Pharmacological Research 111 (2016) 731–739

Contents lists available at ScienceDirect

Pharmacological Research

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

Perspective

Histamine prevents radiation-induced mesenchymal changes in breast cancer cells

Tamara E. Galarza[a], Nora A. Mohamad[a], Mónica A. Táquez Delgado[a], Guadalupe M. Vedoya[a], Ernesto J. Crescenti[b], Rosa M. Bergoc[c], Gabriela A. Martín[a,c,1], Graciela P. Cricco[a,*,1]

[a] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Laboratorio de Radioisótopos, Buenos Aires, Argentina
[b] Instituto de Inmunooncología, Buenos Aires, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form 9 July 2016
Accepted 25 July 2016
Available online 26 July 2016

Chemical compounds studied in this article:
Histamine (PubChem CID: 774)
PP2 (PubChem CID: 4878)

Keywords:
Histamine
Ionizing radiation
Epithelial to mesenchymal transition
Src
Breast cancer

ABSTRACT

Radiotherapy is a prime option for treatment of solid tumors including breast cancer though side effects are usually present. Experimental evidence shows an increase in invasiveness of several neoplastic cell types through conventional tumor irradiation. The induction of epithelial to mesenchymal transition is proposed as an underlying cause of metastasis triggered by gamma irradiation. Experiments were conducted to investigate the role of histamine on the ionizing radiation-induced epithelial to mesenchymal transition events in breast cancer cells with different invasive phenotype. We also evaluated the potential involvement of Src phosphorylation in the migratory capability of irradiated cells upon histamine treatment.

MCF-7 and MDA-MB-231 mammary tumor cells were exposed to a single dose of 2 Gy of gamma radiation and five days after irradiation mesenchymal-like phenotypic changes were observed by optical microscope. The expression and subcellular localization of E-cadherin, β-catenin, vimentin and Slug were determined by immunoblot and indirect immunofluorescence. There was a decrease in the epithelial marker E-cadherin expression and an increase in the mesenchymal marker vimentin after irradiation. E-cadherin and β-catenin were mainly localized in cytoplasm. Slug positive nuclei, matrix metalloproteinase-2 activity and cell migration and invasion were significantly increased. In addition, a significant enhancement in Src phosphorylation/activation could be determined by immunoblot in irradiated cells. MCF-7 and MDA-MB-231 cells also received 1 or 20 μM histamine during 24 h previous to be irradiated. Notably, pre-treatment of breast cancer cells with 20 μM histamine prevented the mesenchymal changes induced by ionizing radiation and also reduced the migratory behavior of irradiated cells decreasing phospho-Src levels.

Collectively, our results suggest that histamine may block events related to epithelial to mesenchymal transition in irradiated mammary cancer cells and open a perspective for the potential use of histamine to improve radiotherapy efficacy.

1. Introduction

Breast cancer is a heterogeneous disease highly predominant among women worldwide. Histological and molecular classification of breast cancer describes four well-defined different phenotypes. The two major molecular classes of breast cancers are: luminal and basal-type tumors [1]. The luminal phenotype generally includes tumors that are estrogen receptor-positive (ER) and comprises about 70% of human invasive mammary tumors. The basal like carcinomas make about 15% of invasive mammary tumors. They are often defined as triple negative breast cancer (TNBC) since most of them are ER negative, progesterone receptor

Abbreviations: EMT, epithelial to mesenchymal transition; ER, estrogen receptor; FBS, fetal bovine serum; GPCR, G-protein coupled receptors; Gy, gray; HA, histamine; HER2, human epidermal growth factor receptor 2; MMPs, matrixmetalloproteinases; P-Src, phosho-Src; PP2, 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine; PR, progesterone receptor; T-Src, total-Src; TBS, tris-buffered saline.

* Corresponding author at: Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Laboratorio de Radioisótopos, Junín 956 (C1113AAB), Buenos Aires, Argentina.
E-mail addresses: gracrico@ffyb.uba.ar, graciela.cricco@gmail.com (G.P. Cricco).
[1] These authors contributed equally as senior authors.

http://dx.doi.org/10.1016/j.phrs.2016.07.039
1043-6618/

(PR) negative and lack human epidermal growth factor receptor 2 (HER2) protein overexpression and gene amplification. In most instances, treatment of early and late stage breast cancers involves surgery followed by radiotherapy and/or chemotherapy. Conventional radiation therapy for breast cancer is delivered in fractionated doses to reduce deleterious effects on healthy tissues. The benefits of therapeutic strategies using ionizing radiation in reducing local-regional recurrence risk and increasing survival are recognized. However, ionizing radiation may paradoxically promote migration and invasion abilities of tumor surviving cells leading to local recurrences (primary tumor site) or metastases (distant organ) via epithelial to mesenchymal transition (EMT) induction [2–6].

EMT is a dynamic and reversible program. It is well documented that cancer cells undergo to EMT with loss or down-regulation of epithelial markers as E-cadherin and up-regulation of mesenchymal markers as vimentin, alpha smooth muscle actin and N-cadherin. The epithelial marker E-cadherin is a glycoprotein involved in cell-cell adhesion. β-catenin is the intracellular binding partner of E-cadherin. E-cadherin and β-catenin form a complex that is required for maintaining epithelial cell-cell contacts. One of the first steps of EMT is the disassembly of this complex with the re-localization and/or degradation of these proteins. Besides, EMT is associated to an enhancement of cell motility, an increase in the expression and activity of matrix metalloproteinases (MMPs) and in the invasiveness and metastatic ability. The Slug zinc-finger protein, a member of the Snail family, is a transcription factor involved in EMT regulation [7].

