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Analysis of expression of MHC class I molecules and TAP genes in malignant human cell lines

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Abstract: TAP proteins (transporters associated with antigen processing) take part in the transport of oligopeptides created in proteasomes from cytoplasm into endoplasmic reticulum. In the endoplasmic reticulum those oligopeptides are bound to MHC class I molecules and transported to the cell surface. TAP proteins consist of two subunits: TAP1 and TAP2. It has been previously shown that TAP protein expression can be decreased in malignant cells, followed by reduced protein expression or complete lack of MHC class I antigens on the cell surface. The aim of the study was to characterize of MHC class I protein expression and TAP mRNA synthesis in twenty human malignant tumor cell lines. MHC class I protein expression was examined by immunohistochemistry and flow cytometry. Expression of TAP genes was studied using RT-PCR and real-time PCR. All tested cell lines expressed MHC class I molecules. Flow cytometry showed different expression of MHC class I protein in tested cell lines. Molecular analysis revealed the presence of TAP1 and TAP2 gene transcripts in all cell lines examined. Quantitative real time PCR analysis showed differences of gene expression among cell lines tested.

Key words: TAP1 - TAP2 - MHC class I - Immunohistochemistry - Flow cytometry - RT-PCR - Real time PCR

Introduction

During the transformation process tumor cells often change their phenotype, what leads to tumor escape from the immune system. Downregulation of the MHC class I antigens is one of the possible strategies of this escape. The mechanisms responsible for that phenomenon include, *i.e.* β2-microglobulin gene mutations, changes in binding of transcription factors and MHC class I mRNA gene expression, allele or haplotype loss caused *i.e.* by a mutation encoding for heavy chain of MHC molecule [1]. However, many other irregularities in a production and functioning of proteins responsible for intracellular peptide transport may appear. These changes can influence functions of proteasome proteins (*i.e.* LMP2, LMP7), as well as transport proteins (TAP 1 and/or TAP2), or chaperone proteins (*i.e.* tapasin) [2]. Before proteasome processed protein will be loaded on MHC molecules it has to be relocated from the cytoplasm into the lumen of endoplasmic reticulum (ER). TAPs belong to an ABC protein family [3]. TAP proteins complex is responsible for the transport of MHC molecules into ER.

Cells with a defect in TAP complex function are not able to present properly an antigen. As a consequence of TAP proteins absence or dysfunction, MHC molecules can be held inside the ER lumen, or empty MHC class I may be presented on a cell surface. Such empty MHC class I molecules have a decreased lifetime. Generally, they are stopped in the ER as only the whole MHC complex consisting of a MHC heavy chain, 2-microglobulin and a bound peptide can be efficiently presented on the cell sufrace [4]. The lack or decreased protein and/or gene expression of MHC class I and the TAP was previously observed in many cancer cells, *i.e.* in the small-cell lung carcinoma [5], colorectal tumor [6], primary cervical carcinoma [7], malignant melanoma [8], renal cell carcinoma [9], squamous cell carcinomas of the head and neck (SCCHN) [10], primary breast carcinomas [11], primary ovarian carcinoma [12], as well as in many

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Table 1. Established tumor cell lines used.

Symbols	Histology	Origin	Site	Source
HEp-2 (CCL-23)	Squamous cell ca.	Larynx	Primary	ATCC (Rockwill, MD, USA)
HepG2 (HB-8065)	Hepatocellular ca.	Liver	Primary	ATCC (Rockwill, MD, USA)
Hep3B (HB-8064)	Hepatocellular ca.	Liver	Primary	ATCC (Rockwill, MD, USA)
MCF7 (HTB-22)	Adenocarcinoma	Breast	Metastatic	ATCC (Rockwill, MD, USA)
MDA-MB 231 (HTB-26)	Adenocarcinoma	Breast	Metastatic	ATCC (Rockwill, MD, USA)
TT (CRL-1803)	Medullary carcinoma	Thyroid	Primary	ATCC (Rockwill, MD, USA)
A549 (CCL-185)	Carcinoma	Lung	Primary	ATCC (Rockwill, MD, USA)
HT29 (HTB-38)	Adenocarcinoma	Colon	Primary	ATCC (Rockwill, MD, USA)
HCT116 (CCL-247)	Carcinoma	Colon	Primary	ATCC (Rockwill, MD, USA)
SW620 (CCL-227)	Adenocarcinoma	Colon	Metastatic	ATCC (Rockwill, MD, USA)
T24 (HTB-4)	Transitional cell ca.	Urinary bladder	Primary	ATCC (Rockwill, MD, USA)
A375 (CRL-1619)	Malignant melanoma	Skin	Metastatic	ATCC (Rockwill, MD, USA)
WM9	Malignant melanoma	Skin	Metastatic	The Wistar Institute, (Philadelphia, USA)
WM35	Malignant melanoma	Skin	Primary	The Wistar Institute, (Philadelphia, USA)
WM239	Malignant melanoma	Skin	Metastatic	The Wistar Institute, (Philadelphia, USA)
WM902b	Malignant melanoma	Skin	Primary	The Wistar Institute, (Philadelphia, USA)
p54	Glandular carcinoma	Kidney	Primary	Transplantationszentrum, Universität (Düsseldorf, Ger.)
p58	Glandular carcinoma	Kidney	Primary	Transplantationszentrum, Universität (Düsseldorf, Ger.)
p66	Glandular carcinoma	Kidney	Primary	Transplantationszentrum, Universität (Düsseldorf, Ger.)
p138	Glandular carcinoma	Kidney	Metastatic	Transplantationszentrum, Universität (Düsseldorf, Ger.)

established cancer cell lines *i.e.* in the cell lines of the neuroblastoma [13], sarcoma [14], pancreas, biliary tract and colon cancer [15], prostate cancer [16], small-cell lung carcinoma [17].

