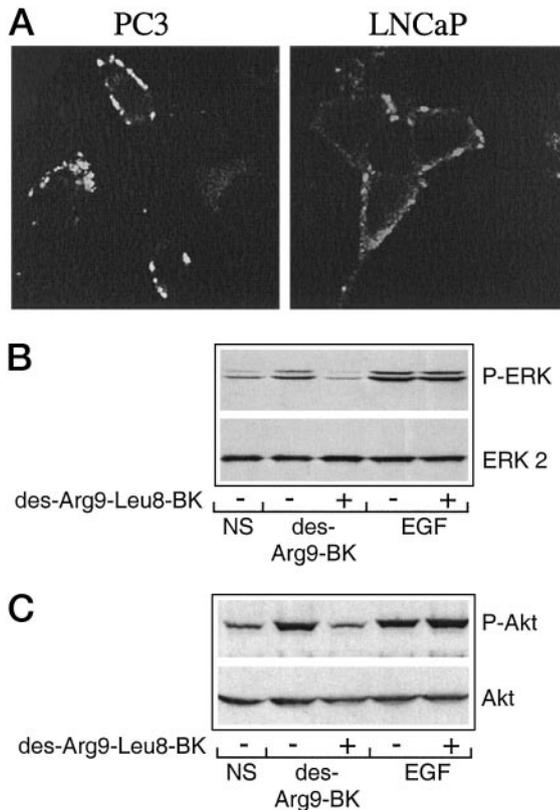


Fig. 2. Expression and function of B1R in androgen-insensitive prostate cancer PC3 cells. **A**, expression of B1R in prostate cancer cells. Cells were fixed in 4% paraformaldehyde and immunostained with anti-B1R antibody (1:200), followed by staining with FITC-conjugated antirabbit antibody. Shown are laser confocal images of cells depicting predominant cell surface localization of B1R. B1R mediates ERK (**B**) and Akt (**C**) phosphorylation in PC3 cells. Serum-starved cells were stimulated with des-Arg⁹-BK (10 nM) for 7 min or EGF (10 ng/ml) for 5 min at 37°C. Monolayers were lysed, and equal amounts of protein were loaded per lane and separated on 4–20% SDS-PAGE. For antagonist treatment, des-Arg⁹-[Leu⁸]BK (1 μM) was added 30 min before agonist stimulation. Proteins were transferred to nitrocellulose filters and immunoblotted with anti-phospho-ERK (**B**) and anti-phospho-Akt (**C**) antibodies. Filters were stripped of immunoglobulins and reblotted for ERK 2 (**B**, bottom panel) and Akt (**C**, bottom panel) to demonstrate equal loading of protein in each lane. On average, des-Arg⁹-BK and EGF induced ERK and Akt phosphorylation 7–8-fold and 3–4-fold, respectively, above unstimulated signal. Data are representative of four independent experiments.

compartment. Analysis of a total of 16 human prostate biopsies obtained from clinically localized prostate adenocarcinoma showed a similar pattern of B1R and B2R expression, suggesting that expression of the B1R protein correlates with the onset of prostate cancer. Because PIN is presumed to be a precursor lesion (17), our findings suggest the potential utility of B1R as an early marker for pathological growth of the prostate.

The finding that B1R is detected only in PIN and cancerous prostate tissues prompted us to investigate whether B1R may be involved in mitogenic signaling and growth of prostate cancer cells. To address this possibility, we first determined the expression profile of B1R in androgen-insensitive and tumorigenic (PC3 and DU145) and androgen-sensitive (LNCaP) prostate cancer cells. Crude plasma membranes were obtained by differential centrifugation (18), and expression of the B1R was determined by immunoblotting with the B1R-specific antibody. Results demonstrated expression of the B1R in all three cell types tested and showed specific expression of one major band that migrated to an apparent molecular mass of 40 kDa. This protein band was not detected in the presence of a neutralizing B1R synthetic peptide but was visible in the presence of a nonspecific B2R synthetic peptide (16). The prostate cancer cells were used to further confirm the expression of B1R and establish its subcellular localization. Con-

