Immune Escape Associated with Functional Defects in Antigen-Processing Machinery in Head and Neck Cancer

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Abstract Recent revival of interest in the role of immune surveillance in the pathogenesis and control of malignant diseases has focused attention on escape mechanisms used by tumor cells to evade immune recognition. Defects in the host's tumor antigen – specific immune responses and abnormalities in tumor cell expression of HLA class I molecules and tumor antigen are known to contribute to tumor progression. However, the mechanism(s) responsible for the lack of tumor cell recognition by functional HLA class I antigen – restricted, tumor antigen – specific CTLs despite expression of the restricting HLA class I allele and targeted tumor antigen by tumor cells remain(s) unexplained. In squamous cell carcinomas of the head and neck (SCCHN), this type of tumor escape is a rule rather than the exception. Here, we discuss evidence pointing to functional defects in the antigen-processing machinery as one mechanism underlying resistance of SCCHN cells to recognition and lysis by HLA class I antigen – restricted, tumor antigen – specific CTL. In addition, based on the restoration by IFN-γ of SCCHN cell sensitivity to recognition by these CTL, we suggest strategies that may improve the clinical course of the disease by enhancing susceptibility of malignant cells to immune recognition.

The limited efficacy of conventional therapies, including surgery, radiotherapy, and chemotherapy, in most malignant diseases has stimulated interest in developing alternative therapeutic strategies. T cell – based immunotherapy for cancer has attracted much attention during the last 10 years (1, 2), largely because of its success in the control of tumor growth in animal model systems (3). The identification of human tumor antigens suitable for immunotherapy and immunomonitoring (4) and the optimistic expectations of basic tumor immunologists and clinical oncologists eager to solve a major problem in current clinical medicine have driven the rapid translation of experimental findings to the clinic. Thus, a rather large number of patients with various types of malignant disease have been enrolled in clinical trials of T cell – based immunotherapy at many clinical centers. Surprisingly, the clinical responses have been disappointing (5), and in general, it has not been possible to correlate clinical responses with tumor antigen – specific CTL immune responses. This discrepancy has prompted investigations into mechanisms underlying the failure of tumor antigen – specific CTL to control tumor growth in cancer patients, especially those treated with immunotherapies.

T-cell dysfunction and abnormalities in molecules crucial for tumor cell recognition, such as HLA antigens and tumor antigen, have been identified as potential mechanisms responsible for disease progression or recurrence in spite of the well-documented presence of HLA class I antigen – restricted, tumor antigen – specific CTL in patients with cancer (6 – 8). However, to the best of our knowledge, no mechanism has been described to account for the lack of tumor cell recognition by HLA class I antigen – restricted, tumor antigen – specific CTL despite the expression of the targeted tumor antigen and of HLA class I molecules by tumor cells. Such an escape mechanism seems to be used by squamous cell carcinomas of the head and neck (SCCHN) cells and could account for disease progression in the presence of circulating tumor antigen – specific CTL in this malignancy (9). Therefore, SCCHN cells represent a useful *in vitro* model to identify the molecular mechanism(s) underlying the lack of correlation between immune and clinical responses in malignant diseases without detectable defects in HLA class I molecule and/or tumor antigen expression or effector cell functions.

In this article, following a short review of the antigen-processing machinery (APM), we will (*a*) describe APM defects as a mechanism underlying *in vitro* resistance of SCCHN cells to recognition by HLA class I antigen – restricted, tumor antigen – specific CTL; (*b*) review evidence supporting the clinical relevance of these *in vitro* findings; and (*c*) discuss strategies to overcome resistance of SCCHN cells to CTL recognition.

APM

Mostly, although not exclusively, endogenously derived antigens are processed via the HLA class I pathway in antigen-

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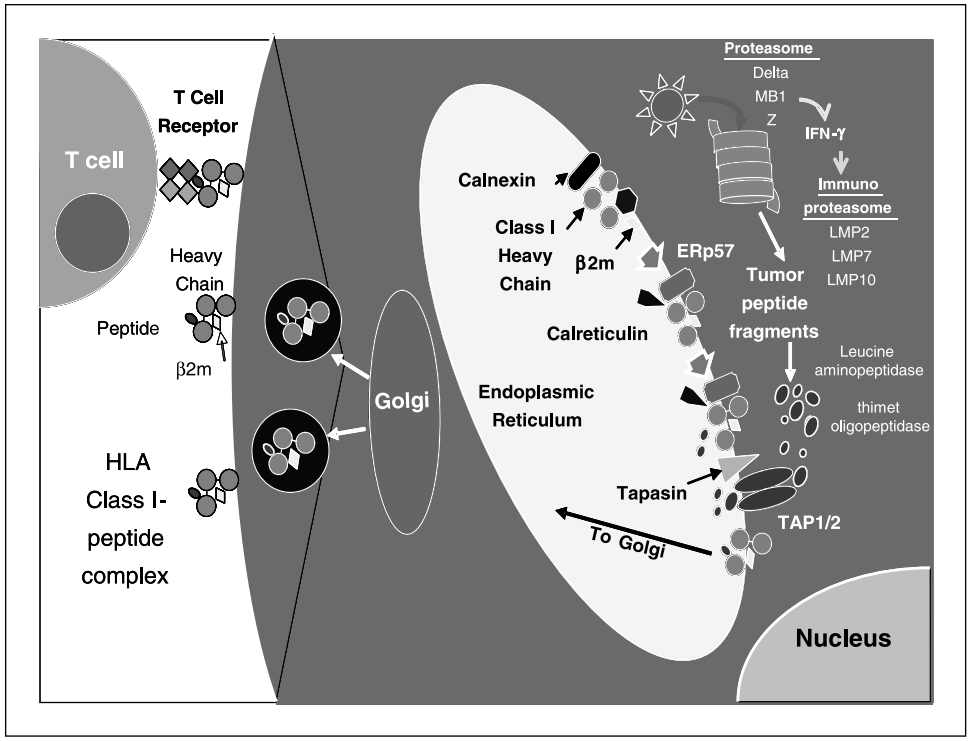