Based on these studies we decided to assess the expression of MHC class I molecules and a molecular analysis of TAP genes expression in selected human cancer cell lines. We tested the cell lines from cancers in which a downregulation of MHC class I, TAP1 and TAP2 was obserwed previously by other authors.

Materials and methods

Cell lines and culture conditions. Studied material consisted of twenty human malignant tumor cell lines (Table 1). All cell lines were obtained from prof. Andrzej Mackiewicz from the Department of Cancer Immunology, University School of Medical Sciences at Great Poland Cancer Center (Poznań, Poland). Cell lines was cultured in standard humidity and temperature conditions in RPMI-1640 medium conditioned with 10% fetal calf serum (FCS), with 2 mM L-glutamine and 1% penicillin and streptomycin (100 μg/ml).

Immunohistochemistry. Presence of surface antigens on the cells was estimated by Alkaline Phosphatase-Anti-Alkaline Phosphatase (APAAP) reaction [18]. Aceton fixed pellets were incubated at 4°C per night with an unconjugated primary monoclonal mouse antibody (anti-human HLA-ABC, clone W6/32, DAKO A/S). After removal of unbound antibodies cell pellets were incubated at room temperature by 30 minutes with the rabbit antibody anti-mouse immunoglobulins (DAKO). After second wash the specimens were incubated for 30 minutes with APAAP complex (DAKO). Two last steps were repeated for complete binding of free epitopes in order to increase sensitivity of the reaction. Preformed complexes were visualized by the incubation with substrate, consisted of alkaline fuchsin (New Fuchsin), naphthol (Naphthol ASBI phosphate) and levamisol (all from Sigma). After staining by Meyer's hematoxylin and mounting in glycerogel the specimens were assessed under light microscope.

Semi-quantitative assessment of immunohistochemical stained specimens was performed in the case of positive reaction. The ratio was established between the number of positive cells and whole cell number in one microscopic field. The intensity of reaction was referred in scores to the four degree scale of reactivity (Appendix for Table 2).

Quantitative image analysis. Microscopic images were recorded by digital camera (Color View) integrated with light microscope (Olympus BX41). Using analySIS^B software the image analysis system was calibrated for the chosen objective (10×), brightness, contrast and color saturation of the microscopic image. Setting parameters were applied for all recorded images from the same specimen. Digital images were recorded at the 2560×1920 resolution. In the next step images were converted to the 640×480 resolution and saved in TIF format. The images prepared in this manner were subjected to the computer morphometric analysis with the spatial visualization. Morphometric survey of the HLA class I molecules was performed by "A4D" software [19]. Using this software the surface area of positive reaction was plotted (S) by pixel counting. Obtained results were referred as percentage contribution of the color reaction in the micro-

scopic visual field. Values were obtained from the ratio between surface area of reaction (S) and image resolution (R):

$$\text{Mean Contribution of the Color Reaction} = \frac{S}{R} \times 100\% \text{ ; R=640×480}$$

Flow cytometry. Expression of MHC class I molecules was assessed by direct immunofluorescence. Antibodies anti-HLA-ABC (clone W6/32, DAKO A/S) used in reaction were conjugated with phycoerythrin (PE). Intracellular staining was performed by the addition of 0.1% the saponine to cell suspension. Data acquisition and analyses were performed by FACScan with the CellQuest (Becton Dickinson) software. Percentage of positive cells estimated quantity of cells presenting analyzed protein. RFI value indicated quantity of analyzed proteins. Protein values were considered positive when at least 5% cells showed its expression. The relative fluorescence intensity (RFI) were obtained by evaluation of mean fluorescence intensity (MFI) of analyzed samples and appropriate isotypic controls.

$$RFI = \left(\frac{\Delta MFI}{MFI} \right) \times 100 \text{ ; } \quad \Delta MFI = MFI - MFI_C$$

MFI - mean fluorescence intensity of an examined sample
MFIC - mean fluorescence intensity of an isotype control

RNA extraction. Total cellular RNA was isolated using RNeasy® Mini Kit (50) (Qiagen) from the cell pellets, according to the manufacturer's instruction. Nucleic acid concentration was measured by spectrophotometr (Perkin-Elmer) at λ=260 nm. For quality estimation isolated RNA was subjected to the electrophoresis on the 1.5% agarose gel with ethidium bromide (0.5 μg/ml).