focal microscopic images were obtained from paraformaldehyde-fixed cells. Fig. 2A shows expression of B1R on the surface of PC3 and LNCaP cells as visualized by staining with FITC-conjugated antirabbit antibody. Control experiments were performed using rabbit isotype antibody and B1R antibody-neutralizing peptide that was added before addition of the B1R antibody. In both cases, no specific signal was detected (data not shown). These data demonstrate specific expression of B1R on the cell surface of prostate cancer cells and justify utility of the PC3 cells as a model to study the function of endogenous B1R in prostate cancer.

Advanced human prostate tumors express elevated levels of activated ERK1 and ERK2 mitogen-activated protein kinases compared with benign specimens (19, 20), suggesting a potential role for ERK in the pathological growth of the prostate. We tested whether specific stimulation of the B1R, which is expressed in PC3 cells, was able to activate ERK. As demonstrated in Fig. 2B (top panel), stimulation of the PC3 cells with des-Arg⁹-BK promoted the phosphorylation of ERK, and blockade of the B1R with des-Arg⁹-[Leu⁸]BK abrogated the ability of the receptor to activate ERK. The magnitude of B1R-mediated activation of ERK was similar to that achieved by the known mitogen EGF and was about 8-fold above basal levels (16). Stimulation of endogenous B1R could also induce phosphorylation of Akt, which is involved in cell survival and was inhibited in the presence of the B1R antagonist (Fig. 2C, top panel). In all cases, the antagonist reduced B1R-mediated signals (phosphorylation of ERK and Akt) but showed no effect on the EGF-mediated responses. The levels of ERK2 and Akt remained unaffected by the different treatments (Fig. 2, B and C, bottom panels), indicating that the observed changes stemmed from differences in protein activation and not in expression levels. Together, these data demonstrate expression of functional B1R in PC3 cells and show that specific activation of the receptor promotes mitogenic and survival signaling pathways in these androgen-insensitive prostate cancer cells.

To test the potential contribution of B1R to the growth of prostate cancer, PC3 cells were directly stimulated with des-Arg⁹-BK or EGF for 36–48 h. Fig. 3 shows that both agonists induced a similar 1.5-fold increases in the proliferation rate of cells, as determined by enhanced synthesis of nascent DNA. This effect was receptor mediated because preincubation of cells with the specific B1R antagonist des-Arg⁹-[Leu⁸]BK abrogated the signal initiated by B1R, but not

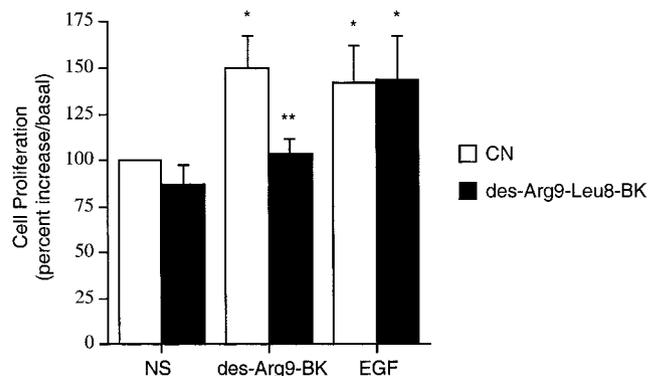


Fig. 3. B1R induces growth of androgen-insensitive PC3 cells. PC3 cells were seeded in 96-well plates and serum-starved overnight. B1R antagonist des-Arg⁹-[Leu⁸]BK (1 μM) was added 30 min before stimulation with the B1R agonist des-Arg⁹-BK (50 nM) or EGF (10 ng/ml) for 48 h at 37°C. Cell proliferation was determined by measuring BrdU incorporation into nascent DNA using an ELISA reader with light absorbance at 450 nm. Values are presented as the percentage increase over baseline by dividing the average of absolute absorbance in each treatment group by the value obtained for unstimulated cells per plate. Each point represents the mean ± SD of values obtained from three independent experiments. Asterisk indicates $P < 0.01$ versus control unstimulated cells, and double asterisk indicates $P < 0.05$ versus des-Arg⁹-BK-treated samples.

