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HLA antigen and NK cell activating ligand expression in malignant cells: a story of loss or acquisition

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Abstract

Malignant transformation of cells is often associated with changes in classical and non-classical HLA class I antigen, HLA class II antigen as well as NK cell activating ligand (NKCAL) expression. These changes are believed to play a role in the clinical course of the disease since these molecules are critical to the interactions between tumor cells and components of both innate and adaptive immune system. For some time, it has been assumed that alterations in the expression profile of HLA antigens and NKCAL on malignant cells represented loss of classical HLA class I antigen and induction of HLA class II antigen, non-classical HLA class I antigen and/or NKCAL expression. In contrast to these assumptions, experimental evidence suggests that in some cases dysplastic and malignant cells can acquire classical HLA class I antigen expression and/or lose the ability to express HLA class II antigens. In light of the latter findings as well as of the revival of the cancer immune surveillance theory, a reevaluation of the interpretation of changes in HLA antigen and NKCAL expression in malignant lesions is warranted. In this article, we first briefly describe the conventional types of changes in HLA antigen and NKCAL expression that have been identified in malignant cells to date. Second, we discuss the evidence indicating that, in at least some cell types, classical HLA class I antigen expression can be acquired and/ or the ability to express HLA class II antigens is lost. Third, we review the available evidence for the role of immune selective pressure in the generation of malignant lesions with changes in HLA antigen expression. This information contributes to our understanding of the role of the immune system in the control of tumor development and to the optimization of the design of immunotherapeutic strategies for the treatment of cancer.

Keywords

Antigen processing machinery; Cancer; Classical HLA class I antigen; Immune escape; Immune selection; HLA class II antigen; MICA; MICB; NK cell activating ligand; Non-classical HLA class I antigen; ULBP

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Introduction

In humans, like in other animal species, malignant transformation of cells is often associated with changes in gene expression and in their antigenic profile. They include changes in classical and non-classical human leukocyte antigen (HLA) class I [1] and class II [2] as well as natural killer cell activating ligand (NKCAL) [3–5] expression. These changes have been convincingly documented in a number of malignant tumors by analyzing cell lines in long-term culture and surgically removed lesions [1–5]. Cell lines have provided the opportunity to identify and characterize the multiple molecular mechanisms underlying changes in HLA antigen and NKCAL expression and to analyze their functional implications. On the other hand, surgically removed lesions have provided the opportunity to prove that the changes found in cell lines are not an in vitro artifact, but reflect in vivo changes. Furthermore, they have allowed investigators to assess the clinical significance of these changes. A number of studies suggest that changes in the expression pattern of these molecules play a role in the clinical course of the disease since they have been associated, in at least some tumor types, with prognosis as well as disease-free interval and survival [1–5]. These associations are likely to reflect the critical role these molecules play in the interactions of tumor cells with components of both innate and adaptive immune system [1–5] (Fig. 1). Nevertheless, the biological and clinical significance of HLA antigen and NKCAL changes remains under debate [6]. The debate has focused on whether HLA antigen and NKCAL changes are simply the by-product of genomic instability or reflect selection of tumor cells with HLA antigen or NKCAL changes secondary to immune selective pressure. This debate also stems, at least in part, from the assumptions investigators have made over the years in terms of changes in HLA antigen and NKCAL expression in malignant lesions. In this regard, changes in classical HLA class I antigen expression in malignant lesions are assumed to represent loss [1, 2] since it has been propagated through textbooks of immunology that classical HLA class I antigens are expressed by all nucleated cells [7, 8]. On the other hand, changes in non-classical HLA class I antigen, HLA class II antigen, and NKCAL expression are assumed to represent appearance [1–5] since these antigens are believed to have a restricted distribution in normal tissues [7–9]. However, there is evidence that dysplastic and malignant cells can acquire classical HLA class I antigen expression and/or lose the ability to express HLA class II antigens. The latter observations challenge our past assumptions regarding the mechanisms underlying changes in the expression of these molecules in malignant lesions.

In light of the experimental evidence demonstrating that in some cases dysplastic and/or malignant cells can acquire classical HLA class I antigen expression and/or lose the ability to express HLA class II antigens as well as of the revival of the cancer immune surveillance theory [10], a reevaluation of the interpretation of changes in HLA antigen and NKCAL expression in malignant lesions is warranted. In this article, we review this topic since this information may contribute to our understanding of the role of the immune system in the control of tumor development and to the optimization of the design of immunotherapeutic strategies for the treatment of cancer. Moreover, this information is likely to contribute to the resolution of some of the conflicting information in the literature regarding the clinical relevance of HLA antigen and NKCAL changes in malignant disease. Specifically, we first briefly describe the types of changes in HLA antigen and NKCAL expression that have been identified in malignant cells to date. Second, we discuss the evidence indicating that, in at least some cell types, malignant transformation may actually be associated with the appearance of classical HLA class I antigens and/ or loss of the ability to express HLA class II antigens. Lastly, we summarize the evidence for the role of immune selective pressure in the generation of malignant lesions with HLA antigen defects.

Analysis of HLA antigen and NKCAL expression in malignant lesions

A conventional view

Beginning in the 1980s and continuing today, a large number of malignant lesions have been tested with classical HLA class I and HLA class II antigen-specific mAb [1, 2]. More recently, these studies have been expanded to include the analysis of the expression of non-classical HLA class I antigens such as HLA-E, -F, and -G, as well as NKCAL including the phylogenetically distant MHC class I related chain (MIC) [1–5] and the UL16-binding proteins (ULBP) [1–5]. At present, the available information regarding non-classical HLA class I antigen and NKCAL expression is still limited since the field is in an early stage. Moreover, progress in this area is hindered by the lack and/or limited availability of non-classical HLA class I antigen- and NKCAL-specific mAb, with the appropriate specificity that is suitable for IHC staining.