Reverse transcription reaction (RT). Samples of total RNA were used for RT reaction. Templates (0.5 μg/μl) were incubated at 65°C and quickly cooled on ice. To the single RNA sample reaction solution was added that contained dNTPs, $MgCl_2$, ribonuclease inhibitor (RNaseOut , Invitrogen), oligo dT and MuLV reverse transcriptase (Invitrogen). The mixture was incubated at 42°C, and then denaturated at 99°C. 3-glycerophosphate aldehyde dehydrogenase gene (GAPDH) was used as a marker gene for constitutive expression. Amplification with primers: F: 5'-GGTCGGAGTCAACGGATTTG-3' and R: 5'-ATGAGCCCCAGCCTTCTCCAT-3' [20] was performed for 30 cycles with AllegroTag DNA Polymerase (Novazym). PCR products (386 bp) were subjected to electrophoresis on 2% agarose gel.

Polymerase chain reaction (PCR). TAP1 and TAP2 mRNA expression was assessed by PCR with AllegroTag DNA Polymerase (Novazym). Primers sequences used for PCR reactions were designed using Primer3, Omiga and BLAST software. Primers sequences for TAP1: F: 5'-TGGTCTGTTGACTCCCTTACAC-3' and R: 5'-AAATACCTGTGGCTCTTGTCC-3' [NCBI GenBank NM_000593], and TAP2: F: 5'-TACAACACCCGCCATCAG-3' and R: 5'-AGGTCTCTCCGCCAATACAG-3' [NCBI GenBank NM_018833]. Annealing temperature optimization was achieved by temperature gradient PCR (56°C TAP1 and 61°C TAP2). Reaction products (301 bp TAP1 and 185bp TAP2) were tested by electrophoresis on 2% agarose gel.

Real-time PCR. Quantitative real-time PCR analysis was performed by LightCycler (Roche Diagnostics). Reaction mixture consisted of QuantiTect SYBR Green PCR Master Mix (Qiagen). After "hot-start" denaturation at 95°C samples were run by 45 cycles reaction with annealing at appropriate temperatures for each gene. To obtain melting curve which defined specificity of the amplification, samples were submitted for 15 second to denaturation at 95°C, cooled at 65°C and re-warmed at 95°C. Finally they were cooled at 40°C to complete product synthesis. Data analysis was performed by Light Cycler Software v3.5 (Roche).

Table 2. Expression of HLA-ABC on the studied cell lines. Results of semi-quantitative and quantitative immunohistochemical studies.

Cell lines	Semi quantitative assessment	Mean Contribution of the Color Reaction (%)
HepG2 (HB-8065)	+++	9,0
MCF7 (HTB-22)	no data	13,1
TT (CRL-1803)	++	0,7
A549 (CCL-185)	+++	14,6
HT29 (HTB-38)	+++	14,4
HCT116 (CCL-247)	++	no data
SW620 (CCL-227)	++	7,6
T24 (HTB-4)	+++	no data
WM9	no data	8,5
WM239	no data	8,3
p54	+++	5,6
p58	+++	no data
p66	+++	2,7
p138	+++	no data

Appendix. Semi quantitative four degree scale of reactivity.

+++	75-100% positive cells
++	25-75% positive cells
+	< 25% positive cells
−	absence of positive cells

Standard curve. Standard curves were drawn for defining the number of analyzed genes copies to total RNA ratio. Results of each analyzed sample were referred to reference values from the standard curve. To obtain standard curve 50 μl PCR products of the each studied genes were submitted to electrophoresis on 2% agarose gel. After electrophoresis and quality control on transiluminator, appropriate products were cut from gel. Products were eluted from the gel by GenElute™ Agarose Spin Column (Sigma) using the manufacturer's protocol. Concentration of the purified products was measured by spectrophotometer. Obtained values were calculated according to the formula:

$$pmol\ dsDNA = \frac{\mu g\ (dsDNA) \times 1{,}515}{N\ bp}$$

In the next step samples were water diluted in appropriate dilution series: 10^4, 10^3, 10^2, 10, 1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} (aM) and added to real-time PCR reactions for indication of the reference values. Standard curves were prepared separately for each of the studied gene and used for calculation of the initial amounts of the cDNA in the analyzed samples. To confirm specificity of the reaction one sample without the cDNA (negative control) were also amplified in each of the analyzed sample set. Lack of the signal in the negative control confirmed specificity of the reaction.