EGF. These data indicate that B1R is a potent mediator of prostate cancer cell growth.

An important characteristic of advanced prostate cancer is the ability of the cancer cells to leave the prostate gland and invade distal organs, such as bone and lymph nodes (21). *In vitro* wound healing assays have commonly been used to measure the ability of cells to migrate in response to stimulation with specific agonists (22). Treatment of wounded PC3 monolayers with des-Arg⁹-BK or EGF (used as a control) induced cells to migrate away from the edge of the cell monolayer and toward the wounded area (Fig. 4, *left column*). The number of cells that migrated to fill the wounded area in four equal, randomly selected fields was counted for each treatment using light microscopy and was as follows: (a) nonstimulated, 47 ± 20 cells; (b) des-Arg⁹-BK, 312 ± 52 cells; and (c) EGF, 265 ± 61 cells. However, when cells were pretreated with des-Arg⁹-[Leu⁸]BK before stimulation with des-Arg⁹-BK, wound filling was inhibited by 67% (102 ± 41 cells). Pretreatment of cells with the B1R antagonist did not exert any effect on the ability of EGF to promote wound filling (Fig. 4).

The characteristic ability to invade surrounding tissue constitutes another parameter for progression to advanced prostate cancer (21). The ability of B1R to mediate PC3 cell invasion was determined using a modified Boyden chamber assay. The dividing membrane was coated with fibronectin, which acts as an artificial matrix that cells must invade to migrate through the membrane pores in response to defined chemotactic stimuli. Fig. 5 shows that stimulation with des-Arg⁹-BK or EGF enhanced PC3 cell invasion approximately 3–4-fold above basal levels. In control wells, agonists were added to both the upper and lower chambers to determine random movement of the cells. Treatment of PC3 cells with the B1R antagonist did not affect the random- or EGF-induced movement of cells. On the other hand, pretreatment with the B1R antagonist abrogated the des-Arg⁹-BK-induced PC3 cell invasion capability. Taken together, these data demonstrate that B1R can mediate PC3 cell migration and invasion and suggest a potential role for B1R in prostate cancer metastasis.

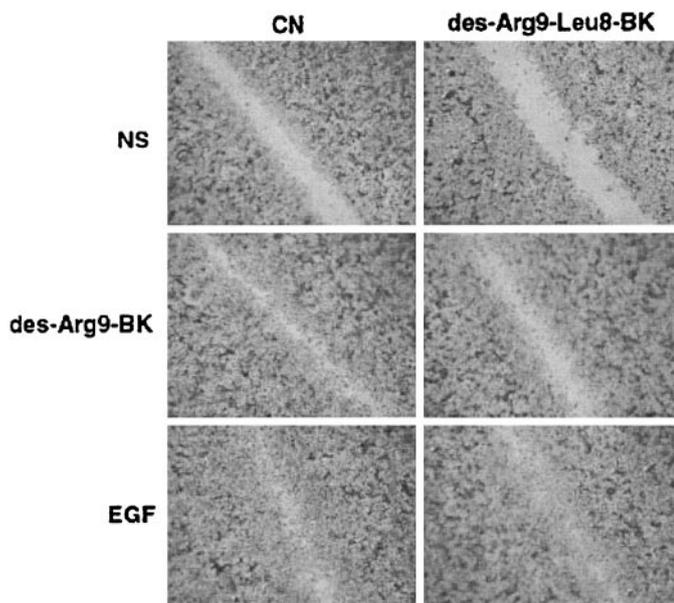


Fig. 4. Effect of B1R stimulation on PC3 cell *in vitro* wound healing migration. PC3 monolayers were wounded with a pipette tip, washed extensively to remove detached cells, and incubated with B1R agonist des-Arg⁹-BK (10 nM) or EGF (10 ng/ml) for 36–48 h at 37°C. Where indicated, B1R antagonist des-Arg⁹-[Leu⁸]BK (1 μ M) was added 30 min before stimulation. Methanol-fixed cells were stained with crystal violet. Phase-contrast images represent initial wounding of cell monolayers and migration in response to agonist stimulation. Data shown are representative of three independent experiments done in duplicate.

