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Histone H1 inhibits the proliferation of MCF 7 and MDA MB 231 human breast cancer cells

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Abstract

Purified histone H1 exerts extracellular functions suggesting novel histone functions. The cytotoxic effects of histone H1 have lead to its choice as a pharmacological tool in breast cancer. Hence the present study was aimed at investigating the effect of exogenous histone H1 on the proliferation of estrogen receptor positive (MCF 7) and estrogen receptor negative (MDA MB 231) human breast cancer cells. Cells were incubated with various concentrations of histone H1 and antiproliferative activity was assessed by MTT assay. Proliferation of breast cancer cells was assessed from the activity of ornithine decarboxylase (ODC) using [^{14}C] labeled ornithine. Histone H1-mediated cellular effects, such as anchorage dependent growth and apoptosis, were assessed by colony formation assay, fluorescence microscopy after acridine orange/propidium iodide staining and DNA fragmentation analysis. Histone H1 was significantly cytotoxic as it inhibited colony formation, ODC activity and induced apoptosis in both estrogen receptor positive and estrogen receptor negative cells. These results suggest that histone H1-induced antiproliferative effects on human breast cancer cells could possibly involve inhibition of ODC.

Keywords: Apoptosis; Breast cancer; Histone H1; MCF 7 cells; MDA MB 231; Ornithine decarboxylase

1. Introduction

Breast cancer is the second most common cause of cancer deaths in women. A major clinical problem is that breast tumors, which are initially responsive to both hormonal and chemotherapeutic approaches, generally progress to more aggressive forms that are poorly responsive to either category of agents. The need for anti-neoplastic agents with novel mechanisms of action is, therefore, inevitable.

As with any drug development, an empirical screening is required to examine the biochemical influences of any agent in order to understand the cytotoxic effects. Until recently the functional role of histone H1 had been viewed in connection with DNA stabilization (Elgin and Weintraub, 1975) and gene expression (Grunstein, 1992). Histone involvement in autoimmune diseases such as rheumatoid arthritis and multiple sclerosis have been documented (Leak and Woo, 1991; Wesierska-Gadek et al., 1990). The therapeutic role of histone H1 in cancer and immune therapy has been reported (Zeppezauer and Reichhart, 1989). The cytotoxic effect of histone H1 has been demonstrated in leukemia cells (Class et al., 1996) and thymocytes in vitro (Abakushin et al., 1999). Extranuclear functions of histone H1 have been identified (Reichhart et al., 1985), including a possible role in the induction of apoptosis (Konishi et al., 2003). Extracellularly histone H1 increases membrane permeability (Kleine et al., 1995), has hormone-like activity (Comsa et al., 1982) and represses ERα transcription (Cheung et al., 2002).

The biological activity of histones is addressed by the interaction of circulating histones and hormonal network. Changes in the level of histones can result in severe failure of the immune system, as higher incidence of lymphatic tumors are

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reported in patients with increase in anti-histone antibodies causing a lowering of histones in circulation (Pettersson et al., 1992). Previous studies in vivo have shown that histone H1 suppresses tumor growth (Vani and Shyamaladevi, 2000) and modulates immune status (Vani et al., 2003). Hence the present study was aimed at assessing the benefits of histone H1 in vitro against breast cancer cells in search of a favorable therapeutic index.

Recently attention has been focused on the polyamine metabolic pathways as potential targets for intervention. The critical role of polyamines in regulation of cell growth has led to the development of a number of inhibitors of key enzymes in the polyamine biosynthetic pathway as therapeutic strategies (Pegg, 1988; Davidson et al., 1993). Natural polyamines − Putrescine, Spermidine, and Spermine are organic cationic proteins essential for cell growth. A highly regulated metabolic pathway finely controls intracellular polyamine concentrations. The rate-limiting enzymes, ODC and *S*-Adenosylmethionine decarboxylase (SAMDC) regulate biosynthesis, whereas catabolism of polyamines is regulated by spermidine/spermine N^1-acetyltransferase (SSAT) and polyamine oxidase h1/spermine oxidase (Casero and Pegg, 1993). Among the three enzymes (ODC, SSAT and SADMC), ODC over expression is the most significant alteration in polyamine metabolism associated with breast cancer progression (Manni et al., 1995a,b,c) and its influence is superior as an independent prognostic factor for both disease free survival and overall survival (Manni et al., 1995a,b,c; Canizares et al., 1999). A decrease in intracellular polyamine levels is associated with apoptosis (Ha et al., 1997) and inhibitors of ODC induce apoptosis (Takahashi et al., 2000).

In the present study, the effect of histone H1 on the proliferation of human breast cancer cells MCF 7 and MDA MB 231 was assessed from the activities of ODC as reports are lacking on its effect in human breast cancer cells. The present study employed MCF 7 cells and MDA MB 231 cells, as the properties of these cells are akin to human breast cancer cells and are successfully used as models for estrogen receptor positive (ER(+)) and estrogen receptor negative (ER(−)) breast cancer research, respectively (Soule et al., 1973; Sheikh et al., 1995). The time course and concentration response relationship of histone H1 on the possible mode of cell death have been examined and the biological relevance is discussed.