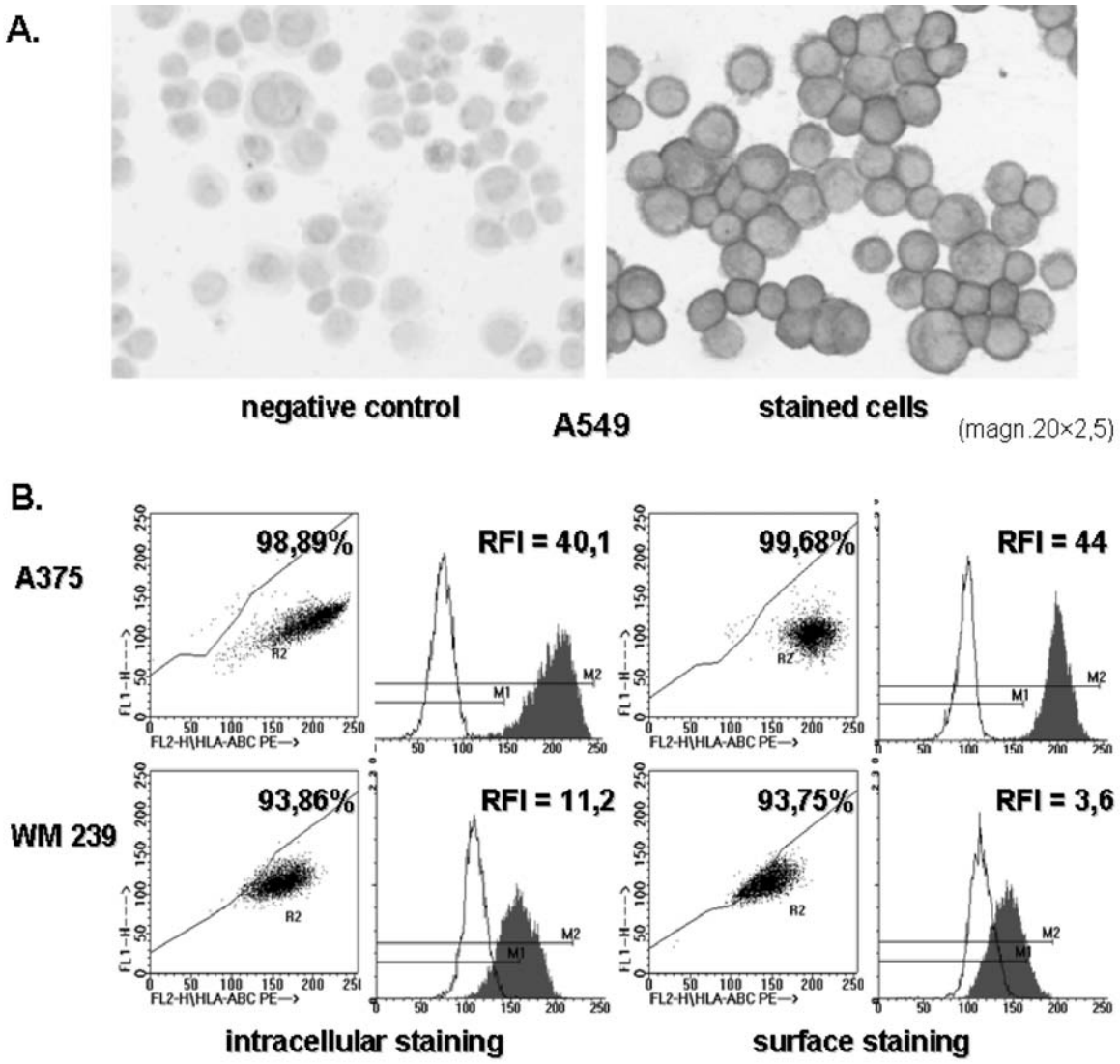

Fig. 1. A. The expression of HLA-ABC antigen on the human lung cancer (A549 cell line). Immunohistochemistry, APAAP reaction. Negative control and positive reaction. **B**. Surface and cytoplasmic expression of HLA-ABC antigen analyzed by flow cytometry. Percentage of positive cells and relative fluorescent intensity (RFI) obtained in the A375 and WM239 melanoma cell lines.

Statistical analysis. Nonparametric Spearman rank correlation tests and nonparametric U Mann-Whitney tests were carried out using Excel (Microsoft) and Statistica (StatSoft) software. All cytometric data were measured in duplicate and are presented in the table as mean.

Results

Assessment of expression of MHC class I molecules

Immunohistochemical analysis showed expression of the HLA class I molecule on the cells all tested cell lines (Fig. 1A). Results of the semi-quantitative assessment of specimens and quantitative image analysis are shown in detail in the Table 2. There was no correlation between semi-quantitative assessment and digital image analysis except for A549 and HT29 cell lines. The highest values of the latter (<14%) in A549 and HT29 cell lines corresponded to +++ scores of the former. In other cases such parallel results were usually not found.

Flow cytometry studies have shown that most of the cell lines studied were able to stabily express of MHC class I molecule both on the cell membrane as well as in the cytoplasm (Fig. 1B). Detailed data has been shown in the Table 3. In the most of studied cases the surface expression of the MHC molecule was higher than its intracellular expression. Almost all cells of renal cancer cell line p54 have shown cell membrane HLA molecule expression, but in cells of other renal cancer p66 this expression was minimal. The percentage of positive cells expressed in cytoplasm was highest in the cells of breast cancer MDA-

Table 3. Expression of HLA-ABC on the studied cell lines. Results of flow cytometry analyzes. Data shown the percentage of positive cells and the Relative Fluorescence Intensity (RFI) measured on the surface and in the cytoplasm of tested cells.

Cell lines	Percentage of positive cells (%)		Relative Fluorescence Intensity (RFI)	
	cytoplasmic staining	surface staining	cytoplasmic staining	surface staining
HEp-2 (CCL-23)	no data	no data	no data	no data
HepG2 (HB-8065)	76,72	87,91	91,2	200,8
Hep3B (HB-8064)	no data	no data	no data	no data
MCF7 (HTB-22)	98,72	98,31	145,9	64,2
MDA-MB 231 (HTB-26)	99,63	99,52	100,3	73,1
TT (CRL-1803)	99,63	98,62	80,2	118,3
A549 (CCL-185)	94,74	98,09	45,8	23,9
HT29 (HTB-38)	68,35	99,26	3,1	49,3
HCT116 (CCL-247)	98,73	99,54	25,4	19,3
SW620 (CCL-227)	88,06	96,48	30,5	17,5
T24 (HTB-4)	91,11	97,17	103,4	146,7
A375 (CRL-1619)	98,89	99,68	40,1	44,0
WM9	93,42	93,01	26,0	30,1
WM35	82,47	99,14	56,8	33,8
WM239	93,86	93,75	11,2	3,6
WM902b	89,67	99,56	34,0	82,1
p54	97,43	99,73	69,4	119,0
p58	95,58	99,60	70,0	132,3
p66	81,92	74,89	353,9	378,3
p138	85,34	97,56	11,5	194,6