Fig. 1. APM. Proteins marked for ubiquitination in the cytosol are degraded by the proteasome. Resulting peptides are then transported into the endoplasmic reticulum by the TAP1/TAP2 heterodimer. Cytosolic proteases (leucine aminopeptidase and thimet oligopeptidase) influence the generation of HLA class I antigen – restricted peptides. In parallel, HLA class I heavy chains are translated and translocated into the endoplasmic reticulum, where they associate with β_2m and with BiP, calnexin, calreticulin, and ERp57. These proteins assist in proper folding of HLA class I antigens. The HLA class I heavy chain-β_2m complex associates with tapasin, which brings the dimeric complex into association with TAP and ensures peptide loading onto class I heavy chain-β_2m complexes. Trimeric HLA class I-β_2m-peptide complexes are transported to the cell surface.

presenting cells, including tumor cells (8). As shown in Fig. 1, proteins are marked for ubiquitination in the cytosol and subsequently degraded by the proteasome. This complex structure is composed of constitutive β subunits of the proteolytic core, delta (δ), MB-1 (β5), and ζ. Their replacement with the β-type subunits LMP2, LMP7, and LMP10, which are induced by IFN-γ, leads to the assembly of the immunoproteasome. The latter generates a distinct set of antigenic peptides with increased affinity to MHC class I antigens. Peptides processed by the proteasome are transported to the endoplasmic reticulum by the transporter associated with antigen processing (TAP), which is composed of the two noncovalently associated subunits TAP1 and TAP2. In the endoplasmic reticulum, HLA class I heavy chain associates with β_2-microglobulin (β_2m). After being properly folded with the assistance of the chaperones BiP, calnexin, calreticulin and ERp57, β_2m-HLA class I heavy chain complexes are brought into association with TAP by tapasin, which ensures their loading with peptides. Finally, trimeric HLA class I heavy chain-β_2m-peptide (class I-β_2m-peptide) complexes are transported to the cell surface, where they are recognized by T cells expressing cognate T-cell receptors.

An orderly progression of tumor antigen – derived peptides through the APM pathway, with all of its components acting in concert, is necessary for the assembly and transport to the cell surface of class I-β_2m-peptide complexes. Defects in expression and/or function of any of the APM components in this pathway may lead to abnormalities in the formation of trimolecular complexes and/or to their ineffective recognition by CTL (10). Thus, interactions of CTL with tumor cells are disrupted, and tumor cells are not recognized by CTL, even when the latter are functional and are present at the tumor site. Thus, alterations in tumor antigen processing and presentation represent one of the major mechanisms used by tumor cells to escape from recognition by HLA class I antigen – restricted, tumor antigen – specific CTL (11).

Escape of SCCHN Cells from CTL Recognition in Spite of HLA Class I Molecule and Tumor Antigen Expression

The *in vitro* model we have used to investigate mechanisms of tumor cell resistance to HLA class I antigen – restricted, tumor antigen – specific CTL is shown in Fig. 2. It consists of (*a*) an HLA-A2$^+$ SCCHN cell line PCI-13 established from a tumor biopsy (12) and expressing HLA-A*0201 molecules and the tumor antigen, MAGE-3 (The PCI-13 cell line is responsive to exogenous IFN-γ, which up-regulates TAP1, TAP2, and tapasin expression in the tumor cells.); (*b*) HLA-A2 antigen – restricted, MAGE-$3_{271\text{-}279}$ peptide – specific CTL lines generated from peripheral blood mononuclear cells of normal donors or SCCHN patients by *ex vivo* stimulation with MAGE$3_{271\text{-}279}$-pulsed autologous dendritic cells; and (*c*) enzyme-linked immunospot assays for IFN-γ secretion by the CTL challenged with HLA-A2$^+$ MAGE-3$^+$ tumor targets. As shown in Fig. 2, the PCI-13 SCCHN cell line is not recognized (i.e., does not induce IFN-γ secretion in CTLs in the enzyme-linked immunospot assay) by semiallogeneic HLA-A2$^+$ MAGE-$3_{271\text{-}279}$ peptide – specific CTL, although PCI-13 cells express the restricting HLA-A2 antigen and the target tumor antigen as detected by flow cytometry and by quantitative reverse transcription-PCR, respectively. Another HLA-A2$^+$, MAGE-3$^+$ SCCHN cell line (PCI-30) was not lysed in 4-hour ^{51}Cr-release assays (Fig. 3G). However, the susceptibility of these SCCHN targets to CTL-mediated recognition was restored either by pulsing them with the cognate tumor antigen – derived peptide MAGE-$3_{271\text{-}279}$ or by incubation with IFN-γ (100 units/mL for 72 hours at 37°C). The recognition was inhibited by HLA-A2- and HLA-A,-B,-C

