Biochemical and Biophysical Research Communications xxx (2017) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Apomorphine suppresses TNF-α-induced MMP-9 expression and cell invasion through inhibition of ERK/AP-1 signaling pathway in MCF-7 cells

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ARTICLE INFO

Article history:
Received 24 April 2017
Accepted 29 April 2017
Available online xxx

Keywords:
Apomorphine
TNF-α
Invasion
MMP-9
AP-1

ABSTRACT

Recent studies have shown that dopamine plays an important role in several types of cancer by inhibiting cell growth and invasion via dopamine receptors (DRs), such as dopamine receptor D2. However, the roles of DR agonists in cancer cell growth and invasion remain unclear. In our study, we found that apomorphine (APO), one of the most commonly prescribed DR agonists, inhibited TNF-α-induced matrix metalloprotease-9 (MMP-9) expression and cell invasion in MCF-7 human breast carcinoma cells through DR-independent pathways. Further mechanistic studies demonstrated that APO suppresses TNF-α-induced transcription of MMP-9 by inhibiting activator protein-1 (AP-1), a well-described transcription factor. This is achieved via extracellular signal-regulated kinases 1 and 2 (ERK1/2). Our study has demonstrated that APO targets human MMP-9 in a DR-independent fashion in MCF-7 cells, suggesting that APO is a potential anticancer agent that can suppress the metastatic progression of cancer cells.

11. Introduction

The neurotransmitter dopamine has been shown to regulate a variety of physiological and pathological functions in multiple organs via dopamine receptors (DRs). These include the brain, in which dopamine plays an important role in reward-motivated behavior and motor control [1]. Consequently, several nervous system disorders, such as Parkinson's disease, are associated with dysfunctions of the dopaminergic system. Treatments targeting this system include the metabolic precursor of dopamine, L-3,4-dihydroxyphenylalanine, and dopamine receptor agonists that directly stimulate dopaminergic neurons [1,2].

Recently, DRs and dopamine have been reported to exert diverse pleiotropic effects through DR-dependent and -independent pathways. For example, dopamine receptors D_1 (DRD1) and D_2 (DRD2) were shown to be involved in cell proliferation and invasiveness in several types of human cancer, including breast cancer, small cell lung cancer, non-small cell lung cancer, and gastric cancer [3–6]. Dopamine has also been shown to increase the efficacy of anticancer drugs in preclinical models of human cancer [7–11]. Additionally, several studies have shown that DR agonists can also suppress proliferation, induce apoptosis, and inhibit invasion in human cancer cells and tumors [3,12,13]. Supporting reports of the anticancer properties of DR and dopamine, epidemiological data have also suggested an association between reduced incidence rates of cancer in patients with Parkinson's disease and a possible link between DR agonists and the risk of cancer [14,15].

However, the anticancer effects of dopamine and DR agonists described in prior studies are mainly DR-dependent, and DR-independent anticancer mechanisms are still largely unknown. In our present study, we have found that apomorphine (APO), a non-selective DRD1/DRD2 agonist, inhibited TNF-α-induced MMP-9 expression and cell invasion via DR-independent pathways in MCF-7 human breast cancer cells. Further mechanistic analysis indicated that APO regulates TNF-induced MMP-9 expression through an ERK/AP-1-dependent mechanism.

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http://dx.doi.org/10.1016/j.bbrc.2017.04.151
0006-291X/

22. Materials and methods

2.1. Cells and materials

MCF-7 cells were obtained from the American Type Culture Collection (Manassas, VA) and cultured in DMEM (Gibco BRL, Rockville, MD) supplemented with 10% fatal bovine serum (FBS) and 5% CO_2 at 37 °C. All chemicals were obtained from Sigma-Aldrich Chemical (St. Louis, MO) unless otherwise indicated. APO was dissolved in dimethyl sulfoxide (DMSO) and the maximum concentration of DMSO was 0.1%. Recombinant human TNF-α was obtained from R&D systems (Boston, MA). Polyclonal antibodies to phospho-MAPK family proteins were purchased from Cell Signaling Technologies (Beverly, MA) and all other antibodies were purchased from Santa Cruz Biotechnology (Dallas, TX).

2.2. Cell viability assay

The cytotoxic effect of APO on MCF-7 cells was investigated using a commercially available proliferation kit (XTT II, Boehringer Mannheim, Mannheim, Germany) as described previously [16].

2.3. Gelatin zymography assay

MCF-7 cells were treated with various concentrations of APO in the presence of TNF-α for 24 h and conditioned medium was electrophoresed in a polyacrylamide gel containing 0.1% (w/v) gelatin. The gel was then washed at room temperature for 30 min with 2.5% Triton X-100 and subsequently incubated at 37 °C for 24 h in a buffer containing 10 mM $CaCl_2$, 0.01% NaN_3, and 50 mM Tris-HCl (pH 7.5). The gel was stained with 0.2% Coomassie brilliant blue and photographed on a light box. Proteolysis was detected as a white zone in a dark blue field.

