Biochemical Pharmacology xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Bradykinin promotes vascular endothelial growth factor expression and increases angiogenesis in human prostate cancer cells

Hsin-Shan Yu [a,1], Shih-Wei Wang [b,1], An-Chen Chang [a], Huai-Ching Tai [c,d], Hung-I Yeh [b,e], Yu-Min Lin [f,g,**], Chih-Hsin Tang [a,h,i,*]

[a] Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan
[b] Department of Medicine, Mackay Medical College, New Taipei City, Taiwan
[c] Department of Urology, National Taiwan University Hospital, Taipei, Taiwan
[d] Graduate Institute of Clinical Medicine, National Taiwan University, Taipei, Taiwan
[e] Department of Internal Medicine, Mackay Memorial Hospital, Taipei, Taiwan
[f] Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
[g] Department of Orthopedic Surgery, Taichung Veterans General Hospital, Taichung, Taiwan
[h] Department of Pharmacology, School of Medicine, China Medical University, Taichung, Taiwan
[i] Department of Biotechnology, College of Health Science, Asia University, Taichung, Taiwan

ARTICLE INFO

Article history:
Received 7 August 2013
Accepted 17 October 2013
Available online xxx

Keywords:
Bradykinin
Angiogenesis
VEGF
Prostate cancer

ABSTRACT

Prostate cancer is the most commonly diagnosed malignancy in men and shows a tendency for metastasis to distant organs. Angiogenesis is required for metastasis. Bradykinin (BK) is an inflammatory mediator involved in tumor growth and metastasis, but its role in vascular endothelial growth factor (VEGF) expression and angiogenesis in human prostate cancer remains unknown. The aim of this study was to examine whether BK promotes prostate cancer angiogenesis via VEGF expression. We found that exogenous BK increased VEGF expression in prostate cancer cells and further promoted tube formation in endothelial progenitor cells and human umbilical vein endothelial cells. Pretreatment of prostate cancer with B2 receptor antagonist or small interfering RNA (siRNA) reduced BK-mediated VEGF production. The Akt and mammalian target of rapamycin (mTOR) pathways were activated after BK treatment, and BK-induced VEGF expression was abolished by the specific inhibitor and siRNA of the Akt and mTOR cascades. BK also promoted nuclear factor-κB (NF-κB) and activator protein 1 (AP-1) activity. Importantly, BK knockdown reduced VEGF expression and abolished prostate cancer cell conditional medium-mediated angiogenesis. Taken together, these results indicate that BK operates through the B2 receptor, Akt, and mTOR, which in turn activate NF-κB and AP-1, activating VEGF expression and contributing to angiogenesis in human prostate cancer cells.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Prostate cancer is a common health problem worldwide and the high cancer mortalities in men [1]. Because prostate cancer shows a weak response to chemotherapy, surgery and radiation treatment are usually used to treat early stage prostate cancer [2]. Similar to the treatment of other cancers, therapy during the advanced disease stages of prostate cancer requires systemic intervention to inhibit the growth and spread of secondary metastasis. Secondary metastasis contributes to the high mortality rate in prostate cancer as a result of lymphatic or hematogenous dissemination of cancer cells [3]. Therefore, improving treatment strategies requires an understanding of the regular mechanisms of metastasis in human prostate cancer and the identification of critical signaling markers as potential targets.

Angiogenesis, the development of new vessels from existing vasculature, plays an important role in solid tumor growth, invasion, and metastasis [4,5]. The formation of a separate blood supply to the tumor for nutrients and oxygen is a necessary step during tumor growth and the metastatic process [6]. Vascular endothelial growth factor (VEGF) is a key mediator in wound healing, embryonic development, growth of certain solid tumors, and angiogenesis [7]. VEGF is reportedly up-regulated by oncogene expression, a variety of growth factors, and hypoxia [8]. On the

* Corresponding author at: Graduate Institute of Basic Medical Science, China Medical University, No. 91, Hsueh-Shih Road, Taichung, Taiwan.
Tel.: +886 4 22052121x7726; fax: +886 4 22333641.
** Corresponding author.
E-mail addresses: yuminlin@gmail.com (Y.-M. Lin), chtang@mail.cmu.edu.tw (C.-H. Tang).
[1] These authors equally contributed to this work.

