Cancer Letters 354 (2014) 164–171

Contents lists available at ScienceDirect

Cancer Letters

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

A novel β-catenin signaling pathway activated by IL-1β leads to the onset of epithelial–mesenchymal transition in breast cancer cells

Eloy Andres Perez-Yepez [a], Jorge-Tonatiuh Ayala-Sumuano [b], Ruth Lezama [c], Isaura Meza [a,*]

[a] Department of Molecular Biomedicine, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Avenida Instituto Politécnico Nacional 2508, San Pedro Zacatenco, Mexico DF 07360, Mexico
[b] Institute of Neurobiology, Universidad Nacional Autónoma de México Campus Juriquilla, Blvd. Juriquilla 3001, Querétaro 76230, Mexico
[c] Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Av. Wilfrido Massieu y Calzada Miguel Stampa s/n, México DF 07738, Mexico

A R T I C L E   I N F O

Article history:
Received 3 June 2014
Received in revised form 14 July 2014
Accepted 4 August 2014

Keywords:
Breast cancer cell
IL-1β stimulation
IL-1RI/β-catenin signaling
Invasiveness

A B S T R A C T

Interleukin 1β has been associated with tumor development, invasiveness and metastasis in various types of cancer. However, the molecular mechanisms underlying this association have not been clearly elucidated. The present study is the first to show, in breast cancer cells, that an IL-1β/IL-1RI/β-catenin signaling pathway induces β-catenin accumulation due to GSK3β inactivation by Akt phosphorylation. Translocation to the nucleus of accumulated β-catenin and formation of the TCF/Lef/β-catenin complex induce sequential expression of *c-MYC*, *CCDN1*, *SNAIL1* and *MMP2*, leading to up-regulation of proliferation, migration and invasion; all of the processes shown to be required, in cancerous cells, to initiate transition from a non-invading to an invasive phenotype.

© 2014 Elsevier Ireland Ltd. All rights reserved.

Introduction

The cellular and molecular mechanisms of cancer generation and progression involve many factors. Until now, it has not been possible to determine which of these are the most important for malignancy [1,2]. Transition from a non-invasive to an invasive phenotype requires that tumor cells undergo multiple molecular, biochemical and structural changes through a process known as epithelial–mesenchymal transition (EMT). During this transition, activation of transcription factors, expression of specific genes and proteins and deregulation of signaling pathways significantly enhance proliferation and migration of the tumor cells and tissue invasion by their degradation of the extracellular matrix [3,4].

Epithelial–mesenchymal transition is controlled by at least five important signaling pathways that normally function in early embryonic development and tissue homeostasis [5]. However, when these pathways are deregulated, as in the case of cancer, they are deviated toward progression of tumor malignancy. These pathways are called: Wnt/β-catenin, TGFβ, Hedgehog, Notch, and the tyrosine kinase receptors (RTKs) [6,7]. Wnt/β-catenin corresponds to the canonical pathway of Wnt signaling, in which β-catenin participates as the main intermediate effector [8]. β-catenin is a multifunctional protein belonging to the Armadillo family; it binds to E-cadherin and α-catenin localized at the adherens junctions in the cell membrane. This protein complex is also associated to the actin cytoskeleton tethered to the cell membrane, in which it plays an important regulatory role in epithelial cell polarity, adhesion, proliferation and differentiation [5]. When binding between β-catenin and E-cadherin is disrupted, β-catenin is released from the membrane complex into the cytoplasm and marked for degradation by GSK3β [9–12]. This kinase inactivation given by its recruitment into Fdz receptors [5] or by its serine 9 phosphorylation by Akt [13] causes an accumulation of β-catenin in the cytoplasm and facilitates its translocation to the nucleus, in which activates expression of cyclin D1, c-Myc, metalloproteases 2 and 9, Twist and Slug, which are proteins involved in cell proliferation, migration, invasion and morphogenesis [11,14–16]. The Wnt/β-catenin pathway is activated by Wnt receptors and the co-receptors Fzd and RLPS [11,15], although the epithelial growth factor receptor (EGFR), belonging to the RTK family, has also been reported to activate β-catenin transcriptional activity through the RTK/PI3K/Akt pathway [17,18].

Epidemiological studies have shown that chronic inflammation predisposes individuals to develop cancer [19,20]. It has been proposed that IL-1β, secreted mainly by macrophages localized in the tumor microenvironment, is involved in several stages of cancer progression [21,22]. Furthermore, elevated levels of IL-1β in the microenvironment of invasive tumors of breast origin have been reported [23] and in murine models it has been shown that IL-1β is involved in various stages of tumor development, invasiveness and metastasis [21,24]. Recent studies by our group, utilizing human breast cancer MCF-7 cell monolayers, have shown that IL-1β binding

Abbreviation: MFA, 2-methyl-2′-F-anandamide.

