Transcription Factors Xbp-1, Blimp-1, and BSAP Are Involved in the Regulation of Plasmacytic Differentiation Induced by 2-Methoxyestradiol in Myeloma Cell Lines

Hua Jiang,a WeiRan Gao,a Daniel Man-Yuen Sze,b Hong Xiong,a Jian Houa

aDepartment of Hematology, Changzheng Hospital, Shanghai, China; bCancer Immunology Group, Faculty of Pharmacy, The University of Sydney, Sydney, Australia

Received July 28, 2006; received in revised form March 15, 2007; accepted August 9, 2007

Abstract

Our previous studies demonstrated that a low concentration of 2-methoxyestradiol (2ME2) could induce the differentiation of myeloma cell lines and CD138+ primary myeloma cells from myeloma patients and up-regulate the expression of messenger RNA (mRNA) and protein for the gene encoding X-box binding protein 1 (Xbp-1) in myeloma cell lines. In the present study, we used phosphorothioated antisense oligodeoxynucleotides (ASODN) to investigate the roles and interactions of transcription factors Xbp-1, B-lymphocyte induced maturation protein 1 (Blimp-1), and PAX-5–encoded B-cell–specific activator protein (BSAP), which are thought to be involved in the regulation of B-lymphocytic or plasmacytic differentiation. Blimp-1 ASODN and Xbp-1 ASODN clearly inhibited myeloma cell differentiation and significantly partially inhibited the differentiation effects induced by 2ME2 at low concentration, whereas PAX-5 ASODN clearly induced myeloma cell differentiation and significantly enhanced 2ME2-induced differentiation effects. Moreover, after incubation with Blimp-1 ASODN, the level of Xbp-1 mRNA clearly declined, whereas the level of PAX-5 mRNA significantly increased in myeloma cells. These results demonstrate that transcription factors Xbp-1, Blimp-1, and PAX-5–encoded BSAP play important roles in the regulation of plasmacytic differentiation and exert their effects on differentiation induced by low 2ME2 concentrations. Our primary study provided the rationale for a promising strategy—the future application of transcription-factor ASODN for clinical patients.

© 2007 The Japanese Society of Hematology

Key words: Xbp-1; Blimp-1; PAX-5; 2-Methoxyestradiol; Cell differentiation; Multiple myeloma

1. Introduction

Plasma cells are post–germinal center end-stage B-cells, each with a uniquely rearranged B-cell receptor [1]. Multiple myeloma (MM) is a neoplastic disorder of these terminally differentiated plasma cells and remains incurable. Although chemotherapy and stem cell transplantation are still the most important therapies for this disease and even though these approaches have somewhat improved the prognoses for some MM patients, new and effective treatments with low toxicity are desirable, especially for elderly patients, who fail to endure transplantation and high-dose chemotherapy.

2-Methoxyestradiol (2ME2), once considered merely an inactive end metabolite of estradiol, has recently emerged as a very promising agent for cancer treatment [2]. Some studies have shown that 2ME2 can suppress the proliferation of myeloma cells and induce their apoptosis [3-5]. The mechanisms by which 2ME2 prevents proliferation and induces apoptosis have been partially studied, and such studies have included examinations of apoptosis induction in rapidly proliferating cells and the inhibition of blood vessel formation at several stages in the angiogenic cascade. Other 2ME2-related activities that have been reported include the inhibition of tubulin polymerization [6,7], sulfonation of 2ME2 [8], and inhibition of superoxide dismutase [9].

Our previous studies have shown that low concentrations of 2ME2 can induce cell differentiation in MM cell lines and in CD138+ primary cells from myeloma patients [10] and that high concentrations (1-16 µmol/L) can induce the
apoptosis of myeloma cells in a dose-dependent manner. These effects are similar to those of arsenic trioxide, which can induce tumor cell differentiation at low doses and apoptosis at high doses. Given what we know about the mechanisms of the antiproliferation effect of 2ME2, the molecular mechanisms of 2ME2-induced differentiation of MM cells remain unclear. In our previous study, we found that low-dose 2ME2 was able to up-regulate the expression of X-box binding protein 1 (Xbp-1) messenger RNA (mRNA) and protein along with the differentiation of myeloma cell lines. We used phosphorothioated antisense oligodeoxynucleotides (ASODN) to investigate the roles of the transcription factors Xbp-1, B-lymphocyte induced maturation protein 1 (Blimp-1), and B-cell-specific activator protein (BSAP), which are generally regarded to be involved in the regulation of B-lymphocytic or plasmacytic differentiation, and we investigated the interactions of these factors during 2ME2-induced cell differentiation of myeloma cell lines LP-1, CZ-1, and NCI-H929.