0006-2952/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bcp.2013.10.016

Please cite this article in press as: Yu H-S, et al. Bradykinin promotes vascular endothelial growth factor expression and increases angiogenesis in human prostate cancer cells. Biochem Pharmacol (2013), http://dx.doi.org/10.1016/j.bcp.2013.10.016

other hand, tumor growth and angiogenesis is significantly attenuated by VEGF antagonist treatment [9]. Therefore, VEGF is central in cancer metastasis and an important target for therapy.

BK is an autocrine growth factor that directly stimulates cancer growth and angiogenesis by stimulating the release of fibroblast growth factor and VEGF [10]. BK also facilitates prostate cancer migration and invasion by stimulating the activity of membrane matrix metalloproteases [11] and intercellular adhesion molecule-1 [12]. The function of BK is regulated by 2 G-protein-coupled BK receptors, B1 and B2, which have been pharmacologically characterized and defined through molecular cloning [13,14]. Recent studies have reported that BK can induce proliferation, invasion, and migration in various cell types via BK receptors, suggesting that BK receptors play critical roles in tumor metastasis [15–17]. In addition, the B2 receptor, more so than the B1 receptor, is commonly involved in most BK-mediated biological actions [17–19].

The activation of Akt regulates several cellular processes including cell proliferation and survival, angiogenesis, and tissue invasion [20]. The Akt signaling pathway is believed to play an important role in the genesis of some human cancers [21]. Downstream of Akt, mammalian target of rapamycin (mTOR) protein kinase is included in 2 distinct multi-protein complexes that regulate different branches of the Akt/mTOR signaling pathway: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [22,23]. Compelling evidence indicates that mTORC2 phosphorylates the pro-survival kinase Akt on Ser^{473}, thereby regulating Akt activity [24]. Increasing evidence from *in vitro* studies has demonstrated that the Akt/mTOR pathway plays a critical role in prostate cancer development and progression [25]. However, the role of the Akt/mTOR signaling pathway in regulating BK-mediated VEGF expression and angiogenesis in human prostate cancer remains unknown.

Angiogenesis is indispensable for the development, growth, and progression of human cancers [26,27]. VEGF is a key factor in tumor angiogenesis [28]. Although BK has been implicated in angiogenesis in some cancer cells [29], the signaling pathway for BK in VEGF expression in human prostate cancer cells has been inadequately studied. We hypothesized that BK regulates the angiogenesis of prostate cancer cells, and our study found that BK increases tube formation capability and VEGF expression in human prostate cancer cells. Moreover, B2 receptor, Akt, mTOR, and NF-κB/AP-1 signaling pathways may be involved in increasing VEGF expression and angiogenesis by BK.

2. Materials and methods

2.1. Materials

Anti-mouse and anti-rabbit immunoglobulin G-conjugated horseradish peroxidase, rabbit polyclonal antibodies specific for VEGF, Akt, p-Akt, mTOR, p-mTOR, c-Jun, p-c-Jun, p-65, p-p-65, β-actin, and small interfering RNA (siRNA) against B2 receptor, mTOR, c-Jun, and control (negative control for experiments using targeted siRNA transfection; each consisted of a scrambled sequence that caused no specific degradation of any known cellular messenger RNA [mRNA]) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). B2 receptor antagonist HOE140 was purchased from Tocris Bioscience (Ellisville, MO, USA). The Akt inhibitor 1L-6-hydroxymethyl-chiro-inositol-2-([R]-2-Omethyl-3-O-octadecylcarbonate), rapamycin, and BK (dissolved in 0.1 M acetic acid and stored concentration was 10 μM) were purchased from Calbiochem (San Diego, CA, USA). NF-κB and AP-1 luciferase plasmids were purchased from Stratagene (La Jolla, CA, USA). An Akt dominant-negative mutant (Akt K179A) was a gift from Dr. W.M. Fu (National Taiwan University, Taipei, Taiwan). Inhibitor of nuclear factor kappa-B kinase (IKK) α and β mutants IKKα (KM) and IKKβ (KM) were gifts from Dr. H. Nakano (Juntendo University, Tokyo, Japan). The pSV-β-galactosidase vector and luciferase assay kit were purchased from Promega (Madison, MA, USA). All other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Cell culture

