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# Correlation between Expression of Major Histocompatibility Complex Class I and That of Antigen Presenting Machineries in Carcinoma Cell Lines of the Pancreas, Biliary Tract and Colon

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**Key words:** gastrointestinal cancer, MHC class I, antigen processing, β2-microglobulin, LMP2

**To elicit a tumor immune response, tumor antigens represented by major histocompatibility (MHC) class I complex on the cell surface is indispensable. Some investigators demonstrated that many cancer cells reduce expression of β2-microglobulin, a transporter of antigen presenting (TAP) or low molecular protein (LMP), due to the deletion mutant or point mutation. We investigated gene expression levels of antigen presenting machineries in 13 cell lines of the pancreas, biliary tract and colon cancer by using real-time quantitative PCR. We also evaluated the correlation between expression of MHC class I and that of antigen-processing molecules in these gastrointestinal cancer cell lines. Flow cytometric analysis showed that expression of MHC class I for the pancreatic cancer cell lines was generally lower than that for the biliary tract or colon cancer cell lines. It was further found that the colon cancer cell lines HCT-15/DLD-1 showed no MHC class I expression and lack of protein expression for β2-microglobulin. Transfection of the wild-type β2-microglobulin gene restored MHC class I antigen expression on the cell surface for DLD-1. Quantitative real-time PCR demonstrated lower expression for TAP1, TAP2, LMP2 and LMP7 gene in five cancer cell lines. Partial correlation analysis demonstrated that LMP2 was the only antigen presenting machinery which was significantly associated with MHC class I expression. Our results suggest that β2-microglobulin and LMP2 are important for the expression of MHC class I in 13 gastrointestinal cancer cell lines, while the combined but complex expression of antigen presenting mechanisms was related to MHC class I expression level on the surface of cancer cells.**

CD8+ cytotoxic T lymphocytes play an important role in the recognition and elimination of tumor cells. To elicit a tumor immune response, the presentation of tumor antigens by the major histocompatibility (MHC) class I complex on the surface of these cells is indispensable. Various intracellular molecules, which influence the antigen processing presentation and surface expression of MHC class I molecules, can serve as tumor antigens on the MHC class I complex. Impairment of the expression of MHC class I antigens, on the other hand, is attributable to structural alteration and/or dysregulation of various components

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of the MHC class I antigen processing mechanisms. These mechanisms include four major components: (a) a large family of integral membrane transporters, including the transporter of antigen presenting (TAP) 1 and TAP2; (b) multicatalytic proteasomes, in particular low molecular proteins (LMP) 2, LMP7 and LMP10; (c) numerous chaperons; (d) MHC class I molecules (1-7). Beta2-microglobulin, also known as water soluble light chain (12kDa), is associated with the MHC class I heavy chain (8). In the absence of β2-microglobulin, the MHC class I heavy chain in the endoplasmic reticulum (ER) degraded rapidly (9, 10). Total loss of MHC class I expression in solid tumor has been attributed to lack of β2-microglobulin or synthesis of truncated β2-microglobulin (11-15). In addition, it was found that lack of TAP or LMP resulted in reduction of MHC class I antigens in various cancers (16, 17). Loss of MHC class I antigen expression on the cell surface is assumed to allow tumors to avoid being killed by cytotoxic T lymphocytes (13, 18, 19). In the malignant melanoma, which has been studied thoroughly in terms of cancer antigens and T cell-based immunotherapy, cell transformation or tumor progression are frequently associated with loss of MHC class I expression (13, 20, 21). However, it is still not clear which antigen processing mechanism is most important for expression of MHC class I in gastrointestinal cancers. In the study presented here, we investigated the gene expression levels of antigen processing machineries, such as β2-microglobulin, TAP and LMP, in 13 gastrointestinal cancer cell lines. In addition, we analyzed the correlation between gene expression of antigen processing machineries and of the MHC class I complex in gastrointestinal cancer cell lines.