As reviewed elsewhere [1, 2], convincing evidence indicates that malignant transformation of cells is frequently associated with changes in classical HLA class I and HLA class II antigen expression (Fig. 2). These studies, which include our own, have concluded that classical HLA class I antigens are lost or downregulated, whereas HLA class II antigens are induced on malignant cells [1, 2]. The molecular mechanisms underlying changes in HLA antigen expression in malignant cells include structural gene abnormalities as well as defective regulation of HLA gene transcription and translation [1, 2]. More recently, epigenetic events associated with tumor development and progression have been found to underlie changes in HLA antigen, antigen processing machinery, co-stimulatory molecule, and tumor antigen (TA) expression in malignant cells [2]. It is noteworthy that in some tumors such as breast, colon, and cervical carcinoma, HLA class II antigen expression is not restricted to cells that have undergone malignant transformation [1, 2]. Among the non-classical HLA class I antigens, HLA-G expression has been studied the most extensively [1, 2]. Although the results in the literature conflict, there is a general agreement that malignant transformation of cells may be associated with the appearance of HLA-G, the frequency being quite different among the various types of tumors analyzed (Fig. 3) [1, 2, 11, 12]. In contrast to HLA-G, less information is available regarding HLA-E and HLA-F expression by malignant cells [1, 2, 13–16]. In surgically removed malignant lesions, HLA-E expression is expressed in glioblastoma, carcinomas of colon and ovary, lymphoma, and melanoma [1, 2, 13–16]. Only cell lines derived from EBV-transformed lymphoid cells as well as monocytic, glioblastoma, liver carcinoma, and transitional cell bladder cells have been found to express HLA-F on the cell surface [1, 2].

Information regarding NKCAL expression by malignant cells is derived from the analysis of only a limited number of surgically removed malignant lesions. Nevertheless, the results of these studies, which have been corroborated by those derived from the analysis of cell lines in long-term culture of different embryological origin [1–5], indicate that MICA and MICB have a much broader distribution in malignant tumors than in normal tissues, as they are expressed in solid tumors derived from the breast, lung, colon, kidney, ovary, and pancreas as well as glioblastoma, neuroblastoma, and cutaneous and uveal melanoma [1–5]. Moreover, both MICA and MICB have been found to be expressed in hematologic malignancies such as acute and chronic lymphatic leukemia, and acute and chronic myeloid leukemia [1–5]. ULBP expression has been examined only in glioma, leukemia, and melanoma [1–5]. In general, ULBP molecules are expressed less frequently than MIC molecules, and MIC and ULBP do not appear to be expressed in a coordinated fashion in the tumor cells examined. In addition, the available information suggests that the frequency of MIC and ULBP expression is independent of classical and non-classical HLA class I antigen expression.

A contemporary view

The view that HLA class I antigens are frequently lost or downregulated on malignant cells is based on the assumption that classical HLA class I antigens are expressed on all nucleated cells except for immunoprivileged tissues (e.g., brain, cornea, anterior chamber of the eye, liver, testis, fetotrophoblast, hair matrix, and proximal nail matrix) [7, 8, 17–25]. The view that malignant transformation of cells may be associated with the appearance of HLA class II antigens is based on the assumption that these antigens are constitutively expressed only by hematopoietic antigen presenting cells (APC) including B lymphocytes, dendritic cells (DC), and macrophages [7–9]. However, a number of studies in the literature which for unknown reasons are ignored by the scientific community argue against the aforementioned assumptions regarding classical HLA class I antigen as well as class II antigen expression in normal tissues. First, classical HLA class I antigens are not expressed on all nucleated cells. In addition to immunoprivileged tissues [17–25], classical HLA class I antigens are not always detected on adipocytes, chondrocytes, hepatocytes, smooth and skeletal muscle cells, epithelial cells of parathyroid, acinar pancreas, biliary duct, nested melanocytes in benign nevi, basal layer melanocytes, ureter endothelia, and sympathetic ganglia [26–39]. Moreover, in some cases, such as epithelial cells of trachea glands, bronchial glands, esophagus, and stomach, classical HLA class I antigens are expressed in the cytoplasm and not on the cell membrane [29]. Along the same lines, HLA class II antigen expression in normal tissues is not restricted to APC. In this regard, it is well known that HLA class II antigen expression can be induced on a number of cell types including, but not limited to, endothelial cells, epithelial cells, keratinocytes, fibroblasts, and mast cells as well as melanocytes upon incubation with interferon-γ (IFN-γ) [40, 41]. Furthermore, as noted above in tumors such as breast, colon, and cervical carcinoma, HLA class II antigens are expressed not only by cells that have undergone malignant transformation but also by normal breast, colon, and cervical epithelial cells [1, 2]. In addition, HLA class II antigen expression has also been observed on several normal nucleated cells of different embryological origin under basal conditions. They include the epithelium of the bronchial glands, gastrointestinal tract, urinary bladder, and thymic reticuloepithelial cells among cells of entodermic origin; epithelium of mammary gland, acinar cells of parotid, and astrocytes among cells of ectodermic origin; breast, glomerular, and peritubular renal endothelium, cervix, and endometrium among cells of the mesoderm, as well as keratinocytes and nested melanocytes in benign and atypical (dysplastic) melanocytic nevi [39, 42, 43]. Second, there are examples in the literature demonstrating that both classical HLA class I antigen and HLA class II antigen expression can be detected in sites of immune privilege under normal conditions [29, 42, 43] as well as in patients with autoimmune and inflammatory conditions such as alopecia areata, hepatitis, dermatomyositis, psoriasis, rheumatoid arthritis, and systemic lupus erythematosus [17, 29, 42–53]. Lastly, there is evidence to suggest that non-malignant, pre-malignant as well as malignant cells can acquire classical HLA class I antigen expression. In this regard, acquisition of classical HLA class I antigen expression has been observed in halo nevi [54] as well as some forms of hepatocellular [1, 2] and testicular [1, 2] carcinomas. More recently, we have performed an analysis of HLA antigen expression in a panel of surgically removed benign and atypical (dysplastic) nevi [39]. We have observed that among benign nevi, only halo nevi express HLA class I heavy chain and HLA class II β chain (Fig. 4). On the other hand, HLA class I heavy chain and HLA class II β chain were expressed in more than 70% of the atypical (dysplastic) nevi (Fig. 4). The latter are associated with an increased risk of melanoma and, at least in some cases, represent a precursor of malignant melanoma. It is noteworthy that the level of HLA class I heavy chain and of HLA class II β chain expression in atypical (dysplastic) nevi correlated with the degree of cytologic atypia and architectural disorder. The latter distinction has important clinical implications since the degree of cytologic atypia and architectural disorder are two criteria employed most often in the histologic diagnosis of atypical (dysplastic) nevi

