

Characterization of Human Prostate and Breast Cancer Cell Lines for Experimental T Cell-Based Immunotherapy

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BACKGROUND. In order to develop experimental immunotherapy for prostate and breast cancer it is of utmost importance to have representative target cell lines that through human leukocyte antigen (HLA) class I molecules present relevant levels of peptides from tumor-associated antigens for cytotoxic T lymphocyte (CTL) recognition.

METHODS. We sequenced the HLA-A and HLA-B loci of eight commonly used prostate and breast cancer cell lines and analyzed the surface expression of HLA-ABC, HLA-DR, CD40, CD80, CD86, and CD54 by flow cytometry. We also analyzed the cell lines for mRNA expression from 25 genes reported to be specifically or preferentially expressed by prostate cells.

RESULTS. Among the analyzed cell lines we found that LNCaP, PC-346C and MCF-7 are HLA-A*0201 positive. However, the HLA-A2 expression level is low and only MCF-7 upregulates HLA-A2 in response to IFN- γ stimulation. MCF-7 also expresses high levels of CD54, which further improve its value as a CTL target cell line. On the other hand, LNCaP and PC-346C express 25 and 23 out of 25 prostate-related genes, respectively, while MCF-7 expresses 16 out of 25 genes.

CONCLUSIONS. None of the analyzed prostate cancer cell lines are optimal CTL target cells. However, MCF-7 could in many cases be used as a complement to HLA-A*0201 positive prostate cancer cells. The LNCaP and PC-346C cell lines are rich sources of prostate-related antigens that may be valuable for cancer vaccine development. *Prostate* 67: 389–395, 2007.

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KEY WORDS: cell line; tumor-associated antigen; HLA; immunotherapy

INTRODUCTION

There are, today, no curative therapies for locally advanced or recurrent metastatic prostate cancer and novel treatment options are urgently needed. Immunotherapy with the aim to activate tumor-associated antigen (TAA)-reactive T cells is particularly well suited for prostate cancer. An important reason is that a large number of proteins with specific or preferential expression in normal prostate and prostate cancer cells have been identified [1]. Since the prostate gland is an organ that one can live without, these proteins constitute suitable targets for T cell-based immunotherapy. Immune responses to prostate-related antigens can be considered tumor-specific in patients with recurrent metastases after radical prostatectomy. Furthermore, serum prostate specific antigen (PSA)

can be used as a surrogate marker to evaluate clinical responses. Breast cancer share many features with prostate cancer and strategies for prostate cancer immunotherapy can in many cases be applied also for breast cancer. For example, PSA and other kallikreins that are expressed by prostate cancer cells are also found in breast cancer cells [2,3].

Grant sponsor: The Swedish Cancer Society; Grant number: 4419-B05-06XBC; Grant sponsor: Swedish Research Council; Grant number: K2005-31X-15270-01A.

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Received 22 May 2006; Accepted 5 July 2006

DOI 10.1002/pros.20498

Published online 11 January 2007 in Wiley InterScience

(www.interscience.wiley.com).

The major cell type that mediates the rejection of solid tumors in preclinical animal models is the cytotoxic T lymphocyte (CTL). CTLs recognize 9–11 amino acid long peptide fragments bound to major histocompatibility complex (MHC) class I molecules, also known as human leukocyte antigen (HLA)-ABC, on the surface of the antigen presenting cell or target cell. Successful experimental cancer immunotherapy requires representative cell lines that can be used as target cells to verify the efficacy of activated CTLs. It is therefore of particular interest to know the HLA-ABC genotype and HLA-ABC expression level of the target cell lines. Cancer cell lines can also be used as antigen presenting cells for T cell activation, that is, as cancer vaccines. It is then of importance to know the expression levels of costimulatory molecules such as B7.1 (CD80), B7.2 (CD86), and CD40 as well as adhesion molecules such as ICAM-1 (CD54) since these molecules highly influence the quality of T cell activation. Furthermore, it is of outmost importance to know whether the laboratory cell lines express TAAs found in clinical cancer. Many of these characteristics have not yet been established and we therefore set out to sequence the HLA-A and HLA-B loci of six commonly used prostate cancer and immortalized normal prostate epithelial cell lines and two breast cancer cell lines. We also analyzed their surface expression of HLA-ABC (MHC class I), HLA-DR (MHC class II), CD40, CD80, CD86, and CD54 and investigated how the expression is influenced by exposure to interferon gamma (IFN- γ). In addition, we have analyzed the cell lines for mRNA expression of a large number of genes reported to be specifically or preferentially expressed by normal prostate and prostate cancer cells.