## MATERIALS AND METHODS

**Cell lines and cell culture.** Nine human pancreatic and colon cancer cell lines were obtained from the RIKEN cell bank (Tsukuba Science City, Ibaraki, Japan) or the American Tissue Type Culture Collection (Rockville, MD). Three biliary tract carcinoma cell lines, SK-ChA-1, MZ-ChA-1 and TGBC-2TKB were kind gifts from Dr. T. Todoroki (Tsukuba University School of Medicine, Ibaraki, Japan). NOZ, a gallbladder cancer cell line, was a kind gift from Dr. S. Nagamori (Jikei University School of Medicine, Tokyo). Four cell lines of the biliary tract carcinoma were cultured in DMEM medium with 10% FCS and penicillin/ streptomycin. Human pancreatic cancer cell lines; MIAPaCa-2 and Panc-1, were kept in DMEM with 10% FCS and penicillin/ streptomycin, two pancreatic cancer cell lines; AsPC-1 and BxPC-3, were cultured in RPMI1640 medium with 10% FCS and antibiotics, and human colorectal cancer cell lines; NCI-H747, HCT-15/DLD-1, Colo-320 and Caco-2, were kept in RPMI1640 medium with 10% FCS and penicillin/ streptomycin. All the cells were kept in a 5% carbon dioxide humidified atmosphere at 37˚C. All the culture medium were used as recommended complete growth medium according to the cell line description of ATCC, RIKEN cell bank or the cell depositors.

**RNA isolation and real-time quantitative RT-PCR.** Total RNA was isolated from cell pellets by using an RNA isolation reagent, RNeasy Mini Kit (QIAGEN, Austin, TX), according to the manufacturer's instructions. Next, 2μg of total RNA was reverse transcribed into cDNA by using random hexamers generated by Superscript II (Invitrogen, Carlsbad, CA). In brief, 20μl reaction volume was used for synthesizing single strand cDNA, into which the following reagents were mixed: 2μg total RNA, 1μl of 10mM dNTP mix, 1μl of 100ng/μl random primers (Invitrogen), and distilled water for a total of 13μl. The mixture was then heated to 65°C for 5 min and quickly chilled on ice. Next, 4μl of 5 x first strand buffer and 2μl of 0.1M DTT were then gently mixed into the tube followed by incubation at 42°C for 2 min. 1μl of Superscript II was then added and mixed in by gentle pipetting, after which the mixture was incubated at 25°C for 10 min prior to incubation at 42°C for 50 min.

The reaction was inactivated by heating at 70°C for 15min. The real-time quantitative RT-PCR analysis was performed with an automated sequence detector system (PRISM 7700 Sequence Detector; Perkin Elmer Applied Biosystems, Foster City, CA) combined with a QuantiTect™ SYBR® Green PCR (QIAGEN) according to the manufacturer's instructions. The forward and reverse primers for real-time PCR are listed in Table 1. The specificity of PCR products was checked by means of agarose gel electrophoresis. 20μl of each synthesized single-strand cDNA was dilated into 80μl ddw, and 2.5μl of dilated cDNA was used for the template for quantitative RT-PCR with PRISM 7700. Concentrations of primers were optimized to 400nM for each forward and reverse primer. Thermal cycling was initiated with a 2-min incubation step after 5 min and 15 min at 95°C to eliminate PCR products resulting from carry-over contamination and activate DNA polymerase. This procedure used 40 PCR cycles at 94°C for 30s, at 62°C for 30s and at 72°C for 30s. Reaction volume was 25μl per tube. The experiments were performed in duplicate for each data point and each PCR run. Standard curves were generated using serial 1: 10 dilations of $10^7$ copies of the pcDNA3.1(-) vector (Invitrogen). The threshold was set at the mid-point of the log-liner phase amplification plot and defined as Ct, which represents the number of cycles at this point. For each experimental sample, the absolute copy numbers of mRNA were determined from the standard curve. The normalized amount of each sample was then determined by dividing the copy numbers of each mRNA by those of GAPDH mRNA.

Table 1. Primer sets for quantitative real-time PCR of β2-microglobulin, TAP1, TAP2, LMP2, LMP7 and GAPDH

| Gene | Primer sequence | |
|---|---|---|
| | Forward primer (5'→3') | Reverse primer (5'→3') |
| β2-microglobulin | CTCGCGCTACTCTCTCTTTCTGG | GCTTACATGTCTCGATCCCACTTAA |
| TAP1 | CTCATGTCCATTCTCACCATAGCCAG | CTCTGAGGAGCCCACAGCCTTCTG |
| TAP2 | GGAGATCCAGGATGCAGTGGCC | GAGAACAGCACAGGCTCCTGCCC |
| LMP2 | CCTTGCAGGGATGCTGCG | GGGAAGGTTCACTCATCA |
| LMP7 | GTGATGCTCATAGGAACC | GCCACCACCACCATTA |
| GAPDH | GGAGCCAAAAGGGTCATCATCTC | AGTGGGTGTCGCTGTTGAAGTC |

