Involvement of HLA class I molecules in the immune escape of urologic tumors

R. Carretero, H. Gil-Julio, F. Vázquez-Alonso, F. Garrido, J. Castiñeiras, J.M. Cózar

Department of Biologia Molecular, Bioquímica III e Inmunologia, Universidad de Granada, Granada, Spain
Servicio de Análisis Clínicos, Hospital Universitario Virgen de las Nieves, Granada, Spain
Servicio de Urología, Hospital Universitario Virgen de las Nieves, Granada, Spain
Servicio de Urología, Hospital Universitario Virgen de la Macarena, Sevilla, Spain

Received 12 April 2013; accepted 2 June 2013
Available online 11 February 2014

KEYWORDS
Human leukocyte antigen class I; Human leukocyte antigen class II; Phenotypes; Bladder cancer; Antigenic processing; Prostate cancer; Renal cell carcinoma; Immunotherapy

Abstract
Context and objective: To analyze the influence of different alterations in human leukocyte antigen class I molecules (HLA I) in renal cell carcinoma, as well as in bladder and prostate cancer. We also study the correlation between HLA I expression and the progression of the disease and the response after immunotherapy protocols.
Evidences acquisition: It has been shown, experimentally, that the immune system can recognize and kill neoplastic cells. By analyzing the expression of HLA I molecules on the surface of cancer cells, we were able to study the tumor escape mechanisms against the immune system.
Evidences synthesis: Alteration or irreversible damage in HLA I molecules is used by the neoplastic cells to escape the immune system. The function of these molecules is to recognize endogenous peptides and present them to T cells of the immune system. There is a clear relationship between HLA I reversible alterations and success of therapy. Irreversible lesions also imply a lack of response to treatment. The immune system activation can reverse HLA I molecules expression in tumors with reversible lesions, whereas tumors with irreversible ones do not respond to such activation. Determining the type of altered HLA I molecules in tumors is of paramount importance when choosing the type of treatment to keep looking for therapeutic success. Those tumors with reversible lesions can be treated with traditional immunotherapy; however, tumor with irreversible alterations should follow alternative protocols, such as the use of viral vectors carrying the HLA genes to achieve damaged re-expression of the protein.
Conclusion: From studies in urologic tumors, we can conclude that the HLA I molecules play a key role in these tumors escape to the immune system.

© 2013 AEU. Published by Elsevier España, S.L. All rights reserved.
Introduction

The molecules of human leukocyte antigen class I (HLA-I), also known as MHC-I, are responsible for the presentation of intracellular peptides to cytotoxic lymphocytes. This process is vital for the detection of intracellular pathogens and tumor cells showing mutated proteins. The loss of this molecule is used by the cancer cells to prevent recognition of the tumor cells as foreign cells, leading to increased risk of tumor recurrence after maintenance therapy. Some of the alterations are reversible with immune system stimulants in order to make it more effective against cancer, reducing the potential risk of recurrence and facilitating elimination; such as the use of interferon γ, the use of antibodies versus tumor cell markers, the use of T lymphocytes or NK cells, vaccinations with tumor cells or peptides, and activation by interleukins such as IFN or IL-2. However, other alterations, such as mutations, are irreversible with the current treatments and we would have to resort to gene therapy to reconstitute the damaged HLA-I molecules. To date, there is no clinical, biochemical, or molecular parameter that can predict the response of urologic tumors to immunotherapy. Our study seeks to understand the alterations involved in tumor escape and suggest for the future selective therapy for responder patients, avoiding toxicity and reducing the costs associated with maintenance therapy in those potentially non-responder patients.

Features of the molecules of the major histocompatibility complex in humans

In the 1940s, Snell et al. found that the transplants between genetically identical mice were viable, while those between mice of different strains were rejected. This study concluded that the recognition of a tissue as its own or foreign has a genetic basis and that the genes responsible for this recognition vary between individuals of the same species, i.e., are polymorphic. This group of genes was called major histocompatibility complex (MHC).

In humans, the studies were based on the analysis of transplants. Dausset et al. found that the patients who rejected transplants had antibodies capable of recognizing proteins of the donor’s leukocytes. Therefore, the MHC molecules are also called human leukocyte antigens (HLA).

