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Progesterone May Prevent Osteosarcoma Cells Proliferation

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Abstract

Background and Aim: Progesterone has been reported to inhibit osteosarcoma cell proliferation; however, its inhibitory mechanism has not yet been clarified. The aim of the present study was to clarify the effects of progesterone on apoptosis in human osteosarcoma (MG-63) cell.

Methods: In this experimental study, cells line MG-63 was divided into two groups, control and groups exposed to effective concentration progesterone (2.5 mg/ml). flow cytometry was used to determine apoptosis effects of the Progesterone.

Results: Flow cytometry results showed apoptosis in the MG-63 cells exposed to effective concentration of progesterone.

Conclusion: Progesterone cytotoxic effect on osteosarcoma cell was mediated by apoptotic pathways. In this context, progesterone triggers extrinsic and intrinsic apoptotic pathways in induces intrinsic apoptotic pathway in MG-63 cell.

Keywords: *Progesterone, MG-63, Apoptosis.*

Introduction

Primary bone tumors are rare and account for less than 0.2% of the malignancies [1]. Osteosarcoma (OS) is a common malignant bone tumor in children and adolescents [2], which are considered as sex steroid hormone-associated tumors [3]-[5]. Prevention of osteosarcoma cells development is partially dependent on apoptosis induction in these cells**.** Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell populations in tissues. The mechanisms of apoptosis are highly complex and sophisticated, involving an energydependent cascade of molecular events [6].It has been reported that progesterone can induce or prevent apoptosis in cancer cells and is associated with many types of cancers including bone tumors [7].Progesterone promotes osteocalcin gene transcription resulting in osteoblast proliferation and differentiation [8]. Progesterone also protects osteoblast against apoptosis through the downstream mitochondrial pathway [9]. In addition, progesterone is involved in osteoporosis [10]-[12] by which plays a crucial role in bone tumors development.

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Regarding few reports concerning with the effects of progesterone on osteosarcoma cells, the present study was exerted to clarify apoptotic effects of progesterone on human osteosarcoma (MG-63) cellsand discriminating between apoptosis and necrosis in MG-63 cells exposed to cytotoxic dose of progesterone.

Materials and Methods

Progesterone

Progesterone was obtained from the Abu Reyhan Pharmaceutical Company (Tehran-Iran) and solved in DMSO, Dulbecco's modified Eagle's medium (DMEM) and phosphate buffered saline (PBS) to produce effective concentration of progesterone (2.5 *mg/ml).*

Cell culture

The MG-63 cells were obtained from National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). The cells were cultured in DMEM supplemented with 10% Fetal Bovine Serum (FBS) and 1% antibiotics (gentamicin). Cells were then preserved in a humidified atmosphere with 5% CO2 at 37°C incubator. Cultured cells at 70-80% confluency were washed with PBS and detached from the flask using trypsin-EDTA with incubation at 37ºC for 3-4 min, followed by addition of culture media containing 10% FBS to neutralize the excess trypsin-EDTA activity. The cell suspension was eventually centrifuged and the cell pellet was re-suspended in fresh culture media to be used in the experiments.

Flow cytometry analysis of cell death

The MG-63 cells were divided in to control, and cells exposed to effective concentration of progesterone (2.5 *mg/ml)***.** The Annexin-V-Fluos Staining Kit (Biolegend, USA) was used to discriminate between apoptosis and necrosis in the given culture system. The assay involves simultaneous staining with both annexin-V and the DNA stain propidium iodide (PI). Three subpopulation of cells were discriminated: (a) PI-negative and (FITC)-negative viable cells (PI–/FITC–) that maintain the typical asymmetry of plasma membrane lipids; (b) PI-negative and FITC-positive early apoptotic cells (PI–/FITC+) capable of transporting PI outside the cell; and (c) PI-positive and (FITC)-positive late apoptotic orm necrotic cells (PI+/FITC+) with a loss of plasma membrane integrity. For analysis, cells were prepared following the manufacturer's protocol. Fluorescence intensity was measured by flow cytometry (FACSCalibur, BD Biosciences, Franklin Lakes, NJ, USA) [13].