2.4. Invasion assay

Matrigel-coated filter inserts (8 μm pore size) that fit into 24-well invasion chambers were obtained from Becton–Dickinson (New Jersey, USA). MCF-7 cells were detached from the tissue culture plates, washed, and resuspended in fresh medium (2×10^4 cells/well). Cells were then added to the upper compartment of the invasion chamber and treated with APO in the presence or absence of TNF-α. Conditioned medium (500 μl) was added to the lower compartment of the invasion chamber. The chambers were incubated at 37 °C for 24 h in 5% CO_2, the filter inserts were removed from the wells, and the cells on the upper side of the filter were removed using cotton swabs. The filters were fixed, stained, and mounted according to the manufacturer's instructions. The cells that invaded through the Matrigel and were located on the underside of the filter were counted. Three invasion chambers were used per condition.

2.5. Western blot analysis

Whole cell lysates were prepared in RIPA lysis buffer (Thermo Scientific, Waltham, MA) supplemented with protease and phosphatase inhibitor cocktails (Gendepot, Katy, TX). To determine the activations of NF-κB and AP-1, nuclear extracts of cells were isolated as described previously [16]. Total cell proteins and nuclear proteins were size fractionated by SDS-PAGE and electrotransferred to Immobilon-P membranes (Millipore Corp., Bredford, MA). Detection of specific proteins was carried out by enhanced chemiluminescence following the manufacturer's instructions (Amersham Biosciences, Piscataway, NJ).

3. Subcellular localization assay

Cells were seeded onto coverslips in 12-well plates, and then treated with APO in the presence of TNF-α. After 6 h, cells were fixed in 1% formalin in phosphate-buffered saline (PBS, pH 7.4) and permeabilized by immersing the cells in 0.2% Triton X-100 solution in PBS for 10 min. Cells were then incubated with anti-c-jun rabbit IgG, followed by anti-rabbit IgG conjugated with FITC (Santa Cruz Biotechnology, Dallas, TX). For nuclear counterstaining, cells were mounted in mounting medium including DAPI (Vector Lab, Burlingame, CA, USA). Fluorescent images were collected and analyzed using a Zeiss Axioplan2 fluorescence microscope (Carl Zeiss, Jena, Germany).

3.1. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted by the RNAzol B reagent (Tel-test, Friendswood, TX, USA) according to the manufacturer's instructions. For RT-PCR, a cDNA was synthesized from 0.5 μg of total RNA using AMV RNA PCR Kit (Takara, Japan) according to the manufacturer's instructions. The cDNA was amplified by PCR with the following primers: MMP-9 (537 bp), 5′-CGGAGCACGGAGACGGGTAT-3′ (sense) and 5′-TGAAGGGGAAGACGCACAGC-3′ (antisense); TIMP-1 (481 bp), 5′-CTGTTGTTGCTGTGGCTGATA-3′ (sense) and 5′-CCGTCCACAAGCAATGAGT-3′ (antisense); TIMP-2 (416 bp), 5′-GTAGTGATCAGGGCCAAAG-3′ (sense) and 5′-TTCTCTGTGACCCAGTCCAT-3′(antisense); β-actin (247 bp), 5′-CAAGAGATGGCCACGGCTGCT-3′ (sense) and 5′-TCCTTCTGCATCCTGTCGGCA-3′ (antisense). PCR products were analyzed by agarose gel electrophoresis and visualized by treatment with ethidium bromide.

3.2. Transient transfection and luciferase reporter assay

The 710 bp fragment from the 5′-promoter region of the MMP-9 gene (pGL2-MMP-9-WT) and its NF-κB mutant (pGL2-MMP-9-NF-κB-mt) and AP-1 mutant (pGL2-MMP-9-AP-1-mt) were cloned as described previously [16]. Cells were plated onto 12-well plates at a density of 1×10^5 cells/well and grown overnight. Cells were cotransfected with 0.1 μg of each luciferase reporter construct and 0.05 μg of the pCMV-β-galactosidase reporter plasmid for 5 h using Lipofectamine 2000 reagent (Invitrogen, San Diego, CA). After transfection, the cells were cultured in 10% FBS medium and incubated with APO in the presence or absence of TNF-α for 18 h. Luciferase and β-galactosidase activities were assayed by using the luciferase and β-galactosidase enzyme assay systems (Promega, Madison, WI). Luciferase activity was normalized with the β-galactosidase activity.

3.3. Statistical analysis

The results are expressed as means ± SD and differences between means for two groups were determined by unpaired Student's *t*-test. The minimum significance level was set at *P* value of less than 0.05 for all analyses. All experiments were performed at least three times.

4. Results and discussion

4.1. APO suppresses TNF-α-induced MMP-9 expression and cell invasion in MCF-7 cells

Metastasis is a complex and multi-step process that involves cell proliferation and growth, cell migration and invasion into the circulatory system through the extracellular matrix (ECM), cell

Please cite this article in press as: Y.-S. Jung, S.-O. Lee, Apomorphine suppresses TNF-α-induced MMP-9 expression and cell invasion through inhibition of ERK/AP-1 signaling pathway in MCF-7 cells, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.04.151