Histamine is a biogenic amine that exerts multiple physiopathological actions through the stimulation of four (H1–H4) histamine subtypes G-protein coupled receptors (GPCR) which are widely distributed in different tissues and organs. For the last 25 years histamine has displayed an important role in cell proliferation and tumor growth in a number of experimental models through the stimulation of different histamine receptors [8].

Increasing evidence has also been collected indicating that histamine might be a player in tumor progression modulating cell adhesion and MMPs activity in pancreatic and mammary tumor cells [9,10]. We have reported that ionizing radiation induced some EMT-related events in the TNBC cells MDA-MB-231 which were blocked upon histamine treatment before cell irradiation [9].

The proto-oncogene Src is a member of the Src family kinases linked to cancer progression and metastatic disease. Its interaction with different signaling proteins is involved in cell adhesion and migration. A growing body of research indicates that GPCR and Src family kinases are thoroughly implicated in multilayered forms of cross-talk that influence a host of cellular processes [11–15]. Recently, it has been reported that activated/phosphorylated Src is involved in the promotion of the radio-induced malignant phenotypes in breast cancer cells [16].

In this work we investigated whether histamine may modify EMT events induced by ionizing radiation in breast cancer cells with different invasive phenotype. The involvement of Src phosphorylation in histamine actions on migration of irradiated cells was also evaluated.

2. Materials and methods

2.1. Cell culture

Human breast carcinoma cells MCF-7 and MDA-MB-231 were from the American Type Cell Culture (ATCC). Cells were routinely maintained in RPMI 1640 medium containing 10% fetal bovine serum (FBS, Gibco, CA, USA), 0.3 g/l L-glutamine and 40 mg/l gentamicin at 37 °C in a humidified atmosphere with 5% CO2. Cells were subcultured according to the ATCC protocol within 2 months of resuscitation and used between passages 5 and 20. Cell cultures were mycoplasma tested monthly using DAPI as a direct DNA stain.

2.2. Irradiation

Tumor cells were seeded and treated or not with 1 μM or 20 μM histamine (Sigma-Aldrich, St Louis, MO, USA). The selective inhibitor of Src-family tyrosine kinases 4-amino-3-(4-chlorophenyl)-1-(*t*-butyl)-1H-pyrazolo[3,4-*d*]pyrimidine, 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine (PP2, Sigma) was added to cell cultures 30 min before histamine in combined treatments. After 24 h cells were gamma-irradiated at a total dose of 2 Gy with an IBL 437C H type irradiator, ^{137}Cs source.

2.3. Morphological changes

Cell morphology and scattering were evaluated five days after irradiation. Cells were fixed with 4% formalin and stained with 0.1% toluidine blue dye solution. Cells were observed by an optical microscope Olympus Bx50 and photographed using an Olympus DP73 camera.

2.4. Immunoblotting

Cells were washed in ice-cold phosphate-buffered saline, taken up in 100 μl of lysis buffer, and heated to 95 °C for 5 min. 30–50 μg of protein were separated on a 12% SDS-PAGE gel and blotted onto a polyvinylidenedifluoride membrane. Membranes were blocked and probed with anti E-cadherin (1:400, Invitrogen, CA, USA), anti vimentin (1:500, Invitrogen), anti β-catenin (1:400, Invitrogen), anti Slug (1:100, Santa Cruz Biotechnology, TX, USA), anti phospho-Src and Src (1:250, Cell Signaling, MA, USA), anti β-actin or α-tubulin (1:2000, Sigma) antibody overnight and with anti-rabbit or anti-mouse horseradish peroxidase-conjugated antibody (1:2000, Sigma) for 1 h at room temperature. The immunoreactive bands were detected by enhanced chemiluminescence (ECL, Amersham Biosciences, Argentina). Densitometric analyses were performed using the software ImageJ 1.42q (NIH, USA).

2.5. Immunofluorescence studies

Cells grown on cover glasses were fixed, permeabilized, blocked in PBS with 1% bovine seroalbumin and incubated overnight at 4 °C with anti E-cadherin (1:100, Invitrogen), anti β-catenin (1:100, Invitrogen), anti Slug (1:50, Santa Cruz Biotechnologies), anti P-Src (1:100, Cell Signaling) or anti vimentin (1:100, Invitrogen) antibody and for 1 h at room temperature with Alexa Fluor 488 dye conjugated anti-mouse (1:400, Invitrogen) or FITC-conjugated anti-rabbit (1:100, Sigma) antibody. Nuclei were stained with 0.1 μg/ml DAPI or propidium iodide. Immunoreactivity was visualized by an immunofluorescence microscope (Olympus Fluo View FV1000).

2.6. Gelatin zymography

After 24 h in serum free RPMI, supernatants from cell cultures were collected, mixed with non-reducing buffer and electrophoresed on 7% sodium dodecyl sulfate-polyacrilamide gels with 0.1% gelatin (Sigma). The gels were washed with 0.5% Triton X-100 (v/v) in Tris-buffered saline (TBS), pH 7.4 for 30 min, rinsed briefly with TBS, pH 7.4, and incubated in TBS, pH 7.4 supplemented with 1 mM Ca^{2+} at 37° C for 24 h. Gelatinolytic activity was visualized by staining zymograms with Coomassie Brilliant Blue G250 (Sigma) and destaining in acetic acid-methanol-H_2O (1:3:6).