* Corresponding author. Tel.: +52(55)57473800 ext. 5000; fax: +52(55)57477002.
E-mail address: imeza@cinvestav.mx (I. Meza).

http://dx.doi.org/10.1016/j.canlet.2014.08.015
0304-3835/© 2014 Elsevier Ireland Ltd. All rights reserved.

to IL-1RI activates PI3K/Rac1 signaling, leading to disorganization of cell adherens junctions and decreased levels of E-cadherin [25,26]. In addition, it was shown that IL-1β stimulation enhanced expression of markers associated with cell migration and invasion such as CXCR4, a receptor linked to metastatic breast cancer cell organotropism [2,25,27] and cell proliferation [26].

Although all these studies have suggested a correlation between IL-1β stimulation and tumor progression in breast cancer cells, the mechanisms underlying this process have not been clearly elucidated [21,24]. This may be due in part to impossibility to follow tumor progression in patients in addition to the high heterogeneity of cancer cell populations in tumors and tumor-derived cell lines.

Our present studies show that in a highly homogeneous and responsive clone of MCF-7 breast cancer cells, activation of a novel IL-1RI/β-catenin pathway induces β-catenin transcriptional activity and the expression of genes involved in malignancy.

Materials and methods

Cell culture

MCF-7 cells (ATCC, Manasas, VA, USA) were grown in DMEM-F12 supplemented with 10% fetal bovine serum, penicillin (5000 U/ml) and streptomycin (5000 μg/ml) from Gibco BRL (Grand Island, NY, USA). Cultures were incubated at 37 °C with 5% CO_2. An MCF-7 cell clone (MCF-7 6D) highly responsive to IL-1β stimulus was obtained and cultured, as previously described, for cell population enrichment [26].

Stimulation of cell cultures with IL-1β

MCF-7 parental cell line (MCF-7) and MCF-7 6D cells were stimulated with 20 ng/ml of human recombinant IL-1β (Peprotech, Rocky Hill, NJ, USA). For all assays, cells were seeded at 6000 cells/cm^2 and cultured for 48 h and then switched to DMEM-F12 supplemented only with 1% FBS for 18 h. Afterwards, cells were incubated with DMEM-F12 containing 20 ng/ml of IL-1β and analyzed every 24 h. Control cells were not stimulated with the cytokine.

Flow cytometry and cell sorting

Cells were harvested with 0.5% EDTA/PBS for 5 min, suspended and washed with ice-cold 2% FBS/PBS and centrifuged at 4 °C, then blocked with 2% FBS containing 16.5 μg/ml of human IgG in PBS for 30 min in ice. Cell suspensions were incubated 1 h at 3 °C with either mouse anti-CXCR4 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or goat anti-IL-1RI (R&D System, Minneapolis, MN, USA). FITC-labeled anti-mouse or anti-goat IgG were used as secondary antibodies, respectively. Fluorescence was assessed in a FACS Calibur flow cytometer (Becton Dickinson, Mountain View, CA, USA). Cell sorting was carried out in a MoFlo cell sorter (Beckman Coulter, Brea, CA, USA). Results, expressed as mean fluorescence intensity (MFI), were analyzed with Summit 5.1 (Beckman Coulter) or FlowJo 7.0 (Tree Star Inc, Ashland, OR, USA) softwares.

Inhibitors

The β-catenin pathway inhibitor 2-methyl-2′-F-anandamide (10 μM) was added 1 h prior to exposure to IL-1β and maintained in the cultures during the experiments. To block IL-1RI signaling, cells were incubated for 24 h with 100 μM of IMG-2005, an inhibitor of MyD88 adaptor (IMGENEX, San Diego, CA, USA). Wortmannin (Sigma Aldrich, St. Louis, MO, USA) was used at 250 μM to inhibit PI3K activity.

Immunofluorescence

Cell immunoflorescence procedures have already been described [26]. Briefly, cells cultured on glass coverslips were fixed and challenged with a mouse anti-human β-catenin antibody (1:100) (Invitrogen, Carlsbad, CA, USA) for 1 h at RT followed by incubation with a FITC-labeled goat anti-mouse IgG (1:100) (Zymed, San Francisco, CA, USA). Nuclei were counterstained with DAPI. Images were acquired with a digital camera Olympus DP72 coupled to an Olympus inverted microscope.

