REVIEW

HLA class I defects in malignant lesions: What have we learned?

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Abstract. Depending on the tumor types, HLA class I antigen downregulation or loss has been found in 16% to 50% of malignant lesions in many malignancies with a clinical association with histopathological markers of poor prognosis of the disease and with reduced free interval and survival. These findings may reflect the escape of tumor cells with HLA class I abnormalities from recognition and destruction by HLA class I-restricted, tumor-associated antigen-specific cytotoxic T lymphocytes. This possibility has stimulated investigations on the mechanisms underlying HLA class I antigen abnormalities in malignant cells. Distinct molecular defects underlying an abnormal HLA class I phenotype have been identified and characterized. These defects range from structural alterations of the genes which encode HLA class I antigen subunits to deregulation of antigen processing machinery components responsible for a functional HLA class I antigen expression. These findings, in conjunction with those of clinical recurrence of lesions with HLA class I antigen loss following T cell-based immunotherapy in patients, suggests that immunoselection may play a role in the generation of malignant lesions with HLA class I antigen abnormalities. This possibility has stressed the need to effectively monitor functional HLA class I antigen expression in malignant lesions in the application of T cell-based immunotherapy as well as to develop strategies to circumvent the negative impact of immunoselection. (Keio J Med 52 (4): 220–229, December 2003)

Key words: HLA class I antigens, malignancy, immunoselection, immunomonitoring

Introduction

HLA class I antigens are part of a highly polymorphic antigenic system and play a crucial role in the interaction of malignant cells with immune cells. They present peptides derived from tumor-associated antigens (TAA) to TAA-specific cytotoxic T lymphocytes (CTL) and modulate the interaction of natural killer (NK) cells and T cells with tumor cells (Fig. 1).[1,2] The appreciation of the major role played by HLA class I antigens in the interaction of tumor cells with host's immune system has stimulated interest in the characterization of the molecular mechanisms, functional significance and clinical relevance of HLA class I antigen abnormalities which are frequently associated with malignant transformation of cells.[3] In this paper, we will first discuss the molecular defects underlying HLA class I antigen abnormalities in malignant cells. Second, we will summarize the available information about the frequency of HLA class I antigen abnormalities in malignant lesions. Third, we will discuss the role of immune selective pressure, imposed by the host's immune system, in the generation of malignant lesions with HLA class I defects. Lastly, we will discuss the potential clinical significance of HLA class I antigen abnormalities in malignant lesions and their impact on the outcome of T cell-based immunotherapy of malignant diseases.

Molecular Abnormalities Underlying HLA Class I Antigen Defects

Distinct molecular mechanisms have been found to underlie the various abnormal HLA class I antigen phenotypes (Fig. 2) which have been identified by immunohistochemical (IHC) staining of surgically removed

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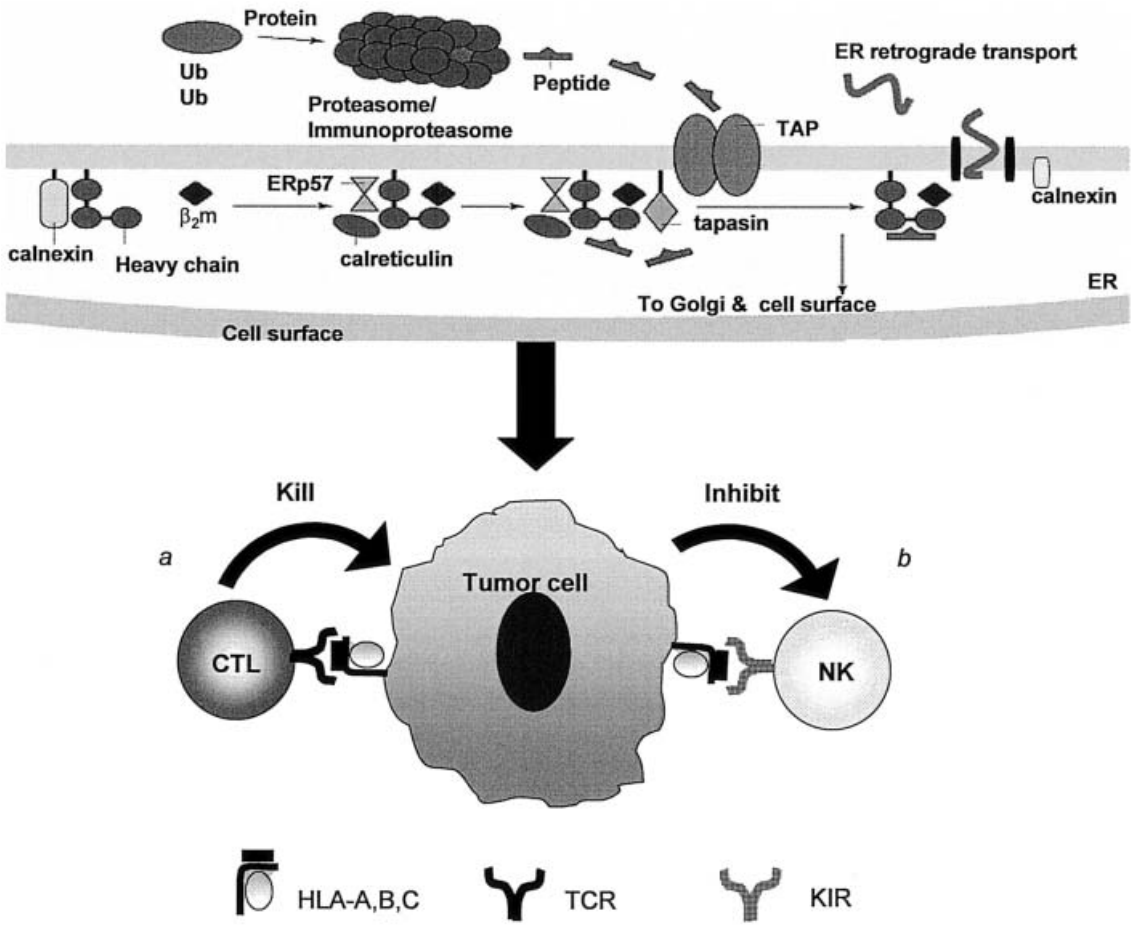