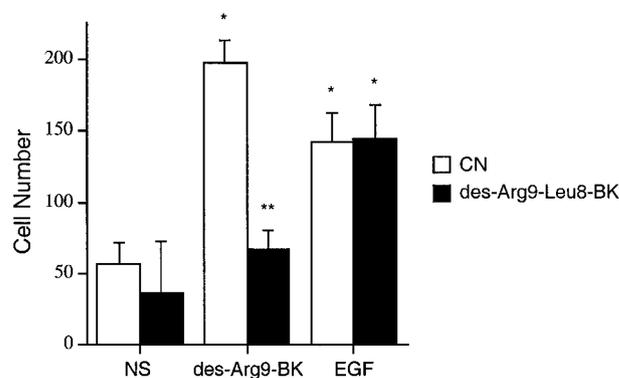


Fig. 5. B1R promotes PC3 cell invasion of fibronectin matrix. Cells were plated onto fibronectin-coated 8- μ m-pore membranes, stimulated with the indicated agonist, and induced to migrate in transwell assays for 4 h in the absence or presence of B1R antagonist. The number of cells that transmigrated to the lower chamber was quantitated as described. Each point represents the mean \pm SD of values obtained from three independent experiments. Asterisk indicates $P < 0.01$ versus control, and double asterisk indicates $P < 0.01$ versus des-Arg⁹-BK-treated samples. NS, nonstimulated.

DISCUSSION

Prostate cancer cells display an altered gene expression profile compared with normal prostate cells. Differences in the expression levels of genes and their products provide an opportunity for cancer diagnosis and treatment. Indeed, increased expression of hK2 and prostate-specific antigen is a widely practiced diagnostic tool for pathological growth of the prostate. In this study, we demonstrate that expression of B1R, which is activated by products of the hK2 enzyme, is detected only in PIN and prostate carcinoma and not in benign epithelial or stromal tissues. In addition, we show that specific stimulation of endogenous B1R promotes prostate cell growth and migration. These observations provide a link between B1R expression and pathological growth of the prostate and have implications for the pathogenesis, diagnosis, and potential treatment of prostate cancer.

Our results demonstrate that both B1R and B2R are expressed in epithelial prostate cancer cells and suggest that both receptor subtypes may contribute to prostate carcinogenesis. However, based on the present results showing expression of B2R in both benign and malignant prostate tissues, we propose that targeted inhibition of B2R may be of limited clinical value for the treatment of prostate cancer. The pan-inhibition of B2R signaling would be anticipated to exert toxic effects and disrupt normal function of all cells, including benign epithelial and stromal prostate cells. Importantly, the present results demonstrate specific expression of B1R only in diseased epithelial prostate cells. The current data also show that specific inhibition of B1R function attenuates mitogenic and survival signaling as well as PC3 cell growth. Based on these observations, we suggest that targeted inhibition of B1R signaling may be of clinical benefit to advanced prostate cancer patients.

PC3 cells, used here as a model for the study of androgen-insensitive prostate cancer, express endogenous B1R proteins that are competent to transduce cell growth signals. The magnitude of B1R-mediated PC3 cell growth was similar to that achieved by the known mitogen EGF (Fig. 3). The exact signaling pathways involved in B1R-mediated cell growth are not clear. Agonist-bound B1R can affect target cells via immediate activation of the heterotrimeric $G\alpha_q$ proteins. Previously, we showed that BK-induced activation of $G\alpha_q$ subunits promotes the growth of PC3 cells via sequential phosphorylation of EGFR and ERK (23). Activated EGFR forms a multiprotein signaling complex that culminates in signal relay from Ras \rightarrow Raf \rightarrow mitogen-activated protein/ERK kinase (MEK) \rightarrow ERK. The identity of intermediates connecting the activated $G\alpha_q$ and EGFR is less