and the degree of histologic atypia in AN has been associated with melanoma risk [55, 56]. In view of the role of lymphocyte-mediated destruction of melanocytes in the development of halo nevi, it is likely that HLA antigen expression renders melanocytes more susceptible to both CD8(+) and CD4(+) T-cell recognition and killing [54, 57]. However, whether the appearance of HLA antigens on melanocytes in halo nevi as well as atypical (dysplastic) nevi reflects their induction via some form of cellular stress and/or is secondary to cytokine release by lymphocytes present within the microenvironment remains to be determined.

The data described above suggests that HLA class I antigens have a more restricted distribution in normal tissues, whereas HLA class II antigens have a broader distribution in normal tissues than originally described. Moreover, there is increasing evidence demonstrating that HLA class II antigens presenting TA-derived peptides can serve as a target for TA-specific CD4(+) T cells and be subject to immune selective pressure [58–60]. Therefore, previous assumptions regarding the HLA antigen phenotype of malignant cells may need to be reevaluated. In fact, a thorough review of the literature demonstrates that classical HLA class I antigen loss and HLA class II antigen induction do not occur in every type of malignant disease. Specifically, HLA class I antigen defects are rarely observed in tumors derived from hepatocytes, uveal melanocytes, testicular germ cells as well as hematologic malignancies [1, 2]. Moreover, colon cancer cells [61] which demonstrate microsatellite instability (MSI) as well as primary mediastinal B-cell lymphoma and classical Hodgkin lymphoma cell lines [62] lose HLA class II antigen expression due to somatic mutations affecting HLA class II antigen-regulatory genes [61, 62]. The loss of HLA class II antigen expression is associated with decreased survival in primary mediastinal B-cell lymphoma (PMBCL) patients [62]. Lastly, the inability to upregulate HLA class II antigen expression has been documented in tumors as well as cell lines derived from carcinoma of breast, stomach, colon, cervix, cutaneous epithelia, plasma cells as well as from T-cell leukemia, neuroblastoma, teratocarcinoma, choriocarcinoma, and uveal and cutaneous melanoma [1, 2].

The findings we have discussed above suggest that the manner in which investigators have depicted the HLA antigen status of malignant cells in the past may not reflect the true biology of HLA antigen expression. Whether this premise may also be applied to non-classical HLA class I antigen and NKCAL expression remains to be determined since this field is in an early stage. Nonetheless, an alternative view of the HLA antigen and NKCAL status may be that in tumors, like in cases of infection [3–5, 63–68], HLA antigen and/or NKCAL expression is upregulated and/or lost depending on the nature of the host's immune response. In this regard, intense selective pressure acting on malignant cells is likely to evoke (via mutation and selection) a wide range of defensive strategies, including changes in HLA antigen and NKCAL expression, which enable them to survive immunological attack. In the following sections, we will review the available evidence for the role of immune selective pressure in the generation of the HLA antigen and NKCAL phenotype of tumor cells.

Role of immune selective pressure in the HLA antigen and NKCAL phenotype of malignant cells

Cancer immune surveillance

In the last decade, the cancer immune surveillance theory has been revived by countless studies in mice supporting the notion that both CD8(+) and CD4(+) T cells and NK cells are engaged in the control of tumor cell growth [4, 6, 60, 69–73]. Specifically, frequencies of spontaneously arising tumors or tumors induced by the chemical carcinogen methylcholanthrene (MCA) are higher in mice that are genetically deficient for key effector

