HISTAMINE HINDERS RADIATION-INDUCED MESENCHYMAL TRANSITION IN EPITHELIAL TUMOR CELLS

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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 tumor 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) were cultured in 10% FCS RPMI 1640 at 37ºC in a 5% CO2 humidified atmosphere with or without histamine for 48h.

IRRADIATION: A ^{137}Cs source irradiator IBL 437C type H with three 189 TBq ^{137}Cs sources was employed (Dose rate 7.7 Gy/min). 2 Gy irradiated cells received the same treatments as non irradiated cells.

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

MMP-2 and MMP-9 gelatinolytic activity (zymography): Cells were cultured in serum free RPMI for 24 h. Obtained conditioned media were electrophoresed in 7% poliacrilamide gels with 1% gelatine and stained with Coomasie Blue. Lytic bands activity was determined by densitometry using ImageJ 1.42 software (NIH, USA). Results are expressed as percentage of lytic activity respect to non irradiated control.

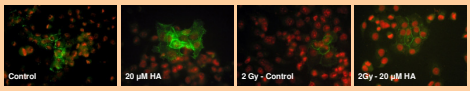
CELL MIGRATION: Migration was evaluated using transwell units with 8 μm pore size polyethylene terephtalate membranes. Cells were seeded in the upper chamber and 20 μM histamine was added in the lower chamber. After 20 h, migrated cells were fixed, hematoxylin stained and counted. Results are expressed as a percentage of migrated cells respect to non irradiated control.

4. RESULTS

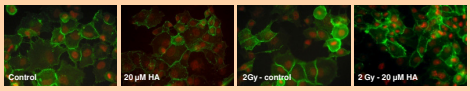
PANC-1 CELLS

EMT markers

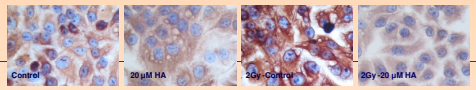
a) E-cadherin expression



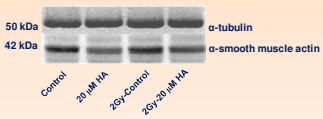
b) N-cadherin expression



c) Vimentin expression



d) Alpha-smooth muscle actin expression

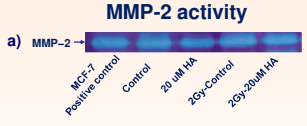


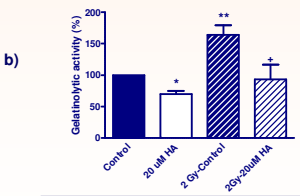
Immunodetection of EMT markers.

Immunofluorescence of E-cadherin (epithelial marker-400x) (a) and N-cadherin (mesenchymal marker-400x) (b). Immunocytochemistry of vimentin (mesenchymal marker-630x) (c). Immunoblotting of alpha-smooth muscle actin (mesenchymal marker (d). **Ionizing radiation (2Gy-Control) decreases E-cadherin and increases the expression of mesenchymal markers as N-cadherin, vimentin and alpha-smooth muscle actin expression. Notably, these responses are reverted when histamine is added before 2Gy irradiation (2Gy-20μM HA).**

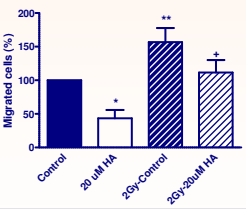
Gelotinolytic activity and cell motility assays

MMP-2 activity





Cell migration



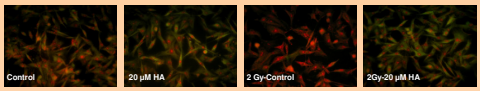
a) A zymography representative of one experiment. b) and c) Bar chart: values are representative of three experiments run in duplicate. *p < 0.05 and **p< 0.01 vs Control; +p < 0.05 vs 2 Gy Control. One-way ANOVA and Bonferroni post test.

Histamine blocks the increase in MMP-2 gelatinolytic activity and cell migration induced by 2 Gy irradiation in the pancreatic cancer cells PANC-1.

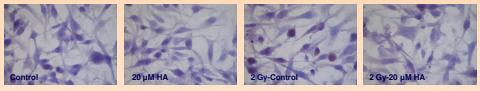
MDA-MB-231 CELLS

EMT markers

a) E-cadherin expression



b) Alpha-smooth muscle actin expression

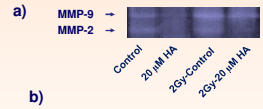


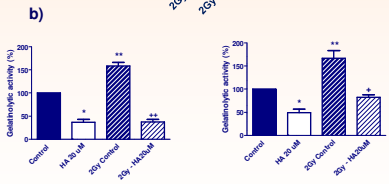
Immunodetection of EMT markers.

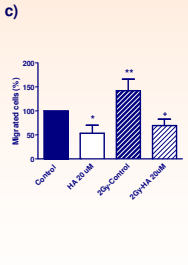
Immunofluorescence of E-cadherin (epithelial marker-400x) (a). Immunocytochemistry of alpha-smooth muscle actin (mesenchymal marker-630x) (b). **Ionizing radiation (2Gy-Control) decreases the expression of E-cadherin and augments alpha-smooth muscle actin expression. Notably, theses responses are counteracted when histamine is added before 2Gy irradiation (2Gy-20μM HA).**

Gelotinolytic activity and cell motility assays

MMP-2 activity | **MMP-9 activity** | **Cell migration**







a) A zymography representative of one experiment. b) and c) Bar chart: values are representative of three experiments run in duplicate. *p < 0.05 and **p< 0.01 vs Control; +p < 0.05 and ++p<0.01 vs 2Gy Control. One-way ANOVA and Bonferroni post test.

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.