Fig. 1 Generation and interaction of HLA class I antigen-peptide complexes with T cells and NK cells. Intracellular protein antigens, which are mostly endogenous, are marked for ubiquitination within the cytosol and subsequently degraded into peptides by proteasomal cleavage. The constitutive proteasome subunits delta, MB1 and Z and the interferon-γ inducible immunosubunits LMP2, LMP7 and LMP10 are responsible for the catalytic activity of the proteasome. Once generated, peptides are transported into the endoplasmic reticulum through the dimeric transporter associated with antigen processing, TAP1 and TAP2. TAP is responsible for both qualitative and quantitative peptide translocation. Nascent HLA class I antigen heavy chains are synthesized in the ER and associate with the chaperone immunoglobulin heavy chain binding protein (BiP), a universal ER chaperone involved in the translation and insertion of proteins into the ER. Following insertion into the ER, the HLA class I heavy chain associates with the chaperone calnexin and the thiol-dependant reductase ERp57. Calnexin dissociation is followed by HLA class I heavy chain association with β_2m, tapasin and the chaperone calreticulin. Calnexin, calreticulin and ERp57 play a role in folding of the HLA class I heavy chain. Subsequently, tapasin brings the HLA class I heavy chain, β_2m, chaperone complex into association with TAP and plays a role in both quantitative and qualitative peptide selection. The trimeric HLA class I-β_2m-peptide complex is then transported to the plasma membrane where it plays a major role in the interactions of target cells with (*a*) peptide-specific CTL through TCR and with (*b*) NK cell through inhibitory receptor KIR.

malignant lesions with monoclonal antibodies (mAb) (for review, see ref. 3).

Total HLA class I antigen loss is caused by loss of β_2-microglobulin (β_2m) expression and/or function. As a result, the HLA class I heavy chain-β_2m-peptide complex is not formed and not transported to the cell membrane (Fig. 2A). The mutations identified thus far in β_2m genes range from large deletions to single nucleotide deletions, which in most cases inhibit the translation of β_2m mRNA.[4–7] Recently, a novel point mutation resulting in a single amino acid substitution in codon 25 of β_2m (C25W) has been identified in the melanoma cell line Vmm5B with total HLA class I antigen loss (manuscript in preparation). This mutation abolishes the disulfide linkage required for the native structure of β_2m and prevents its binding to HLA class I heavy chains. Although the mutations are distributed randomly in β_2m genes, a mutation hotspot has been suggested to be located in the CT repeat region in exon 1 of the β_2m gene. Mutations in this region have been identified in more than 75% of tumor cells with total HLA class I antigen loss[4] and have been found to parallel the mutator phenotype in tumor cells,[8] reflecting the increased genetic instability of this region during

