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5. Histamine in the cardiovascular, gastrointestinal and reproductive systems

Effect of histamine on the expression of metalloproteinases and cell adhesion in breast cancer cell lines

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Introduction

Changes in cell adhesion and matrix metalloproteinases production (MMPs) are pivotal for tumor progression to occur. The MMPs that degrade extracellular matrix, MMP-2 and MMP-9, are associated with the invasive potential of cancer cells. Tissue inhibitors of metalloproteinases (TIMPs), small molecules which form high affinity complexes with active MMPs, regulate their proteolytic activity. E-Cadherin, a cell-cell adhesion molecule, plays an important role in the separation of cells from original tumors and may also regulate MMP-2 enzymatic activity [1]. Histamine (HA) has been demonstrated to be a well-established growth factor in several human and experimental neoplasms. We have recently reported that HA modulates cell survival and invasiveness in the human pancreatic adenocarcinoma cell line, PANC-1 [2]. Our aim was to study the effect of HA on cell adhesion and expression of MMP-2, MMP-9, TIMP-1 and TIMP-2 in human tumorigenic and non-tumorigenic mammary cell lines, MDA-MB-231 and HBL-100, respectively.

Materials and methods

Cell culture: HBL-100 and MDA-MB-231 cells obtained from ATCC (Manassas Va, USA) were cultured in 10 % FBS-RPMI medium at 37°C in humidified atmosphere (95 % air/ 5 % CO_2).

Gelatinolytic Activity: MMPs activities were evaluated by zymography [2].

Cell Adhesion: A colorimetric assay was employed to evaluate cell adhesion as described [2].

RT-PCR: mRNA levels were evaluated in cell cultures after 24 h treatment. Total RNA was extracted with Trizol®. Retrotranscription was carried out with MMLV enzyme. cDNA was amplified by PCR, using the appropriate primers for MMP-2, MMP-9, TIMP-1 and TIMP-2. Resulting products were run in 2 % agarose gels containing ethidium Bromide and semi-quantified by the ImageJ 1.36b software (NIH, Bethesda Md. USA).

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Results and discussion

During progression to malignancy, human mammary epithelial cells exploit the invasive potential altering the pattern of expression of many molecules, as is the case with cell adhesion molecules (cadherins and integrins), MMPs and TIMPs [1]. Several lines of evidence indicate that HA and HA antagonists may modify cell adhesion and metalloproteinases production [2, 3, 4].

In the present work we evaluated the gelatinolytic activity of MMP-2 and MMP-9 in HBL-100 and MDA-MB-231 cells by zymography. Results show that HA treatment produced a significant decrease in gelatinolytic activity of MMP-2 in HBL-100 cells while reduced both MMP-2 and MMP-9 activity in MDA-MB 231 cells (Table 1). MMPs mRNA expression levels were modified by HA in the same way as enzymatic activities (data not shown). Furthermore HA significantly augmented cell adhesion in non- tumorigenic HBL-100 cells. In contrast, HA produced a slight decrease in MDA-MB 231 cell adhesion, which could be correlated with the reduction in E-Cadherin expression exerted by HA (data not shown). These results are in agreement with the increase in cell motility recently reported by us [5] and with literature on the role of E-Cadherin in epithelial tumor progression [6]. In addition, there was a basal mRNA expression of TIMP-1 and TIMP-2 in both cell lines and HA treatment only decreased TIMP-2 level in MDA-MB-231 cells (30 %, $p<0.05$). Probably the balance between activated MMPs and TIMPs may be crucial for extracellular matrix degradation involved in malignant progression [7, 8]. These data suggested that HA might modify the invasive phenotype in MDA-MB-231 breast cancer cells by dimi-

Table 1. Effect of HA on HBL-100 and MDA-MB-231 gelatinolytic activities and cellular adhesion. *Gelatinolytic activity:* Equal number of cells was cultured in serum-free RPMI medium for 24 h in absence (control) or presence of 10 μM HA. Supernatants were then mixed with non-reducing buffer and run on SDS-polyacrylamide gels with 0.2 % gelatine after which the gels were incubated at 37°C for 24 h, stained with 0.1 % Coomassie Brilliant Blue and destained. Activity of lytic bands was determined by densitometry employing ImageJ 1.32j software (NIH, Bethesda Md. USA). Results were expressed as % of optical density of control cells.
n.d.: not detectable (too low to be quantified)
Cell adhesion: Cells were grown in 10 % FBS-RPMI medium for 48 h in the absence (control) or presence of 10 μM HA. After detaching, cells were re-seeded in equal number and incubated for 4 h at 37°C. Then adherent cells were fixed, stained with 1 % methylene blue and eluted by addition of 1 M HCl. The intensity of staining was measured spectrophotometrically at 620 nm.
Cellular adhesion (%) = (Optical density (OD) for HA treatment / OD for control) x 100.
Data represent mean values ± SEM (n = 5 experiments). *$p<0.05$ vs control (t-test)

Cell line	**MMP-2 Gelatinolytic Activity (%)**	**MMP-9 Gelatinolytic Activity (%)**	**Cellular Adhesion (%)**
HBL-100	77 ± 3*	n.d.	138 ± 8*
MDA-MB 231	63 ± 6*	78 ± 3*	83 ± 4

nishing cell adhesion and by altering the balance between MMP-9 and TIMP-2.

In summary, HA may differentially modulate two events involved in malignant progression in breast cells: proteolysis and cell adhesion. However, both are complex processes so further studies will be required to completely elucidate the role of HA in their modulation.

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