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Different Expression Levels of the TAP Peptide Transporter Lead to Recognition of Different Antigenic Peptides by Tumor-Specific CTL

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Decreased antigenicity of cancer cells is a major problem in tumor immunology. This is often acquired by an expression defect in the TAP. However, it has been reported that certain murine Ags appear on the target cell surface upon impairment of TAP expression. In this study, we identified a human CTL epitope belonging to a TAP category. This epitope is derived from preprocalcitonin (ppCT) signal peptide and is generated within the endoplasmic reticulum by signal peptidase and signal peptide peptidase. Lung cancer cells bearing this antigenic peptide displayed low levels of TAP, but restoration of their expression by IFN-γ treatment or TAP1 and TAP2 gene transfer abrogated ppCT Ag presentation. In contrast, TAP upregulation in the same tumor cells increased their recognition by proteasome/TAP-dependent peptide-specific CTLs. Thus, to our knowledge, ppCT16–25 is the first human tumor epitope whose surface expression requires loss or downregulation of TAP. Lung tumors frequently display low levels of TAP molecules and might thus be ignored by the immune system. Our results suggest that emerging signal peptidase-generated peptides represent alternative T cell targets, which permit CTLs to destroy TAP-impaired tumors and thus overcome tumor escape from CD8+ T cell immunity. The Journal of Immunology, 2011, 187: 5532–5539.

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D8+ T lymphocytes constitute major effectors in host defense against viral infection and malignant transformation. Most antigenic peptides recognized by CD8+ T cells are derived from degradation of intracellular mature proteins by proteasomes and are translocated to the lumen of the endoplasmic reticulum (ER) by the TAP1–TAP2 heterodimeric complex (for a review, see Refs. 1, 2). The resulting 8- to 10-aa peptides are then loaded onto MHC class I (MHC-I) molecules and conveyed to the surface of target cells or APCs for T cell recognition. Defects in processing molecules, such as proteasome or TAP subunits, have been described as a strategy for countering the host T cell response. Indeed, viruses are able to interfere with MHC-I–viral peptide complex formation by inhibiting TAP so as to evade CTL recognition and destruction of infected cells (3–10). TAP deficiencies have also been observed in a wide variety of human cancers, including cervical carcinoma (11), head and neck carcinoma (12), melanoma and gastric cancer (13–15), and are associated with tumor escape from immune system control. Therefore, better knowledge of proteasome–TAP processing regulation and the discovery of alternative pathways for tumor Ag degradation may improve antitumor immune responses and immunotherapy approaches.

Proteasome–TAP-independent tumor-specific CTL epitopes generated either by the cystosol metallopeptidase insulin-degrading enzyme or the cystosolic endopeptidases nadilysin and thimet oligopeptidase have been identified (16, 17). TAP-independent presentation of peptides can also be mediated by the so-called secretory pathway in which the proteolytic enzyme furine releases C-terminal peptides (18). Moreover, CTLs specific for Ag-processing mutants have been described in humans and in mouse models and were found to recognize epitopes processed by a TAP-independent mechanism (19–21). Peptide elution experiments indicated that these epitopes can be derived from signal peptide domains of cellular proteins (22–26). Moreover, an artificial H3 molecule signal sequence containing an HLA-A2–restricted T cell epitope resulted in efficient presentation of this signal sequence–derived epitope to HLA-A2–restricted T cells (27). Among signal peptide-derived tumor peptides, melanoma-associated tyrosinase epitope 1–9 is presented independently of TAP and proteasomes (28). However, little is known of the exact processing mechanisms of these antigenic peptides. We recently identified a shared tumor epitope derived from the C-terminal region of the preprocalcitonin...
Cytotoxicity assay, cytokine release, and immunofluorescence analyses

The cytotoxic activity of the T cell clones was measured by a conventional 4-h 51Cr release assay (36). The autologous IGR-Heu tumor cell line, treated or untreated with IFN-γ and pulsed or not for 30 min at room temperature with the antigenic peptide (100 μM), was used as a target.