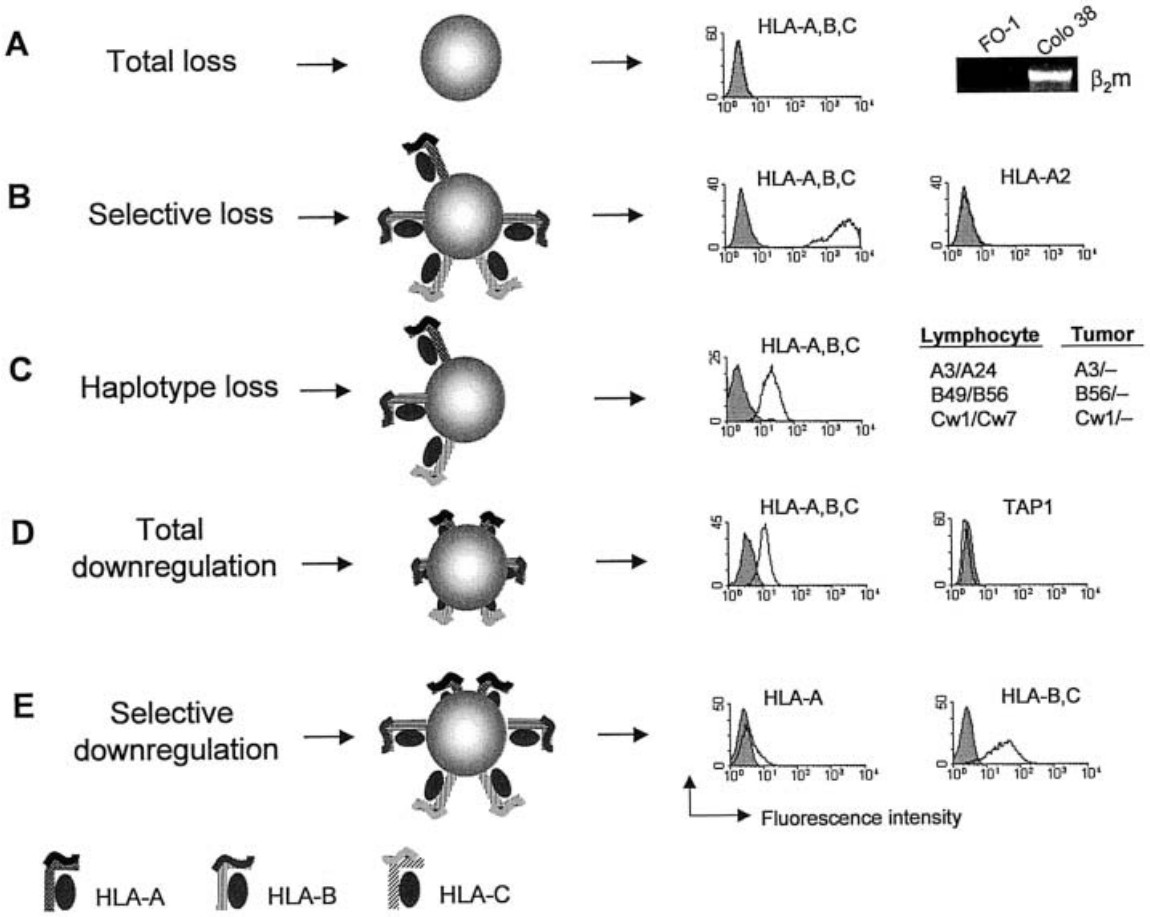

Fig. 2 Molecular mechanisms underlying abnormal HLA class I antigen phenotypes identified in malignant cells. (A) Total HLA class I antigen (HLA-A, -B and -C) loss is caused by loss of β_2m expression and/or function. An example is represented by the large deletion of the $\beta_2 m$ gene detected by PCR in the melanoma cell line FO-1 as compared to the HLA class I-positive melanoma cell line Colo38. (B) Selective HLA class I allospecificity loss is caused by loss of the gene(s) which encode the lost HLA class I allele(s) or by mutations which inhibit their transcription or translation. An example is represented by the lack of HLA-A2 antigen-specific mAb reactivity with the melanoma cell line T372A which has selective HLA-A2 antigen loss. (C) Total loss of one HLA class I haplotype is caused by total or partial loss of one copy of chromosome 6 which encodes the genes for HLA class I heavy chains. An example is represented by the loss of HLA-A24, -B49, -Cw7 in the melanoma cell line COPA-159, as determined by comparative HLA class I genotyping of the tumor cells and the patient's lymphocytes. (D) Total HLA class I antigen downregulation is caused by loss or downregulation of antigen processing machinery components. An example is represented by TAP1 loss in the melanoma cell line SK-MEL-19, as determined by intracellular cytofluorograghic analysis. (E) Selective HLA class I locus downregulation may be caused by locus-specific defects in HLA class I gene transcription. An example is represented by HLA-A antigen downregulation on the melanoma cell line Om431, as assessed by flow cytometry.

malignant transformation of cells.[8] It is noteworthy that β_2m defects leading to total HLA class I antigen loss by malignant cells result from two events: mutations in one copy of the β_2m gene and loss of the other copy. The latter event is often referred to as loss of heterozygosity (LOH), a notable genetic abnormality frequently found in malignant cells (Fig. 3A).[9] The requirement of two events to generate total HLA class I antigen loss in malignant cells accounts for the lower frequency of this phenotype than that of most of the other phenotypes found in malignant cells.[3]

Selective HLA class I allospecificity loss, *e.g.* HLA-A2 loss, is caused by loss of the gene(s) which encode the heavy chain of the lost HLA class I allele(s) or by mutations which inhibit its (their) transcription or translation (Fig. 2B).[4] Unlike total HLA class I antigen loss which requires two mutational events in the β_2m gene, selective HLA class I antigen loss results from only one mutational event in a heterozygous allelic background (Fig. 3B). Whether a mutation hotspot in the genes encoding HLA class I heavy chains exists remains to be determined.

