Cellular Signalling 36 (2017) 1–13

Contents lists available at ScienceDirect

Cellular Signalling

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

Calcitonin receptor increases invasion of prostate cancer cells by recruiting zonula occludens-1 and promoting PKA-mediated TJ disassembly

Ahmed Aljameeli, Arvind Thakkar, Girish Shah*

Pharmacology, University of Louisiana College of Pharmacy, Monroe, LA 71209, USA

ARTICLE INFO

Keywords:
Calcitonin receptor-protein kinase A-tight junctions-invasion-prostate cancer

ABSTRACT

Almost all primary prostate cancers (PCs) and PC cell lines express calcitonin (CT) and/or its receptor (CTR), and their co-expression positively correlates with their invasiveness. Activation of the CT–CTR axis in non-invasive LNCaP cells induces an invasive phenotype. In contrast, silencing of CT/CTR expression in highly metastatic PC-3M cells markedly reduces their tumorigenicity and abolishes their ability to form distant metastases in nude mice. Our recent studies suggest that CTR interacts with zonula occludens 1 (ZO-1) through PDZ interaction to destabilize tight junctions and increase invasion of PC cells. Our results show that CTR activates AKAP2-anchored cAMP-dependent protein kinase A, which then phosphorylates tight junction proteins ZO-1 and claudin 3. Moreover, PKA-mediated phosphorylation of tight unction proteins required CTR-ZO-1 interaction, suggesting that the interaction may bring CTR-activated PKA in close proximity of tight junction proteins. Furthermore, inhibition of PKA activity attenuated CT-induced loss of TJ functionality and invasion, suggesting that the phosphorylation of TJ proteins is responsible for TJ disassembly. Finally, we show that the prevention of CTR-ZO-1 interaction abolishes CT-induced invasion, and can serve as a novel therapeutic tool to treat aggressive prostate cancers. In brief, the present study identifies the significance of CTR-ZO-1 interaction in progression of prostate cancer to its metastatic form.

1. Introduction

Epithelial-to-mesenchymal transition (EMT) occurs during normal development and at the beginning of epithelial cancer during which epithelial cells acquire invasive or migratory phenotype [1]. This change is characterized by the loss of cell-cell adhesion and rearrangement of cytoskeleton. Epithelial cell-cell adhesion is predominantly maintained by the apical junction complex [2], which is formed by tight junctions (TJs) and adherens junctions (AJs) [3]. AJC regulates cell polarity, mechanical integrity and cell and junctional adhesion molecules (JAMs) [4]. Each strand of tight junctions is formed from a row of transmembrane proteins embedded in both plasma membranes of adjoining cells, with extracellular domains joining one another directly. The major types of transmembrane proteins that form the barrier are the claudins and the occludins, which interact with adaptor proteins of the zonnula occludens family, ZO-1, ZO-2 and ZO-3 to link TJs with the actin cytoskeleton [5,6].

Calcitonin receptor (CTR) is a member of the class B family of G Protein-coupled receptors (GPCRs), which contain numerous drug targets [7–9]. CTR plays an important role in the maintenance of calcium homeostasis in the bone and the kidneys [10,11]. Our recent studies suggest that the expression of CT and its receptor is up-regulated in metastatic prostate cancer (PC) [12]. Activation of CT-CTR axis induces EMT in multiple PC cell lines, and invasive phenotype in non-invasive prostate cells [13–17]. Human prostate CTR (hCTR2) is dually coupled to stimulatory G protein (Gαs) as well as Gαq, but also activates non G protein-mediated signaling networks such as PI-3K-Akt-survivin and Wnt/β-catenin signaling [15,17–19]. CTR destabilizes TJs as assessed by transelectric epithelial resistance (TER) and paracellular permeability (PCP) of multiple PC cell lines [13,20]. We have recently identified the presence of a type 1 PDZ-binding motif (-ESSA) on CTR-C tail, and showed that CTR-C PDZ-binding motif interacts with the PDZ3 domain of ZO-1. This interaction is critical for proinvasive actions CTR on PC cells. Deletion of either CTR-C PDZ-binding motif or PDZ3 domain of ZO-1 abolishes the ability of PC cells to form distant metastases in orthotopic xenograft model [20].

