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Focal adhesion kinase is required for bombesin-induced prostate cancer cell motility

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Abstract

Clinical evidence links neuroendocrine differentiation (NED) to prostate cancer progression. In the prostate carcinoma PC-3 cell model, the action of the gastrin releasing peptide (GRP) analog, bombesin (BN), on the activation of focal adhesion kinase (FAK) and invasiveness suggests that this kinase might favor metastasis. Given that components of the FAK signalling pathway are also up regulated in prostate cancer, the aim of the present investigation was to test if FAK function is required for BN-induced motility in PC-3 cells. In wound assays designed to investigate the fate of FAK in cells undergoing BN-induced motility, it was observed that BN treatment resulted in relocalization of FAK in focal contacts concomitantly with its tyrosine phosphorylation on residue 397 (FAK [pY^{397}]) and with the formation of actin lamellipodia. Moreover, BN-induced cell motility was significantly reduced in the presence of FAK inhibitors (either anti-FAK [pY^{397}] antibody or FRNK, the FAK-related non-kinase). Altogether, these observations point towards a critical role for FAK in the action of BN on PC-3 cell motility.

Keywords: Bombesin; FAK; Focal adhesion kinase; Human prostate cancer; Motility; Neuroendocrine cells

1. Introduction

Death from cancer is generally associated with metastasis, a process linked with the tumor cell's ability to migrate, seed, and colonise distant sites. While molecular mechanisms responsible for the invasive phenotype of malignant cells are mostly intrinsic to their genetic program, underlying signalling pathways are also largely influenced by microenvironmental factors, a concept well described in the prostate. Indeed, neuroendocrine (NE) cells are believed to play a paracrine regulatory role in the prostate. These cells are scattered throughout acini, extending neuron-like membrane processes to adjacent basal and secretory cells (Bonkhoff, 2001). Prostatic NE cells contain abundant secretory granules filled with numerous bioactive compounds collectively named NE products (NEP) (Bonkhoff, 2001). The functional role of NEP is unclear but their expression together with that of specific markers, such as chromogranin-A, allow the detection of NE cells in both normal and malignant tissues. In prostate cancer, an increased number of NE cells are detected, and this increased NED correlates with the progression of metastatic and hormone-refractory disease (Hansson and Abrahamsson, 2001). In parallel, the growth- and motility-promoting properties of several NEP, including vascular endothelial growth factor (VEGF) concentrated in prostatic NE cells (Harper et al., 1996; Borre et al., 2000), has been established using human prostate carcinoma cell lines (Chevalier et al., 2002; Hansson and Abrahamsson, 2001). Together, these observations support a deregulation of the NEP-secreting microenvironment and association of increased NED with poor clinical outcome in prostate cancer.

NEP have been implicated in diverse biological processes, such as embryogenesis of various organs, cell growth and differentiation, and tumorigenesis. In particular, members of the GRP family, with GRP and its analogue BN as the most documented, have been implicated in the biology of several human malignancies including lung, colon, breast and prostate (Aprikian et al., 1998; Bonkhoff, 2001; di Sant'Agnese, 2001;

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Hansson and Abrahamsson, 2001). In prostate cancer, several clinical and experimental observations in tissues and/or cell models point to an important contribution of BN/GRP (Aprikian et al., 1998). Notably, a role in metastases was suggested by the stimulatory effect of BN/GRP on the matrix invasiveness capacity of the PC-3 cell line (Hoosein et al., 1993; Aprikian et al., 1997; Nagakawa et al., 1998).