antigen – specific monoclonal antibodies (mAb) but was not affected by HLA-DR antigen – specific mAb. These results show that HLA-A2 molecules are not only expressed on PCI-13 cells but are also able to present the exogenous cognate peptide to CTL. Furthermore, these findings imply that CTL recognition of PCI-13 targets following incubation with IFN-γ is not the result of HLA-A2 antigen up-regulation but is likely to reflect other mechanisms, such as increased expression of APM component expression. This conclusion is corroborated by the results derived from the analysis of the mechanisms underlying the lack of recognition of the SCCHN PCI-30 cell line by HLA-A2 antigen – restricted, $HER2_{369-377}$ peptide – specific CTL.[3] Therefore, the lack of recognition of SCCHN cells by CTL in the absence of exogenously added tumor antigen peptide or IFN-γ probably reflects defect(s) in the generation, transport, and/or loading of the endogenous peptide on HLA-A2 molecules, most likely because of APM component down-regulation and/or dysfunction. This mechanism that has been occasionally described in small cell lung carcinoma, renal cell carcinoma, and melanoma (13 – 15) is supported by the following lines of evidence. The lack of PCI-13 cell recognition by CTL is associated with a low level of TAP1, TAP2, and tapasin expression, as shown by the results of flow cytometry analysis of cells intracellularly stained with APM component – specific mAb. Furthermore, up-regulation of APM components LMP2, TAP1, TAP2, and tapasin in four HLA-A2$^+$ SCCHN cell lines incubated with IFN-γ (Fig. 3) was associated with restoration of their recognition by HLA-A2 antigen – restricted, tumor antigen – specific CTL.[4] Lastly and more conclusively, transfection of PCI-13 cells with wild-type TAP1 cDNA or with TAP1 and TAP2 cDNA restored their recognition by HLA class I antigen – restricted, tumor antigen – specific CTL (16).

These conclusions based on the *ex vivo* analysis of interactions between a SCCHN target and tumor antigen – specific CTL have two important implications. First, they provide a mechanism for the unexpected clinical finding that the disease progresses despite the presence of functional tumor antigen – specific CTL and the lack of detectable defects in HLA class I molecule and/or in targeted tumor antigen expression in malignant lesions (15). Second, they emphasize the limitations of the analysis of HLA class I antigen expression on tumor cells for evaluating their susceptibility/resistance to recognition by HLA class I antigen – restricted, tumor antigen – specific CTL. Monitoring of the level of the trimolecular class I-β_2m-peptide complexes on tumor cells is likely to be more informative to establish tumor cell sensitivity/resistance to immune intervention, because HLA class I molecule expression may not correlate