clear and may be cell type specific. In vascular smooth muscle cells, $G\alpha_q$ induces the Ca^{2+} -dependent activation of matrix metalloproteinases that promote shedding of plasma membrane-anchored HB-EGF (24). Soluble HB-EGF binds EGFR in an autocrine or paracrine fashion and activates it exactly as if it were directly stimulated with exogenously added HB-EGF. Another potential mechanism for the $G\alpha_q$ -mediated activation of EGFR involves the nonreceptor tyrosine kinase c-Src (25), which can directly phosphorylate EGFR on Tyr⁸⁴⁵ and activate it (26). Our data demonstrate that BIR activates the mitogenic ERK pathway and induces prostate cancer cell growth. The exact signal transduction pathway(s) used by BIR to activate ERK in PC3 cells remains unclear. Significantly, antagonism of BIR attenuates prostate cancer cell growth and may be considered as an effective option for treatment of prostate cancer.

The cell migration and invasion assays provide direct evidence for involvement of BIR in chemotactic responses of prostate cancer cells. How does BIR function to positively regulate cellular chemotaxis? Although one can only speculate, recent advances suggest several possibilities. First, BIR may regulate cell migration via activation of heterotrimeric $G_{12/13}$ proteins, which can be activated by kinin receptors (27). Recent data demonstrate that activated $G_{12/13}$ proteins inhibit cadherin function to promote cell adhesion and that expression of constitutively active $G_{12/13}$ promoted breast cancer cell migration in the wound healing assay (28). Second, BIR can induce cell migration via β -arrestin proteins, which are recruited to the plasma membrane to participate in many G protein-coupled receptor-regulated signal transduction events (29). For example, targeted disruption of β -arrestin 2 in mice leads to defective T-lymphocyte chemotaxis (30). More recent work showed that β -arrestin 2 associates with Ral GDP dissociation stimulator, which is involved in chemoattractant-induced cytoskeletal reorganization (31). Third, BIR can promote prostate cell migration via the activation of focal adhesions. Preliminary data show that stimulation of BIR in PC3 cells induces the tyrosine phosphorylation of the focal adhesion kinase FAK, which is important in cytoskeletal reorganization and cell migration (32). Finally, BIR could regulate prostate cancer cell movement via activation of matrix metalloproteinases, which promote degradation of the extracellular matrix, an early event in cell migration and metastasis (33, 34). Nonetheless, our data show that BIR can promote cell migration and invasion, which are critical determinants for progression to advanced-stage prostate cancer.

In summary, we demonstrated specific expression of BIR in PIN and prostate cancer cells and showed that stimulation of BIR regulates androgen-insensitive prostate cancer cell growth and migration. The practical outcome of this study is identification of BIR as an early marker for detection of prostate cancer and as a potential target for adjunctive therapy of hormone-refractory prostate cancer cells.