TNF-β release was detected by measuring the cytotoxicity of the culture supernatants on the TNF-sensitive WEHI-164/13 cells with an MTT colorimetric assay (37). Briefly, T cells were cultured for 24 h in the absence or presence of stimulator cells; then, TNF-β production was tested in culture supernatants. IFN-γ secretion was measured using ELISA (eBioscience).

Surface expression of MHC-I and HLA-A*0201 on the IGR-Heu cell line was quantified by indirect immunofluorescence using W6/32 or B7.2 mAb, respectively, as described (36).

RNA interference and TAP viral inhibitors

Gene silencing of TAPI expression in allogeneic cancer cell lines was performed using sequence-specific small interfering RNA (siRNA-TAPI) purchased from Qiagen (5’-GGCGAUACCUCACUGCAAdTdT-3’ and 5’-UUCGAAGUAAGGUAUCGGCdTdT-3’). Briefly, cells were transfected by electroporation with 400 nM siRNA in a gene pulser Xcell electroporation system (Bio-Rad) at 300 V, 500 μF using electroporation cuvettes (Eurogentec). A second electroporation was performed after 24 h, and cells were then cultured for 48 h. Luciferase siRNA (siRNA duplex 5’-CGUACGGGAAUACUGAdTdT-3’ and 5’-UCGAAGAUUCCCGGUACGdTdT-3’, included as a negative control (siRNA-control), was purchased from Sigma-Proligo.

293-EpNB cells (Invitrogen) were electroporated twice at a 24-h interval with specific siRNA as described earlier and then cotransfected (30,000 cells/well) 24 h later with either pcDNA3.1 or pCEP4 expression vector (Invitrogen) containing cDNA 150 together with pcDNA3.1 containing an HLA-A*0201 cDNA. After 24 h, Heu161 (3000 cells/well) was added, and after another 24 h, half of the medium was collected and its TNF-β content measured.

To inhibit TAPI transiently in HLA-A2* dendritic cells (DCs) isolated from healthy donor PBMCs, a plasmid construct of the immediate-early protein ICP47 of HSV type 1 (38) was used as reported (29).

Results

TAP and SP expression in lung cancer cells

To investigate the prevalence of TAP downregulation in human lung cancer, we analyzed the expression levels of TAPI mRNA in several SCLC and NSCLC cell lines by quantitative RT-PCR. These cell lines include IGR-Heu (NSCLC), which generates mutated α-actinin–4 (actn4) and ppCT tumor-specific CTL epitopes, and DMS53 (SCLC), DMS454 (SCLC), and TT (MTC), which generate the ppCT epitope. The TAP-deficient cell line T2 was used as a negative control, and the human bronchial epithelial cell line 16HBE and PBMCs from healthy donors were included as positive controls. Results indicated that most tumor cell lines expressed low levels of TAPI compared with those of PBMCs and that several cell lines, including A549, IGR-B2, IGR-Heu, Ludlu, Sk-Mes, H1355, and PE-ChA, expressed lower levels of TAPI than that of the 16HBE cell line (Fig. 1A). Low expression levels of TAP2 mRNA were also observed in several cell lines, including IGR-Heu (Supplemental Fig. 1). Importantly, primary human tumors from several NSCLC patients (patients 2, 4, 5, 6, 9, 10, and 11) also displayed low TAPI mRNA expression compared with that of autologous normal lung tissues (Fig. 1B). Moreover, Western blot analysis confirmed low TAPI protein expression in several lung cancer cell lines compared with that of the B-EBV cell line used as a positive control, in particular A549, IGR-B2, H460, IGR-Heu, Ludlu, IGR-Pub, and SK-Mes, except for DMS53 and ADC-Tor (Fig. 1C).

Next, experiments were performed to assess SP and SPP mRNA expression in lung tumor cell lines. SCLC cell lines DMS53 and DMS454, NSCLC cell line IGR-Heu, and MTC cell line TT expressing the CALCA gene that encodes the ppCT tumor

Materials and Methods

Tumor cell lines and T cell clones

The IGR-Heu cell line was derived from a large cell carcinoma (LCC) lesion of patient Heu as described (30). For TAP induction, IGR-Heu cells were either treated with IFN-γ (500 IU/ml) or stably transfected with plasmid constructs bearing human TAPI or TAP2 (generous gift from F. Momborg, Heidelberg, Germany).