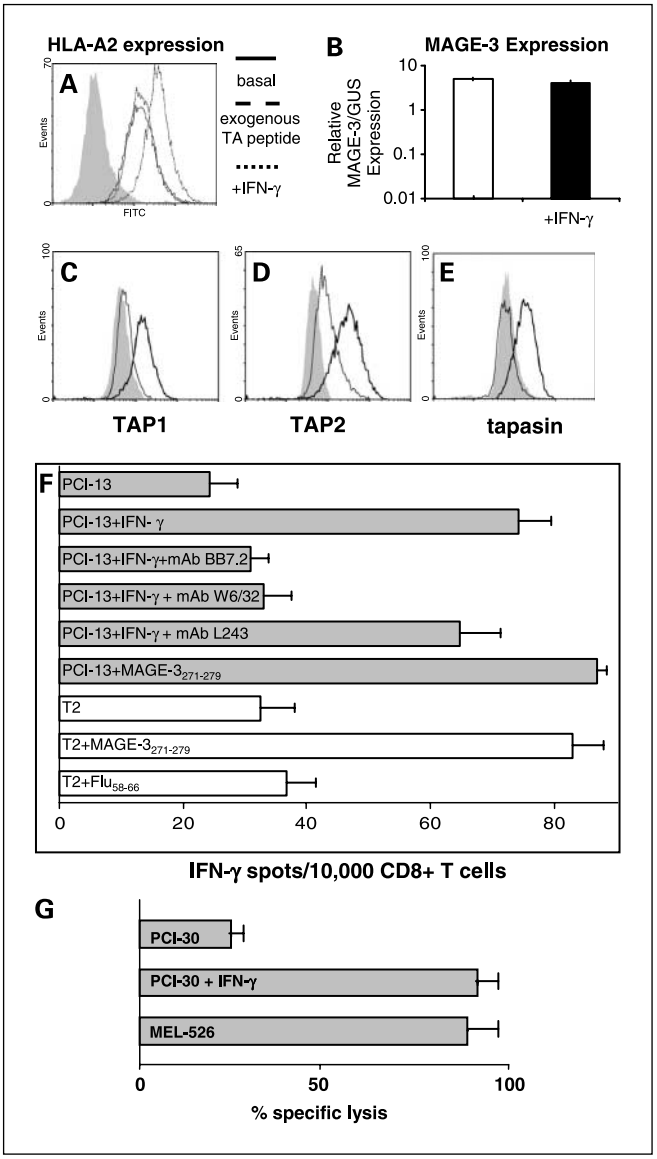


Fig. 2. HLA class I molecule and APM component up-regulation in SCCHN PCI-13 cells and restoration of their tumor antigen – specific CTL recognition following incubation with IFN-γ or pulsing with exogenous MAGE-$3_{271-279}$ peptide. *A,* HLA-A2 molecule up-regulation on SCCHN PCI-13 cells following incubation with IFN-γ but not following pulsing with tumor antigen peptide. Untreated PCI-13 cells (*solid line*) and PCI-13 cells incubated with IFN-γ (100 units/mL for 72 hours at 37°C; *finely dotted line*) and PCI-13 cells pulsed with the exogenous tumor antigen – derived peptide $MAGE_{271-279}$ (10 μg/mL for 1 hour at room temperature) were stained with HLA-A2 antigen – specific FITC-conjugated mAb BB7.2 and analyzed by flow cytometry. Cells incubated with an isotype control mAb (*shaded histogram*) were used as a control. *B,* lack of MAGE-3 modulation by IFN-γ in SCCHN PCI-13 cells. mRNA was isolated from untreated (*white column*) and IFN-γ-treated (100 IU/mL for 72 hours at 37°C; *black column*) PCI-13 cells. Expression of MAGE-3 and endogenous control gene (*β-glucuronidase*, *GUS*) was measured by quantitative reverse transcription-PCR (36). Relative expression of MAGE-3 to β-glucuronidase. *C* to *E,* up-regulation by IFN-γ of APM component expression in PCI-13 cells. Untreated (*light solid line*) and IFN-γ-treated (100 IU/mL for 72 hours at 37°C; *solid black line*) cells were intracellularly stained with TAP1-specific mAb NOB1 (S. Ferrone, unpublished data), TAP2-specific mAb NOB2 (S. Ferrone, unpublished data), and tapasin-specific mAb TO-3 (19) followed by FITC-conjugated anti-mouse IgG xenoantibodies and analyzed by flow cytometry (37). Cells incubated with an isotype control mAb (*shaded histogram*) were used as a control. *F,* restoration by IFN-γ or by pulsing with exogenous MAGE-$3_{271-279}$ peptide of SCCHN PCI-13 cell recognition by MAGE-$3_{271-279}$ peptide – specific CTL. IFN-γ-treated (100 IU/mL for 72 hours at 37°C) or exogenous MAGE-$3_{271-279}$ peptide – pulsed (10 μg/mL for 1 hour at room temperature) PCI-13 cells were incubated with effector cells at the target/effector ratio of 1:10 in 24-hour enzyme-linked immunospot assays. The number of IFN-γ spots per 10,000 $CD8^+$ T cells plated reflects the frequency of CTL responding to the cognate peptide by IFN-γ secretion. Untreated PCI-13 cells and T2 cells pulsed with the MAGE-$3_{271-279}$ peptide or with the unrelated Flu_{58-66} peptide were used as controls. The HLA class I antigen restriction of PCI-13 cell recognition by CTL was monitored by testing the ability of HLA-A2 antigen-specific mAb BB7.2, HLA class I antigen – specific mAb W6/32, and HLA-DR antigen – specific mAb L243 (final concentration, 10 μg/mL) to block target recognition. *G,* CTL-mediated lysis of MAGE-3^+ SCCHN PCI-30 cells following incubation with IFN-γ. PCI-30 cells incubated with IFN-γ (100 IU/mL for 72 hours at 37°C) were labeled with ^{51}Cr and incubated for 4 hours at 37°C with HLA-A2 antigen – restricted, MAGE-specific CTL at an effector/target cell ratio of 10:1. ^{51}Cr release assays were done in triplicate. MEL-526 cells, which express MAGE-3, were used as positive controls. % Specific lysis was calculated as experimental (^{51}Cr release − spontaneous ^{51}Cr release) / (maximal ^{51}Cr release − spontaneous ^{51}Cr release) × 100. Background (spontaneous) ^{51}Cr release was <20% in this assay.

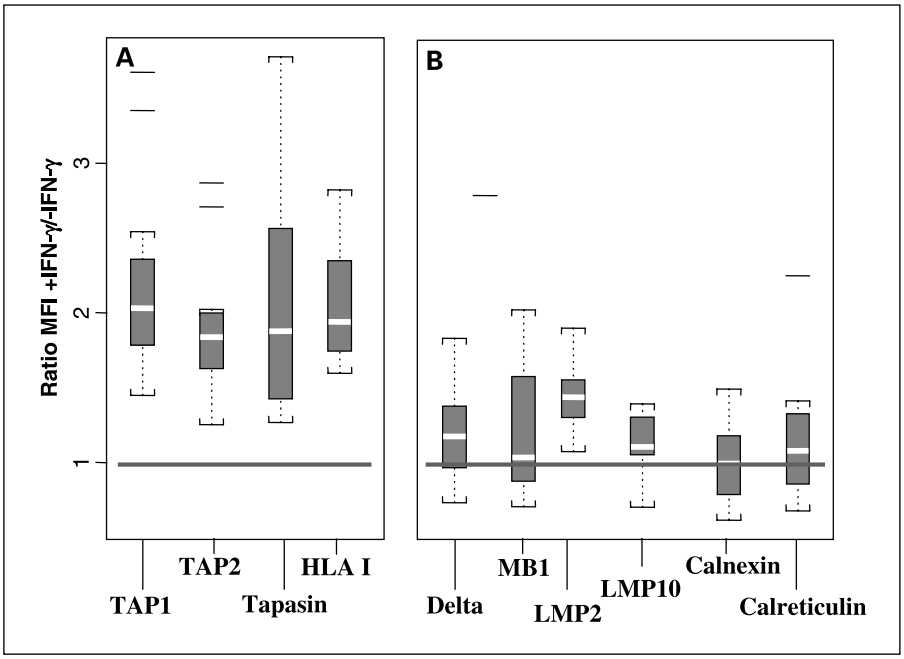
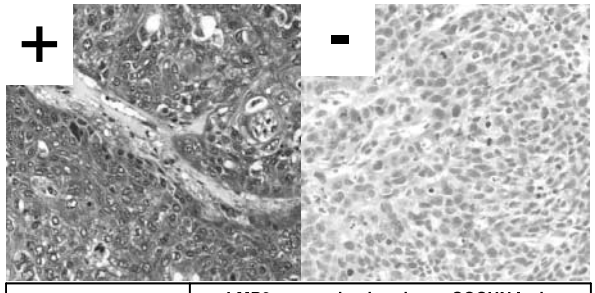