molecules of T and/or NK cells or their respective receptors, i.e., IFN-γ R1−/−[74], IFN-γ−/− [75], perforin (pfp)−/− [75–78], RAG-2−/−[79, 80], JAK2 [81, 82], ABL [79–81], STAT1−/− [74, 80], TCRJα281−/− [80, 83], TCRβ−/− [80, 84], and TCRδ−/−[80, 83]. Along similar lines, treatment of mice with MCA induces some cancers of the occult type that grow out only in the presence of concomitant immunosuppression [85]. Additional studies have provided evidence that the anti-tumor activities of NK cells may not only directly lead to tumor eradication by means of cytolysis or IFN-γ secretion but may also indirectly contribute to tumor control by inducing an efficient TA-specific immune response [86]. In humans, evidence for a role of the immune system in preventing tumor growth is derived from correlative studies in patients with solid organ and bone marrow transplants, HIV/AIDS, and primary immune deficiencies. These studies have shown that in these patients, the incidence of a variety of malignancies originating from brain, thyroid, breast, colon, liver, pancreas, kidney, prostate, cervix, bone, connective tissue as well as cutaneous squamous cell carcinoma, basal cell carcinoma, melanoma, Kaposi's sarcoma, and hematologic malignancies is increased [87–106]. These correlative studies have been further supported by the association between infiltration of tumors with T or NK cells and positive prognosis in patients with different types of malignancies [107–112]. More recently, convincing evidence for a beneficial role of NK cells in control of human malignancies comes from the association between (1) low NK cell-like cytotoxicity of peripheral blood lymphocytes and an increased risk for cancer [108] and (2) leukemia patients receiving alloreactive NK cells in the course of allogeneic hematopoietic stem cell transplantation and an increase in disease-free relapse and survival [113, 114].

Immune selective pressure

Given the mounting evidence of cancer immune surveillance along with the observation that dysplastic and malignant cells can acquire classical HLA class I antigen and/or lose HLA class II antigen expression, it is likely that the actual status of HLA antigen as well as NKCAL expression on malignant cells reflects the complex interplay between tumor microenvironment, host's immune system, and tumor cells. In this regard, it is assumed that tumors arise from a single normal cell by a series of cumulative genetic and epigenetic changes through a sequential evolutionary process of mutation and selection. Malignant cells within a tumor may harbor different mutations in a number of critical genes at various stages during the evolution of the tumor, providing some malignant cells with a selective advantage [115, 116]. Although genetic and epigenetic alterations drive cellular transformation, multiple signals delivered within the microenvironment through the release of intracellular components termed DAMPs (damage-associated molecular patterns) from damaged or dying malignant cells [117–120] as well as the release of soluble factors by stromal, endothelial, and immune cells are critical factors in determining the progression versus dormancy or destruction of a dysplastic or malignant cell. Therefore, this complex interplay between tumor cell heterogeneity and tumor microenvironment not only determines but also shapes the phenotype of malignant cells towards generation of mutant resistant variants [121, 122]. To this end, tumor evolution is thought to adhere to Darwinian principles by escaping both non-immune (intrinsic) and immune (extrinsic) responses against self-altered malignant cells.

The notion that the type of host immune response elicited by a tumor may determine the HLA antigen or NKCAL phenotype of a malignant cell is not novel. During the 1920s, Little and Snell [123, 124] demonstrated selection of tumor cell variants with MHC class I antigen loss in inbred mice. Moreover, metastatic tumor variants derived from transplants into normal mice regularly lose MHC class I antigen expression, while cells from similar transplants into immunocompromised (athymic nude) mice do not [125]. More recently, Harding's group has shown that immune selective pressure targeting a H2-restricted

cytotoxic T lymphocyte (CTL) epitope can isolate tumor cells lacking the targeted epitope by failing to express the MHC-anchored peptide from a tumor cell population [126]. Specifically, using matched panels of TAP1(+) and TAP1 (−) tumor cell lines generated from a parental transformed murine fibroblast line, Harding and co-workers demonstrated that both TAP1(+) and TAP1(−) cells produce tumors in athymic mice, while only TAP1(−) cells form persistent tumors in the immunocompetent autologous C57BL/6 mice [126]. Moreover, inoculation of C57BL/6 mice with mixtures of TAP1(+) and TAP1(−) cells produced tumors composed exclusively of TAP1(−) [126]. These data suggest that the selection pressure applied by MHC class I antigen-restricted, antigen-specific CTLs favors the outgrowth of cells with defective presentation of antigen-derived peptides because of TAP defects. In other words, the tumor's MHC phenotype has been "immunoedited" in the course of the disease, resulting in the survival of tumor variants with defective presentation of antigen-derived peptides by MHC class I antigens. Along similar lines, both we [Ferrone unpublished] and others [127] have observed that adoptive transfer of antigen-specific CTL to SCID mice implanted with autologous melanoma cells leads to immunoselection of HLA class I antigen and TA loss variants. In parallel with HLA antigens, it is worth noting that there is evidence to suggest that NKCAL may also be a target of immune selection. In this regard, a higher frequency of NKCAL MICA/B loss has been observed in metastatic melanoma lesions than in primary lesions [128]. Further, experimental data in vitro have shown that MICA loss was associated with resistance to NK cell-mediated lysis of two human melanoma cell lines isolated from recurrent metastases in spite of lack of HLA class I antigen expression [129, 130].