Non-small cell lung carcinoma (NSCLC) cell lines IGR-B2 (LCC), adenocarcinoma (ADC)-Coco (31), IGR-Pub (ADC), LCC-M4 (32), squamous cell carcinoma (SCC)-Coco, ADC-Tor, and ADC-Let were derived from tumor specimens as described (30, 33, 34). Pleural effusions (PE)-Cha, PE-Deb, and PE-Ga1 were established from PE of lung cancer patients. PE-Cha was generated from an epidermal growth factor receptor (EGFR)-mutated former smoker ADC female patient. PE-Deb was generated from a light-smoker (less than 3 pack-years) EGFR-mutated ADC male patient. PE-Gal was generated from an EGFR-amplified former-smoker ADC female patient. H460, H1155 (LCC), and H1355 (ADC) were previously described (35). A549 (ADC), SK-Mes, Ludlu (SCC), DMS53, DMS454 (small cell lung carcinoma; SCLC), TT (MTC), and T2 cell lines were purchased from American Type Culture Collection.

Heu161 and Heu171 CTL clones were derived from TILs of patient Heu as reported (32, 34).

Real-time quantitative RT-PCR analysis

RNA was extracted with TRIzol reagent (Invitrogen), reverse transcribed using random hexamers, and then subjected to real-time quantitative PCR analysis (TaqMan; Applied Biosystems) as described (29). PCR primers and probes for TAPI, TAP2, SP, SPP, and CALCA genes were designed by Applied Biosystems (TAPI: Hs00184465_m1; TAP2: Hs00241066_m1; SP: Hs00264468_m1; SPP: Hs00604897_m1; CALCA: Hs00266142_m1) and used according to the manufacturer’s recommendations. Healthy donor PBMCs and the 16HBE human bronchial epithelial cell line (a generous gift of Dr. D.C. Gruenert, San Francisco, CA) were used as controls.

Western blot analysis

Total cellular extracts were prepared by cell lysis in ice-cold lysis buffer (HEPES 10 mM pH 7.4, NaCl 150 mM, 1% CHAPS, 1% glycerol) supplemented with a mixture of antiproteases (Roche) and orthovanadate (2 mM) for 30 min at 4°C. Equivalent amounts of protein extracts (30 μg) were denatured in Laemmli buffer, separated by SDS-PAGE on 4–20% precision gel (Thermo Scientific), and transferred to a nitrocellulose membrane (Pierce/Perbio). After saturation of nonspecific binding sites by incubating the blot for 1 h in TBS containing 20 mM Tris-HCl, 5% nonfat dry milk, and 0.1% Tween 20, the membrane was probed with primary Ab specific for human TAPI (a generous gift from E. Wiertz, Utrecht, The Netherlands), proteasome subunit β2 (Abcam), SPP (Abcam), immunoproteasome subunit LMP7 (Abcam), or β-actin (Santa Cruz) proteins followed by appropriate secondary HRP-conjugated Ab (Santa Cruz) and then revealed by chemiluminescence using SuperSignal WestPico substrate (Pierce/Perbio). The Heu-EBV (30) B cell line (B-EBV) was used as a positive control.

(ppCT) signal sequence and recognized on human lung and mediastinal thyroid carcinomas (MTCs) by a CTL clone isolated from tumor-infiltrating lymphocytes (TILs) of a lung cancer patient (29). ppCT peptide processing is independent of the proteasomes–TAP pathway and involves signal peptidase (SP) and the aspartic protease signal peptide peptidase (SPP). In this report, we analyzed regulation of this novel Ag-processing mechanism and its potential cross-talk with the classical mechanism involving proteasomes. Our results indicate that the SP–SPP pathway is effective in all cells tested when TAP expression levels decrease. In contrast, this pathway is overruled by the proteasome pathway in normal APCs or in TAP-transduced cancer cells. Thus, competition between proteasome- and SP-dependent pathways may occur in cancer cells and is determined by TAP expression levels. Our data suggest that the SP–SPP pathway corresponds to an alternative mechanism of Ag processing exploited by the immune system to eliminate TAP-deficient tumor variants.
Ag were included. Quantitative RT-PCR analyses indicated that all cell lines with overexpression of the CT transcript (Fig. 2A) also expressed high levels of SP mRNA (Fig. 2B). In contrast, the SPP transcript was similarly expressed in all tumor cell lines tested (Fig. 2C). These results suggested that an alternative SP-dependent processing pathway of available protein signal peptides may relay the proteasome pathway in TAP-deficient tumors.