Loss of one HLA class I haplotype, *e.g.* HLA-A24, -B56, -Cw7, appears to be frequently caused by loss of segments of the short arm of chromosome 6 where HLA class I genes reside (Fig. 2C).[10] This phenotype

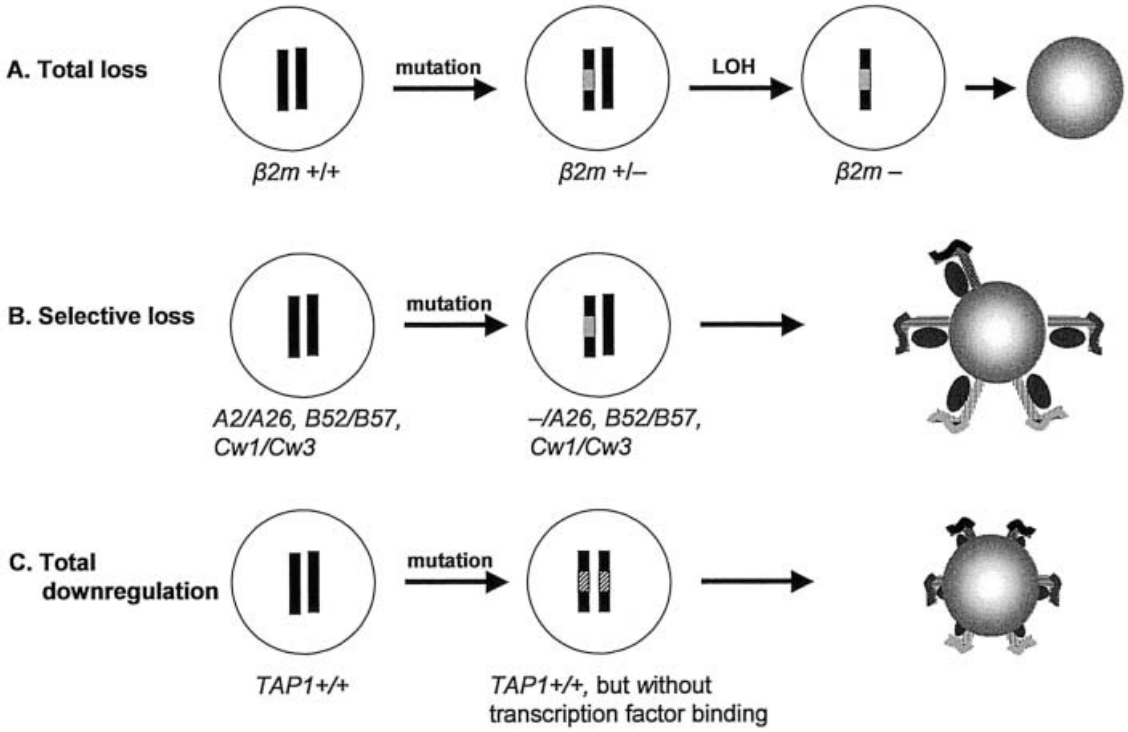

Fig. 3 Contributions of loss of heterozygosity (LOH) to the generation of abnormal HLA class I phenotypes in malignant cells. (A) Total HLA class I antigen loss as a result of $\beta_2 m$ gene mutations requires two genetic events, one of which involves a mutation in one copy of the wild-type $\beta_2 m$ gene and the other involves loss of the non-mutated copy, *i.e.* LOH. (B) Selective HLA class I allospecificity loss, *e.g.* HLA-A2 antigen loss, however, requires only one genetic event which involves mutations of the HLA-A2 gene. The other allele, HLA-A26, remains intact; no LOH is required. (C) Total HLA class I downregulation as a result of TAP1 downregulation usually involves transcription repression in both copies of the *TAP1* gene, presumably by lack of transcription factor binding to the *TAP1* promoter. This phenotype requires neither structural defects nor LOH of the gene.

is often identified by HLA class I genotyping and LOH analysis of chromosome 6. LOH at chromosome 6 appears to represent a frequent mechanism contributing to selective HLA haplotype loss in tumors.[11] This finding may reflect the frequent genetic recombination events at the human *MHC* located at chromosome 6p21.3, which carries the highest density of genes among all gene loci in human chromosomes.[12]