**Flow cytometric analysis.** Flow cytometry was used to examine the MHC class I level of 13 gastrointestinal cancer cells with or without interferon gamma (IFN-γ). For expression of MHC class I cancer cells treated with IFN-γ, the cells were cultured with 1000 units/ml recombinant human IFN-γ (Peprotech, Rocky Hill, NJ) for 40-48 h. Flow cytometric analysis for FITC and PE fluorescence was performed using a 4-color FACS Calibur (Becton Dickinson, Franklin Lakes, NJ). Electronic compensation was used for the fluorescence channels to remove residual spectral overlap, and fluorescence data were represented logarithmically. For each sample 50,000 events were collected and MHC class I antigen cell surface expression was analyzed by using cells harvested from subconfluent cultures grown in 60mm plates. Cultured tumor cell lines were harvested with Trypsin/ EDTA, washed with

phosphate-buffered saline, and then washed again with a wash buffer (phosphate-buffered saline with 3% BSA and 0.01% sodium azide). After washing, the cells were stained with the PE conjugated anti-human HLA class I ABC monoclonal antibody (Becton Dickinson) for 15 min on ice, after which the cells were washed again with wash buffer. A FACScan flow cytometer was used for two-color analysis. Dead cells and debris were eliminated from analysis after their detection by forward and sideways light scattering and staining with 1μg/ml propidium iodide. The mean channel fluorescence intensity (MFI) was recorded.

**Western blot analysis.** Western blot analysis was used for determining β2-microglobulin expression. Freshly harvested tumor cells were lysed in lysis-buffer (phosphate-buffer saline with 1% NonidetP-40, 0.5% sodium deoxycholate, 0.1% SDS, 10μl/ml PMSF, 30μl/ml Aprotinin, and 100mM sodium orthovanadate). After centrifugation at 15,000 x g at 4˚C for 10 min, the protein contents of the supernatants were determined with the Lowry method using DC protein assay kits (Bio-Rad, Hercules, CA). Each sample (10μg per lane) was subjected to 10-20% gradient SDS-PAGE gel electrophoresis and transferred onto a polyvinyl membrane (Immobilon-P; Millipore, Billerica, MA). The membrane was blocked for 1 hr in TTBS (pH7.2) containing 1% BSA, followed by washing with TTBS and incubation of the membrane with anti- β2-microglobulin (sc-8362, Santa Cruz Biotechnology Inc., Santa Cruz, CA), or anti beta-actin (Sigma-Aldrich, St. Louis, MO). After another washing with TTBS, the membranes were incubated with the second antibody for 30 min.

**Mammalian expression vectors and gene transfection.** The wild type β2-microblobulin cDNA was PCR-amplified from healthy donor peripheral blood monocytes with primers (CGC GGA TCC GCC GAG ATG TCT CGC) and (GGG GTA CCT TAC ATG TCT CGA TCC CAC) and subcloned into pcDNA3.1 (-) (Invitrogen) between BamH I / Kpn I site (all restriction enzymes were purchased from TaKaRa, Otsu, Japan). For the transient expression study, DLD-1 cells were used, which had been grown in 100-mm culture dishes to 70% confluency. After the cells had been collected by means of trypsinization and washed once with PBS, they were transfected with 10μg of plasmid DNA. 1.0 x $10^7$ cells were then resuspended in 500μl of DMEM without FCS. The cell suspension was transiently transfected by electroporation at 300V and 950 microfarads in a 0.4-cm Gene Pulser cuvette by a Gene Pulser II (Bio-Rad) together with 10 μg of an expression vector. We also co-transfected the GFP expression vector (TaKaRa) with the β2-microglobulin expression vector for the marker gene. The transfected cells were grown in DMEM 10% FBS with penicillin/streptomycin for 48 h prior to FACS assay or western blotting assay.

**Statistical analysis.** All data are presented as mean±SD. Pearson's correlation was used for all statistical analyses and Dr. SPSS II software (SPSS Japan Inc., Tokyo, Japan) for partial correlation. P values $<0.05$ were considered statistically significant.