MB 231 and in those of thyroid cancer TT, but lowest in HT29 cells. In addition, relative fluorescence intensity (RFI) value evaluating the expression level of the examined protein, its intracellular and surface quantity, had the highest HLA class I molecule expression on the cells of p66 line, both in the cytoplasm and on the cell surface. The lowest value of the RFI was detected in the cytoplasm of colon cancer HT29 cells and on the cell surface of WM239 melanoma cells (Table 3).

In the statistical analysis the Spearman rank test showed correlations between percentage of positive cells expressed HLA-ABC intracellular and on the cell surface, the correlation coefficient was 0.5121 at the $p<0.05$ (Fig. 2A) and between RFI of HLA-ABC measured intracellular and on the cell surface, the correlation coefficient was 0.5851 at the $p<0.05$ (Fig. 2B) for all tested cell lines. The Percentage of positive cells and RFI of HLA-ABC was not dependent on the type of malignancy (epithelial origin or malignant melanoma) and on the source of its malignant origin (primary origin or metastasis).

Molecular analysis of TAP1 and TAP2 genes expression

In molecular terms the RT-PCR revealed the presence of mRNA transcripts for TAP1 (Fig. 3A) and TAP2 (Fig. 3B) genes in all human malignant tumor cell lines examined.

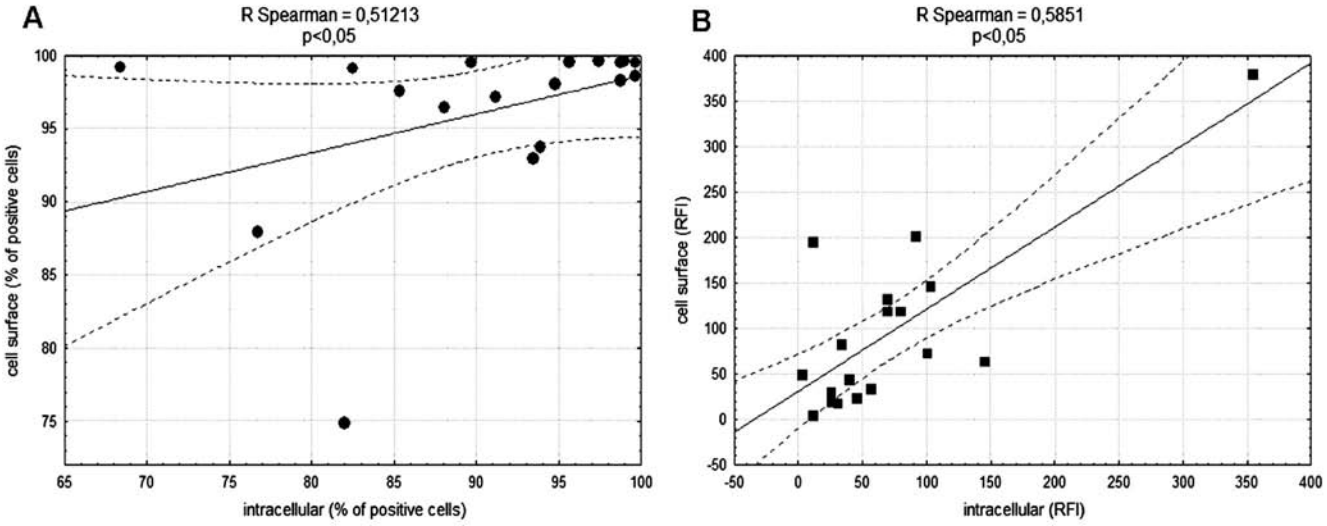

Fig. 2. Correlation between percentage of positive cells expressed HLA-ABC intracellular and on the cell surface - Spearman rank test (**A**). Correlation between RFI of HLA-ABC intracellular and on the cell surface - Spearman rank test (**B**).