Total HLA class I downregulation is caused by multiple mechanisms. First, transcriptional activity of HLA class I heavy chain genes can be suppressed by the presence of silencer located at the distal promoter[11] or by epigenetic mechanisms such as hypermethylation and/or altered chromatin structure of the HLA class I heavy chain gene promoters.[14,15] Second, the level of HLA class I antigens expressed on cells can be reduced by downregulation or loss of antigen processing machinery components (Fig. 2D).[16] These components play a crucial role in the assembly and expression of functional HLA class I antigen-peptide complexes (Fig. 1). Among the antigen processing machinery components, TAP1 has been most extensively investigated. TAP1 downregulation or loss has been found in head and neck squamous cell carcinoma (HNSCC), in carcinomas of the breast, small cell lung (SCLC), colon, kidney, cervix and prostate and in cutaneous melanoma with a frequency ranging from 10–84% (Fig. 4).[16,17]

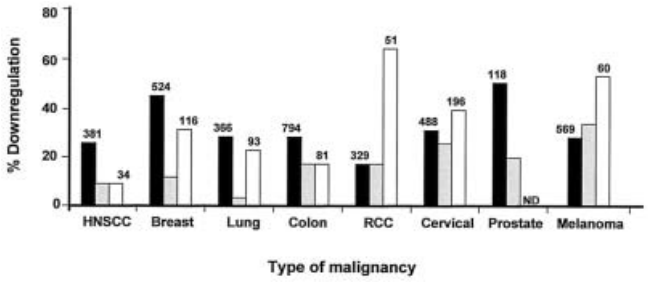

Fig. 4 Frequency of HLA class I antigen and TAP1 downregulation in malignant lesions of different embryological origin. The most common types of solid tumors for which more than 300 or 30 lesions have been analyzed for HLA class I antigen or TAP1 expression, respectively, are shown. (■) Indicates total HLA class I antigen downregulation; (□) indicates selective HLA class I allospecificity loss; and (□) indicates TAP1 downregulation. Figures indicate the number of lesions analyzed. ND: not determined. Data has been adapted from ref. 3.

TAP1 downregulation or loss is likely to be caused by abnormalities in regulatory mechanisms (Fig. 3C), since in some instances they can be corrected by *in vitro* administration of cytokines, such as IFN-γ and TNF-α, and is accompanied by an increase in HLA class I antigen expression.[18,19] To the best of our knowledge, structural defects in TAP1 as a result of mutations have been observed only in two human tumor cell lines.[20,21] The role played by other antigen processing machinery components in HLA class I antigen downregulation in tumor cells is under investigation and remains to be elucidated.

Selective downregulation of the gene products of one HLA class I locus can be caused by alterations in the transcription factors for genes encoding HLA class I heavy chains.[22,23] However, there is limited information regarding selective downregulation of the gene products of one HLA class I locus, since the expression of some HLA class I allospecificities in malignant lesions has not been assessed because of the lack of appropriate probes.

Detection and Frequency of HLA Class I Antigen Abnormalities in Malignant Lesions

A large number of lesions removed from patients with different types of tumors have been analyzed over the years. The most common types of solid tumors for which more than 100 surgically removed primary lesions have been analyzed include HNSCC, carcinomas of the breast, lung, colon, kidney, cervix and prostate and cutaneous melanoma (Fig. 4).[3] The frequency of total HLA class I antigen loss and/or downregulation in primary lesions ranges from 16% for cutaneous melanoma to 50% for prostate lesions.[3] The frequency of selective HLA class I allospecificity loss or downregulation ranges from 4% to 35% for lung carcinoma and cutaneous melanoma, respectively.[3] It should be noted that the reported frequency of HLA class I allospecificity loss or downregulation may be an underestimation, since at present only a limited number of mAb to HLA class I allospecificities suitable for use in IHC staining of malignant lesions are available.