Our earlier results have shown that proinvasive actions of CTR were mimicked by constitutive activation of Gαs, and were blocked by the inhibitors of cAMP-dependent protein kinase (PKA) [19,21,22]. Therefore, we investigated the role of PKA on CTR-induced TJ destabilization and invasion of PC cells. The results suggest that both CTR actions, *i.e.* the interaction with ZO-1 and PKA activation, are necessary for TJ

* Corresponding author.
E-mail address: shah@ulm.edu (G. Shah).

http://dx.doi.org/10.1016/j.cellsig.2017.04.008
Received 20 February 2017; Received in revised form 11 April 2017; Accepted 12 April 2017
Available online 17 April 2017
0898-6568/ © 2017 Elsevier Inc. All rights reserved.

destabilization and invasion. CTR, by interacting with ZO-1, activates localized PKA in TJ complex to phosphorylate ZO-1 and claudin 3. PKA-mediated phosphorylation of these proteins leads to rapid TJ disassembly and endocytosis of TJ proteins.

2. Materials and methods

2.1. Materials

PKA inhibitor 14–22 amide peptide, cell-permeable, myristoylated (mPKI) was purchased from EMD Millipore, Billerica, MA. PKA inhibitor H-89 was purchased from Sigma-Aldrich Corporation, St. Louis, MO. Stearated (St) form of the peptide Ht-31, AKAP-inhibitory peptide and the control peptide were purchased from Promega Corporation, Madison, WI.

The following antibodies were purchased and used for immunocytochemistry and Western blotting. FLAG antibody was purchased from Sigma-Aldrich Corporation, St. Louis, MO. Anti-Calcitonin Receptor polyclonal antiserum was purchased from Acris Antibodies US, Rockville, MD. Antisera against tight junction proteins zonula occludens-1, occludin and claudin 3 were purchased from Invitrogen Corporation, Grand Island, NY. Antisera against phosphoserine, phosphothreonine, beta actin and alpha tubulin were purchased from Abcam Inc., Cambridge, MA. All the antibodies have been previously characterized and used for the subsequent studies including the present study.

2.2. Cell culture

PC-3 cells (CT +, CTR −) were obtained from ATCC, Manassas, VA. PC-3 sublines stably expressing CTR-wt or CTR-ΔESS were generated and characterized as described recently [20]. The cell lines were cultured in complete medium (RPMI 1640 medium supplemented with 10% fetal calf serum, 100 IU/ml penicillin G and 100 μg/ml streptomycin) under previously described culture conditions.

2.3. Measurement of transelectric epithelial resistance (TER) and paracellular permeability (PCP)

Cells were plated on six well Transwell filters (0.4 μm pore size) and grown to confluence in complete medium. TER of the cultures (in duplicate wells) was measured at various time intervals with EVOM volt-ohm meter as previously described [13]. The readings were corrected for blank (TER values from the filter and bathing medium). The integrity and cell density of monolayers were carefully monitored during these studies.

For PCP measurements, cells were plated on 12-well Transwell filters, and grown to confluency in complete medium. Tetra methyl rhodamine-dextran (1 mg/ml, average molecular mass of 4 kDa, Sigma) was added to the upper chamber. At various time intervals, 100 μl aliquots from the lower chamber were collected and checked for fluorescence.

2.4. In vitro invasion assay

Invasion experiments were conducted in Matrigel™ invasion chambers as previously described [19].

2.5. Immunofluorescence of TJ proteins

Approximately 1×10^5 cells were grown to confluence on 0.4 μm 12-well Transwell filters (Costar, MA). After overnight serum starvation, cells were treated with/without 50 nM CT for 60 min, fixed with methanol and incubated with previously authenticated primary antisera (rabbit anti-ZO-1 IgG, Zymed, Calrsbad, CA; claudin-3, CTR-FLAG). Immunostaining was visualized after incubation with TRITC-labeled secondary antibody (1:500). Controls with either non-immune goat IgG or no primary antisera were used in all studies. Digital photographs were taken with Retiga 1300 camera connected to a Nikon Optiphot-2 microscope equipped for epifluorescence, the images were captured at different wavelengths for TRITC and DAPI, were given red and blue pseudo colors respectively, and analyzed using IP Lab™ image analysis program.