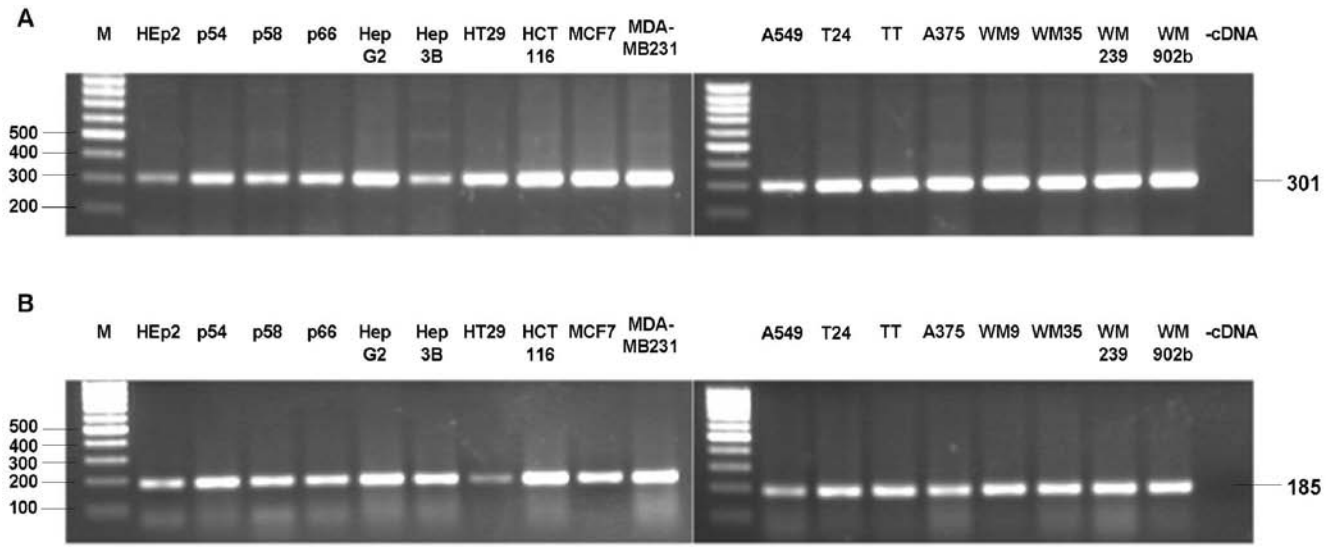

Fig. 3. Expression of the genes coding TAP1 (**A**) and TAP2 (**B**) proteins. Electrophoretic analysis of RT PCR. cDNA from analyzed cell lines, line M: mass marker, GeneRuler^{TM} 100 bp DNA Ladder (Fermentas), line - cDNA: no cDNA, negative control PCR. Products size of the PCR products: TAP1 301 bp., TAP2 185 bp.

Quantitative evaluation of the expression of both TAP genes by real-time PCR was performed in five selected cell lines: p66, HepG2, A375, WM9 and WM239. Based on the standard curve the amounts of transripts were estimated. The obtained values were used to calculate the number of gene copies within the total RNA isolated from the cells. Results indicate lower level of both TAP1 and TAP2 genes expression in comparison with GAPDH used as a housekeeping gene. Within the total RNA isolated from the tested cell lines the number of GAPDH gene copies were one log higher than those encoding for the both subunits of TAP heterodimer (10^5:10^4). Moreover, TAP2 gene expression was lower than that of TAP1 (Fig. 4A).

Table 4. Quantitative real-time PCR for TAP1 and TAP2 gene expression level in tested cancer cell lines. Data are shown as ratio between the number of gene copies of TAP1 and TAP2 in g of total RNA to copies of GAPDH in g of total RNA for duplicate assays.

Cell lines	TAP1/ GAPDH	TAP2/ GAPDH
HepG2 (HB-8065)	$51,66\times10^4$	$19,73\times10^4$
A375 (CRL-1619)	$9,93\times10^4$	$1,81\times10^4$
WM9	$83,75\times10^4$	$27,43\times10^4$
WM239	$19,88\times10^4$	$10,00\times10^4$
p66	$63,14\times10^4$	$12,53\times10^4$

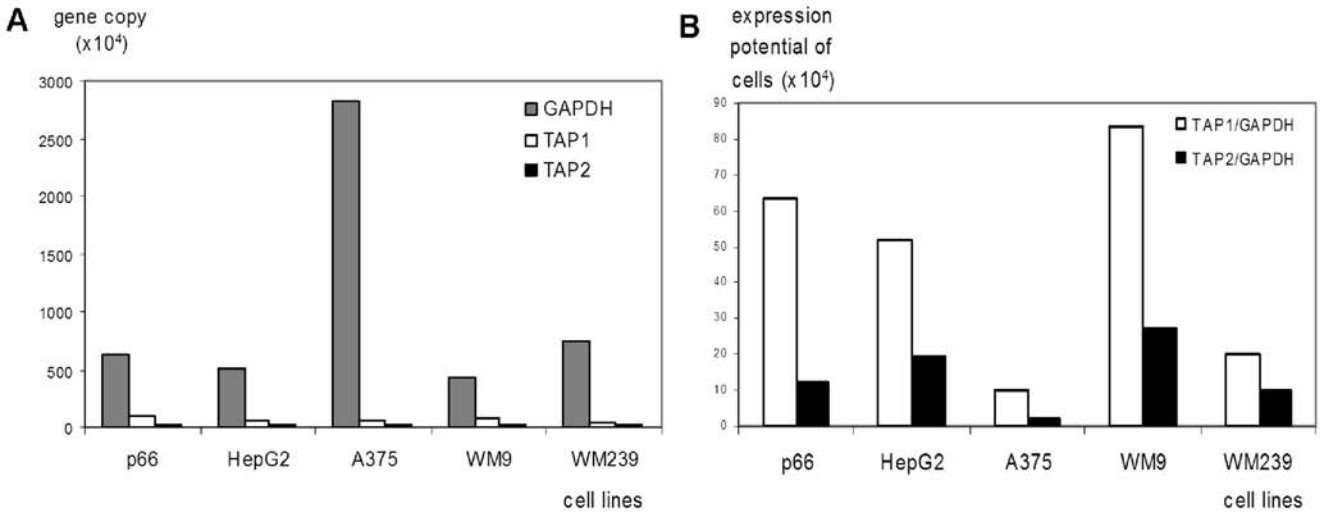

Fig. 4. Copy number of the studied genes to the total RNA (μg) isolated from cultured cells ratio (**A**). The TAP1 and TAP2 genes expression level - a ratio between the number of gene copies of TAP1 or TAP2 in μg of total RNA and copies of GAPDH in μg of total RNA (**B**).