Subcellular fractionation

Nuclear and cytoplasm fractions were obtained as described [28]. IL-1β-stimulated cells (with or without inhibitors) were harvested in buffer A (150 mM NaCl, 10 mM $MgCl_2$, 1% Nonidet P-40, 10 mM Tris–HCl pH, 7.5) supplemented with Complete® protease inhibitor cocktail, incubated in ice for 10 min, vortexed and centrifuged for 5 min at 1000 × *g*. Recovered supernatants kept as cytoplasmic fractions. Pellets were resuspended in ice-cold buffer B (20 mM hepes, 25% glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 1 mM DTT pH, 8.0) for 20 min, centrifuged for 5 min at 1000 × *g*. Recovered supernatants corresponded to nuclear fractions. Possible cross contamination and purity of fractions were evaluated in western blots, using anti-histone 4 and anti-GAPDH antibodies.

SDS-PAGE and Western blot

Protein extracts obtained by cell lysis with a 50 mM Tris–HCl, pH 7.5, 5 mM EDTA, 150 mM NaCl, 1% Triton X-100 and Complete® buffer were sonicated for 10 s, centrifuged 5 min at 10,000 rpm and quantified by micro Bradford. Proteins were SDS-PAGE-separated in 10% gels and blotted onto nitrocellulose membranes, blocked with 5% non-fat milk/TBS-0.1% Tween 20. Membranes were exposed to goat anti-human β-catenin (1:3000), anti-phosho-ser9 GSK3β (1:1000) or anti-GSK3β (1:1000) antibodies (Cell Signaling Technology, Danvers, MA, USA). Anti-β-actin monoclonal antibody, donated by Dr. J. M. Hernández (CINVESTAV-IPN), was utilized as protein load control. Secondary antibodies used were HRP-labeled anti-goat IgG (1:8000) or anti-mouse IgG (1:5000) and bands revealed by chemiluminiscence. Densitometry analyses were performed with ImageJ software.

Luciferase assay

One million cells of each line were transfected with 1 μg of TOPFlash vector containing the luciferase gene regulated by the β-catenin-sensitive TCF/LEF responsive elements (Addgene, Cambridge, MA, USA). Cells were co-transfected with 0.1 μg of vector pRL-CMV, which constitutively expresses renilla luciferase as transfection control. A parallel group of cells were transfected with FOPFlash, which is not responsive to β-catenin transactivation as negative control. After 24 h, luciferase expression was assessed in each experimental condition. Luciferase activity was measured using the Dual-Luciferase Assay kit (Promega, Madison, WI, USA).

Gene expression

Cell total RNA was checked for integrity in agarose gels and 1.0 μg used for reverse transcriptase reactions. Gene expression was carried out using the FastStart SYBR Green Master (Rox) kit (Roche Applied Science, Manheim, Germany), using a 7500 Real Time Thermal Cycler (Applied Biosystems). Specific primers for β-catenin target genes are shown in Appendix: Supplementary Table S1. Relative gene expression was normalized to the constitutive expression of *RPLP0*. Gene expression values were determined using the $2^{-\Delta\Delta CT}$ formula.

Cell migration and invasion

Cells cultured and stimulated with IL-1β with or without inhibitors were harvested at 48 and 96 h as indicated earlier and resuspended in serum-free DMEM-F12. Migration and invasion assays were performed in Transwell® chambers and evaluated as previously described [26].

Cell proliferation

Cells suspended in PBS were incubated for 15 min with 2 μM of carboxyfluorescein (Molecular Probes, Grand Island, NY, USA) at 37 °C and seeded at 10^4 cells/cm^2. Forty eight hours later, cells were serum-starved for 18 h and then stimulated with IL-1β with or without inhibitors. Cells harvested every 24 h were measured for fluorescence intensity. Doubling time was calculated from MFI values at the different times.

Protease activity

Cells were cultured with IL-1β with or without inhibitors. Culture media recovered at 48 and 96 h were concentrated using 5000-kDa cutoff Amicon Centricon filters (Millipore, Billerica, MA, USA). Protease activity from these media was revealed in 8% gels co-polymerized with 1 mg/ml gelatin after non-denaturing PAGE. After electrophoresis, gels were rinsed three times with a 2.5% Triton X-100 in 50 mM Tris–HCl, pH 7.4, 4.5 mM $CaCl_2$ buffer at 37 °C and incubated in fresh buffer for 24 h. Gels were stained with coomassie blue. Densitometry analyses were performed as indicated earlier.

Statistical analysis

Data are presented as mean ± standard deviation. Statistical analyses were performed by Mann–Whitney test or Kruskal–Wallis test. *P* values ≤ 0.05 were considered significant.

Download English Version:

https://daneshyari.com/en/article/10902405

Download Persian Version:

https://daneshyari.com/article/10902405

Daneshyari.com