Fig. 3. Differential susceptibility of APM components and HLA class I antigens to up-regulation by IFN-γ in SCCHN cells. Untreated and IFN-γ-treated (100 units/mL for 72 hours at 37°C) SCCHN cell lines were intracellularly stained (37) with Delta-specific mAb SY-5, MB1- specific mAb SJJ-3, LMP2-specific mAb SY-1, LMP10-specific mAb TO-7, TAP1-specific mAb NOB1, TAP2-specific mAb NOB2, calnexin-specific mAb TO-5, calreticulin-specific mAb TO-11, and tapasin-specific mAb TO-3 (19, 20) followed by FITC-conjugated anti-mouse IgG xenoantibodies. Untreated and IFN-γ-treated (100 units/mL for 72 hours at 37°C) SCCHN cell lines were surface stained with HLA class I antigen – specific FITC-conjugated mAb W6/32. Stained cells were analyzed by flow cytometry. Ratio of mean fluorescence intensity (*MFI*) between IFN-γ treated cells and untreated cells for each individual APM component. *A,* APM components TAP1, TAP2, tapasin, and HLA class I molecules were clearly up-regulated by IFN-γ. *B,* the other APM components were not significantly up-regulated by IFN-γ. The box plots show mean fluorescence intensity values for APM component and HLA class I molecule expression in 12 SCCHN cell lines. The box plots show the interquartile ranges of 25% to 75%; the white columns are medians, and the whiskers extend to 1.5 times the interquartile range.

with that of the trimolecular complex recognized by CTL. This possibility emphasizes the need for probes to measure HLA class I antigen-tumor antigen peptide complexes on tumor cells.

Clinical Significance of Abnormalities in HLA Class I Antigen or APM Component Expression in SCCHN Lesions

The question remains whether the described *in vitro* findings have an *in vivo* counterpart and are of clinical significance. Information about APM component expression in SCCHN lesions and in other types of tumors is scanty. Only a limited number of malignant lesions have been investigated by immunohistochemistry, and the analysis has been restricted to a few APM components in the majority of the studies (17, 18). The paucity of information reflects, at least in part, the limited availability of mAb suitable for immunohistochemical detection of APM components in tissues and, especially, in formalin-fixed, paraffin-embedded tissue sections. Only recently, a panel of APM component-specific mAb, which meet these requirements has become available (19, 20). These reagents have facilitated the analysis of APM component expression in tumor biopsies, and the studies done thus far have shown down-regulation of several APM components in malignant lesions (17, 18, 21–23). A representative example of the staining of SCCHN lesions with the LMP-2-specific mAb SY-1 is shown in Fig. 4. The frequency of APM component down-regulation varies in SCCHN lesions, ranging from 18% to >80% for LMP2, TAP1, TAP2, and tapasin (21–23). Furthermore, an association has been found between APM component down-regulation in SCCHN lesions and clinical course of the disease (21) as well as patient survival, as illustrated in Fig. 5 (22, 23). This association is likely to reflect the negative effect of APM dysfunction on the recognition of tumor cells by HLA class I antigen–restricted, tumor antigen–specific CTL because of defects in the generation and/or expression of the trimolecular class I-β_2m-peptide complexes. This hypothesis is supported by the association found in maxillary sinus carcinoma between APM component down-regulation and reduced patients' survival as well as reduced $CD8^+$ T-cell infiltration into malignant lesions (21). Furthermore, our



	LMP2 expression in primary SCCHN lesions			
Cervical nodes	<25%	25-75%	>75%	Total:
Benign nodes	3	4	3	**10**
Metastatic nodes	8	2	0	**10**
Total :	**11**	**6**	**3**	**20**

Fig. 4. Association of LMP2 down-regulation in primary SCCHN lesions with cervical lymph node metastases. Representative immunohistochemical staining patterns of primary SCCHN lesions by LMP2-specific mAb SY-1 (19). Positive staining (*left*) and negative staining (*right*). LMP2 expression was scored as positive, heterogeneous, or negative when the % stained tumor cells was >75%, 25% to 75%, and <25%, respectively. Analysis of LMP2 expression in 20 primary SCCHN lesions showed a significant ($P = 0.005$) correlation of LMP2 down-regulation with lymph node metastases.

[3] R.L. Ferris, unpublished data.
[4] R.L. Ferris, unpublished results.