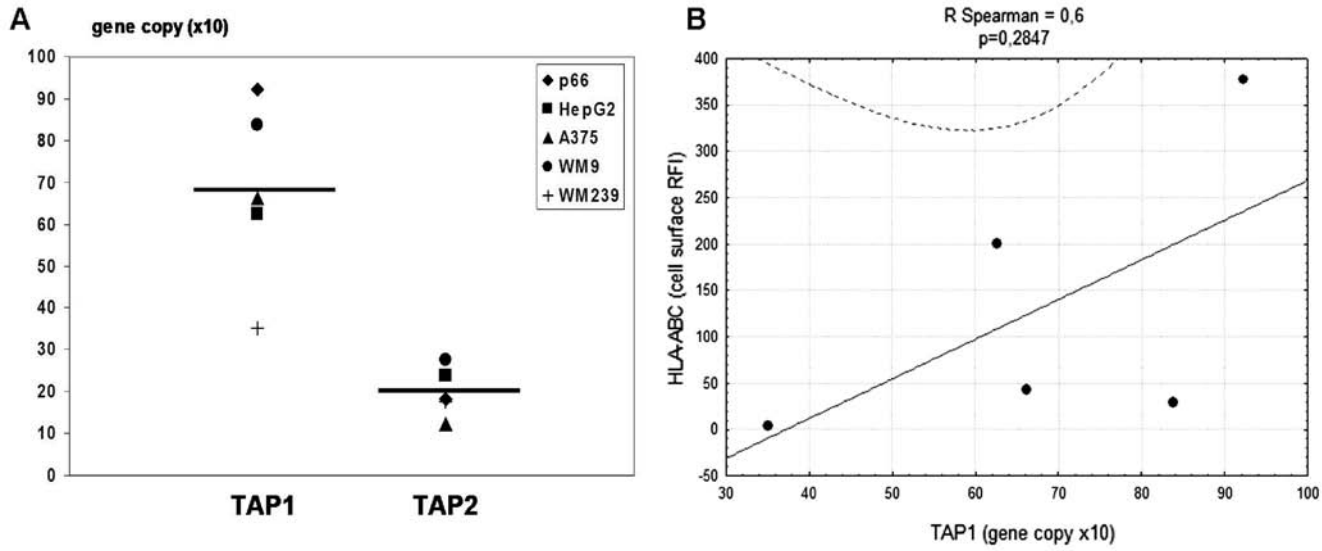

Fig. 5. The significant statistical difference between the TAP1 and TAP2 genes expression (lines indicated mean values) - nonparametric U Mann-Whitney's test (**A**). Correlation between the number of gene TAP1 copies and RFI of MHC class I molecules on the cell surface of the studied cells - Spearman rank test (**B**).

Based on the data obtained, the expression potential of studied cells could be estimated (gene expression level). This value represents a ratio between the number of gene copies of TAP1 or TAP2 in μg of total RNA and copies of GAPDH in μg of total RNA. Calculated expression potential showed differences between analysed genes in cell lines studied (Table 4). The highest ratio was found for TAP1 gene in cells of malignant melanoma WM9. The lowest number was observed in the cells of other melanoma cell line A375. Similar to TAP1 gene expression the highest expression of TAP2 genes was observed in cells of melanoma WM9, and the lowest in cells of A375 (Fig. 4B).

A nonparametric U Mann-Whitney's test showed statistically significant differences ($p<0.05$) in the gene expression between the control gene GAPDH and the TAP1 and TAP2 genes in the cell lines tested. The significant difference at the p value <0.05 was also found between the TAP1 and TAP2 genes expression in the cell lines examined (Fig. 5A). Spearman correlation test has shown trend suggesting the number of gene TAP1 copies proportional to RFI of MHC class I molecules of the studied cells (Fig. 5B).

Discussion

The key component of sufficient antigen binding and presentation by major histocompatibility complex are the interactions between the TAP protein complex and MHC class I molecules [21]. Several previous studies have shown that one of the main strategies of cancer's escape from immune system control is partial or total loss of its ability to synthesize proteins taking part in antigens processing, transport and presentation [13,22-28]. In this study we demonstrated the expression of HLA class I molecule in all cell lines tested. We also revealed in these cell lines the presence of mRNA coding TAP1 and TAP2 proteins on molecular level. Our results are in line with the studies of the other authors who indicated previously the presence of MHC class I molecules and/or TAP proteins in the HepG2 [29], HCT116 [30], MCF-7, MDA-MB231 and A549 [31], HT29 [31,32] cell lines.

