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The Effect of Histone Hyperacetylation on Viability of Basal-Like Breast Cancer Cells MDA-MB-231

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Abstract

Background: The Basal-Like breast cancer, is always known for lack of expression of estrogen receptor (ER), progesterone receptor (PR) and as well, absence of epidermal growth factor receptor 2 (HER2) gene amplification. Improper expression pattern of ER, PR, and Her2, makes Basal-Like breast tumors resistant to the current hormonal and anti HER2 treatments. In recent decades, several studies have been conducted to investigate the regulatory role of chemical modifications of core histones in gene expression. Their results have shown that histone acetylation is involved in regulation of cell survival. Acetylation of core histones is regulated by the epigenetic-modifying enzymes named Histone Deacetylases (HDACs). As a new approach to control the viability of breast tumor cells resistant to the hormonal and anti-HER2 treatments, we have targeted the HDACs. Using Trichostatin A (TSA) as a known HDACs inhibitor, we have tried to hyperacetylate the core histones of MDA-MB-231 cells as an in vitro model of Basal-Like breast tumors. Then we have investigated the effect of histone hyperacetylation on viability of MDA-MB-231 cells.

Methods: MDA-MB-231 cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and were incubated at 37°C, in a humidified incubator with 5% CO2 atmosphere. Then cells were treated with different concentrations of TSA including: 50, 100, 200, 400, 800 and 1000 nM or control (1% DMSO). After 24 and 48 hours, viability of cells was evaluated by MTT assay.

Results: After 24 and 48h exposure to different concentrations of TSA, MDA-MB-231 cells showed a maximum tolerable dose. At higher concentrations, TSA decreased the percentage of cell viability through a time-dose dependent manner. IC50 value for 48h treatment was 600 nM.

Conclusions: Our results indicate that HDACs inhibition and subsequently hyperacetylation of histones, leads to cytotoxic effects on breast tumor cells resistant to the current treatments. Following this pilot research we are trying to suggest molecular mechanisms underlying the anti-proliferative effects triggered by HDACs inhibition.

Keywords: Basal-Like, HDACs, Trichostatin A, MDA-MB-231, Cell Survival

1. Background

Breast cancer is well-known as the most prevalent malignancy among women (1). Tumors of the breast tissue are divided into several types based on the expression pattern of genes encoding cell surface proteins (2-4). Among these proteins, estrogen receptor (ER), progesterone receptor (PR), and epidermal growth factor receptor 2 (HER2) are three immunohistochemical markers in molecular classification of breast tumors (5) and expression pattern of these receptors is a key criteria in classifying tumor types and also to determine the prognosis (6). Tamoxifen is an effective treatment prescribed in patients with breast cancer, which its metabolites act as an estrogen antagonist (7). Also treatments targeting HER2 receptors, are other therapeutic approaches against breast tumors (8). However, these treatments are effective only in tumors expressing ER and amplifying the gene encoding HER2 receptor. "Basal-Like" (also called "triple negative") is a kind of breast tumor which does not express ER, PR and HER2 receptors. So this subtype of breast cancer does not benefit from hormonal therapies such as Tamoxifen or anti HER2 treatments (9). So it seems to be necessary to investigate mechanisms controlling cell cycle, to find out an effective approach to control cell proliferation of Basal-Like breast tumors.

In recent years, two enzymatic protein complexes have been identified as master regulators of histone dynamics during the gene transcription. These complexes, including: histone deacetylases (HDACs) and histone acetyltransferases (HATs), adjust the compression level of nucleosomes by acetylating and deacetylating of -amino groups in Lysyl residues of histones (10, 11). This process can be considered as an important factor in regulation of gene expression, since compression level of chromatin, affects transcriptional activity of the genes (12, 13). Many studies have been conducted to investigate the regulatory role of histone acetylation in cell cycle control. Previous researches findings, suggested that HDACs inhibition results in cell cycle arrest in G1 and G2 phase and also stimulates the cell differentiation (14-20). In addition, hyper-

acetylation of histones followed by the HDACs inhibition, induces apoptosis in transformed cells (21). Increased expression and activity of HDACs, is associated with key events of tumorigenesis such as epigenetic silencing of CDKN1A (the gene encoding "p21waf1/cip1" protein which is a suppressor of Cyclin Depended Kinases (CDKs)) (22) and results in reduced expression of genes encoding DNA repair enzymes such as BRCA1, ataxia telangiectasia and RAD3 (ATR) (23). Therefore, aberrant activity of HDACs may lead to progression of tumorigenic events such as uncontrolled cell proliferation.

Nowadays, several chemical compounds have been identified that are able to inhibit HDACs activity. Trichostatin A (TSA) is one of these compounds (PubChem: 444732). TSA is a carboxamic acid which inhibits HDACs reversibly and non-competitively (24). Using TSA, several studies have been conducted to find the biological functions of HDACs. MDA-MB-231 cells, lack the expression of ER, PR and HER2 are considered as an in vitro model of breast tumors resistant to the anti-HER2 and hormonal therapies (25). In this study we used TSA as a HDACs inhibitor to hyperacetylate the core histones of MDA-MB-231 cells. Then we compared viability and proliferation of treated groups with control group to determine the effect of histone hyperacetylation on viability of Basal-Like breast tumors.

