Cell Cycle-dependent Expression of TAP1, TAP2, and HLA-B27 Messenger RNAs in a Human Breast Cancer Cell Line

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Abstract

Tumor cells are generally poorly responsive to immunotherapy. The results presented here suggest that antigen presentation of somatic tumor cells may be diminished greatly in quiescence and may be determined in part by growth regulation. Peptides produced by proteasomes are transported into the endoplasmic reticulum by transporter proteins TAP-1 and TAP-2, where they bind and stabilize MHC class I molecules required for antigenic presentation on the cell surface. TAP-1 and TAP-2 mRNAs were undetectable in serum-deprived human breast cancer cells (2IPT). They appeared 10 h after serum induction, near the G1-S boundary. In contrast, HLA-B27 mRNA was biphasically up-regulated. These mRNAs were significantly down-regulated in most tissues that contain mainly terminally differentiated, nonproliferating cells. All of the investigated breast cancer cell lines showed lower expression levels of these mRNAs than did the corresponding normal cells.

Introduction

To identify growth-related genes in human breast cancer cells, we recently compared mRNA expression by the method of differential display (1,2) in cells synchronized at quiescence (G0) and late G1 (3). From among several differentially expressed mRNAs, we identified and cloned a cDNA that revealed 99% identity to human TAP-1 mRNA. MHC class I molecules present short peptides on the cell surface for recognition by CD8+ CTLs (4). The short peptides are processed by degradation of self- and foreign proteins in the cytosol by a multisubunit protease called the proteasome (4-6). Short peptides produced by the proteasome are transported into the endoplasmic reticulum by transporter proteins TAP-1 and TAP-2, which bind and stabilize MHC class I molecules in the endoplasmic reticulum (4,7). Proteasome subunits LMP-2 and LMP-7 and peptide transporters TAP-1 and TAP-2 are the products of genes that are located in the class II region of chromosome 6 in humans. MHC class I heavy-chain genes, including HLA-B27, are located in the MHC class I region in the same chromosome (8). Inhibition of expression (9), inactivation of TAP proteins (10), mutation (11), and proteasome inhibitor drugs (5,11) were shown to result in insufficient presentation of antigenic peptides with MHC class I molecules required for antigen presentation on the cell surface. This failure of antigen presentation may result in escape of virus-infected and transformed cells (viral or spontaneous) from immune recognition by CTLs in vivo (12).

Proceeding from this finding, we now have analyzed TAP-1, TAP-2, and HLA-B27 mRNA expression during the cell cycle. We report here that the expression of these mRNAs is cell cycle dependent; they are down-regulated in quiescence created by serum deprivation and are up-regulated in late G1 following serum induction in the human breast cancer cell line 2IPT. Moreover, an average 3-fold suppression in the expression levels of these mRNAs compared with levels in normal breast epithelial cells was observed in a set of investigated primary and metastatic breast cancer cell lines.

Materials and Methods

Cell Culture and Synchronization. The normal human mammary epithelial cell line 81N and human breast cancer cell lines 2IPT, 21NT, and 21MT2 used in this study were established as described (13) and kindly provided by Ruth Sager (Harvard Medical School, Boston, MA). Other breast cancer cell lines are from the American Type Culture Collection (Rockville, MD). To create time course experiments, 2IPT cells were starved in 0.5% fetal bovine serum for 85 h. At time 0, cells were released into complete medium containing 10% fetal bovine serum, and samples were taken for total RNA extraction at indicated time points. Cell synchronization and cell cycle progression following serum induction were monitored by flow cytometry as described (14). For the time course experiments, 2IPT cells were grown in α-MEM as described (15). For growing normal mammary epithelial cells and breast cancer cells, Dana-Farber Cancer Institute-1 (DFCI-1) medium was used as described (16).

cDNA Probes. TAP-1, TAP-2, and HLA-B27 full-length cDNAs were kindly provided by Hidde Ploegh (Massachusetts Institute of Technology, Cambridge, MA).