Regulation of tumor Ag-processing pathways by IFN-γ

To investigate the influence of the TAP expression level on HLA-A2–mediated presentation of antigenic peptides of different origins and processing mechanisms, we used the TAP-deficient tumor cell line IGR-Heu treated or not with IFN-γ. Two autologous CTL clones, Heu171 recognizing a proteasome-dependent mutated actn4 peptide (actn491–100) and the Heu161 clone recognizing an SP-dependent/proteasome-independent ppCT16–25 peptide (29), were used as effector cells. As expected, the results shown in Fig. 3A indicate that treatment of IGR-Heu with IFN-γ resulted in a strong increase in TAP1 mRNA expression. In contrast, it did not influence SP, SPP, or CALCA gene expression (Fig. 3A, left panel). Western blot analysis confirmed upregulation of the TAP1 and LMP7 immunoproteasome subunit by IFN-γ, but not SPP and β2 proteasome subunit protein expression. We then assessed the influence of IFN-γ on tumor cell recognition by specific CTL clones. Chromium release assay indicated that whereas treatment of IGR-Heu for 3 d with IFN-γ strongly increased Heu171 CTL clone-mediated lysis, it dramatically inhibited cytotoxicity by ppCT-specific CTL Heu161 (Fig. 3B). In particular, sensitivity of tumor cells to anti-ppCT CTL was restored by pulsing the IFN-γ–treated target with the antigenic peptide, providing evidence of its capacity to present Ag (Fig. 3B).

To corroborate further the inhibitory effect of IFN-γ on tumor cell recognition by the ppCT-specific T cell clone, kinetic experiments were performed by measuring TNF-β secretion by effector cells. Results indicated that incubation of IGR-Heu with IFN-γ induced a prompt decrease in TNF-β production by the Heu161 CTL clone starting from day 3 and lasting at least until day 11 (Fig. 3C). In contrast, the same treatment resulted in a strong increase in cytokine secretion by the mutated actn4-
specific Heu171 CTL clone. This increase correlated with up-regulation of TAP1 (Fig. 3A) and HLA-A2 surface molecule expression (Fig. 3C). These data suggest that TAP expression levels may influence antigenic peptide presentation by target cells.

**Transduction of tumor cells by TAP disrupts the antigenic peptide repertoire**

To determine whether the consequence of IFN-γ treatment on tumor cell recognition by CTL clones was directly due to its effect on TAP expression, we transfected IGR-Heu with TAP1- and TAP2-encoding plasmids. Two tumor cell lines, IGR-Heu-TAPlow and IGR-Heu-TAPhigh, were selected on the basis of their TAP1 and TAP2 expression levels quantified by RT-PCR (Fig. 4A). Remarkably, cytotoxicity experiments indicated that whereas tumor cell transduction with TAP strongly increased Heu171-mediated lysis, it impaired sensitivity to Heu161 CTL in a manner proportional to TAP expression level (Fig. 4B). As expected, reestablishment of TAP expression in IGR-Heu induced enhancement of MHC-I and HLA-A2 surface expression on transduced cells (Table I).