Results

Our results study showed that in the early stages of apoptosis, phosphatidylserine (PS) is translocated from the inner side of the plasma membrane to the outer layer. Annexin V, a calcium dependent phospholipid-binding protein with a high affinity for PS, can therefore be used as a sensitive probe for the exposure of PS on the cell membrane and hence as a marker of apoptosis. Figures1.a1 and is representative of control MG-63 cells, which almost no apoptotic cells were detected. However, in progesterone treated MG-63 cells (1. a2), a significant increase in early and late apoptotic cells and significant decrease in live cells were shown. As shown in figures 1. a2, analysis of the cell population had distinct sets of population. Annexin V and propidium iodide-negative cells increased significantly by the treatment of MG-63 cells with effective dose of progesterone compared to control, indicating the translocation of phosphatidyl serine, an early

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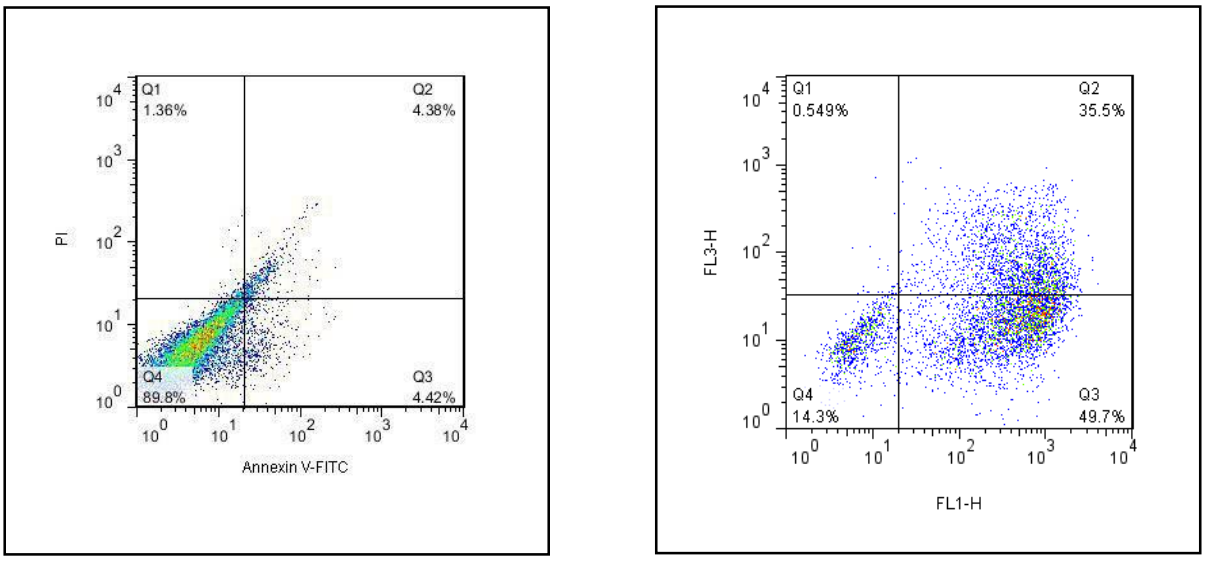
event of the apoptotic process.



1 a1 **1 a2**

Fig.1: Apoptosis in MG-63 cell lines induced by progesterone: Q1: Necrosis; Q2: Late Apoptosis; Q3: Early Apoptosis; Q4: Viable cells. (**a1**) Control (non-treated) MG-63 cells, (**a2**) MG-63 cells treated with progesterone. Percentage of apoptotic increased in progesterone treated cells compared with control groups.

Discussion

The findings of the present study showed that progesterone can induce apoptosis in osteosarcoma cells. Previous studies have shown that sex steroids, particularly estrogen and progesterone, are considered to play important roles in the regulation of cell proliferation in human osteosarcoma [14], [15]. Progesterone may have the potential to reduce the burden of osteoporosis [10] indicating the progesterone direct effect on bone cells proliferation. Progesterone therapy has been reported to prevent the postovariectomy bone loss in aged rats [11]. The results of the research have shown that progesterone promotes osteocalcin gene transcription by stimulating the expression of c-fos and c-jun, resulting in osteoblast proliferation and differentiation [8]. However, there are studies suggesting that progesterone receptors signaling in is not essential for bone growth [16].

In line with the findings of this researchthe data have shown that sex steroids induce apoptosis in bone tumors [17]-[19].The data suggest that the effects of progesterone on osteoblast apoptosis may contribute to the mechanisms by which progesterone exerts its action on bone formation [9].

Conclusion

Progesterone can induce apoptosis in osteosarcoma cells. Further research are required to clarify the exact effects of progesterone on osteosarcoma cells at cell and molecular level.

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