MATERIALS AND METHODS

Cell Lines

The human prostate adenocarcinoma cell lines LNCaP [4], PC-3 [5] and DU145 [6], and the immortalized normal human prostate epithelial cell lines BPH-1 [7] were obtained from ATCC (Rockville, MD). The prostate adenocarcinoma cell line PC-346C [8] was obtained from Dr. C Bangma, Erasmus University, Rotterdam, The Netherlands. The immortalized normal human prostate epithelial cell lines PNT2-C2 [9] and the human breast carcinoma cell line T47D [10] were obtained from Dr. N Maitland, York University, York, UK. The human breast carcinoma cell line MCF-7 [11] was obtained from Dr. J Berg, Karolinska Institute, Stockholm, Sweden. LNCaP, PC-346C, PC-3, DU145, BPH-1, PNT2-C2, and MCF-7 were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 10 mM HEPES and 1 mM sodium pyruvate while T47D was cultured in DMEM supple-

mented with 5% FBS and 2 mM L-glutamine. All cell culture reagents were from Invitrogen (Carlsbad, CA).

Sequencing of the HLA-A and HLA-B Loci

The HLA-A and HLA-B loci of the immortalized prostate, prostate cancer, and breast cancer cell lines were sequenced using previously described methods [12].

Flow Cytometry Analysis

Prostate and breast cancer cell lines were analyzed by flow cytometry (FACSCaliburTM, BD Biosciences, San Jose, CA) using the following antibodies: FITC-labeled anti-HLA-ABC (BD Biosciences), FITC-labeled anti-HLA-A2/A28 (One Lambda, Canoga Park, CA), PE-labeled anti-HLA-DR (BD Biosciences), FITC-labeled anti-CD40 (BD Biosciences), PE-labeled anti-CD80 (BD Biosciences), APC-labeled anti-CD86 (BD Biosciences), and APC-labeled anti-CD54 (BD Biosciences). A fraction of cells were pretreated with 1,000 IU/ml IFN- γ (ImukinTM, Boehringer-Ingelheim, Ingelheim, Germany) for 72 hr. Unspecific antibody bindings were analyzed by staining with isotype-match FITC-, APC-, and PE-labeled control antibodies (BD Biosciences).

Reverse-Transcription (RT)-PCR

To evaluate expression of 25 prostate-associated antigens, total RNA was isolated from all cell lines using TRIzol (Invitrogen) and cDNA was synthesized using Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's instructions. Five micrograms of total RNA were used for each cDNA synthesis and an oligo-dT primer and a random hexamer primer were used in parallel reactions. One-tenth of the cDNA obtained from the oligo-dT cDNA synthesis and one-tenth of the cDNA obtained from the random hexamer cDNA synthesis were pooled and used as template for 35 cycles of PCR using the ExpandTM polymerase enzyme mix (Roche, Mannheim, Germany). In order to amplify cDNA only and not trace amounts of genomic DNA the primer pairs for each transcript were designed to generate PCR products spanning at least two exons. The primer sequences, annealing temperatures, and expected PCR product sizes are shown in Table I. PCR products were visualized by ethidium bromide staining on 2% agarose gels.

RESULTS

HLA-A and HLA-B Loci Sequences of Prostate and Breast Cancer Cell Lines

Prostate and breast cancer cell lines are often used as target cells for experimental immunotherapy and in

TABLE I. Oligonucleotide Primers for RT-PCR Analyses of Prostate Tissue Antigen Expression