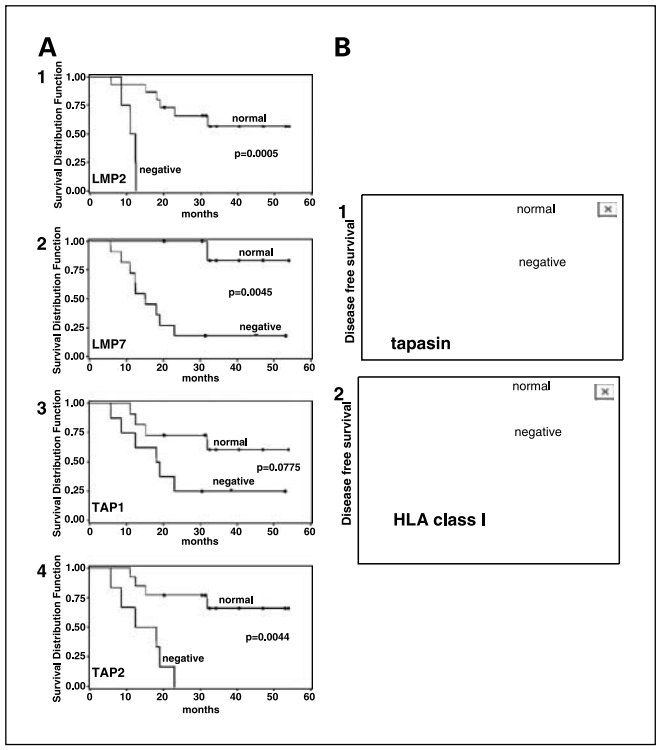


Fig. 5. Association of APM component down-regulation in primary SCCHN lesions with reduced patients' survival. *A,* LMP2 (*1*), LMP7 (*2*), TAP1 (*3*), and TAP2 (*4*) down-regulation was correlated with reduced patients' survival. Adjusted *P*s are shown for the log-rank test used to compare the groups. Reproduced with permission from Meissner et al. (22). *B,* tapasin (*1*) and HLA class I antigen (*2*) down-regulation in primary maxillary sinus squamous cell carcinoma with reduced patients' disease-free survival. The disease-free survival of patients with lesions stained with a positive score (*solid lines*) was compared with that of patients with lesions stained with a heterogeneous or a negative score (*dotted lines*), using the Kaplan-Meier's method. Differences in disease-free survival were analyzed with a log-rank test. Reproduced with permission from Ogino et al. (23).

studies in progress indicate that LMP2 down-regulation is significantly ($P < 0.005$) correlated with the extent of $CD8^+$ T-cell infiltration into the tumor and poor prognosis (18). Taken together, these findings argue in favor of the possibility that APM component defects responsible for *in vitro* resistance of SCCHN cells to CTL recognition also represent the mechanism underlying the association between APM component expression in SCCHN lesions and clinical course of the disease. Thus, APM defects in tumor cells may be emerging as a prognostic biomarker of disease.

Strategies for Counteracting the Lack of SCCHN Cell Recognition by CTL

T-cell recognition of tumor cells plays a major role in the control of tumor growth by the host's immune system and in the success of T cell–based immunotherapy. Therefore, the development of strategies to counteract the escape of tumor cells from T-cell recognition is a priority. The *in vitro* data we have described indicate that the lack of SCCHN cell recognition by HLA class I antigen–restricted, tumor antigen–specific CTL reflects defects in the presentation of the targeted tumor antigen–derived peptides because of APM dysfunction. The latter most likely results from abnormalities in peptide transport and/or loading on β_2m-associated HLA class I heavy chains, because transfection of SCCHN cells with wild-type TAP1 cDNA is effective in restoring their T-cell recognition (16).

As observed in almost every other type of malignant cell described in the literature (13, 14), the defects in APM components identified in SCCHN cells are functional and not structural, because TAP function can be restored by cytokines, especially IFN-γ (17, 18). These findings suggest the potential use of intralesional IFN-γ administration as a strategy to up-regulate APM component expression in SCCHN cells *in vivo* and to restore their recognition by HLA class I antigen–restricted, tumor antigen–specific CTL, provided that the tumor-associated microenvironment does not counteract the effect of IFN-γ. The concentration of IFN-γ required to up-regulate APM component expression *in situ* is not known at present, although 100 units/mL of IFN-γ seem to be sufficient to correct APM dysfunction in SCCHN cells *in vitro*. Because previous clinical trials have not reported significant side effects associated with the systemic administration of IFN-γ (24, 25), we would predict no or few side effects associated with the intralesional administration of the dose of IFN-γ, which is expected to be required to up-regulate APM components in SCCHN cells. Moreover, the validity of this therapeutic approach is supported by *in vivo* studies in experimental tumor models (26) and by the up-regulation of APM components we have observed in human SCCHN lesions transplanted in nonobese diabetic/severe combined immunodeficient mice following intralesional IFN-γ administration.[5]

