HISTAMINE HINDERS RADIATION-INDUCED MESENCHYMAL TRANSITION IN EPITHELIAL TUMOR CELLS

Mohamad NA¹, Cricco GP¹, Porretti, JC¹, Ventura C¹, Núñez M¹, Cocca C¹,², Rivera ES¹, Martin GA¹,².
¹Laboratory of Radioisotopes, School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956 (1113) Buenos Aires, Argentina. ²National Research Council (CONICET)

Contact: gamartin@fify.uba.ar

1. INTRODUCTION

Epithelial-mesenchymal transition (EMT), a physiologic process during which epithelial cells acquire mesenchymal features, is also required for converting tumors into aggressive and highly invasive cancers. Changes in cell adhesion and motility, positive regulation of metalloproteinases (MMP-2/MMP-9), and enhanced expression of mesenchymal markers (N-cadherin, vimentin, alpha-smooth muscle actin) are observed. Evidence indicates that ionizing radiation may increase the proliferative, invasive and metastatic capacities in the surviving tumor cells of irradiated neoplasias.

2. OBJECTIVE

The aim of this work was to study the effect of histamine on ionizing radiation-induced EMT in two epithelial cell lines whose proliferation is inhibited by histamine over 10 µM.

3. METHODS

CELLS: Human pancreatic adenocarcinoma (PANC-1) and breast cancer (MDA-MB-231) cell lines (ATCC). MMP-2 and MMP-9 activity measured by 10% PAGE gelatinase gel (zymography) in 10% FCS RPMI 1640 at 37°C in a 5% CO2 humidified atmosphere with or without histamine for 48h.

IRRADIATION: A 125I source irradiator RII 417C type H with three 189 TBR 125I sources was employed (Dose rate 7.7 Gymin). 2 Gy irradiated cells received the same treatments as non irradiated cells.

IMMUNOCYTOCHEMISTRY/IMMUNOFLORESCEIN: After 24h treatment, cells grown on coverglasses were fixed and permeabilized. POMP expression was evaluated employing the specific primary antibody and a secondary antibody conjugated to horseradish peroxidase (plus DAB) or to fluorescein, using hematoxylin or ethidium bromide as counterstaining respectively.

4. RESULTS

Gelatinolytic activity and cell motility assays

MMP-2 activity

a) \[ \text{MMP-2 activity} \]

b) [Graph showing MMP-2 activity]

c) [Graph showing cell migration]

MMP-9 activity

a) \[ \text{MMP-9 activity} \]

b) [Graph showing MMP-9 activity]

c) [Graph showing cell migration]

Gelatinolytic activity and cell motility assays

MMP-2 and MMP-9 gelatinolytic activity (zymography) ImageJ 1.42 software (NIH, USA). Results are expressed as percentage of lytic activity respect to unirradiated cells.

Histamine blocks the increase in gelatinolytic activity (MMP-2 and MMP-9) and cell migration induced by 2 Gy irradiation in the breast cancer cells MDA-MB-231.

5. CONCLUSIONS

Our results show that histamine over 10 µM is able to counteract ionizing radiation-induced EMT in epithelial tumors cells supporting the idea that histamine may play a promissory role as an adjuvant for the management of radiotherapy reactions.