HLA class I antigen downregulation or loss has also been described in other tumor types. However, the number of lesions that have been analyzed is too low for one to draw definitive conclusions. These types of tumors include stomach,[24] pancreatic,[25] bladder,[26] germ cell,[27] and basal cell[28] carcinomas. Liver carcinoma[29–31] and leukemia[32] are exceptions to the general finding of HLA class I antigen downregulation or loss in malignant lesions. In the latter, defects in HLA class I antigen expression in malignant cells have been only occasionally identified. This finding is not likely to reflect a lack of genetic instability in leukemic cells, since like solid tumor cells, leukemic cells harbor many genetic and/or epigenetic alterations in their DNA.[33] Furthermore, in view of the role of immunoselection in the generation of malignant cell populations with HLA class I defects,[34,35] as it will be discussed later, lack of immune responses against leukemic cells is unlikely to be the mechanism. This possibility is supported by the higher frequency of HLA class I antigen abnormalities in sporadic diffuse large cell lymphoma than in immunodeficient and transplant-related lymphomas.[36] Therefore, we favor the possibility that the lack of defects in HLA class I antigen expression identified in leukemia reflects the time interval between the onset of leukemia and its diagnosis, which is likely to be shorter than that of solid tumors. A short time interval between the onset of leukemia and diagnosis may not allow sufficient time for cells to acquire mutations in the gene(s) involved in HLA class I antigen expression and for selective pressure to facilitate the expansion of malignant cells with HLA class I abnormalities. In liver, normal hepatocytes, which do not express or express very low levels of HLA class I antigens,[33] acquire the expression of these antigens during malignant transformation. The results obtained with liver carcinoma cell lines suggest that HLA class I antigen upregulation may result from the induction of antigen processing machinery components by cytokines secreted by immune cells infiltrating malignant lesions.[19]

Role of T Cell Selective Pressure in the Generation of Lesions with HLA Class I Antigen Defects

Generation of cells with HLA class I antigen defects results from mutations in the gene(s) which are involved in the expression of HLA class I antigens. It is likely that these mutations occur randomly due to increased epigenetic changes and genomic instability in the early stages of tumor development.[8] One important question to ask is which mechanism(s) play(s) a role in the expansion of cells with HLA class I defects in malignant lesions. In view of the continuous exposure of tumor cells to the host's immune response,[34,35] one might ask whether immune selective pressure plays a major role in the expansion of cells with HLA class I antigen defects so that they become the major population in a lesion. As shown in Fig. 5, one can envision two possible scenarios: (i) if immune selective pressure plays a major role, then tumor cells with HLA class I defects expand because of escape from host's immune response which targets tumor cells without HLA class I antigen defects; (ii) if on the other hand, immune selective pressure does not play a role, then the expansion of tumor cells with HLA class I antigen defects is independent of the development of an immune response in the host. The available evidence derived from studies

in animal model systems and in patients treated with T cell-based immunotherapy argues in favor of a major role played by immune selective pressure in the generation of malignant lesions with HLA class I antigen defects.[37–39]

If immune selective pressure does play a major role in the generation of malignant lesions with HLA class I antigen defects, how does the host's immune system react when tumor cells have developed an escape mechanism to the ongoing immune response? Does the immune system change the target or is the immune system unable to mount an immune response to a different target? To the best of our knowledge, no studies have addressed these questions. The frequent detection of multiple HLA class I antigen defects in cell lines isolated from malignant lesions argues in favor of the possibility that a patient's immune response changes its target when a tumor cell population develops an escape mechanism. As illustrated in Fig. 6, when tumor cells develop an escape mechanism to evade an ongoing immune response targeted on immunodominant antigens, the host's immune system redirects its immune response toward subdominant antigens. This change in selective pressure will favor the expansion of a new population of tumor cells capable of escaping the new immune response. Subsequently, tumor escape variants, which have been generated in the early stages of immune response, would now be targeted by immune response at a later stage. Following these sequential phases of immune response, tumor cells with multiple defects would eventually be generated. If this interpretation is correct, a tumor would grow only when it has developed enough escape mechanisms to avoid the range of immune responses a patient has been able to mount against his own tumor.

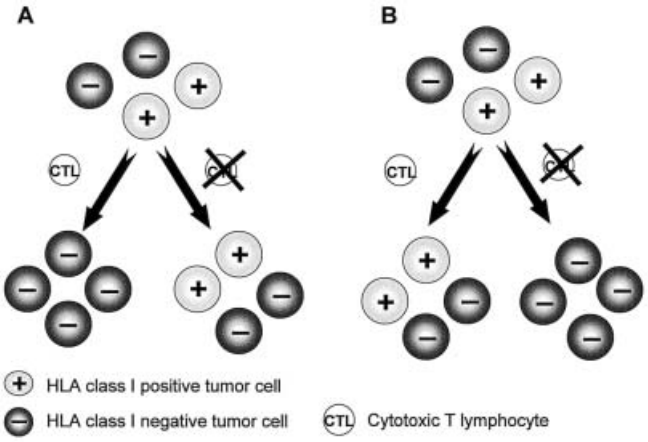

Fig. 5 Models of the role of T cell immunoselection in the generation of malignant lesions with HLA class I antigen abnormalities. (A) If T cell selective pressure plays a role in the generation of malignant lesions with HLA class I antigen loss, it is expected that tumor cells with HLA class I antigen expression will be selectively targeted and destroyed with the emergence and outgrowth of tumor cells without HLA class I antigen expression. (B) If T cell selective pressure does not play a role in the generation of malignant lesions with HLA class I antigen loss, then the emergence of tumor cells without HLA class I antigen expression will be independent of the presence of T cell selective pressure.