The results obtained in the aforementioned animal studies are paralleled by those obtained in clinical settings. Jager et al. [131] have demonstrated total HLA class I antigen loss and selective HLA-A2 antigen loss in three of five and one of five, respectively, melanoma lesions which progressed in spite of the expression of the targeted TA and of the presence of a TA-specific T-cell response in patients immunized with MART-1- and tyrosinase-derived, HLA-A2-binding peptides. Similar results have been described by Khong et al. [132] who have characterized melanoma metastases that recurred after an initial dramatic clinical response in a patient immunized with gp100-, MART-1-, and tyrosinase-derived peptides. T-cell clones specific to these TA were present in the patient's peripheral blood as well as in the isolated tumor-infiltrating lymphocytes (TILs). In one recurrent melanoma metastasis, multiple TA had been lost, but HLA class I antigen expression was retained [132]. In another recurrent metastasis, HLA class I antigens were not detectable, while TA continued to be expressed [132]. Restifo et al. [133] have described total HLA class I antigen loss in recurrent melanoma metastases in five patients who experienced an initial clinical response following T-cell-based immunotherapy. Rosenberg's group have demonstrated loss of TA as well as HLA class I antigens in patients treated with TA-peptide- [132] and autologous cell transfer-based [134] therapies. More recently, Garrido et al. have demonstrated an increased incidence of alterations in HLA class I antigen expression in patients with recurrent bladder cancer treated with the Bacillus Calmett–Guerin (BCG) immunotherapy, whereas mitomycin treatment did not change the pattern of HLA class I antigen expression [135]. It is noteworthy that a number of molecular mechanisms have been found to underlie defects in HLA class I antigen expression [1]. Among them is loss of HLA class I antigen expression by tumor cells due to mutations in one copy of the beta-2-microglobulin (β2m) gene associated with loss of the other copy [1, 132, 136]. These findings reflect the critical role β2m plays in the expression of classical HLA class I antigens on the cell membrane [137]. Additional studies have revealed a mutational hotspot in the β2m gene that may be associated with immune selective pressure introduced by T-cell-based immunotherapy [136]. The presence of this mutational hotspot implies a relationship between the modified tumor microenvironment during immunotherapy and the type of genomic instability and/or DNA repair capacity possessed by tumor cells. The latter relationship is further supported by

an elevated frequency of β2m gene CT dinucleotide deletion mutations identified in MSI (+) colon carcinoma lesions [61, 138]. The β2m gene mutations may be preferentially selected by T cell selective pressure. In this regard, the impairment of HLA class I antigen expression is a frequent even in MSI (+) colon carcinoma [140] and predominantly mediated by frameshift mutations of the β2m gene [137, 141, 142] likely reflecting immunoselective pressure. In view of these findings, the outgrowth of MSI (+) colon carcinoma cells that lack HLA class I as well as class II antigen expression is compatible with the selection of HLA class I and class II antigen-deficient tumor cells during MSI (+) colon carcinoma tumorigenesis. Although the exact mechanisms of such a selection process are not known at present, it is tempting to speculate that tumor-infiltrating CD8(+) as well as CD4(+) T cells recognize MSI (+) colon carcinoma cells that expression TA-derived peptides on the cell surface via HLA class I and class II antigens. This hypothesis is in line with the observation that MSI (+) tumors are usually infiltrated with large numbers of activated CTLs presumably recognizing neoepitopes generated by the tumor's genomic mutator phenotype [61, 138, 139] and a recent report of a HLA-DR-restricted CD4(+) T-cell response in a MSI (+) colon carcinoma patient [143].

Conclusion

The results we have summarized demonstrate that dysplastic and malignant cells can acquire classical HLA class I antigen expression and/or lose the ability to express HLA class II antigens. Given the mounting evidence that immune selection most likely underlies the generation of immunoresistant tumor variants, our past assumptions regarding HLA antigen as well as NKCAL expressions in malignant lesions may not be accurate. It seems more likely that the status of HLA antigen as well as NKCAL expression on malignant cells reflects the complex interplay between tumor microenvironment, host's immune system, and tumor cells. Therefore, it is our belief that more appropriately defining the HLA antigen and NKCAL phenotype of malignant cells may provide insights into the actual type of immune response generated as well as potential mechanism of immune selection in different malignancies. Previous assessments of the status of HLA antigen and NKCAL expression on tumor cells most likely do not give the "whole" picture since these studies only provide a static account of the level of expression of these molecules. From a clinical standpoint, this static picture most likely clouds our ability to determine the true clinical relevance of HLA antigen as well as NKCAL expression on tumor cells since their level is likely to change throughout the course of the disease.

On the basis of the aforementioned experimental results and clinical data, immune selection may be viewed as conceptually equivalent to the theory of evolution proposed by Darwin more than a century ago [144, 145] (Fig. 5). During an early stage of tumor development, HLA antigen or NKCAL expression on pre-malignant or malignant cells may be increased to reflect the genetic instability of these cells and the activation of the intrinsic DNA damage response pathways [3–5]. At this stage, whether the immune system is activated most likely depends on the nature of the tumor microenvironment. In this regard, the tumor microenvironment can have potential positive or negative impacts on the ability of the host's immune system to recognize tumor cells and to control tumor growth [146]. If a favorable tumor microenvironment exists, the net change at this stage is a shift in the "immune balance" toward activation and immune surveillance is initiated. Since HLA class I antigens play a crucial role in the control of tumor growth by CTLs in the tumor microenvironment, HLA antigen abnormalities in tumor cells may be envisioned as the result of immune selection advantageous to tumor cell survival in situations where T cells play a major role in controlling tumor growth. On the other hand, because the activity of NK cells is inhibited by MHC class I molecules [3–5], in situations where NK cells provide the major source of selective pressure, HLA antigen expression may be indeed advantageous to tumor survival.