Target	Primers (5' to 3')	Annealing temperatures	Product size
PAP	CATTGACACCTTTCCCACTG (F) GTTCTTGAAAACGAGGGCAG (R)	51°C	353 bp
PSA	AGCCCCAAGCTTACCACC (F) GCTGACCTGAAATACCTG(R)	53°C	322 bp
hKLK4	GAACGGCAGAATGCCTACC (F) TTAACTGGCCTGGACGGTTTTT (R)	51°C	294 bp
PSP94	CACCATGAATGTTCTCCTGGGCAGCG (F) TATCCATTCACTGACAGAACAGG (R)	51°C	347 bp
STAMP1	GCAGTCACTTCTATCCCTTC (F) TCACATTACTGTGACCCTCTCCGG (R)	51°C	351 bp
Cten	AGAGAGAGAACTGGGAGGTGC (F) CTACATCCTTTCTGCGTCCTGC (R)	51°C	482 bp
hKLK2	CGAACCCAGAGGAGTTCTTGC (F) TCAGGGGTTGGCTGCCATGGTG (R)	51°C	307 bp
TARP	GGGAACACCATGAAGACTAACGACAC (F) TTTCTCTCCATTGCAGCAGAAAGCCG (R)	60°C	339 bp
PDEF	AAAGAGCGGACTTCACCTG (F) TCAGATGGGGTGCACGAACTGG (R)	51°C	373 bp
PSGR	CACCATGAGTTCCTGCAACTTCACAC (F) TCACTTGCCTCCACAGCCTGC (R)	51°C	962 bp
PART1	CACCATGCAATGTCAGCTATTAGGAC (F) CTAAGTGATTGGCTGGCTCTGG (R)	51°C	179 bp
AlbZIP	CAGACCAGCACTTGTGTTTTG (F) TCACATCTCATCTGCATGCAGC (R)	51°C	312 bp
Trp-p8	CATTGTGTGTTTTGCCAAG (F) TGAGGACATCATGGGTGAGA (R)	51°C	580 bp
POTE	CAAGTGGTGCTGTCTGCT (F) ATGTACGCCAAGCAAAAAGTGGT (R)	51°C	545 bp
PMEP A1	CACCATGGCGGAGCTGGAGTTTGTTC (F) CTAGAGAGGGTGTCTTTCTGT (R)	51°C	758 bp
PCTA1	CGTAGTGTCTTTGGACACG (F) CTACCAGCTCCTTACTTCCAGT (R)	51°C	420 bp
Prostein	CCTTCACGCTGTTTTACACG (F) CTACGCTGAGTATTTGGCCAAG (R)	51°C	799 bp
PSMA	CCAGGTTTCGAGGAGGGATGGTG (F) GCTACTTCACTCAAAGTATCTG (R)	51°C	450 bp
PSDR1	CAACATCCTTTCACCCAGG (F) TTAGTCTATTGGGAGGCCCAGC (R)	51°C	333 bp
TGM4	CATCATTGCGGAAATTGTGG (F) CTACTTGGTTGATGAGAACAATC (R)	50°C	364 bp
PSCA	TGCTTGCCCTGTTGATGGCAG (F) CCAGAGCAGCAGGCCGAGTGCA (R)	53°C	320 bp
STEAP	CACCATGGAAAGCAGAAAAGACATCA (F) CTACAACCTGGGAACATATCTCA (R)	53°C	1,019 bp
NGEP	ACAGCACCGTCCTGATCGATGTGAGC (F) TGTCTAGCTTCAGGTCCTCCTCCCAA (R)	60°C	176 bp
PrLZ	GTAGAGAGATGGACTTATATGAGG (F) TCACAGGCTCTCCTGTGTCTTTT (R)	55°C	668 bp
NKX 3.1	CAGAACGACCAGCTGAGCAC (F) CGTTCAGGGGCCGACAGGTAC (R)	53°C	227 bp
β-actin	CTGGCACCCAGCACAATGAAGATCAA (F) TCAAAGTCCTCGGCCACATTGTGAAC (R)	51°C	392 bp

Direction of primers: F, forward; R, reverse.

TABLE II. HLA-A and HLA-B Typing of Prostate and Breast Cancer Cell Lines

Cell line	HLA-A*	HLA-B*
LNCaP	0101, 0201	0801, 3701
PC-346C	0101, 0201	0801, 4001
PC-3	0101, 2402	1801
DU145	0301, 3303	5001, 5701
BPH-1	0301, 1101	1501, 1801
PNT2-C2	0101, 3201	0702, 5701
MCF-7	0201	1801, 4402
T47D	3301	1402

many cases the full HLA genotypes of the cell lines are not known. We sequenced the HLA-A and HLA-B loci in four prostate cancer cell lines (LNCaP, PC-346C, PC-3, and DU145), two immortalized normal prostate epithelial cell lines (BPH-1 and PNT2-C2) and two breast cancer cell lines (MCF-7 and T47D). The results are presented in Table II. HLA-A*0201 is the most commonly targeted human MHC class I molecule in T cell-based immunotherapy. Of the cell lines sequenced herein, LNCaP, PC-346C, and MCF-7 have an HLA-A*0201 genotype.