Clinical Significance of HLA Class I Abnormalities in Malignant Lesions

Abnormalities in HLA class I antigen expression in malignant lesions appear to have clinical significance, since they are associated with histopathological characteristics of the lesions and/or with clinical parameters in several malignant diseases.[3] As already mentioned, in general the frequency of HLA class I antigen defects in metastatic lesions is higher than that in primary

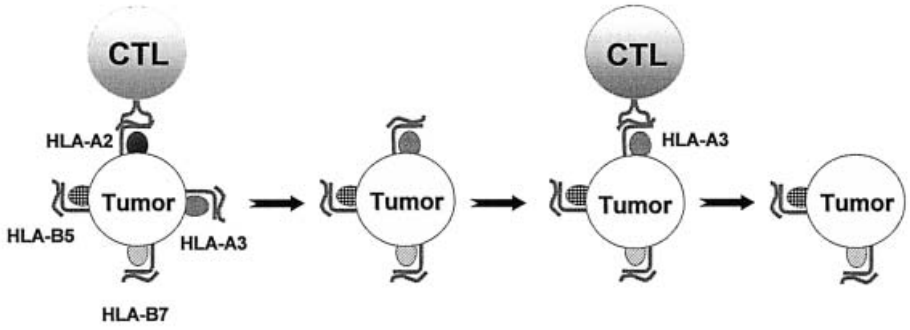

Fig. 6 Sequential immunoselection as a proposed mechanism for the generation of malignant cells with multiple HLA class I antigen defects. In the early stages of T cell-mediated immune response, tumor cells with the immunodominant HLA-A2-TAA peptide complex expression are targeted and destroyed, which results in the emergence and expansion of tumor cells without HLA-A2-TAA peptide complex expression. In response, the immune system redirects its immune response toward the subdominant HLA-A3-TAA peptide complex expressed by this tumor cell population, which results in the selection and expansion of tumor cells with neither HLA-A2-TAA peptide complex nor HLA-A3-TAA peptide complex expression. Eventually, tumor cells with multiple HLA class I antigen defects are generated.

Table 1 Association of HLA Class I Antigen Defects with the Clinical Course of Malignant Diseases

Poor prognosis	Favorable prognosis	No association
HNSCC	Uveal melanoma	NSCLC
Breast carcinoma	Colon carcinoma[a]	Pulmonary adenocarcinoma
SCLC		Adenocarcinoma of the cervix
Prostate carcinoma		Cutaneous squamous cell carcinoma
Bladder carcinoma		Large cell lymphoma[b]
Squamous cell carcinoma of the cervix[a]		Large cell immunoblastic lymphomas[b]
Cutaneous melanoma		

[a] Conflicting reports in the literature. [b] B- or T-lymphomas.

and premalignant lesions.[3] However, depending on the tumor type, HLA class I antigen defects can be associated, inversely correlated or not associated with disease progression and/or poor clinical outcomes (Table 1).[3] The reasons for these discrepancies are not known but may reflect differences in the characteristics of the patient population, the methods of analysis and/or the system used to score HLA class I antigen expression. In addition, these findings may be attributed to differences in types of immune response elicited by tumors of different tissue or differences in routes of metastasis. An example is represented by the opposite association of HLA class I antigen downregulation with the clinical outcome in cutaneous and uveal melanomas.[40] HLA class I antigen downregulation is associated with a poor prognosis in cutaneous melanoma, where CTL are believed to control the metastatic tumor spread via the lymphatics.[40] In contrast, HLA class I antigen downregulation is associated with a favorable clinical outcome in uveal melanoma, where NK cells, which tend to kill tumor cells with a low HLA class I antigen expression,[41,42] have been suggested to limit metastasis via the blood.

The major role played by the HLA class I-TAA peptide complex in the recognition of tumor cells by CTL can be further illustrated by the association found between abnormalities in the expression and/or function of antigen processing machinery components and poor clinical course of the disease in some malignancies.[3] This association most likely reflects the importance of these components in the generation of functional HLA class I-TAA peptide complexes. Notably, TAP1 downregulation has been reported to associate with tumor staging and reduction in patients' survival in breast carcinoma, SCLC, cervical cancer and cutaneous melanoma.[3] An increased frequency of TAP1 downregulation in metastatic lesions when compared to primary lesions has also been reported in breast carcinoma, cervical carcinoma and cutaneous melanoma.[3] Most recently, the role of tapasin in the clinical course of malignant diseases has been suggested by Ogino *et al.* who reported that tapasin downregulation in conjunction with HLA class I antigen downregulation was associated with reduced survival in patients with maxillary sinus squamous cell carcinoma.[43] It remains to be determined whether this finding applies to other types of tumors. Nevertheless, all of these findings are likely to reflect the crucial role of TAP1 and tapasin in the generation of HLA class I antigen-TAA peptide complexes and suggest that alterations in the repertoire of peptides presented by HLA class I antigens may provide an alternate route of immune escape for malignant cells. This possibility highlights the need to monitor specific HLA class I antigen-TAA derived peptide complex expression in malignant lesions. To this end, we have begun to develop probes capable of recognizing allospecific HLA class I antigen-TAA derived peptide complex expression on malignant cells (manuscript in preparation).