This possibility is supported by the high HLA class I antigen expression level in uveal melanoma [1, 2], carcinomas of the breast [147], lung [148] and liver [1, 2] as well as leukemia [1, 2] and lymphoma [1, 2] where NK cells are thought to control tumor progression. Nonetheless, it must be stressed that NKCAL expression is not always beneficial to immune surveillance. In this regard, non-classical HLA antigen expression by tumor cells may induce apoptosis of NK cells upon ligation of activating receptors via a Fas–FasL-dependent mechanism [1, 2, 149]. Moreover, sustained membrane-bound as well as soluble NKG2D ligand expression in vivo, either systemically or locally, actually impairs NKG2D-mediated immune control of tumor growth by downregulating the NKG2D receptor and inhibition of NK-cell-mediated killing [149]. At a later stage in tumor development, the tumor site is most likely dominated by escape variants expressing low levels of HLA antigens and medium to low levels of NKCAL on the plasma membrane, but shedding and/or secreting large amounts of soluble NKCAL or non-classical HLA class I molecules. The net influence in this period is sustained NKCAL receptor downregulation, resulting in impaired NK cell control of tumor growth, and inability of CD4(+) or CD8(+) T cells to recognize and destroy tumor cells.

The challenge for the tumor immunologists now is to more appropriately characterize HLA antigen and NKCAL expression on benign, dysplastic, and malignant cells in order to understand the mechanisms by which tumors become refractory to the immune system. The mechanisms underlying the ability of cells to up- or downregulate HLA antigen and/or NKCAL expression are most likely due to multiple variables. Changes in HLA antigen expression have been attributed to defects in β2m synthesis, loss of the gene(s) encoding HLA antigen heavy chain(s), mutations which inhibit HLA antigen heavy chain transcription or translation, defects in the regulatory mechanisms which control HLA antigen expression, and/or abnormalities in one or more of the antigen processing machinery components [1, 2]. More recently, epigenetic events associated with tumor development and progression have been found to underlie changes in HLA antigen, antigen processing machinery, co-stimulatory molecule, and TA expression in malignant cells [2]. In this regard, the ability of epigenetic drugs to restore the defective HLA antigen, APM component and co-stimulatory molecule expression, and the consequent increase in immune recognition of malignant cells provides us with new therapeutic tools that may improve the clinical efficacy of active-specific immuno-therapy for the treatment of malignant disease. Nonetheless, to date very little attention has been focused on the regulation of HLA antigens in malignant lesions following treatment with DNA methyltransferases (DNMT) and histone deacetylases (HDAC) inhibitors. Future studies aimed at identifying the cell-type-specific molecular mechanisms underlying the transcriptional and post-transcriptional regulation of HLA antigen and NKCAL in benign and malignant cells should be undertaken. Moreover, investigations should be directed at determining the ability of epigenetic pharmacologic agents to modulate HLA and NKCAL expression in malignant lesions. In addition, investigations need to be tailored towards characterizing the interplay between tumor microenvironment and immune effector cells. This information may suggest strategies to overcome the barriers posed by the microenvironment to an effective destruction of tumor cells mediated by immunological mechanisms. Lastly, as investigators, we must keep in mind that the status of HLA antigen and NKCAL expression on malignant cells is not static. The levels of these antigens most likely fluctuate throughout the course of a patient's immune response. Studies should be directed towards characterizing not only the status of HLA antigen and NKCAL expression on malignant cells but also the nature of TA-specific immune responses in patients at different stages of tumor development. A better understanding of the mechanisms influencing the status of HLA antigen and NKCAL expression in malignant cells will likely translate into the optimization of the design of immuno-preventative as well as immuno-therapeutic strategies for the treatment of cancer. Moreover, the combination of immunization strategies with approaches that counteract

changes in HLA antigen as well as NKCAL expression may enhance the clinical efficacy of immunotherapy.

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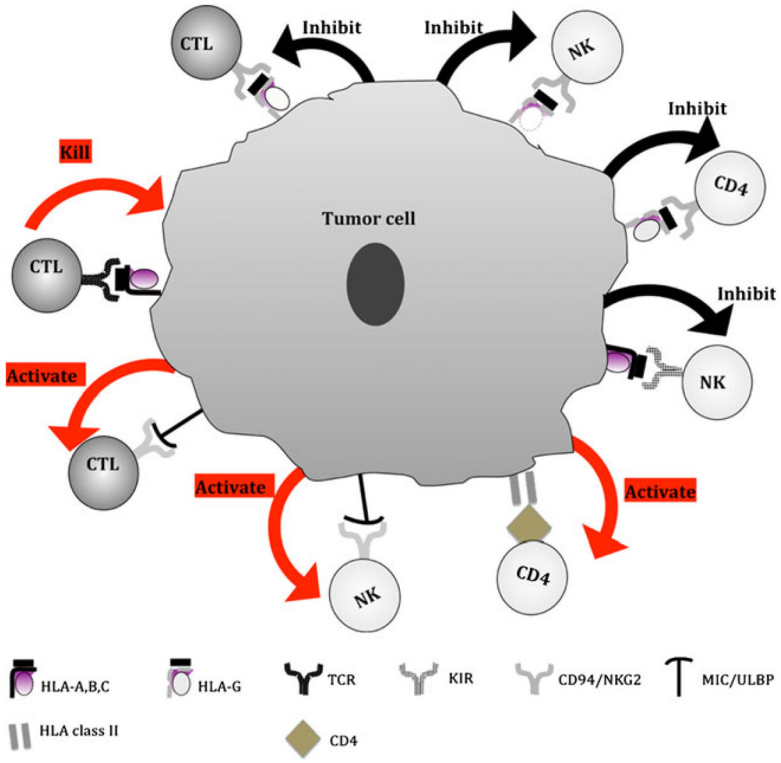

Fig. 1.