Next, we questioned whether the susceptibility of allogeneic tumor target cells expressing the CALCA gene might be improved by inhibition of TAP expression. Knockdown of TAP1 in HLA-A2+ MTC cell line TT using a specific siRNA, siRNA-TAP1 (Fig. 5A, left panel), enhanced its sensitivity to Heu161-mediated lysis (Fig. 5A, right panel) to levels similar to those of the IGR-Heu autologous target. Moreover, incubation of siRNA-TAP1–treated TT cells with IFN-γ resulted in inhibition of ppCT epitope presentation (data not shown). In contrast, siRNA control had no effect on TT recognition by the anti-ppCT T cell clone. Similarly, inhibition of TAP1 in SCLC cell line DMS53 with specific siRNA (Fig. 5B, left panel), followed by its transient transfection with an HLA-A*0201 construct, resulted in increased TNF-β secretion by Heu161 CTLs compared with that of tumor cells transduced with HLA-A2 alone or together with an siRNA control (Fig. 5B, right panel). However, this production remained lower than that induced by IGR-Heu autologous tumor cells used as a positive control. These results further emphasized that TAP expression levels regulate mechanisms of Ag processing and thereby the antigenic peptide repertoire presented on the target cell surface.
Our previous results suggested that overexpression of the \textit{CALCA} gene is required for tumor cell recognition by autologous CTLs (29). To investigate whether TAP expression levels influence the threshold of tumor Ag detection by the immune system, we knocked down TAP in 293-EBNA cells using siRNA-TAP1 before their transfection 3 d later with HLA-A*0201, together with the cDNA 150 clone encoding ppCT (29). Transfer of cDNA 150 was done by pcDNA3.1 and pCEP4 plasmids chosen for their capacity to induce differential Ag expression levels. The results in Fig. 6A show that whereas the cDNA 150-pCEP4 vector, which promotes high ppCT expression, induced TNF-\(\beta\) secretion by the Heu161 clone at both selected concentrations and in the absence of siRNA-TAP1, cDNA 150-pcDNA3.1 was inefficient. Importantly, knockdown of TAP1 in 293-EBNA cells using specific siRNA before their transfection with cDNA 150 cloned into the pCEP4 vector, which produces higher amounts of tumor Ag than those by pcDNA3.1, is able to activate the ppCT-specific clone even during normal TAP expression (Fig. 6B). These data suggest an additional selective mechanism used by the immune system to specifically recognize and eliminate tumor cells with impaired TAP expression.

**Discussion**

Peptide elution experiments clearly demonstrated that peptides derived from leader sequences of proproteins emerge at the surface of tumor cells with impairment of the classical Ag-processing pathway (23, 25, 26). Some such peptides can be targeted by CTLs and are processed by as yet poorly defined TAP-independent mechanisms. T cell reactivity to TAP-inhibited tumors (whereas TAP-positive counterparts are not recognized) has been described for CTL-recognizing TEIPP (T cell epitopes associated with impaired peptide processing) and could be induced in vivo in mouse cancer models through TAP\(^{2/2}\) DC vaccination (21, 39). Moreover, CD8\(^+\) T cells specific for TAP-inhibited APCs were detected in healthy human PBMCs (19), but the nature of these peptides and their processing mechanisms remain unknown. In this study, to our knowledge, we identified ppCT\(_{16-25}\) antigenic peptide, derived from the signal sequence of the calcitonin hormone precursor and processed within the ER by SP and SPP, as the first molecularly characterized human TEIPP Ag. Indeed, we showed that IGR-Heu NSCLC cells bearing this epitope in an HLA-A2 context exhibited low levels of TAP1 and TAP2 and that down-regulation of TAP is required for CTL recognition. Moreover, our results indicated that knockdown of TAP in ppCT-expressing

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**TAP expression levels fine-tune the tumor Ag detection threshold**

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We then transfected immature DCs, derived from blood monocytes of a healthy HLA-A2\(^+\) donor, with pcDNA3.1 or pCEP4 constructs coding for the antigenic peptide, combined or not with the immediate-early protein ICP47 of HSV type 1, which binds to and inhibits human TAP. The results shown in Fig. 6B indicate that inhibition of TAP in normal DCs using the pBJi-neo vector containing ICP47 cDNA, together with transfer of the cDNA 150 clone with the pcDNA3.1 plasmid (75 ng), triggered secretion of IFN-\(\gamma\) by Heu161 T cells. The results also indicate that transfer of cDNA 150 cloned into the pCEP4 vector, which produces higher amounts of tumor Ag than those by pcDNA3.1, is able to activate the ppCT-specific clone even during normal TAP expression (Fig. 6B). These data suggest an additional selective mechanism used by the immune system to specifically recognize and eliminate tumor cells with impaired TAP expression.