Conclusion

During the past ten years our understanding of the mechanisms underlying a tumor-specific immune response and tumor escape has substantially increased. HLA class I antigen defects remain an important tumor escape mechanism since they affect the interactions of tumor cells with HLA class I-restricted, TAA-specific T cells in the course of a malignant disease. Although this has not been formally proven yet, increasing findings of HLA class I antigen loss variants in recurrent metastatic lesions in patients who had experienced clinical responses following T cell-based immunotherapy[37,38] are compatible with a major role played by HLA class I antigen abnormalities in tumor cell escape from TAA-specific CTL lysis. The region of chromosome 6, which carries the human *MHC*,[10] is unstable during malignant transformation of cells [3]. Mutations may lead to downregulation or loss of the HLA class I antigen-TAA derived peptide complex which plays a key role in the interaction of tumor cells with CTL. The selective pressure imposed by CTL on tumor cell populations may result in the expansion of tumor cell subpopulations which are not recognized by host's immune system.

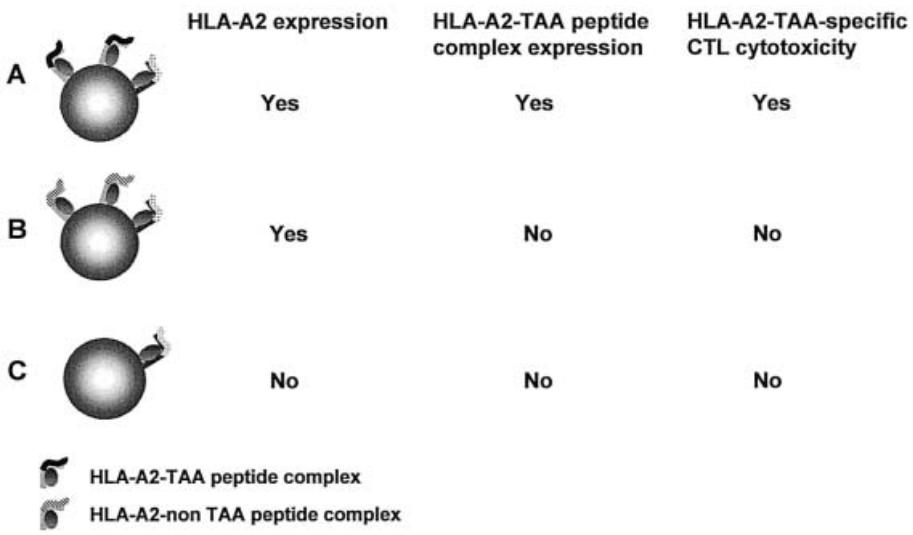

Fig. 7 Lack of absolute correlation between expression of a HLA class I alloantigen and that of HLA class I-TAA peptide complex on tumor cells. Detection of HLA-A2 antigen does not always correlate with expression of the HLA-A2-TAA peptide complex, which is recognized by HLA-A2-restricted, TAA-specific CTL. (A) Both HLA-A2 antigen and HLA-A2-TAA peptide complex are detected on a tumor cell. (B) HLA-A2-TAA peptide complexes are not expressed in spite of the detection of HLA-A2 antigen on a tumor cell. (C) Neither HLA-A2 antigen nor HLA-A2-TAA peptide complex are expressed on a tumor cell.

From a practical viewpoint, the possible role played by immune selective pressure in the generation of malignant lesions with HLA class I antigen defects suggests that the use of T cell-based immunotherapy for the treatment of malignant diseases may only be successful in a limited number of cases. Even when successful, it is likely that the selective pressure imposed by T cell-based immunotherapy will facilitate the emergence and expansion of tumor populations with HLA class I defects and eventually the recurrence of malignant lesions. Therefore, it will be important to combine T cell-based immunotherapy with other types of immunological and non-immunological strategies which utilize distinct mechanisms to control tumor growth. Furthermore, monitoring HLA class I antigen expression in malignant lesions during the course of T cell-based immunotherapy is important but may be misleading if merely expression rather than function of HLA class I antigens is assessed (Fig. 7). The latter can be accomplished by utilizing probes which recognize membrane-bound HLA class I-TAA derived peptide complexes. The development of these probes is underway in a number of laboratories including ours.[44–46] If proven useful in patients, these probes may greatly increase the efficacy of T cell-based immunotherapy for the treatment of malignant diseases.

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