Molecular mechanisms underlying the functional properties of HLA antigen and NKCAL expressed by malignant cells. Once the classical HLA class I-β_2m-peptide complex is transported to the plasma membrane, it plays a major role in the interactions between target cells and (a) activation of peptide-specific CTL through TCR; and (b) inhibition of T-cell subpopulations through inhibitory receptors KIR. In contrast to classical HLA class I antigens, the non-classical HLA class I antigens, HLA-G, inhibit CTL, CD4(+) T cells and NK cells through their interaction with the NK cells receptor CD94/NKG2. HLA class II expression by tumor cells may be potentially beneficial to TA-specific immune responses through their interaction with CD4(+) T cells, resulting in the activation of CD4(+) T-cell-mediated killing, macrophage through release of Th1 cytokines; B cells and eosinophils through release of Th2 cytokines, and CTL through release of Th1 cytokines

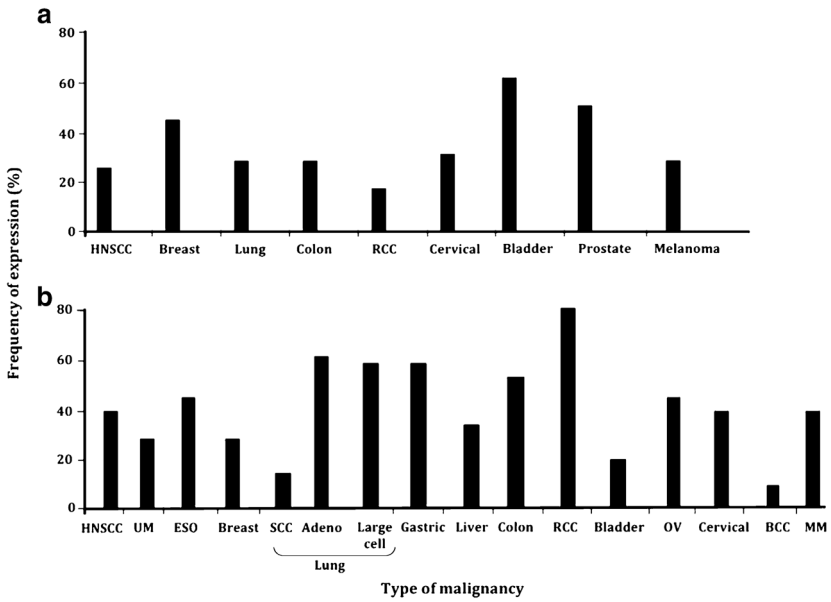

Fig. 2.

Frequency of classical HLA class I antigen downregulation and HLA class II antigen expression in malignant lesions of different embryological origin. Solid tumors for which more than 50 primary lesions have been analyzed for **a** classical and **b** HLA class II expression are shown. *BCC* cutaneous basal cell carcinoma, *ESO* esophageal carcinoma, *HNSCC* head and neck squamous cell carcinoma, *MM* malignant melanoma, *OV* ovarian carcinoma, *RCC* renal cell carcinoma, *SCC* squamous cell carcinoma, *UM* uveal melanoma

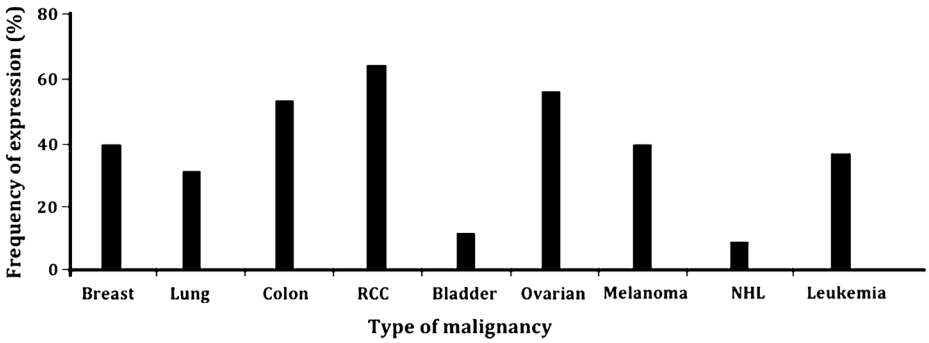

Fig. 3.

Frequency of non-classical HLA class I antigen, HLA-G, expression in malignant lesions of different embryological origin. Solid tumors for which more than 50 primary lesions have been analyzed for non-classical HLA class I antigen, HLA-G, expression are shown. *NHL* non-Hodgkin's lymphoma, *RCC* renal cell carcinoma