Cell Surface Expression of Immune Cell Interacting Molecules

The expression level of MHC class I molecules (HLA-ABC) on the surface of target cells is crucial for CTL-mediated cell lysis. Therefore, we analyzed the expression level of HLA-ABC and HLA-A2 by flow cytometry on the panel of cell lines. The results are presented in Figure 1. We observed that the HLA-ABC expression varied substantially among cell lines with LNCaP and PC-346C displaying the lowest and PC-3 the highest HLA-ABC expression, Figure 1A. We also found that the HLA-ABC expression on LNCaP and PC-346C was not effected by IFN- γ stimulation while PC-3, DU145, BPH-1, PNT2-C2, MCF-7, and T47D substantially upregulated HLA-ABC cell surface expression upon IFN- γ stimulation. The HLA-A2 expression correlated well with the overall HLA-ABC expression on the HLA-A*0201 positive cell lines, Figure 1B.

The expression levels of costimulatory molecules and adhesion molecules are important for T cell activation and to some extent for CTL-mediated target cell lysis. We therefore analyzed the cell surface expression of CD40, CD80, CD86, and CD54 as well as HLA-DR by flow cytometry on the panel of cell lines. The results are presented in Table III. The expression of the costimulatory molecules CD40, CD80 and CD86

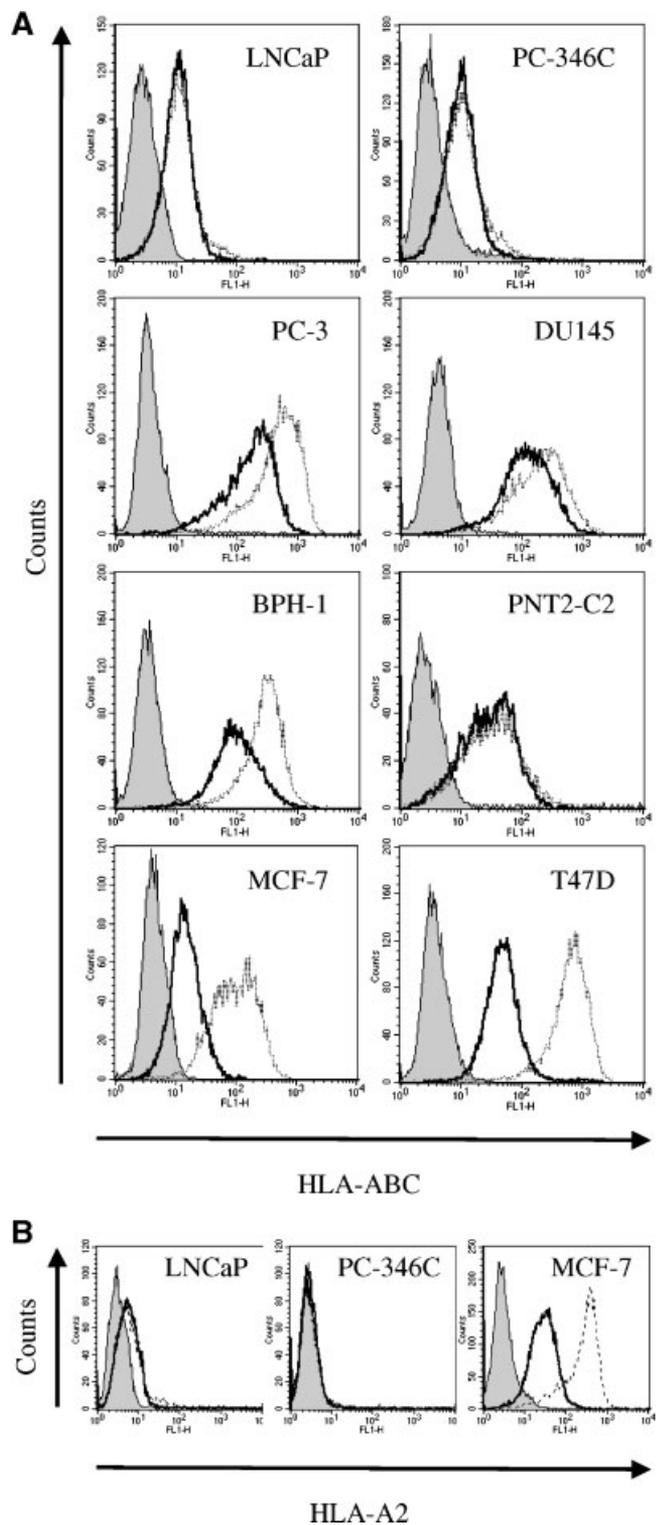


Fig. 1. HLA-ABC and HLA-A2 cell surface expression on prostate and breast cell lines. The cell lines were cultured in the presence (thin line) or absence (thick line) of 1,000 IU/ml IFN- γ for 72 hr. They were stained with an anti-HLA-ABC antibody (A) or an anti-HLA-A2 antibody (B) and analyzed by flow cytometry. Filled curves represent isotype-matched background controls.

TABLE IV. mRNA Expression of Prostate Tissue Antigens in Prostate and Breast Cancer Cell Lines

	LNCaP	PC-346C	PC-3	DU145	BPH-1	PNT2-C2	MCF-7	T47D
PAP	+	+	-	-	-	-	-	-
PSA	+	+	-	-	-	-	-	-
hKLK4	+	+	+	+	+	-	+	-
PSP94	+	+	-	-	-	-	+	-
STAMP1	+	+	+	+	+	+	+	+
Cten	+	+	+	+	+	+	+	+
hKLK2	+	+	-	-	-	-	-	-
TARP	+	+	-	-	-	-	+	-
PDEF	+	-	+	-	-	-	+	+
PSGR	+	+	-	-	-	-	+	-
PART1	+	+	-	+	+	+	+	+
AlbZIP	+	+	+	+	+	+	+	+
Trp-p8	+	+	-	-	-	-	-	-
POTE	+	+	-	-	-	+	+	-
PMEPA1	+	+	-	-	+	+	-	+
PCTA1	+	+	+	+	+	-	-	+
Prostein	+	+	-	-	-	+	-	-
PSMA	+	+	-	-	-	+	-	-
PSDR1	+	+	+	+	+	+	+	+
TGM4	+	+	+	-	+	+	-	-
PSCA	+	+	+	+	+	+	+	-
STEAP	+	+	+	+	+	+	+	-