2. Materials and methods

2.1. Chemicals

All chemicals used were of tissue culture grade purchased from GIBCO (Grand Island, NY, USA). Histone H1 (Type III Calf thymus) was obtained from Sigma, St Louis, MO, USA. ^{14}[C] Ornithine was purchased (Sp.Act. 52.7 Ci/mol) from BRIT, Mumbai, India. Unlabelled ornithine and hyamine hydroxide were purchased from SRL, India.

2.2. Culture

Human breast cancer MCF 7 and MDA MB 231 cells were obtained from National Centre for Cell Science, Pune, India and maintained at King's Institute of Preventive Medicine, Guindy, Chennai, India. MCF 7 cells and MDA MB 231 cells were at the 32nd and 87th passages, respectively. MCF 7 cells and MDA MB 231 cells were maintained in Eagle's MEM and L-15 (Leibovitz) medium, respectively, supplemented with 5% fetal bovine serum, 2 mM glutamine, and 100 units/ml penicillin/streptomycin. MCF 7 cells were incubated at 37 °C in a 5% CO_2 atmosphere, whereas MDA MB 231 cells were incubated in CO_2 free atmosphere. Histone H1 was dissolved in PBS at a concentration of 1 mg/ml.

2.3. Cell viability by MTT assay

Cells (MCF 7 and MDA MB 231) were seeded in 96-well microtitre plates (100 μl/well) at a concentration of 1×10^6 cells/ml culture medium for 24 h. The next day culture medium was replaced with 100 μl of serum free medium containing different concentrations of histone H1 (50−300 μg/ml) and cell viability was assayed using MTT (3 (4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) (Mossmann, 1983). For time-dependent assays cells were incubated with histone H1 for 24 h. The cytotoxic index (CI) of cells are expressed as percentage calculated according to the equation

$$\mathrm{CI} = 1 - \frac{\mathrm{OD}_{540} \text{ of (treated cells)}}{\mathrm{OD}_{540} \text{ of (control cells)}} \times 100$$

2.4. Anchorage dependent assay

MCF 7 and MDA MB 231 cells were suspended in complete media (MEM and L-15 media, respectively) supplemented with 4% FCS, 0.3% agar and plated in 35 mm Petri dishes at 1×10^4 cells per dish. The concentration of histone H1 ranged from 0.5 to 3 μg/ml for 10^4 cells (≅50−300 μg/ml for 10^6 cells) and were added in 50 μl portions. After 14 days, colonies were counted using an inverted microscope and cell viability was assessed after MTT staining (Yuhas et al., 1977). The anchorage dependent growth is expressed as number of colonies.

2.5. Analysis of intracellular ODC activity

ODC activities were measured using cellular extracts as described (Beaven et al., 1987). Briefly, treated and untreated cells were pelleted and cell suspension of 10 μl was mixed with reagent (100 mM phosphate buffer, pH 6.8; 0.1 mM EDTA, 0.4 mM [^{14}C] ornithine, unlabelled ornithine, 0.01 mM pyridoxal phosphate) in a microfuge that was placed in a 20 ml scintillation vial. Hyamine hydroxide (20 μl in methanol) was placed at the bottom of glass vial away from the microfuge. The vials were tightly capped and incubated at 37 °C for 30 min. The vials were placed in ice and uncapped one at a time and 10 ml of 2 N perchloric acid was added. Glass vials were quickly recapped and reincubated for 1 h. At the end of second incubation microfuges were removed and discarded. Liquid scintillation fluor (toluene based) was added directly to the counting vials for the assay of $^{14}CO_2$ released. The enzyme activity is expressed as nmoles of $^{14}CO_2$ released/min/10^6 cells.

2.6. Acridine orange/PI staining of nuclear chromatin

Propidium iodide and acridine orange staining was performed as described (Duke and Cohen, 1992). Briefly, 2 μl of a combined dye of 100 μg/ml acridine orange and 100 μg/ml of propidium iodide were added to 20 μl of the cell suspension, and 5 μl of stained suspension was transferred to glass slide for immediate analysis using an ultraviolet fluorescence microscope. Cells were scored into four categories as follows: C_1 − large, green, non-condensed nuclei as non-apoptotic; C_2 − cells with green nuclei showing signs of nuclear condensation as apoptotic viable; C_3 − cells with red/orange nuclei showing nuclear bead formation as apoptotic non-viable cells; C_4 − cells with large red nuclei that showed no signs of nuclear condensation as necrotic. At least 200 cells/sample were counted and scored. The apoptotic index was calculated as follows: apoptotic index = $(C_2 + C_3) \times 100$/total number of cells.