Clues on the underlying BN/GRP signalling mechanisms have emerged from numerous molecular studies in different systems. The BN/GRP group of peptides bind to the same family of heptahelical membrane receptors (GRP-R, BN-receptor subtype 3 and neuromedin-B receptor) (Ji et al., 1998), whose action requires subsequent coupling to heterotrimeric GTP-binding proteins, in particular Gαq or Gα12, and hence named G proteins-coupled receptors (GPCRs) (Rozengurt, 2002). Among downstream effectors reflecting GPCR-mediated activation are phospholipase C, protein kinase C, the Rho family of small GTP-binding proteins, paxillin and the cytoplasmic tyrosine kinases FAK and Src (Rozengurt, 2002). The signalling of GRP-R to FAK is particularly interesting, as our group and others have reported high expression levels of GRP-R (Reile et al., 1994; Aprikian et al., 1996; Bartholdi et al., 1998; Markwalder and Reubi, 1999) and FAK (Tremblay et al., 1996; Zheng et al., 1999; Slack et al., 2001; Rovin et al., 2002) in human prostatic tissues from patients with advanced disease and in tumorigenic cell lines. In contrast, low levels of GRP-R and FAK expression were detected in normal and benign prostatic hyperplastic tissues, and in less tumorigenic cells (Aprikian et al., 1996; Tremblay et al., 1996; Bartholdi et al., 1998; Mark walder and Reubi, 1999; Zheng et al., 1999; Slack et al., 2001; Rovin et al., 2002). Moreover, the presence of high levels of tyrosine phosphorylated FAK found in signalling complexes with downstream effectors (Tremblay et al., 1996) suggested an operational FAK pathway in human prostate cancer. In addition, NEP such as BN and VEGF rapidly activate tyrosine phosphorylation of FAK in PC-3 cells and, in both instances, this was associated with increased invasiveness through matrigel and/or motility (Aprikian et al., 1997; Chevalier et al., 2002). BN treatment also enhanced the formation of complexes between FAK, integrins and effectors, such as paxillin and Csk (Tremblay et al., 1996). This is of interest because FAK, a component of the integrin-based focal contacts, serves as the major transmitter of extracellular matrix (ECM)-triggered signals, and thereby modulates integrin-mediated cell adhesion and motility (Hynes, 2002; Parsons, 2003). This may explain why GRP-R activation leads to enhanced prostate cancer cell motility (Hoosein et al., 1993; Aprikian et al., 1997; Nagakawa et al., 1998, 2001).

The fundamental question of whether FAK activation is directly linked to prostate cancer cell motility in response to NEP, as secreted in the tumor microenvironment, was addressed in the present study. BN was used as the prototypical NEP and tested on PC-3 cells as the model, based on their aggressive phenotype and high expression levels of FAK and GRP-R (Reile et al., 1994; Aprikian et al., 1996; Tremblay et al., 1996; Bartholdi et al., 1998; Markwalder and Reubi, 1999; Zheng et al., 1999; Slack et al., 2001; Rovin et al., 2002). Results indicate that a functionally active FAK in focal contacts was associated with the formation of lamellipodia and was required for BN-induced prostate PC-3 cell motility. Therefore these observations, coupled to clinical evidence, support an implication of FAK in the NEP-induced motility of the prostate cancer cells and, hence, in the paracrine action of NED in prostate cancer progression and metastasis.

2. Materials and methods

2.1. Cell culture and reagents

The prostate carcinoma PC-3 and LNCaP cell lines (obtained from the American Type Culture Collection and used between passages 30 and 50) were maintained in RPMI 1640 culture media containing 10% fetal bovine serum and 50 μg/ml gentamycin. Lab-Tek® Permanox® Chamber Slide™ were from Nalgene Nunc International Corp. (Naperville, IL). [Tyr^4]-bombesin and TRITC-phalloidin (a red fluorochrome for the detection of polymerized actin) were from Sigma (Oakville, Ont., Canada). Monoclonal anti-FAK antibodies (clone 4.47 against the N-terminus and clone 2A7 against the C-terminus) were purchased from UBI (Lake Placid, NY). The rabbit polyclonal anti-FAK [pY^{397}] phosphospecific antibody and corresponding peptides (±tyrosine phosphorylated) were obtained from Biosource International (Camarillo, CA). The green fluorochrome Alexa Fluor™ 488 immunoglogulin type G (IgG) conjugates (anti-mouse or anti-rabbit) were from Molecular Probes (Eugene, OR). 4′,6-Diamidine-2′-phenylindole dihydrochloride (DAPI) was used for nuclear staining and was purchased from Roche Diagnostics (Laval, Que., Canada). Normal rabbit IgG, the red fluorochrome Cy3™ IgG conjugates (anti-mouse or anti-rabbit) and the peroxidase-linked anti-mouse IgG were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). The mounting medium Immuno-Fluore™ was from ICN Biomedicals (Montreal, Que., Canada). Peroxidase-linked protein A and enhanced chemiluminescence (ECL) reagents were from Amersham Pharmacia Biotech (Montreal, Que.). BCA protein assay reagents were purchased from Pierce (Rockford, IL). pEGFP, hereafter named “for green fluorescent protein” (GFP), was obtained from BD Biosciences (Mississauga, Ont.). The GFP-FRNK construct was kindly provided by Dr. J.T. Parsons (University of Virginia, Charlottesville, VA) and engineered by cloning the FRNK cDNA into pEGFP, as described (Taylor et al., 2000).

2.2. Migration assays

A wound assay was designed to simultaneously monitor BN-dependent cell motility, FAK intracellular localization