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Effect of all-*trans*-retinoic acid on mRNA binding protein p62 in human gastric cancer cells

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Abstract

p62 is a cancer-associated antigen binding to mRNA encoding insulin-like growth factor II that was isolated by immunoscreening a cDNA expression library with autoantibodies from patients with hepatocellular carcinoma (HCC). In the present study, multiple methods including flow cytometry, confocal laser-scanning microscope, electron microscope were used to characterize the effect of ATRA on BGC-823 cells, which presented two phenotypes of differentiation and apoptosis in cells treated with 1.0 and 50 μM ATRA, respectively. Interestingly, we found that p62 was cytoplasmic in location, but it significantly decreased in cytoplasm and appeared in nucleus of cells when the cells were treated with 50 μM all-*trans* retinoic acid (ATRA) for 5 days. Furthermore, proteomics approach on differential nucleus proteins showed that the up-regulation and/or down-regulation of cell cycle proteins and IGF binding proteins were involved in the apoptosis of BGC-823 cells induced by ATRA. These results suggest that there is a significant association between expression and distribution of p62 and the growth arrest of tumor cells, in which p62 is associated with cell apoptosis induced by ATRA.

Keywords: p62; All-*trans* retinoic acid; Apoptosis; Insulin-like growth factor II

1. Introduction

Recently, we have undertaken a serial of studies on p62, a tumor-associated autoantigen. p62 was originally identified by immunoscreening a cDNA expression library with autoantibodies from patients with hepatocellular carcinoma (HCC) (Zhang, Chan, Peng, & Tan, 1999). The autoantigen contains two types of RNA-binding motifs, one set of the RNA recognition (Query, Bentley, & Keene, 1989) and four hnRNP K homology (KH) domains (Adinolfi et al., 1999; Siomi, Choi, Siomi, Nussbaum, & Dreyfuss, 1994; Siomi, Matunis, Michael, & Dreyfuss, 1993) and belongs to the family of IMPs (insulin-like growth factor IImRNA-binding proteins) (Nielsen et al., 1999). Autoantibody to p62 was found in 21% of patients with

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HCC but not in the precursor conditions: chronic hepatitis and liver cirrhosis (Zhang et al., 1999). p62 is developmentally regulated, expressed in fetal, but not in adult liver (Lu et al., 2001). The aberrant expression of p62 in 30% of unselected HCC suggested that it could play a role in HCC and other tumors by up-regulating expression of growth factor IGF-II in the milieu of other oncogenic factors (Zhang & Chan, 2002). However, information regarding the association of p62 with cell phenotypes is not available.

Accumulating evidences indicate that all-*trans*-retinoic acid (ATRA) has profound effects on many biological processes including the modulation of cell growth, differentiation and apoptosis (Bollag & Holdener, 1992; Grubbs, Moon, Sporn, & Newton, 1977; Lippman, Kessler, & Meyskens, 1987a; Liu et al., 1996; Lotan, 1981). It can induce clinical complete remission in over 85% of acute promyelocytic leukemia (APL) patients by a differentiation process and an anti-proliferative effect of retinoic acid associated with induction of apoptosis in myeloid leukemia (Zheng et al., 2000), hepatoma (Wan, Cai, Cowan, & Magee, 2000), and lung cancer cells (Manna & Aggarwal, 2000). These processes are associated with a dose of the drug, showing that lower and higher doses induce differentiation and apoptosis, respectively. Therefore, ATRA is an ideal drug to understand the behaviors of p62 in cells.

The results reported here was the use of immunochemistry for detection of the expression and location of p62, and proteomic methodologies for analysis of nucleus proteins when p62 entered nuclei in ATRA-induced BGC-823 cells with different phenotypes of differentiation or apoptosis identified by of flow cytometry and electron microscope. Our results indicate that down-regulation and translocation of p62 are associated with apoptosis of BGC-823 cells induced by ATRA.

2. Materials and methods

2.1. Cell culture

Human gastric cancer cell line BGC-823 was from the Institute of Cell Biology, Shanghai, China. The cells were cultured at 37 °C under a humidified 5% CO_2 atmosphere in RPMI 1640 medium (Sigma Chemical Company, St Louis. MO, USA) supplemented with 10% fetal bovine serum (FBS) and 2% penicillin–streptomycin (100 μg/ml penicillin and 10 μg/ml streptomycin).

2.2. Flow cytometry

BGC-823 cells were plated at 0.5×10^5 cells in six-well plates in 2 ml of RPMI 1640 medium supplemented with 10% FBS. After overnight adherence, ATRA was separately added to cells at final concentration of 1.0 and 50 μM in subdued light. Cells were separately harvested at 1, 3, 5 days of incubations. The harvested cells were divided into two groups with and without supernatant. Cells in the group without supernatant were washed with PBS, resuspended in 500 μl of PBS, and fixed in 500 μl of ice-cold absolute ethanol at −20 °C. After an incubation for 30 min, cell pellets were collected by centrifugation, resuspended in 0.5 ml of PBS containing 100 μg/ml RNase, and incubated at 37 °C for 30 min. Then 0.5 ml of propidium iodide (PI) solution (50 μg/ml in PBS) was added, and the mixture was allowed to stain on ice for 30 min. The cells were analyzed with FACScan flow cytometer (Becton Dickinson, San Jone, CCA, USA). Cell cycle phase distribution was analyzed using ModFit LT software. Cells in the group with supernatant were used as apoptotic rate analysis. Harvested cells were directly incubated with PI solution (5 μg/ml in PBS) at 4 °C for 10 min. Then the cells were analyzed as above. There were two parameters in this protocol, including front scatter (FS) and PI concentration. This dual-parameter analysis was performed directly with trypsinized PI-stained cells. Populations of cells can be identified because of their different abilities in permeabilization to PI. Necrotic cells on the top of the histogram are completely permeable to PI and survival cells with intact membrane on the bottom of the histogram are impermeable to PI. Apoptotic cells locate on the amorphous region we defined because they are semi-permeable to PI.

2.3. Electron microscopy (EM) technique

The harvested BGC-823 cells were fixed with 2% glutaraldehyde in PBS for 2 h. After washing in PBS, the cells were treated with 1% OsO_4 in 0.1 M PBS overnight at 4 °C, washed three times with PBS, dehydrated with a graded series of acetone and embedded