Total RNA Extraction and Northern Blot Hybridization. Total cellular RNA extraction was performed with RNeasy-B RNA extraction solution (Biotecx Lab., Inc., Houston, TX) according to the manufacturer's instructions. Northern blot analyses were performed as described with minor variations (17). Briefly, 20 μg total RNA were resolved on a 1.1% agarose-1.7 M formaldehyde gel and transferred to nylon membranes (MSI, Westboro, MA). cDNAs used as probes were labeled with [32P]dCTP by a random prime labeling kit (Boehringer Mannheim Biochemicals, Indianapolis, IN) according to the manufacturer's instructions, and hybridizations were performed as described (17). For the analysis of mRNA expression in different tissues, a human multiple-tissue Northern blot membrane (Clontech, Palo Alto, CA) containing 2 μg polyadenylated RNA/lane was used. Northern blots were quantitated by optical densitometric analysis (Bio-Rad GS-700 imaging densitometer) and normalized with regard to β-actin blots.

Results

TAP-1, TAP-2, and HLA-B27 mRNA Expression during the Cell Cycle. Total cellular RNA extraction was performed with RNeasy-B RNA extraction solution (Biotecx Lab., Inc., Houston, TX) according to the manufacturer's instructions. Northern blot analyses were performed as described with minor variations (17). For the time course experiments, 2IPT cells were starved in α-MEM and grown in complete medium containing 10% fetal bovine serum. At time 0, cells were released into complete medium containing 10% fetal bovine serum, and samples were taken for total RNA extraction at indicated time points. Cell synchronization and cell cycle progression following serum induction were monitored by flow cytometry as described (14). For the time course experiments, 2IPT cells were grown in α-MEM as described (15). For growing normal mammary epithelial cells and breast cancer cells, Dana-Farber Cancer Institute-1 (DFCI-1) medium was used as described (16).

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CELL CYCLE-DEPENDENT EXPRESSION OF TAPI, TAP2, AND HLA-B27 mRNA

Figure 1. Cell cycle-dependent expression of TAPI, TAP2, and MHC class I (HLA-B27) heavy-chain mRNAs. Total RNA was extracted at the indicated time points following serum induction, and 20 µg RNA were loaded on each lane. Northern blot analysis was performed with TAPI (A), TAP2 (B), and HLA-B27 (C) cDNAs as described in “Materials and Methods.” D, ethidium bromide-stained rRNA from the agarose gel shown for equal loading.

Expression of TAP-1, TAP-2, and Class I mRNAs in Various Human Tissues and Breast Cancer Cell Lines. The mRNA levels of TAP-1, TAP-2, and class I were down-regulated after serum deprivation in culture (Fig. 1). We observed that the class I heavy-chain mRNA expression pattern was significantly different from TAP-1 and TAP-2 during the cell cycle and showed biphasic up-regulation following serum induction (Fig. 1C). The first up-regulation was observed in early G1, at 4 h after serum induction, and this mRNA maintained a steady-state level until 10 h. A second elevation was observed at 10 h, and it stayed elevated in the rest of the cell cycle. From these data, we concluded that TAP-1, TAP-2, and class I heavy-chain (HLA-B27) mRNA expression was cell cycle dependent in 21PT cells.

We also analyzed the expression of TAP-1, TAP-2, and B-27 in proliferating normal breast epithelial cells (81N) compared with various primary (MDA-157, 21PT, and 21NT) and metastatic (21MT2, BT549, MDA-231, MDA-435, ZR75-1, MCF-7, and T47D) breast cancer cell lines. The highest expression levels were detected in the normal breast epithelial cell line (Fig. 3A). As quantitated by densitometry of the original films (Fig. 3B), the detected primary and metastatic breast cancer cell lines showed various degrees of suppression (one-third on average) in the expression levels of these mRNAs compared with normal breast epithelial cells in culture. We could not find a significant difference between the expression in primary and metastatic cell lines, except that in metastatic breast cancer cell line ZR75-1, all three mRNAs were strongly down-regulated compared with other cell lines. In contrast, another metastatic breast cancer cell line, T47D, showed higher expression levels compared with other breast cancer cells.

Discussion

According to the hypothesis of immune surveillance, cancer cells arise frequently in normal individuals and are eliminated by the immune system (20). Virus-infected cells are also subject to immune surveillance. When some tumor or viral antigens are specifically expressed and presented on the cell surface by MHC molecules, these cells are recognized and eliminated by CTLs (21). Thus, MHC molecules are critical for immune surveillance. TAP genes are located in the MHC class II region and express transporter proteins that are and nonproliferating cells (Fig. 2). Particularly, TAP-1 and TAP-2 expression was undetectable in brain tissue, whereas class I was moderately detectable. This observation confirms previous studies reporting the lack of expression of MHC class I molecules on neural cell surfaces (18, 19) and supports our hypothesis of the inverse relationship between growth and expression of TAP and class I heavy-chain genes.