## RESULTS

**Expression of MHC class I antigen on the surface of various gastrointestinal cancer cells.** The expression of MHC class I antigen on the cell surface was analyzed by flow cytometory using the monoclonal antibody specific for HLA-ABC. As shown in Table 2, the expression level was high in SK-ChA-1 and NCI-H747 under normal culture condition. None of the cancer cell lines for pancreatic cancer presented the high MHC class I levels observed in SK-ChA-1 or NCI-H747. However, there were no significant differences among MHC class I expressions in pancreatic, biliary tract and colon cancer cell lines. It was also found that HCT-15/DLD-1 had no detectable MHC class I expression on its cell surface, nor showed any significant difference in expression level between cells cultured with or without

INFγ. Flow cytometry also showed that INFγ treatment up-regulated the expression of MHC class I for AsPC-1, BxPC-3, Mz-ChA-1 and Colo-320, while these cancer cells presented lower-level MHC class I without INFγ. The other seven cell lines also showed higher expression for MHC class I when treated than when not treated with INFγ.

Table 2. MHC class I expression of various gastrointestinal cancers with or without IFNγ stimulation

| Cancer cell line | control | MHC class I | |
|---|---|---|---|
| | | IFNγ (-) | IFNγ (+) |
| Pancreas | | | |
| AsPC-1 | 3.8±0.2 | 8.5±1.1 | 24.9±3.4 |
| BxPC-3 | 3.5±0.1 | 4.7±0.7 | 8.7±0.9 |
| PANC-1 | 3.9±0.4 | 10.5±0.4 | 42.9±1.3 |
| MIAPaCa-2 | 4.2±0.2 | 19.6±0.8 | 25.2±1.8 |
| Biliary tract | | | |
| SK-ChA-1 | 3.6±0.3 | 45.3±2.1 | 74.8±4.1 |
| NOZ | 4.0±0.5 | 11.8±1.3 | 21.0±1.4 |
| MZ-ChA-1 | 3.7±0.1 | 9.8±0.6 | 21.7±1.7 |
| TGBC-2TKB | 3.5±0.3 | 17.2±3.2 | 34.6±3.0 |
| Colon | | | |
| DLD-1 | 3.8±0.3 | 3.4±0.2 | 3.6±0.1 |
| HCT-15 | 3.5±0.2 | 3.3±0.4 | 3.7±0.1 |
| NCI-H747 | 3.9±0.5 | 52.3±2.4 | 112.0±4.5 |
| COLO-320 | 3.9±0.4 | 8.3±1.9 | 29.7±1.1 |
| CACO-2 | 4.0±0.2 | 15.2±2.2 | 28.2±3.2 |

Data are shown as mean±SEM for triplicate assays

**Expression of β2-microgloulin gene and protein.** Messenger RNA, isolated form 13 gastrointestinal cancer cell lines, was reverse-transcribed to single strand DNA, followed by evaluation of β2-microglobulin gene expression levels with the real-time quantitative PCR system. Furthermore, the expression of β2-microglobulin protein in 13 cancer cell lines was also studied with a western blotting assay, for which 10 μg of each cell lysis was used. As Figure 1 shows, for β2-microglobulin expression in gastrointestinal cancer cell lines, the gene level correlated well with the protein level. Beta2-microglobulin showed high expression levels in MZ-ChA-1 and NCI-H747, both in real-time quantitative PCR and western blot studies. It was also found that β2-microblobulin gene and protein expression was reduced in Panc-1, TGBC-2TKB and Colo-320, while in HCT15/DLD1, the β2-microblobulin gene level was strongly reduced (Fig. 1A), and no β2-microblobulin protein was detected in western blot analysis (Fig. 1B). β2-microglobulin gene expression was about three times lower for DLD-1 than for Panc-1 cells, which expressed lower levels for both β2-microglobulin gene and protein.

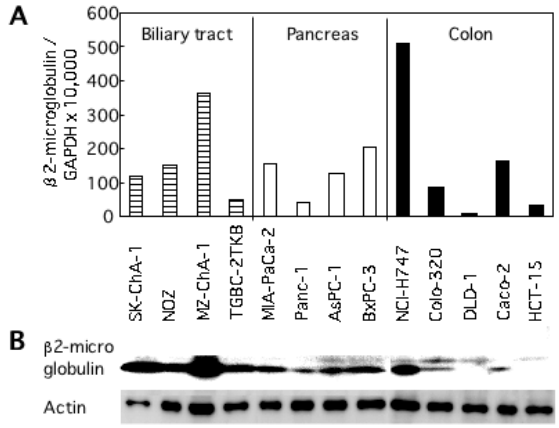



**Fig.1.** Gene and protein expression of β2-microglobulin for various gastrointestinal cancer cell lines. (A) Reduced gene expression was detected for β2-microglobulin in HCT-15/DLD-1 cells. (B) For β2-microglobulin expression, the gene level correlated well with the protein level in gastrointestinal cancer cell lines.