NGEP	+	-	-	-	-	-	+	-
PrLZ	+	+	+	-	+	+	+	-
NKX 3.1	+	+	+	+	+	+	+	-
β -actin	+	+	+	+	+	+	+	+

peptide epitopes may be too low for efficient CTL lysis. On the other hand, our data indicate that MCF-7 could, in many cases, be used as a complement to HLA-A*0201 positive prostate cancer target cells for in vitro T cell analysis. The drawback with MCF-7 is that it does not express all relevant prostate TAAs.

It has recently been shown that interaction between lymphocyte function-associated antigen-1 (LFA-1) on the CTL and CD54 (ICAM-1) on the target cell leads to a larger contact and a unique positioning of granules near the interface. This interaction directs the release of cytolytic granules to the surface of the antigen-expressing target cell and it mediates effective destruction of these cells by CTLs [16]. The fact that LNCaP and PC-346C express low levels of CD54 may render them poor target cells for CTL lysis. On the other hand DU145, BPH-1, PNT2-C2, MCF-7, and T47D express high levels of CD54 that would improve their value as target cells.

Despite the identification of TAAs and their immunogenic peptide epitopes for use as cancer vaccines, there is a steady interest in developing allogeneic (MHC-disparate) cancer vaccines by using tumor cell lines [17,18]. For example, irradiated LNCaP and OnyCap23, a subclone of PNT2-C2, have been used as allogeneic prostate cancer vaccines [19]. However, the

low expression levels of HLA-DR and costimulatory molecules (CD40, CD80, CD86) indicate that they are not optimal as antigen presenting cells for T cell activation. Therefore, to be efficient such therapeutic cancer vaccines would need to be targeted to professional antigen presenting cells such as dendritic cells. In addition, total RNA from LNCaP has recently been used as a source of prostate-related antigens in a phase I clinical trial [20]. Due to the wide expression of prostate-related antigens most prostate cancer cell lines analyzed in this article may be good sources of antigens for vaccination studies if combined with dendritic cells.

ACKNOWLEDGMENTS

This study was supported by the Swedish Cancer Society (Grant No. 4419-B05-06XBC) and the Swedish Research Council (Grant No. K2005-31X-15270-01A). ME is a recipient of the Göran Gustafsson's Award.

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