2. Materials and Methods

2.1. Cell Lines and Cell Culture

The human myeloma cell lines used in this study (CZ-1, LP-1, and NCI-H929) were stored in liquid nitrogen in our laboratory. Before experiments, cells were immediately recultured after thawing. Cells were cultured in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mmol/L L-glutamine and were grown at 37°C in humidified air containing 5% carbon dioxide. All experiments used cells that were in the logarithmic growth phase, and we renewed the medium every 3 days. The CZ-1 cell line, which secretes the λ light chain protein, was established from the bone marrow of a patient with advanced-stage MM classified as the mature class. The LP-1 cell line, which secretes the IgA light chain, was a generous gift of Dr. Michael Hallek (Clinic I for Internal Medicine, University Hospital Cologne, Cologne, Germany), and the NCI-H929 cell line, which secretes the IgGκ light chain, was a kind gift of Dr. Margaret H. L. Ng (Prince of Wales Hospital, Chinese University of Hong Kong).

2.2. Reagents

2ME2, dimethyl sulfoxide (DMSO), and RPMI 1640 were purchased from Sigma-Aldrich, CD49e, other primary mouse antihuman monoclonal antibodies, and phycoerythrin-conjugated rabbit antimonoclonal antibodies were purchased from Southern Biotechnology Associates (Birmingham, AL, USA). Rabbit antihuman Xbp-1 antibody was purchased from BioLegend (San Diego, CA, USA). The Human Kappa ELISA Quantitation Kit and Human Lambda ELISA Quantitation Kit were purchased from Bethyl Laboratories (Montgomery, TX, USA). A 1-mmol/L solution of 2ME2 (molecular weight, 302.4) was prepared in DMSO, stored at 4°C, and diluted with RPMI 1640 culture medium immediately before use. The final DMSO concentration in the culture system was less than 0.1% and had no significant effects on cell growth.

Phosphorothioated ASODN and phosphorothioated sense oligodeoxynucleotides (as controls) for Xbp-1, Blimp-1, and PAX-5 were synthesized in terms of the complementary DNA (cDNA) sequences of Xbp-1 (5′-TCTCCAGGCTGCCT-3′), Blimp-1 (5′-ATGGCGACCTGCGGAGA-3′), and PAX-5 (5′-CCGCGATGCTGCTCGTACGT-3′) and purified by polyacrylamide gel electrophoresis by Shanghai Sangon Biological Engineering Technology and Service Company (Shanghai, China). The ASODN were diluted with RPMI 1640 medium to the required concentrations immediately before use.

2.3. Morphologic Evaluation and Staining Procedures

Cells in the logarithmic growth phase were collected, centrifuged, suspended in RPMI 1640 medium, and cocultured for 72 hours with the following different reagents: group A, DMSO control (with the same final DMSO concentration as group B); group B, 0.5 µmol/L 2ME2; group C, 10 µmol/L ASODN; group D, 0.5 µmol/L 2ME2 + 10 µmol/L ASODN. Cells were sampled from the cultures, and their morphologies were evaluated. The cells were assessed by a Cytospin procedure, stained with Wright-Giemsa, and examined by light microscopy at 400× magnification. Photographs were taken with the aid of a Bone Marrow Evaluation System (Tongji Hunter Corporation, Shanghai, China).

2.4. Flow Cytometric Analysis

Although MM is a malignancy of terminally differentiated B-cells, these myeloma cells can be further classified into mature and immature groups. The neoplastic cells of MM patients constituted various proportions of cells at 3 stages of development: plasmablasts (CD38−CD138−CD45RA−CD19+CD27+CD86+CD45RO−CD20−CD138−CD38−CD49e−), intermediate plasma cells (CD38−CD138+CD45RA−CD19+CD27−CD86−CD45RO−CD20−CD138−CD38−CD49e−), and mature plasma cells (CD38+CD138+CD45RA−CD19+CD27−CD86−CD45RO+CD20−CD138+CD38+CD49e−) [12]. The expression of CD49e can be regarded as indicating the presence of a more mature class of myeloma cells.

An indirect technique of immunofluorescence staining was used to analyze differentiation antigens on the cell surface. Following the various treatments described above, we collected the myeloma cells and washed them once with phosphate-buffered saline (PBS), pH 7.4. Suspensions of individual cells (1 × 10⁶/mL; >95% survival according to staining with 0.2% trypan blue) were incubated for 30 minutes on ice with mouse antihuman CD49e monoclonal antibody (5 µg/mL), washed twice with PBS, and labeled for 30 minutes in the dark at 4°C with phycoerythrin-conjugated rabbit antimouse antibody (20 µg/mL; Open Biosystems, Huntsville, AL, USA). After washing twice with PBS, we analyzed labeled cells (a minimum of 10,000 cells/sample) by flow cytometry with CellQuest software (version 1.2; BD Biosciences, San Jose, CA, USA). In each test, an irrelevant isotype-matched monoclonal antibody was used as a negative control. The values for individual negative-control groups were subtracted from the values for the corresponding experimental groups. All tests were performed in triplicate and replicated 3 times.