**MHC class I expression restored on the cell surface by transfection of wild type β2-microglobulin.** To confirm whether the absence of MHC class I expression on the cell surface is attributable to a lack of wild type β2-microglobulin protein expression, wild type β2-microglobulin expression vector was transiently transfected into DLD-1 cells. After transfection, wild type β2-microglobulin protein expression was demonstrated in a western blotting assay (Fig. 2A). To determine whether MHC class I expression is up-regulated only at the cells transfected with the wild type gene, the DLD-1 cells were co-tranfected with 2.0 μg of GFP expression vector and 8.0 μg of wild type β2-microglobulin expression vector. MHC class I presentation on the surface of the β2-microglobulin transfected DLD-1 cells increased compared with that on the cells transfected with GFP expression vector alone or that on non-transfected cells (Fig. 2B). In DLD-1 cells transfected with the control vector alone, as in non-transfected cells, no up-regulation of MHC class I presentation was detected.

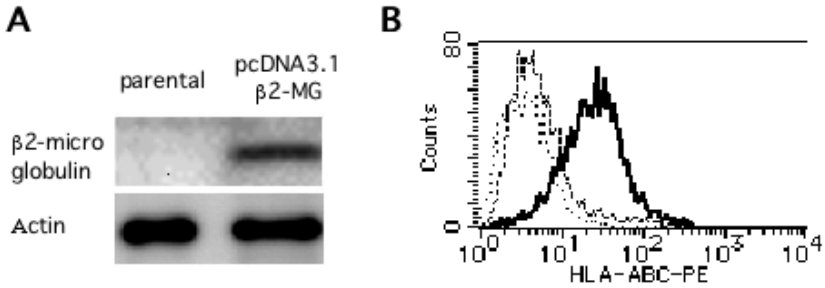



**Fig.2.** Western blot and flow cytometric analysis of DLD-1 cells that were trasfected with wild-type β2-microglobulin gene. (A) In the DLD-1 cells transfected with wild-type β2-microglobulin gene, protein expression was detected by western blot. (B) In flow cytometry study, MHC class I expression was observed in the DLD-1 cells transfected with wild-type β2-microglobulin gene (solid line), while parental cells (dotted line) or the DLD-1 cells transfected with control GFP vector alone (dashed line) demonstrated no class I expression.

**Expression of TAP and LMP in gastrointestinal cancer cells.** Quantitative real-time PCR data for TAP1, TAP2, LMP2 and LMP7 in gastrointestinal cancer cell lines are shown in Table 3. NCI-H747 cells showed high expression levels for all antigen presenting mechanisms in the quantitative real-time PCR study. Gene expression levels for TAP1 were low in HCT-15/DLD-1 and Colo-320 cells, while MIAPaCa-2 showed the highest TAP1 level (6.1/3.4 and 3.6 vs. 62.7, respectively). Expression levels for TAP2 were high in MIAPaCa-2, NOZ and TGBC-2TKB, and low in Colo-320 (209.7, 111.2 and 264.7 vs. 18.4). It was also found that LMP2 and LMP7 levels in Colo-320 cells were the lowest of all cell lines (0.3 and 0.4, respectively). In Panc-1, MZ-ChA-1 and HCT-15/DLD-1, LMP2gene expression was lower than with that in other cells (10.6, 14.8 and 12.6/4.4, respectively). These findings suggest that the gene expression level of the antigen presenting mechanisms was generally lower for Panc-1, MZ-ChA-1 Colo-320 and HCT-15/DLD-1 cells.