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allogeneic cancer cells using specific siRNA resulted in a strong increase in their recognition by ppCT-specific Heu161 T cells. Notably, all tumor cell lines overexpressing the CALCA gene also displayed high levels of SP expression. These data suggest that peptides derived from signal sequences of available secreted self-proteins and processed by SP represent a substantial pool of epitopes presented by TAP-deficient tumors. This implies that normal cells, or tumor cells expressing high levels of TAP, rarely present peptides derived from leader sequences and are unlikely to be recognized by CTLs such as Heu161.

Peptides derived from signal sequences contribute to stabilizing MHC molecules in TAP-deficient cells, even though the expression of peptide–MHC complexes remains weak (40). As expected, treatment of IGR-Heu cells with IFN-γ led to an increase in MHC-I surface expression but did not enhance presentation of the ppCT peptide. The plausible explanation is that in the absence of other peptides entering from the cytosol, there is always a consistently high concentration of available MHC-I molecules within the ER to be loaded with signal peptide-derived epitopes (41). Conversely, an IFN-γ-mediated increase in TAP expression, together with MHC-I proteins, triggered competition with other peptides entering the ER via TAP, leading to decreased loading of the SP-processed ppCT peptide. This was emphasized by increased loading of the proteasome-processed peptide actn491–100 and enhanced reactivity of the specific Heu171 clone. In contrast, a decrease in SP-degraded peptide loading led to decreased recognition of IFN-γ-treated IGR-Heu tumor cells by the ppCT16–25-specific Heu161 clone.

These findings suggest that SP-degraded peptides are competed away from MHC-I presentation by the large flow of TAP-transported peptides in IFN-γ–treated tumor cells. Accordingly, transduction of NSCLC cells with TAP1 and TAP2 resulted in inhibition of ppCT16–25 epitope loading, as reflected by a decrease in target cell recognition by the specific T cell clone, and in contrast optimization of actn491–100 presentation by reestablishment of the proteasome-dependent pathway. Failure of ppCT peptide loading and presentation by HLA class I molecules in TAP-transfected cells could also be explained by the presence in the ER of very limited quantities of this peptide compared with the overwhelming amounts of competing peptides pumped into the ER by TAP. These limitations could be the result of weak expression of the CALCA gene product and/or poor efficiency of the SP–SPP peptide processing mechanism. The relatively low affinity of the ppCT16–25 epitope for HLA-A2 binding (29) may also prevent its presentation under normal TAP expression circumstances. These results suggest that a competition between pro-
for detection in CTL assays, peptides derived from human signal presented on their surface.

used by cancer cells and thereby the antigenic peptide repertoire TAP expression levels determine the Ag-processing mechanism and emerge at the cell surface only after alterations in MHC-I Ag peptide sequences only emerge at the surface of normal cells if there is a defect in the classical Ag presentation pathway. Indeed, presentation of the ppCT16–25 peptide occurred in normal non-transformed cells such as 293 cells and DCs after knockdown of TAP either by specific siRNA or a viral inhibitor without need of CT cDNA overexpression. Moreover, our results indicated that TAP expression levels influence the threshold of tumor Ag detection by specific effector T cells. Indeed, transfection experiments with the pcDNA3.1 plasmid bearing cDNA 150 indicated that higher levels of the CALCA gene product, such as those obtained with the pCEP4 vector, may not be required for recognition by Heu161 T cells when TAP1 is downregulated in recipient cells. This implies that normal cells with proficient TAP and CALCA gene expression, such as normal thyroid C cells and neuronal cells, would not be recognized by T cells like Heu161. This was certified by the observation that patient Heu mounted a spontaneous CTL response to ppCT Ag without clinical autoimmunity. Moreover, CTLs specific to the ppCT16–25 epitope were detected at the tumor site but not in patient PBL (Ref. 34 and data not shown). It is possible that CTLs directed to epitopes associated with impaired TAP function are responsible for protection from tumor growth in vivo.