Immunohistochemical analysis performed in our study localized expression of the HLA class I molecule both on the surface and intracellularly in all tested cell lines. Semi-quantitative methods based on score range are subjective and dependent on researchers experience. Digital imaging analysis was used therefore for the assessment of specimens in objective and reproducible manner [33]. The possibility of visualization of the proteins presented in the cell membrane with their simultaneous quantitative analysis increase the information about topography and amount of analyzed molecule. Standardization should refer to preparation and staining of tissue, size of specimen, number of photographed fields, microscopy resolution and lens enlargement [34]. Lack of correlation between results of cytometric and morphometric analyzes in the performed studies may suggest insufficient stability of some above-mentioned conditions.

Tumor's capability to present its antigens in concert with MHC class I molecules on the cell surface is substantial to create specific adaptive response of cytotoxic T cells (CTL) [35]. Flow cytometry revealed presence of MHC molecules both on the surface and in the cytoplasm of tested cells. Relative fluorescence intensity of MHC class I molecules was higher in the cell membrane than that in the cytoplasm. This finding may be explained by fast migration of antigen presenting complex to the cell surface which remain for long time in cell membrane.

Our molecular studies have shown the ability of all cell lines tested synthesize mRNA molecules for both TAP1 and TAP2 subunits. Clinical symptoms of TAP protein deficiency include chronic inflammation of the respiratory tract and granulomatous skin lesions [36]. Lack of transcripts encoding TAP subunits were previously reported in some of the cancer cell lines [9,37]. However, the presence of TAP transcripts does not always substantiate the synthesis of functional TAP protein heterodimer.

In our study, expression of TAP1 and TAP2 mRNA was detected in the hepatocellular carcinoma cell line Hep3B, which shows permanent hepatitis B virus presence. Proper functioning of TAP heterodimer can be diminished by various agents like viral proteins which may inhibit TAP heterodimer activity [38-41]. We could deduce that TAPs mRNA synthesis in Hep3B cells is not inhibited by virus. This observation correspond with results of Chen *et al.* [42] and Wang *et al.* [43] in which they demonstrated that HBV can induce the expression of HLA class I molecule and TAP1 in other hapatocellular carcer cell line HepG2.

In further steps, we performed quantitative assessment of expression levels of both TAP genes using real- time PCR for collecting information on the number of mRNA copies in comparison to total RNA isolated from tumor cells. Based on this, we could precisely define expression level of genes of interest in selected tumor cell lines tested. High expression of both TAP subunits in WM9 cell line corresponds to the presence of TAP transcripts in primary melanoma correlated with tumors regression [8]. Low levels of TAP genes expression in A375 and WM239 melanomas are in line with decrease or lack of expression of one or both TAP genes in malignant melanomas [28]. Decrease in the expression of TAP1 and TAP2 genes in melanoma WM239 cell line may correspond with previously established functional deficiencies of cells with melanoma origin [22]. In renal cancer cell line p66 number of TAP1 gene copies was fairly high in comparison to the number of gene copies in A375 and WM239 melanoma cell lines. TAP2 gene expression level was not significantly different in the above mentioned cell lines. Moreover, we showed the presence of TAP transcripts in kidney tumor cell lines though previous findings of the lack of expression of this genes in renal cancers [44]. Presence of TAP1 and TAP2 mRNA in HepG2 corresponded to the literature data [29]. Nonstimulated cancer cell lines had much lower expression of TAP genes level in comparison with the GAPDH gene. We found some correlation in the expression potential between TAP2 and GAPDH genes in some of the cell lines tested. No such correlation could be found for TAP1 gene expression. In generally, our results indicated that TAP1 gene expression level was higher than TAP2, unlike than Imanishi *et al.* [15] who showed that ratio of copy numbers of mRNA for TAP1 and TAP2 genes to GAPDH housekeeping gene was higher for TAP2 than TAP1 in tested pancreas, biliary tract and colon cancer cell lines. Our data rather confirm observations of Dissemond *et al.* [8] who showed higher TAP1 protein expression in comparison to TAP2 in the cells from primary melanoma lesions. This may suggest a dominant role of TAP1

gene expression in formed TAP heterodimer and may indicate that TAP1 protein stabilizes TAP2 protein, and both subunits are regulated in parallel. Because of TAP1 is an constitutively expressed gene and its expression may be regulated by signal transducer and activator of transcription 1 alpha (STAT1) and interferon regulatory factor 1 (IRF1) [45] observed discrepancies in its expression between different types of cancers may occur because of different cancer microenvironment.

Advances in the recent understanding of tumor immunology have revealed several different mechanisms by which tumors have escaped immune surveillance. Such mechanisms include the weak expression of MHC class I molecules of cancer cells [46]. Several immunotherapeutic strategies for antigen transporting protein deficiency comprise transfer of genes encoding for distinct subunits in order to reconstitute TAP proteins [47,48]. Other approach is transfer of the genes coding the INF and TNF into the cells expresing low expression level of TAPs for their upregulation [44]. Another suppose is downregulation of the TAPs expression by IL-10 for increase of the cancer cells sensitivity to NK cells action [49]. The established cell lines are the model of the experimental environment so that the results of our study may be helpful to further exploration of the antigen processing and presentation machinery function *in vitro* conditions. Precise examination of mechanisms involved in processing, transport and antigen presentation on MHC class I molecules by tumor cells leads to further understanding of tumors biology and may in future show the way to more effective tumor therapies.

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