Table 3. Quantitative real-time PCR data for TAP1, TAP2, LMP2 and LMP7 in various gastrointestinal cancer cells

| Cancer cell line | TAP1 | TAP2 | LMP2 | LMP7 |
|---|---|---|---|---|
| Pancreas | | | | |
| AsPC-1 | 20.2 | 42.5 | 47.4 | 192.7 |
| BxPC-3 | 21.6 | 56.4 | 29.1 | 156.4 |
| PANC-1 | 16.5 | 28.6 | 10.6 | 22.2 |
| MIAPaCa-2 | 62.7 | 209.7 | 80.1 | 181.1 |
| Biliary tract | | | | |
| SK-ChA-1 | 45.2 | 60.9 | 86.0 | 215.5 |
| NOZ | 24.2 | 111.2 | 55.9 | 253.1 |
| MZ-ChA-1 | 30.2 | 47.3 | 14.8 | 55.6 |
| TGBC-2TKB | 21.8 | 264.7 | 23.6 | 170.5 |
| Colon | | | | |
| DLD-1 | 3.4 | 28.0 | 4.4 | 1.2 |
| HCT-15 | 6.1 | 13.8 | 12.6 | 23.4 |
| NCI-H747 | 35.2 | 82.8 | 219.6 | 168.5 |
| COLO-320 | 3.6 | 18.4 | 0.3 | 0.4 |
| CACO-2 | 31.6 | 45.9 | 32.4 | 105.9 |

Data are shown as copy numbers of mRNA for each molecule / those of GAPDH mRNA x 10,000 for triplicate assays

**Correlation between expression of antigen presenting mechanisms and that of MHC class I.** Statistical analysis of correlations among β2-microglobulin, TAP1, TAP2, LMP2, LMP7 and MHC class I expressions showed that β2-microglobulin, TAP1 and LMP2 correlated with MHC class I expression in gastrointestinal cancer cells (P values: 0.046, 0.031 and <0.001, respectively). Moreover, TAP1 was found to show significant association with both LMP2 and LMP7 (P values: 0.048 and 0.020, respectively). LMP2 also correlated with β2-microglobulin ($r$=0.732; P=0.020). However, partial correlation analysis results shown in Figure 3 demonstrated that LMP2 was the only antigen presenting mechanism which was significantly associated with MHC class I ($r$=0.779; P=0.013).

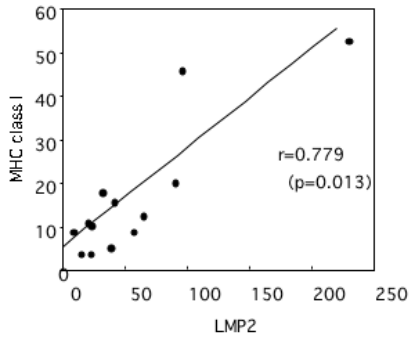



**Fig.3.** Correlation between MHC class I and LMP2 in 13 gastrointestinal cancer cell lines. LMP2 was the only antigen presenting mechanism which was significantly associated with MHC class I

## DISCUSSION

The avoidance by tumor cells of immune recognition is a central problem in tumor immunology. An 8- to 10-residue peptide with an allele-specific consensus motif can bind to the groove of the MHC class I – β2-microgbulin heterodimer (22). When MHC class I molecules represent short peptides derived from cancer specific antigen on cell surface, specific cytotoxic T cells can attack the cancer cells (23). The biochemistry and cell biology of antigen processing and presentation by MHC class I molecules has been analyzed in detail in recent years (24, 25). In order to serve as tumor antigens on the MHC class I complex, it was found that various intracellular molecules influence the antigen processing, presentation, and surface expression of MHC class I molecules. Gene expression levels of various antigen processing mechanism molecules were investigated in numerous cancer cell lines (16, 26-28). In gastrointestinal malignancies, however, it is still not known what the association is between lack or reduction of MHC class I molecule and tumor progression. We therefore investigated MHC class I antigen presentation and gene expression for antigen processing mechanisms of β2-microglobulin, TAP1 and TAP2, and LMP2 and LMP7, in 13 gastrointestinal cancer cell lines. We used two different medium for cell culture in the present study. However, it was suspected that different culture medium have not changed antigen processing mechanism of cancer cell lines, because medium contents have no antigenecity against cell lines.

In malignant melanomas, which have been thoroughly studied in terms of the mechanism of tumor antigen presentation and T-cell based immunotherapy, tumor progression is frequently associated with loss of MHC class I antigens (20, 21). In the case of colorectal cancer, however, the loss of MHC class I expression did not correlate with tumor stage or differentiation (19, 29). Prognosis for pancreatic cancer is very poor compared with that for other gastrointestinal cancers. Flow cytometry analysis showed that there were no pancreatic cancer cell lines which demonstrated high MHC class I expression levels. However, we could not find significant differences between MHC class I expression for pancreatic cancer cell lines and that for biliary tract or colon cancer cell lines. Additionally, when cell lines were stimulated with IFNγ, there were no significant differences for MHC class I expression between pancreatic, biliary tract and colon cancer cell lines. Our data therefore indicate that there cannot be any association between MHC class I expression and prognosis in gastrointestinal cancers.