Expression of TAP-1, TAP-2, and Class I mRNAs in Various Human Tissues and Breast Cancer Cell Lines. The mRNA levels of TAP-1, TAP-2, and class I were down-regulated after serum deprivation in culture (Fig. 1). To confirm this observation for in vivo conditions, we analyzed TAP-1, TAP-2, and class I mRNA expression in various normal human tissues by Northern blot analysis. Supporting the results obtained from cell culture experiments, these mRNAs were relatively down-regulated in brain, skeletal muscle, and liver (to one-fourth), which contain mostly terminally differentiated
important for the presentation of antigenic peptides by MHC class I molecules on the cell surface. In this study, we provide evidence for the cell cycle-dependent expression of steady-state levels of TAP-1, TAP-2, and MHC class I heavy-chain (HLA-B27) mRNAs, which were elevated following growth induction by serum (Fig. 1). IFN-γ also stimulates TAP production (5). These levels could depend on either increased transcription or greater stabilization of these mRNAs (22, 23). Moreover, there was a general trend for these mRNAs to be down-regulated in quiescent tissues in vivo. And they were considerably lower in proliferating cultured breast cancer cells compared with normal mammary epithelial cells (Fig. 3B).

Induction of TAP genes and class I mRNA by serum leads us to speculate that host cells evolved a defense mechanism in which MHC genes are induced simultaneously by the signaling pathways that induce cell proliferation. CTLs recognize and kill proliferating cells presenting non-self-peptides, whereas cells presenting only self-peptides survive. Thus, because of these TAP inductions, the immune system may discriminate against proliferating cells activated by oncogenic transformation or viruses. Oncogenic transformation results in induced proliferation of tumor cells. In the life cycle of DNA viruses and retroviruses, nucleic acid synthesis is dependent on host cell enzymes. But most normal target cells for viral infection in vivo are nonproliferating (arrested in G0) and do not express sufficiently active enzymes for viral replication. Therefore, host cell proliferation must be stimulated to induce the cell machinery needed for viral replication. Induction of TAP and class I genes in parallel to growth induction increases the presentation of self- and non-self-peptides derived from proteins that are degraded in the cytosol.

Although physically, chemically, and virally induced tumors present tumor-specific antigens, spontaneously arising tumors often do not display detectable immunogenicity (21, 24). The current understanding is that immunogenicity depends on the etiological origin of the tumor (21). In cancer, the control of proliferation is defective (25). This feature is gained by the selection in several steps of cells released from host controls as the results of selective growth advantages (24, 26). During this multistep transformation, cancer cells also may have undergone a particularly severe selective pressure to escape CTL cytotoxicity through loss of MHC expression, which favors evasion from host immunity (27). Influences of oncogenes, oncogenic viruses, and altered MHC gene methylation might be responsible for down-regulation of MHC gene expression (19). Our observations confirm previous reports (22, 23, 26, 28, 29) and suggest that down-regulation of TAP-1, TAP-2, and class I mRNAs might be advantageous for cancer cells to survive and metastasize by escaping from immune recognition. In particular, Restifo et al. (22) showed that three small cell lung carcinoma cell lines failed to process endogenously synthesized proteins for presentation to T cells. They suggested that low antigen processing may permit tumors to escape immune surveillance.
Kaklamanis et al. (28) found that many breast tumors, especially metastatic, were defective in production of components of peptide processing and HLA class I activity.

Interestingly, some viruses also evolved counter-defense mechanisms against host immunity by inhibiting antigen presentation by class I molecules, despite their inducing host cell proliferation. Adenovirus 12 infection of cells resulted in suppression of antigen presentation by class I molecules through the transcriptional inhibition of TAP-2. Transfection of the TAP-2 gene into these infected cells restored the antigen presentation (9). Herpes simplex virus expresses an immediate early protein, ICP47, in infected cells, which associates with and inhibits the function of TAP-1 transporter protein and results in the virus evading host immunity by inhibition of antigen presentation (10).

Because TAP genes are important in antigen presentation of MHC class I molecules, their cell cycle-dependent expression patterns should affect the antigen presentation of class I molecules on the cell surface. Thus, these results suggest that antigen presentation of somatic cells is controlled in part by growth regulation.

References


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