Besides the modulation of APM components, IFN-γ is expected to have proapoptotic effects on tumor cells and to bias immunologic responses toward cytolytic and T helper type 1 (Th1) immunity, which seems to be more effective at inducing inflammatory and antitumor effects. Direct proapoptotic effects of IFN-γ on tumor cells have been long recognized (27). IFN-γ mediates tumor cell death by the induction of a number of apoptosis-related genes, and it also sensitizes cancer cells to signals delivered by the tumor necrosis factor family members (27, 28). More recent evidence indicates that caspase-8 is cleaved in IFN- γ-treated cancer cells, and that together with cisplatin, IFN-γ exerts more powerful antiproliferative effects (29). Previous demonstrations that human IFN-γ can directly inhibit the growth of human tumor cells *in vitro* and in human cancer xenografts in nude mice have led to its clinical application in patients with malignancy (30). A number of clinical studies have shown that IFN-γ has activity against advanced cancer, especially in patients with ovarian carcinoma or melanoma (24, 25, 30–32). When used in combination with chemotherapy, IFN-γ administration was associated with a significant increase in progression-free survival and only mild toxicity (30). When systemic IFN-γ (100 $\mu g/m^2$ s.c. once weekly for up to 6 months) was used for adjuvant therapy of metastatic melanoma, it was found to be well tolerated, induced up-regulation of HLA class I and II molecules on the tumor in up to 43% of the 19 patients, and resulted in three complete clinical responses (31). In head and neck cancer, IFN-γ has shown some early clinical promise (25) but has not been evaluated more extensively in clinical trials.

[5] A.B. DeLeo and R.L. Ferris, unpublished data.

Clinical grade human recombinant IFN-γ is now commercially available (Actimmune, Intermune, Brisbane, CA) and is Food and Drug Administration approved to treat patients with the immunodeficiency syndrome, chronic granulomatous disease. Therefore, IFN-γ is available for human clinical trials in cancer patients with Food and Drug Administration approval. Our data indicate that the induction of SCCHN cells recognition by CTL occurs after pretreatment with as little as 100 IU IFN-γ for 72 hours, a dose easily achievable clinically. In an orthotopic murine tumor model established in nude mice, using s.c. implanted PCI-13 cells, four intralesional injections of 50,000 IU per injection of IFN-γ led to detectable up-regulation of TAP1 and TAP2 in tumor cells.[5] The doses of recombinant IFN-γ used in our *in vitro* and *in vivo* experiments to up-regulate APM component expression are well within the pharmacologic range of the doses used previously (1,000,000 IU) for s.c. therapy of patients with melanoma (31). Indeed, this study used a 100-fold higher dose that we believe is necessary for delivery to the tumor site. Thus, intralesional injections of IFN-γ seem to be a feasible approach for achieving sufficient local levels of the cytokine to restore APM component expression *in vivo* and to reproduce the biological effects we have observed in our experiments.

We would predict that the combination of intralesional IFN-γ administration with T cell–based immunotherapy should improve its efficacy by counteracting an escape mechanism used by tumor cells to avoid T-cell recognition. Our predictions are supported by reports of tumor regression in mice following the systemic administration of TAP (33–35). All the available preclinical and clinical information justify proof-of-principle clinical studies to prove that intralesional IFN-γ administration may have a beneficial effect on the clinical course of SCCHN through multiple mechanisms.