Quantitative RT-PCR and Western blot analyses indicated that several human lung cancer cell lines and primary tumors display weak expression of TAP1 molecules. TAP downregulation has also been observed in several SCLC and NSCLC specimens by immunohistochemical analysis (42–44). This suggested that human cancers, including lung cancers, represent poor targets for MHC-I–restricted CTLs and thus correspond to poor candidates for tumor-associated Ag-based immunotherapy (42–46). However, we show in this study that tumor Ag can be presented on cancer cells after degradation by at least two parallel mechanisms, which together contribute to the diversity of antigenic peptides displayed at the surface of malignant cells. Indeed, IGR-Heu NSCLC cells were able to present distinct antigenic peptides, which are processed either by proteasome- or SP-dependent pathways. Our results also show that susceptibility of tumor cells to CTL clone-mediated lysis was directly correlated with TAP expression levels and that TAP downregulation promotes the SP-dependent pathway. This indicates that the immune system can take advantage of this alternative SP-mediated processing mechanism to eliminate tumor immune escape variants with TAP expression defects.

Introduction of the TAPI gene in TAP-negative murine lung carcinoma cell lines resulted in an increase in cancer cell antigenicity and antitumor immune responses (47). However, immunotherapy strategies combining both TAP-dependent and TAP-independent epitopes may reinforce tumor-specific T cell responses and improve current cancer vaccines. This is supported by the finding that patient Heu with long-term survival developed conventional proteasome-dependent peptide-specific CTLs in conjunction with SP-dependent peptide-specific T cells to prevent immune escape by cancer cells as a result of processing defects. The presence of effector T cells capable of eliminating both processing-deficient and -proficient tumors is of a major importance for the development of more efficient anticancer immunotherapy approaches. The fact that these TAP-independent self-peptides are not presented by cells with normal processing status and emerge at the cell surface only after alterations in MHC-I Ag processing machinery might explain their immunogenicity and ability to induce an efficient antitumor CTL response. Thus, signal sequence-derived peptides correspond to attractive candidates for specific cancer immunotherapy against TAP-deficient tumor variants.

FIGURE 6. Downregulation of TAP in normal APCs results in an increase in SP-dependent epitope recognition. A. Effect of TAPI knockdown on 293-EBNA cell recognition by the anti-ppCT clone. Left panel, 293-EBNA cells, electroporated or not with an siRNA-TAPI or siRNA-control, were cotransfected with an HLA-A2 construct and with two different amounts of either pCEP4 or pcDNA-3.1 vectors containing cDNA 150. The CTL clone Heu161 was then added at a 1:10 E:T ratio. TNF-β released after 24 h of culture was determined as described earlier. Controls include untransfected 293-EBNA cells or 293-EBNA cells transfected with pcDNA3.1 or pCEP4 empty vectors alone, and HLA-A2–bearing pcDNA3.1 plasmid alone or with cDNA 150 alone cloned in either pcDNA3.1 or pCEP4 inducing differential expression levels of ppCT. The autologous IGR-Heu cell line was used as a positive control. Data shown correspond to one of three independent experiments. Right panel, Quantitative RT-PCR analysis of TAPI transcript in 293-EBNA cells electroporated or not with siRNA-TAPI or siRNA-control. B. Effect of TAPI inhibition on DC recognition by the anti-ppCT clone. Monocytes were isolated from the blood of an HLA-A2 healthy donor and cultured for 6 d in the presence of IL-4 (100 ng/ml) and GM-CSF (250 ng/ml). DCs (30,000 cells/well) were then transfected with cDNA 150 cloned in pcDNA3.1 and a pBJi-neo vector containing ICP47 cDNA (1 μg) and pCEP4 bearing cDNA clone 150 and IGR-Heu tumor cells were used as positive controls. The amount of IFN-γ released by Heu161 CTLs (3000 cells/well) was measured 24 h later by ELISA.

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B
Acknowledgments

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Disclosures

The authors have no financial conflicts of interest.

References

Supplementary Figure 1: Relative TAP2 gene expression in tumor cell lines. Quantitative RT-PCR analyses of TAP2 transcript in lung cancer cell lines. T2 was used as negative control, 16HBE and healthy donor (HD) PBMC were used as positive controls. Expression levels of TAP2 transcript relative to 18S transcript are shown.