2. Methods

2.1. Chemicals and Reagents

TSA was purchased from R & D Systems (Cat#: 1406). Considering the molar weight of 302.37 (gr/Mole), one milligram of TSA was dissolved in 33 mL of Dimethyl sulfoxide (DMSO) solvent, to obtain 100 (μM) solution of TSA in DMSO as the main stock. Cell culture reagents including: RPMI 1640, fetal bovine serum (FBS), Pen-Strep Antibiotic and Trypsin-EDTA were products of "Life Technologies" (Gibco-USA). Methyl Thiazolyl Tetrazolium bromide (MTT) powder was purchased from "Sigma Aldrich" (Sigma- USA). Stock solution of MTT was prepared by dissolving 5 (mg) of MTT powder in 1 (mL) phenol red-free RPMI 1640 (5 mg/mL).

2.2. Cells and Cell Culture

MDA-MB-231 cell line (ATCC: HTB-26) was purchased from "Pasteur Institute of Iran". Complete growth medium was prepared by combination of 10% FBS and 1% of Pen-Strep in RPMI 1640. MDA-MB-231 cells were cultured with complete growth medium in sterile flasks and were incubated at 37°C in a humidified incubator with 5% CO_2 atmosphere until reaching the desired confluency.

2.3. MTT Assay

Effect of TSA on viability of cells, was determined using MTT assay. Initially, cultured cells reaching the confluency of more than 80 percent, were trypsinized and counted using Trypan Blue and haemocytometer. Then cells were seeded in 96-well sterile plates at the density of 4×10^3 cells/well. After 12 hours of incubation, cells were treated with different concentrations of TSA (50, 100, 200, 400, 800 and 1000 nM) and control medium containing 1% DMSO. MTT test was performed according to the protocol provided by ATCC. MTT solution was added to each well at concentration of 0.5 mg/mL and the micro plates were incubated for 2 hours. Formazan crystals formed at the bottom of the wells, dissolved in 100 ml of DMSO and the absorbance of each well determined using an ELISA plate reader. MTT assay was repeated in both biological and technical replicates.

2.4. Statistical Analysis

MTT test was repeated in technical and biological replicates for both 24 and 48 hours treatments. The means were compared by one way-ANOVA test since there was more than two groups and the data were normally distributed. Using post hoc tests the significance of results were determined and the P-value of less than 0.05 considered as significant.

3. Results

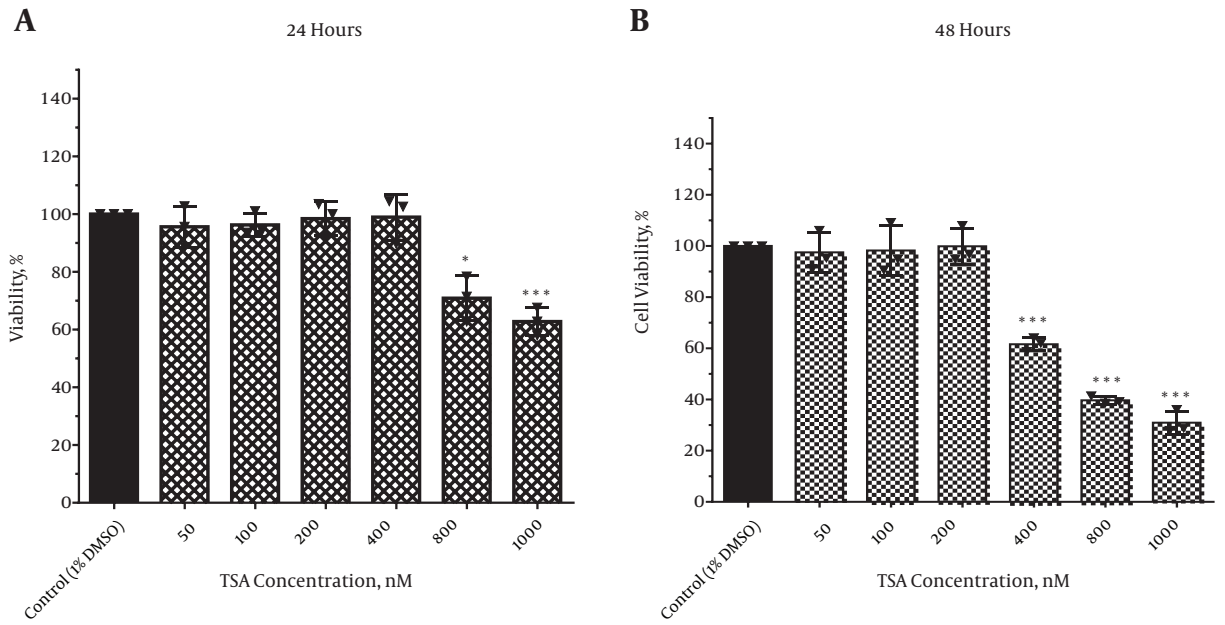
TSA as a classical HDACs inhibitor, decreases the viability of Basal-Like breast cancer cells.

