Abstract

Mucin1 (MUC1) is a class of high molecular weight glycoproteins found in the cell membranes of human epithelial cells. Epithelial glycoprotein 40 (EGP40) is a homophilic cell-cell adhesion molecule and expressed on the surface of most simple epithelial cells and the majority of carcinomas. We analyzed the expression of MUC1 and EGP40 in human bone marrow (BM) and peripheral blood (PB) by reverse transcriptase polymerase chain reaction (RT-PCR) and immunocytochemistry (ICC). Eight BM and 95 PB samples from healthy donors, 39 BM and 17 PB samples from patients with hematological malignancies and 45 BM samples from patients with breast cancer were analyzed. MUC1 mRNA and EGP40 mRNA and protein were detected in BM samples and PB samples from healthy donors and from patients with multiple myeloma (MM), non-Hodgkin’s lymphoma (NHL) and chronic myelogenous leukemia (CML). The positive cells showed erythroblast-like and plasmacell-like morphology by immunocytochemistry. The MUC1 and EGP40 nested PCR were positive in 83.3% (10/12) and in 100% (33/33) respectively of BM from patients who had no evidence of distant metastases. It is concluded that MUC1 and EGP40 is expressed in hematopoietic tissues and hematological malignancies.

Introduction

Mucin1 (MUC1) present in the apical membrane of epithelial cells and is highly expressed in breast cancer [17]. Epithelial glycoprotein 40 (EGP40) is a transmembrane glycoprotein on the surface of epithelial cells [8] and epithelial carcinoma cells [4].

A number of studies have used MUC1 or EGP40 as an epithelial marker for the detection of breast cancer in bone marrow and peripheral blood by RT-PCR and immunocytochemistry [3, 7, 9, 13]. However, MUC1 and EGP40 expression in bone marrow, peripheral blood and lymph nodes (LNs) has been also reported [5, 14, 16]. Antibodies (EMA, BM2 and BM7) against MUC1 have been successfully used for diagnosis of carcinoma cells in bone marrow [3] and as indicators of tumor prognosis [7, 9]. Amplification of MUC1 mRNA by RT-PCR can be used for detection of occult micrometastases in lymph nodes of patients with breast cancer [13]. However, Delsol et al. found EMA-positive plasma cells in 46 of 53 axillary lymph node samples by immunohistochemistry [5]. Expression of MUC1 in hematopoietic tissues was detectable by RT-PCR and Western blots [1, 6].

Antibody HEA125 against EGP40 has been successfully used for detecting breast cancer cell in peripheral blood progenitor cells [2]. In contrast, expression of EGP40 in normal bone marrow was found by RT-PCR [16].
Based on these contradictory data, we analyzed the expression of MUC1 and EGP40 in bone marrow and peripheral blood by RT-PCR, and by immunocytochemistry using specific antibody BM7 against MUC1 and specific antibody HEA125 against EGP40. We found MUC1 mRNA, EGP40 mRNA and antibody positive cells were detected in control bone marrow and control peripheral blood.

**Materials and methods**

Cell preparation

Eight BM and 95 PB samples from healthy donors, 39 BM and 17 PB samples from patients with haematological malignancies, 45 BM samples from patients with breast cancer and breast carcinoma cell lines (T47D, MCF7, SKBr3) were examined by MUC1, EGP40 RT-PCR and/or by immunocytochemistry.

The mononuclear cells (MNCs) from bone marrow or peripheral blood were isolated by density centrifugation through Ficoll/Hypaque (Serva, Berlin) at 1,000 × g for 10 min. The cells of the interphase were washed twice in Dulbecco’s Modified Eagle’s medium containing 5% fetal calf serum.

RNA preparation

Total cellular RNA was extracted with phenol/chloroform/isoamyl alcohol and washed twice with 75% ethanol. RNA was eluted with 250 µl of diethylpyrocarbonate (DEPC)-treated water. The quantity of the isolated RNA was determined by absorbency at 260 nm.

Reverse transcriptase-polymerase chain reaction

The primers for amplification of β2-microglobulin was used to confirm the quality of the cDNA.

RT-PCR was performed using the kit from Stratagene (Stratagene, Heidelberg). Mixture of 5 µg total RNA and 300 ng oligo(dT)20 was incubated at 65°C for 5 min. cDNA was synthesized in a 50 µl reaction mixture containing 40 µl of RNA-oligo(dT)20- complete, 5 µl of 10× first strand buffer, 1 µl of RNase inhibitor, 2 µl of 100 mM dNTPs and 2 U Moloney Murine Leukaemia Virus reverse transcriptase at 37°C for 1 h, stopped at 90°C for 5 min and then placed on ice. From this cDNA solution, 1 µl was removed for subsequent use in PCR amplification by adding each sample to 19 µl of a PCR Master Mix solution (10 mM of Tris-HCl, pH 8.3, 1.5 mM of MgCl2, 0.1% gelatin, 100 mM each dNTPs, primer for β2-microglobulin PCR or MUC1 or EGP40, and 0.5 U of Taq DNA polymerase).

For the β2-microglobulin PCR, the samples were amplified for 20 cycles (30 s at 95°C, 30 s at 60°C and 30 s at 72°C). For MUC1 and EGP40 RT-PCR, the samples were amplified for 20 cycles (30 s at 94°C, 30 s at Tm and 30 s at 72°C). For the second round of MUC1 and EGP40 amplification, a 0.5 µl aliquot of first-round PCR product was added to 19.5 µl of a PCR Master Mix solution in the presence of the nested upstream and downstream primers for 30 cycles. In every procedure, water as sample for control of background, and samples from T47D cells for positive control were performed.

5 µl aliquots of the amplified products were analysed by electrophoresis on 1.5% agarose I gels (Appligene, Heidelberg, Germany).

**Results**

We analyzed expression of MUC1 and EGP40 in BM, PB and in breast cancer cell lines by RT-PCR and/or immunocytochemistry. The results were summarised in Tables 2 and 3.

MUC1 and EGP40 expression in normal BM and PB

MUC1 RT-PCR signals were found in 7 of 8 BM samples and in 28 of 40 PB samples from healthy donors. BM7-positive cells were detected in 4 of 8 BM samples and in 7 of 30 PB samples. EGP40-mRNA was found in all 8 of the analysed BM samples and in 16 of the 40 PB samples from healthy donors by nested PCR. HEA125-