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REFERENCES

- Greenlee, R. T., Hill-Harmon, M. B., Murray, T., and Thun, M. Cancer statistics, 2001. *CA Cancer J. Clin.*, *51*: 15–36, 2001.
- Henry, R. Y., and O'Mahony, D. Treatment of prostate cancer. *J. Clin. Pharm. Ther.*, *24*: 93–102, 1999.
- Isaacs, W., De Marzo, A., and Nelson, W. Focus on prostate cancer. *Cancer Cells*, *2*: 113–116, 2002.
- Small, E. J. Update on the diagnosis and treatment of prostate cancer. *Curr. Opin. Oncol.*, *10*: 244–252, 1998.
- Rittenhouse, H. G., Finlay, J. A., Mikolajczyk, S. D., and Partin, A. W. Human kallikrein 2 (hK2) and prostate-specific antigen (PSA): two closely related, but distinct, kallikreins in the prostate. *Crit. Rev. Clin. Lab. Sci.*, *35*: 275–368, 1998.
- Diamandis, E. P., Yousef, G. M., Luo, L. Y., Magklara, A., and Obiezu, C. V. The new human kallikrein gene family: implications in carcinogenesis. *Trends Endocrinol. Metab.*, *11*: 54–60, 2000.
- Charlesworth, M. C., Young, C. Y., Miller, V. M., and Tindall, D. J. Kininogenase activity of prostate-derived human glandular kallikrein (hK2) purified from seminal fluid. *J. Androl.*, *20*: 220–229, 1999.
- Cloutier, S. M., Chagas, J. R., Mach, J. P., Gygi, C. M., Leisinger, H. J., and Deperthes, D. Substrate specificity of human kallikrein 2 (hK2) as determined by phage display technology. *Eur. J. Biochem.*, *269*: 2747–2754, 2002.
- Bhoola, K. D., Figueroa, C. D., and Worthy, K. Bioregulation of kinins: kallikreins, kininogens, and kininases. *Pharmacol. Rev.*, *44*: 1–80, 1992.
- Sawant, S., Snyman, C., and Bhoola, K. Comparison of tissue kallikrein and kinin receptor expression in gastric ulcers and neoplasms. *Int. Immunopharmacol.*, *1*: 2063–2080, 2001.
- Regoli, D., Jukic, D., Gobeil, F., and Rhaleb, N. E. Receptors for bradykinin and related kinins: a critical analysis. *Can. J. Physiol. Pharmacol.*, *71*: 556–567, 1993.
- Pierce, K. L., Premont, R. T., and Lefkowitz, R. J. Seven-transmembrane receptors. *Nat. Rev. Mol. Cell. Biol.*, *3*: 639–650, 2002.
- Leeb, T., Mathis, S. A., and Leeb-Lundberg, L. M. The sixth transmembrane domains of the human B1 and B2 bradykinin receptors are structurally compatible and involved in discriminating between subtype-selective agonists. *J. Biol. Chem.*, *272*: 311–317, 1997.
- Phagoo, S. B., Reddi, K., Anderson, K. D., Leeb-Lundberg, L. M., and Warburton, D. Bradykinin B1 receptor up-regulation by interleukin-1 β and B1 agonist occurs through independent and synergistic intracellular signaling mechanisms in human lung fibroblasts. *J. Pharmacol. Exp. Ther.*, *298*: 77–85, 2001.
- Kue, P. F., and Daaka, Y. Essential role for G proteins in prostate cancer cell growth and signaling. *J. Urol.*, *164*: 2162–2167, 2000.
- Barki-Harrington, L., Bookout, A. L., Lamb, M. E., Leeb-Lundberg, L. M. F., and Daaka, Y. Requirement for direct cross-talk between B1 and B2 kinin receptors for the proliferation of androgen-insensitive prostate cancer PC3 cells. *Biochem. J.*, *371*: 581–587, 2003.
- Bostwick, D. G. Prostatic intraepithelial neoplasia (PIN). *Urology*, *34*: 16–22, 1989.
- Krueger, K. M., Daaka, Y., Pitcher, J. A., and Lefkowitz, R. J. The role of sequestration in G protein-coupled receptor resensitization. Regulation of β 2-adrenergic receptor dephosphorylation by vesicular acidification. *J. Biol. Chem.*, *272*: 5–8, 1997.