As shown in Figure 1, TSA reduced the viability of MDA-MB-231 cells through a time-dose dependent manner so that inhibition of cell viability was proportional to the concentration and exposure time of cells to the TSA. Exposure to TSA for 24 hours at the concentrations of 800 and 1000 nM caused 29.0% and 37.2% (respectively) decrease in viability of cells ($P < 0.01$ compared with control) (Figure 1A). After 48 hours exposure to TSA, viability of cells was significantly reduced at doses of 400, 800, and 1000 nM (38.3%, 60% and 69.3% respectively) ($P < 0.001$ compared with control) (Figure 1B). However, there was no significant decrease in cell viability at the concentrations of 50, 100 and 200 nM TSA after 24 and 48 hours treatment.

After 24 hours treatment, the IC50 value was not computable because even the highest concentration of TSA caused less than 50% reduction in cell viability. After 48 hours treatment, the IC50 value for anti-proliferative activity of TSA against MDA-MB-231 cells, was 600 nM (Figure 2).

Also after both 24 and 48 hours treatment, MDA-MB-231 cells showed maximum tolerable doses of 400 nM and 200

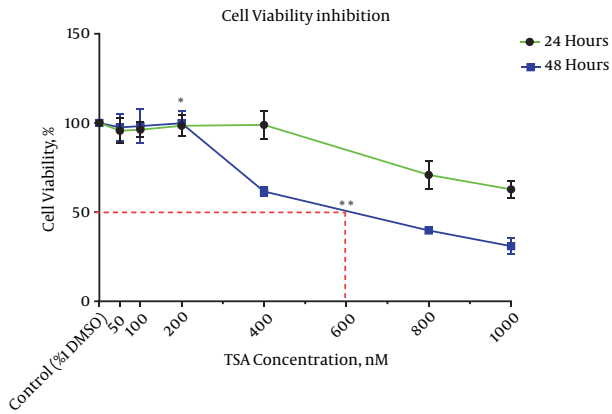
Figure 1. Viability Percentage of MDA-MB-231 Cells Treated with Different Concentrations of TSA Compared to the Control (1% DMSO) for 24 (A) and 48 hours (B)



P values are determined by comparison of each treated group to the control group. *P < 0.05, **P < 0.01, ***P < 0.001.

nM for 24 and 48 hours respectively. These concentrations can be considered as treatment doses for those investigatory approaches which need the treatments which do not affect the percentage of cell viability, such as assessing the effect of the drug on mobility of the cells (Figure 2).

Figure 2. Cell Viability Inhibition Curve After 24 and 48h Treatment with Different Concentrations of TSA Compared to the Control (0.1% DMSO)



*Maximum tolerable dose after 48 hours treatment, **IC50 for 48 hours treatment

4. Discussion

In recent years, HDACs inhibitors (HDACIs) have been introduced as the new generation of anti-cancer drugs. HDAIs include a wide range of chemical compounds and at least 12 different types of them have been studied in clinical trials (26, 27). According to the results of these clinical trials, inhibition of HDACs in patients with lymphoma and leukemia and some solid tumors such as prostate carcinomas and non-small cell lung cancer, leads to the therapeutic responses (26, 27). As a classical HDACs inhibitor, TSA has been widely studied. Since the inhibitory effect of TSA on HDACs has been proven, in this study we have used it, to inhibit HDACs and to hyperacetylate the core histones of MDA-MB-231 cells subsequently. Then we tried to examine the impact of acetyl modifications of core histones and other HDACs regulated proteins on viability of MDA-MB-231 cells.

Poor clinical outcome and high mortality rate are the most known futures of Basal-Like breast tumors, because the lack of expression of ER, PR and HER2 receptors makes this subtype of breast cancer resistant to the current treatments such as tamoxifen and trastuzumab (28-32). In the present study, exposure to the nano-molar concentrations of TSA for 24 and 48 hours, caused to significant reduction in viability of MDA-MB-231 cells. Accordingly, it may be possible to control the proliferation of Basal-Like breast

tumors by hyperacetylation of core histones. Other important future of Basal-Like breast tumors, is their invasive behavior that in most cases leads to the distant metastasis (33). So the impact of histone hyperacetylation on cell mobility and signaling pathways associated with cancer cell invasion, can be assessed by the maximum doses of TSA that do not affect the percentage of cell viability since according to our results, MDA-MB-231 cell line shows a maximum tolerable dose against TSA treatment as described above (Figure 2).

However, anti-proliferative mechanisms triggered by HDACs inhibition, are not well understood. So in an ongoing survey we are trying to suggest a molecular mechanism for anti-proliferative and anti-metastatic effects of HDACs inhibitors.

4.1. Conclusion

In summary, the results of the present study indicate that, TSA may cause significant decrease in viability of MDA-MB-231 cells. 48 hours exposure to the complete growth medium containing 600 nM TSA led to the 50% decrease in viability of MDA-MB-231 cells. Therefore, after further investigations it may be possible to achieve more effective treatments in patients with Basal-Like breast tumors by targeting HDACs and histone acetylation.

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