To serve as tumor antigens on the MHC class I complex, however, foreign proteins must be processed by the multiple catalytic proteasome complex to provide the necessary antigenic epitope carrying peptides. The incorporation of LMP2, LMP7 and LMP10 into proteasomes alters the proteasome activity and enhances the proteolytic production of certain

peptides, depending on the nature of the amino acid immediately preceding the cleavage site (5). These results support the concept of a role for LMP2 and LMP7 in enhancing the proteasomal generation of MHC class I binding peptides (5, 30). It was futher reported that the expression level of MHC class I is associated with the loss of LMP7 gene expression (16). However, in our study, LMP2 was significantly related with MHC class I in the gastrointestinal cancer cell lines, while LMP7 had no correlation with class I expression. One possible examination could be that most of cancer cell lines exhibited high and similar expression levels of LMP7 in real time PCR analysis. Following protein degradation, a peptide antigen consisting of 8-12 amino acids is transported by the transporter associated with antigen processing (TAP) into the lumen of the endoplasmic reticulum where they bind to nascent MHC class I molecules. The TAP1-TAP2 heterodimer belongs to a large family of integral membrane transporters that possess a cytosolics ATP-binding cassette and of multiple hydrophobic regions that are thought to form a transmembrance channel. Lack of TAP results in reduction of MHC class I antigens and is thought to cause avoidance by cancer cells from the immune system (17). In our study, TAP1 was associated with MHC class I expression in gastrointestinal cancer cells by Peason correlation analysis, while no relationship was observed between TAP2 and MHC class I. One explanation could be that TAP2 levels for SK-ChA-1 or NCI-H747 were lower than those for other cells, such as MIAPaCa-2, of which MHC class I was lower than that of SK-ChA-1 or NCI-H747.

β2 microglobulin, also known as water soluble light chain (12kDa), is associated with the MHC class I heavy chain. In the cells which lack β2-microglobulin expression, newly synthesized MHC class I heavy chain molecules do not attain a mature structure and are inefficiently transported to the cell surface. It has been reported that various cancer cell lines and surgically removed cancers feature a reduction or lack of β2 microglobulin protein expression and possess a gene mutation, which affects the MHC class I molecule maturation and the presentation of tumor antigens on the cell surface. It has been shown that human colon cancer cell lines lack MHC class I expression and β2-microglobulin protein expression, while another previous study reported that HCT15/DLD1 showed loss of β2-microglobulin due to mutations (14, 15).

In our study, real time quantitative RT-PCR, used to examine DLD1/HCT15 indicated that β2-microglobulin gene expression was reduced, while TAP and LMP gene expression was also lower than that of other gastrointestinal cancers. Our study also established that transfection with wild type β2-microglobulin restored MHC class expression in DLD1 cells, while complete loss of MHC class I expression and lack of INFγ response were observed in HCT15/DLD1 cells. This suggests that wild type β2-microglobulin expression could be more essential for MHC class I expression in gastrointestinal cancer cell lines than that of other antigen processing molecules, such as TAP or LMP. Statistical analysis showed that TAP1, LMP2 and β2-microglobulin could be associated with MHC class I expression, while partial correlation analysis demonstrated that LMP2 was the only antigen processing molecule which correlated with MHC class I expression on gastrointestinal cancer cells. It was also found with the aid of real time quantitative RT-PCR that LMP2 expression was significantly associated with TAP1 and β2-microglobulin. The TAP1 and LMP2 genes are transcribed from a shared bidirectional promoter containing an IFN response factor element (31). These data suggest that the combined but complex expression of antigen presenting mechanisms could be related to the level of MHC class I expression on the cell surface of various gastrointestinal cancer cell lines.

We conclude that β2-microglobulin and LMP2 could be the most important molecules for MHC class I expression in gastrointestinal cancer cell lines, and that gene expressions for

other antigen processing machineries, such as tapasin, MECL or PA28, should be the subject of future studies. In addition, further study is needed of the gene expressions of antigen processing mechanisms for gastrointestinal cancer cells cultured with IFNγ.

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