References

1. Blattman JN, Greenberg PD. Cancer immunotherapy: a treatment for the masses. Science 2004; 305:200–5.
2. Rosenberg SA. Development of effective immunotherapy for the treatment of patients with cancer. J Am Coll Surg 2004;198:685–96.
3. Ostrand-Rosenberg S. Animal models of tumor immunity, immunotherapy and cancer vaccines. Curr Opin Immunol 2004;16:143–50.
4. Novellino L, Castelli C, Parmiani G. A listing of human tumor antigens recognized by T cells: March 2004 update. Cancer Immunol Immunother 2005;54: 187–207.
5. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med 2004;10:909–15.
6. Seliger B, Cabrera T, Garrido F, Ferrone S. HLA class I antigen abnormalities and immune escape by malignant cells. Semin Cancer Biol 2002;12:3–13.
7. Marincola FM, Wang E, Herlyn M, Seliger B, Ferrone S. Tumors as elusive targets of T cell-based active immunotherapy. Trends Immunol 2003;24:335–42.
8. Whiteside TL. Immune suppression in cancer. Effects on immune cells, mechanisms and future therapeutic intervention. Sem Cancer Biol 2006;16:3–15.
9. Chikamatsu K, Albers A, Stanson J, et al. $p53_{110-124}$-specific human $CD4^+$ T-helper cells enhance *in vitro* generation and anti-tumor function of tumor-reactive $CD8^+$ T-cells. Cancer Res 2003;63:3675–81.
10. Sant A, Yewdell J. Antigen processing and recognition. Curr Opin Immunol 2003;15:66–8.
11. Seliger B, Maeurer MJ, Ferrone S. Antigen-processing machinery breakdown and tumor growth. Immunol Today 2000;21:455–64.
12. Heo DS, Snyderman C, Gollin SM, et al. Biology, cytogenetics, and sensitivity to immunological effector cells of new head and neck squamous cell carcinoma lines. Cancer Res 1989;49:5167–75.
13. Singal DP, Ye M, Bienzle D. Transfection of TAP1 gene restores HLA class I expression in human small-cell lung carcinoma. Int J Cancer 1998;75:112–6.
14. Kallfelz M, Jung D, Hilmes C, et al. Induction of immunogenicity of a human renal-cell carcinoma cell line by TAP1-gene transfer. Int J Cancer 1999;81:125–33.
15. Meidenbauer N, Zippelius A, Pittet MJ, et al. High frequency of functionally active MELAN-A-specific T cells in a patient with progressive immunoproteasome-deficient melanoma. Cancer Res 2004;64: 6319–26.
16. Lopez-Albaitero A, Nayak JV, Ogino T, et al. Role of antigen processing machinery (APM) in the *in vitro* resistance of squamous cell carcinoma of the head and neck (SCCHN) cels to recognition by cytotoxic T lymphocytes (CTL). J Immunol 2006;176:3402–9.
17. Campoli M, Chang CC, Oldford SA, Edgecombe AD, Drover S, Ferrone S. HLA antigen changes in malignant tumors of mammary epithelial origin: molecular mechanism and clinical implications. In: Wei WZ, Lopez D, editors. Immunology of breast cancer, in Breast Disease series. Vol. 20. Amsterdam (the Netherlands): IOS Press; 2004. p. 105–26.
18. Chang CC, Campoli M, Ferrone S. Classical and non-classical HLA class I antigen and NK cell activating ligand changes in malignant cells: current challenges and future directions. Adv Cancer Res 2005; 93:189–234.
19. Ogino T, Wang X, Kato S, Miyokawa N, Harabuchi Y, Ferrone S. Endoplasmic reticulum chaperone-specific monoclonal antibodies for flow cytometry and immuno-histochemical staining. Tissue Antigens 2003;62:385–93.
20. Bandoh N, Ogino T, Cho HS, et al. Development and characterization of human constitutive proteasome and immunoproteasome subunit-specific monoclonal antibodies. Tissue Antigens 2005;66:185–94.
21. Ferris RL, Hunt JL, Ferrone S. Human leukocyte antigen (HLA) class I defects in head and neck cancer: molecular mechanisms and clinical significance. Immunol Res 2005;33:113–34.
22. Meissner M, Reichert TE, Kunkel M, et al. Defects in the human leukocyte antigen class I antigen processing machinery in head and neck squamous cell carcinoma: association with clinical outcome. Clin Cancer Res 2005;11:2552–60.
23. Ogino T, Bandoh N, Hayashi T, Miyokawa N, Harabuchi Y, Ferrone S. Association of tapasin and HLA class I antigen down-regulation in primary maxillary sinus squamous cell carcinoma lesions with reduced survival of patients. Clin Cancer Res 2003;9: 4043–51.
24. Kirkwood JM, Bryant J, Schiller JH, Oken MM, Borden EC, Whiteside TL. Immunomodulatory function of interferon-gamma in patients with metastatic melanoma: results of a phase II-B trial in subjects with metastatic melanoma, ECOG study E4987. J Immunother 1997;20:146–57.
25. Richtsmeier WJ, Koch WM, McGuire WP, Poole ME, Chang EH. Phase I-II study of advanced head and neck squamous cell carcinoma patients treated with recombinant human interferon gamma. Arch Otolaryngol Head Neck Surg 1990;116:1271–7.
26. Dominiecki ME, Beatty GL, Pan ZK, Neeson P, Paterson Y. Tumor sensitivity to IFN-gamma is required for successful antigen-specific immunotherapy of a transplantable mouse tumor model for HPV-transformed tumors. Cancer Immunol Immunother 2005; 54:477–88.
27. Ossina NK, Cannas A, Powers VC, et al. Interferon-gamma modulates a p53-independent apoptotic pathway and apoptosis-related gene expression. J Biol Chem 1997;272:16351–7.
28. O'Connell J, Bennett MV, Nally K, et al. Interferon sensitizes colonic epithelial cell lines to physiologic and therapeutic inducers of colonic apoptosis. J Cell Physiol 2000;185:331–8.
29. Barton C, Davies D, Balkwill F, Burke F. Involvement of both intrinsic and extrinsic pathways in IFN-γ-induced apoptosis that are enhanced with cisplatin. Eur J Immunol 2005;41:1474–86.
30. Windbichler GH, Hausmaninger H, Stummvoll W, et al. Interferon-gamma in the first-line therapy of ovarian cancer: a randomized phase III trial. Br J Cancer 2000;82:1138–44.
31. Propper DJ, Chao D, Braybrooke JP, et al. Low-dose IFN-γ induces tumor MHC expression in metastatic malignant melanoma. Clin Cancer Res 2003;9: 84–92.
32. Schiller JH, Pugh M, Kirkwood JM, et al. Eastern Cooperative Group trial of interferon-gamma in metastatic melanoma: an innovative study design. Clin Cancer Res 1996;2:29–36.
33. Alimonti J, Zhang QJ, Gabathuler R, Reid G, Chen SS, Jefferies WA. TAP expression provides a general method for improving the recognition of malignant cells *in vivo*. Nat Biotechnol 2000;18:515–20.
34. Leitch J, Fraser K, Lane C, et al. CTL-dependent and -independent antitumor immunity is determined by the tumor not the vaccine. J Immunol 2004;172: 5200–5.
35. Lou Y, Vitalis TZ, Basha G, et al. Restoration of the expression of transporters associated with antigen processing in lung carcinoma increases tumor-specific immune responses and survival. Cancer Res 2005;65: 7926–33.
36. Ferris RL, Xi L, Raja S, et al. Molecular staging of cervical lymph nodes in squamous cell carcinoma of the head and neck. Cancer Res 2005;65:2147–56.
37. Ogino T, Wang X, Ferrone S. Modified flow cytometry and cell-ELISA methodology to detect HLA class I antigen processing machinery components in cytoplasm and endoplasmic reticulum. J Immunol Methods 2003;278:33–44.