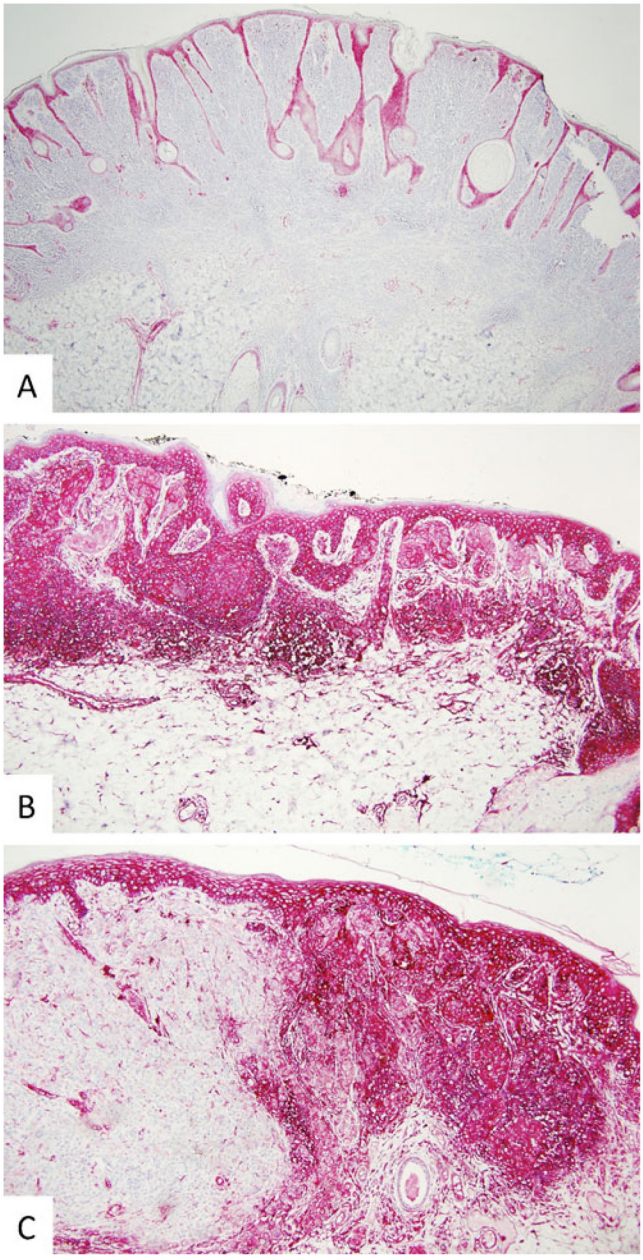

Fig. 4.

Differential HLA antigen expression in benign and atypical melanocytic nevi, in cutaneous melanoma and in surrounding normal cells. **a** Only normal keratinocytes, endothelial cells, and antigen-presenting cells were marked by HLA class I heavy chain-specific mAb HC-10 in the immunoperoxidase reaction in an intradermal nevus (×10). **b** Normal keratinocytes, endothelial cells, antigen-presenting cells, and melanocytes were marked by HLA class I heavy chain-specific mAb HC-10 in the immunoperoxidase reaction in a severely atypical nevus (×10). **c** Normal keratinocytes, endothelial cells, and malignant melanocytes but not intradermal nested melanocytes or vertical growth phase melanoma cells were marked by HLA class I heavy chain-specific mAb HC-10 in the immunoperoxidase reaction in a superficial spreading melanoma arising within an intradermal nevus (×10)

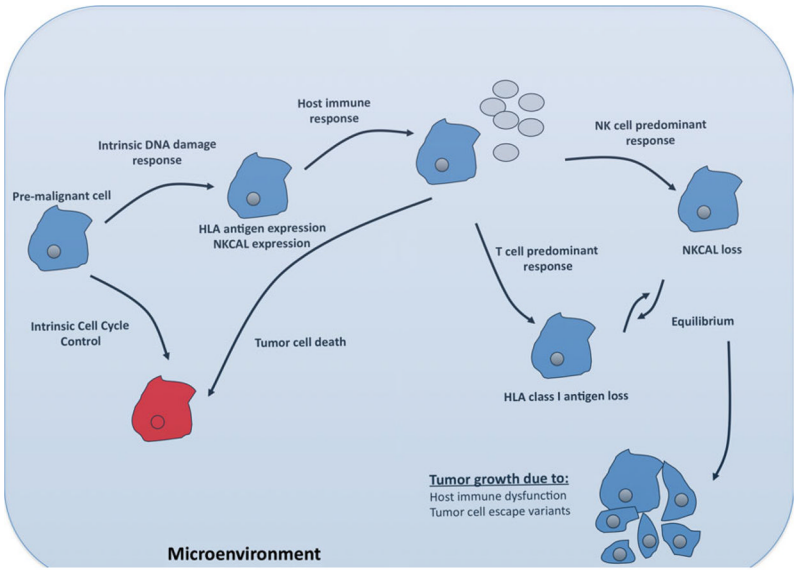

Fig. 5.

Immunoselection and microevolution of tumors. Tumor evolution is thought to adhere to Darwinian principles by escaping both non-immune (intrinsic) and immune (extrinsic) responses against self-altered malignant cells. Pre-malignant cells that acquire genetic mutations may undergo apoptosis or in parallel, acquire the expression of HLA as well as NKCAL through intrinsic cell-cycle control or DNA damage response mechanisms. Those cells resistant to apoptosis may be targeted by the host's immune system. The type of immune response elicited by each tumor is most likely dependent upon a number of factors including the etiology of the tumor, patient characteristics as well as tumor microenvironment. During this immune response, the selective pressure facilitates the outgrowth of tumor cells that have lost the molecule(s) targeted by the ongoing immune response. Equilibrium most likely develops between the tumor cell escape variants and the adapting host's immune response. At some point, the immune response is unable to adapt to the changing tumor cell population resulting in tumor growth. It is noteworthy that a cause–effect relationship between multiple rounds of immune selection and the appearance of multiple HLA or NKCAL abnormalities has not been proved yet. Nevertheless, if correct, our view about the role of immune selection in the generation of a malignant cell phenotype implies that a tumor will grow only when it has developed enough escape mechanisms to avoid the range of immune responses a patient's immune system is able to mount