Human prostate cancer cell lines (PC3 and DU145) were purchased from the American type culture collection (Manassas, VA, USA). The cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 20 μM of 2-(4-[2-hydroxyethyl]piperazin-1-yl)ethanesulfonic acid (HEPES), 10% heat-inactivated fetal bovine serum (FBS), 2 μM glutamine, penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37 °C in 5% CO_2.

Human umbilical vein endothelial cells (HUVEC) (ScienCell Research Laboratories, San Diego, CA, USA) were grown to confluence on 1% gelatin, and maintained in MV2 complete medium consisting of MV2 basal medium and growth supplement (PromoCell, Heidelberg, Germany) supplied in 20% defined FBS (HyClone, Logan, UT, USA).

2.3. Measurement of VEGF production

Human prostate cancer cells were cultured in 24-well culture plates. After reaching 70% of confluence, cells were changed to serum-free medium for 24 h. The cells were then treated with BK and incubated in a humidified incubator at 37 °C for 24 h. To examine the downstream signaling pathways involved in BK treatment, we pretreated cells with various inhibitors for 30 min before adding BK (100 nM). After incubation, the medium was removed and stored at −80 °C until the assay was performed. VEGF in the medium (the VEGF concentration was normalized by total protein) was assayed using VEGF enzyme immunoassay kits according to the manufacturer procedure.

2.4. Reverse transcription quantitative real-time polymerase chain reaction

Total RNA was extracted from prostate cancer cells using a TRIzol kit (MDBio Inc., Taipei, Taiwan). The reverse transcription reaction was performed using 2 μg of total RNA (in 2 μL RNase-free water) that was reverse transcribed into complementary DNA with a Moloney murine leukemia virus reverse transcription (MMLV RT) kit (Promega, Madison, WI, USA) following manufacturer procedures [30,31]. Quantitative real-time PCR (qPCR) analysis was carried out with TaqMan one-step PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Complementary DNA template (2 μL) was added to each 25-μL reaction with sequence-specific primers and TaqMan probes. All target gene primers and probes were purchased commercially (β-actin [Applied Biosystems] was used an internal control). qPCR assays were carried out in triplicate on a StepOnePlus sequence detection system. The cycling conditions were 10-min polymerase activation at 95 °C followed by 40 cycles at 95 °C for 15 s and 60 °C for 60 s. The threshold was set above the non-template control background and within the linear phase of target gene amplification to calculate the cycle number at which the transcript was detected (denoted as C_T).

2.5. Isolation and cultivation of endothelial progenitor cells (EPCs)

Ethical approval was granted by the institutional review board of Mackay Medical College (New Taipei City, Taiwan; reference number: P1000002). Informed consent was obtained from healthy donors before the collection of peripheral blood (80 mL). Peripheral blood mononuclear cells were fractionated from other

Please cite this article in press as: Yu H-S, et al. Bradykinin promotes vascular endothelial growth factor expression and increases angiogenesis in human prostate cancer cells. Biochem Pharmacol (2013), http://dx.doi.org/10.1016/j.bcp.2013.10.016

Download English Version:

https://daneshyari.com/en/article/5823637

Download Persian Version:

https://daneshyari.com/article/5823637

Daneshyari.com