- Gioeli, D., Mandell, J. W., Petroni, G. R., Frierson, H. F., Jr., and Weber, M. J. Activation of mitogen-activated protein kinase associated with prostate cancer progression. *Cancer Res.*, *59*: 279–284, 1999.
- Price, D. T., Rocca, G. D., Guo, C., Ballo, M. S., Schwinn, D. A., and Luttrell, L. M. Activation of extracellular signal-regulated kinase in human prostate cancer. *J. Urol.*, *162*: 1537–1542, 1999.
- Scher, H. I., and Heller, G. Clinical states in prostate cancer: toward a dynamic model of disease progression. *Urology*, *55*: 323–327, 2000.
- Hauck, C. R., Hsia, D. A., and Schlaepfer, D. D. Focal adhesion kinase facilitates platelet-derived growth factor-BB-stimulated ERK2 activation required for chemotaxis migration of vascular smooth muscle cells. *J. Biol. Chem.*, *275*: 41092–41099, 2000.
- Barki-Harrington, L., and Daaka, Y. Bradykinin induced mitogenesis of androgen independent prostate cancer cells. *J. Urol.*, *165*: 2121–2125, 2001.
- Eguchi, S., Dempsey, P. J., Frank, G. D., Motley, E. D., and Inagami, T. Activation of MAPKs by angiotensin II in vascular smooth muscle cells. Metalloprotease-dependent EGF receptor activation is required for activation of ERK and p38 MAPK but not for JNK. *J. Biol. Chem.*, *276*: 7957–7962, 2001.
- Luttrell, L. M., Daaka, Y., and Lefkowitz, R. J. Regulation of tyrosine kinase cascades by G-protein-coupled receptors. *Curr. Opin. Cell Biol.*, *11*: 177–183, 1999.
- Biscardi, J. S., Maa, M. C., Tice, D. A., Cox, M. E., Leu, T. H., and Parsons, S. J. c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr⁸⁴⁵ and Tyr¹¹⁰¹ is associated with modulation of receptor function. *J. Biol. Chem.*, *274*: 8335–8343, 1999.
- Gohla, A., Offermanns, S., Wilkie, T. M., and Schultz, G. Differential involvement of $G\alpha_{12}$ and $G\alpha_{13}$ in receptor-mediated stress fiber formation. *J. Biol. Chem.*, *274*: 17901–17907, 1999.
- Meigs, T. E., Fedor-Chaiken, M., Kaplan, D. D., Brackenbury, R., and Casey, P. J. $G\alpha_{12}$ and $G\alpha_{13}$ negatively regulate the adhesive functions of cadherin. *J. Biol. Chem.*, *277*: 24594–24600, 2002.
- Perry, S. J., and Lefkowitz, R. J. Arresting developments in heptahelical receptor signaling and regulation. *Trends Cell Biol.*, *12*: 130–138, 2002.
- Fong, A. M., Premont, R. T., Richardson, R. M., Yu, Y. R., Lefkowitz, R. J., and Patel, D. D. Defective lymphocyte chemotaxis in β -arrestin2- and GRK6-deficient mice. *Proc. Natl. Acad. Sci. USA*, *99*: 7478–7483, 2002.
- Bhattacharya, M., Anborgh, P. H., Babwah, A. V., Dale, L. B., Dobransky, T., Benovic, J. L., Feldman, R. D., Verdi, J. M., Rylett, R. J., and Ferguson, S. S. β -Arrestins regulate a Ral-GDS Ral effector pathway that mediates cytoskeletal reorganization. *Nat. Cell Biol.*, *4*: 547–555, 2002.
- Hauck, C. R., Sieg, D. J., Hsia, D. A., Loftus, J. C., Gaarde, W. A., Monia, B. P., and Schlaepfer, D. D. Inhibition of focal adhesion kinase expression or activity disrupts epidermal growth factor-stimulated signaling promoting the migration of invasive human carcinoma cells. *Cancer Res.*, *61*: 7079–7090, 2001.
- Kue, P. F., Taub, J. S., Barki-Harrington, L., Polakiewicz, R. D., Ullrich, A., and Daaka, Y. Lysophosphatidic acid-regulated mitogenic ERK signaling in androgen-insensitive prostate cancer PC-3 cells. *Int. J. Cancer*, *102*: 572–579, 2002.
- Coussens, L. M., Fingleton, B., and Matrisian, L. M. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science (Wash. DC)*, *295*: 2387–2392, 2002.

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