2.6. Preparation of Triton X-100-soluble and -insoluble cell lysates and Western blotting

Confluent 100 mm plates of each PC sublines were serum starved overnight, and treated with/without 50 nM CT for various periods. Soluble extract (cytosolic) was obtained by incubating cells with 10 mM Tris-HCl, pH-7.4 (containing 150 mM NaCl, 2 mM $CaCl_2$, 1 mM PMSF, 40 U/ml aprotinin, 15 μg/ml leupeptin, 1% Nonidet P-40 and 1% Triton-X-100) for 30 min with occasional agitation. After washing the plates with TBS (containing protease inhibitors), the insoluble fraction (plasma membrane-associated) was scraped out from the plates with TBS containing 0.5% SDS, 1% Nonidet P-40, 40 U/ml aprotinin and 15 μg/ml leupeptin. The triton X-100-insoluble fraction was collected after homogenizing and centrifuging at 14,000 × *g* for 5 min at 4 °C, and protein content of the supernatants was determined using Bio-Rad Reagent (Bio-Rad, Hercules, CA).

The lysate fractions were boiled for 5 min in 2 × Laemmli solution, proteins were fractionated proteins polyacryl amide gel electrophoresis, and were electrically transferred to a nitrocellulose membrane. The blots were incubated the appropriate antisera and processed as previously described [23]. The blots were then washed and reprobed for α-actin (insoluble) or β-tubulin (soluble). The same experiment was repeated two more times. The density of the bands was quantitated by densitometry.

2.7. DuoLink in situ proximity ligation assay [24]

To observe the interaction between CTR and ZO-1 in PC cells, we utilized the *in situ* Proximity PLA (Olink Bioscience, Uppsala, Sweden). PC cells, cultured in multiwall chambers, were fixed and immunolabeled with two primary antibodies. The secondary antibodies with attached PLA probes are supplied with the Duolink kit. The interaction, as indicated by a red fluorescent dot, was observed by confocal microscopy at 400X. The number of dots per cell was determined by Blobfinder™ image analysis software [25].

2.8. PKA assays

The lysates of untreated/CT-treated cells were incubated with PKA substrate peptide (Kemptide) and ^{32}P-ATP as described in the protocol provided by the manufacturer (Promega, Madison, WI). In brief, the cells treated with CT or the vehicle for 10 min (2×10^6 cells per 100-mm dish) were harvested in lysis buffer (50 mM Tris, pH 7.5, containing 5 mM EDTA, 50 mM NaF, 1 mM sodium pyrophosphate, 0.5 mM EGTA, 10 mM β-mercaptoethanol, 1 μg/ml leupeptin, and 1 μg/ml aprotinin), sonicated, and debris was removed by centrifugation. The extracts were then incubated for 5 min at 30 °C in the reaction buffer [final concentration was 50 mM Tris, pH 7.5; 10 mM $MgCl_2$; 100 μM ATP; 4 nM of [^{32}P] ATP; 0.25 mg/ml BSA; and 50 μM PKA substrate peptide (Promega Corp., Madison, WI)]. 10 μM cAMP (total PKA activity), served as a positive control and 100 nM PKI plus cAMP served as a negative control for PKA assays (total background activity). Triplicates of each sample were assayed, and blotted on SAM2 biotin capture membranes at the end of incubation (Promega Corp., Milwaukee, WI). The membranes were then washed extensively with 2 M NaCl as well as 2 M NaCl 1% H_3PO_4, and the bound phosphorylated substrate on a filter disc was quantified in a scintillation counter. PKI-inhibitable kinase activity was calculated, and the data were reported as picomoles ATP per min per g protein.

Download English Version:

https://daneshyari.com/en/article/5509210

Download Persian Version:

https://daneshyari.com/article/5509210

Daneshyari.com