The products of HLA genes, in humans, are encoded by genes located on the short arm of chromosome 6. The HLA system is a gene region which is organized into different subregions linked to each other, so being inherited in a Mendelian codominant way, giving rise to the most polymorphic existing gene system in humans. This region contains multiple genes, many of which lead to molecules expressed on the surface of cells (Fig. 1). Nowadays we know the existence of 2 major groups of molecules: MHC class I, represented in humans by the classic HLA-A, B and C molecules; and MHC class II which includes the HLA-DP, DQ, and DR...
molecules. In this study, we will refer to the genes which give rise to the classical HLA class I molecules.

HLA I molecules are surface glycoproteins composed of a heavy chain and a small, non-polymorphic protein called β 2-microglobulin (β 2-m). The heavy chain in its outermost surface part forms a cave that the antigenic peptide binds.

**Biological function of the major histocompatibility complex**

For 20 years, HLA molecules were studied in the context of transplants. However, the HLA system must have had a different biological function, as the organ grafts are an artificial process.

The function of HLA I molecules is to collect endogenous peptides from degraded proteins and show them to the T lymphocytes of the immune system. Through a process called antigenic processing, small intracellular peptides (of 10–11 amino acids) are collected and transported to the cell membrane, where they stay in a cave of the HLA molecule. Cytotoxic T lymphocytes are capable of recognizing the HLA protein and the peptide that it leads through its TCR receptor, if the displayed peptide comes from a foreign protein, the lymphocyte will be activated and will result in death of the infected cell, thus preventing reproduction of the pathogen. This process is the specific defense mechanism that the immune system has against intracellular pathogens.

The role of HLA II molecules is similar; however, the peptides that are collected are of extracellular origin, obtained from phagocytosis of foreign elements. The peptides are joined to the HLA II molecule and they are recognized by the TCR of the T helper lymphocytes. When these lymphocytes detect a foreign peptide, they release cytokines activators of the immune response.

The recognition of antigens by T lymphocytes is only performed when these are bound to the HLA molecule. This process is called MHC restriction. Antigen recognition by the T lymphocyte is, thus, a trimolecular interaction between the TCR present in the T lymphocyte membrane and the antigenic peptide along with the MHC molecule, which form a complex in the cell membrane to be recognized by the T lymphocyte. This triple interaction leads to T lymphocyte activation, which will be antigen-specific and MHC restricted. CD8 cytotoxic T lymphocytes identify the antigen presented in conjunction with the HLA I, and their response will be to exert cytotoxic activity against cells presented by the foreign antigen.

**Involvement of human leukocyte antigen molecules in tumor recognition**

The notion that the immune system could protect the host from neoplastic disease was initially proposed by Ehrlich and it was formally established as the immunosurveillance hypothesis 50 years later by Burnet and Thomas.

In animal models, there is a great amount of data that show that the immune system maintains immunosurveillance against cancer cells. In addition, we now know that the incidence of tumors is higher in immunocompromised patients, and although this increase is attributed to a viral induction, it is being observed that in these patients tumors of non-viral origin also increase. The discovery of tumor-associated antigens (TAA) reinforces the theory of the immune system involvement in tumor recognition. In 1991, the Boon Group described, for the first time, the recognition of a tumor cell by a cytotoxic lymphocyte. This recognition is because the tumor cell expresses by means of its HLA a tumor peptide, named MAGE, which is recognized as foreign by the T lymphocyte. Currently, there are
Involvement of HLA class I molecules in the immune escape of urologic tumors

numerous TAA described and new ones are constantly being described.\textsuperscript{14}

Alterations in the expression of human leukocyte antigen molecules class I

The immune system cannot always control the growth of tumor cells. This is due to the development of different mechanisms of immune escape by the transformed cells.\textsuperscript{11} Our research group has studied, for several years, the HLA I expression in different tumors, and we found that immune evasion is a common mechanism.\textsuperscript{15-18} Alterations in the expression of HLA genes prevent the recognition of tumor antigens by T lymphocytes (Fig. 2). Altered antigen presentation is also used by some viruses as a mechanism of evading the immune response.\textsuperscript{19}

The studies carried out have made it possible to classify these alterations into phenotypes according to the HLA I molecule present or absent on the cell surface (Fig. 3).\textsuperscript{20,21}

- Phenotype I: complete loss of HLA class I molecules.
- Phenotype II: loss of HLA class I haplotype.
- Phenotype III: loss of HLA class I locus.
- Phenotype IV: loss of HLA class I allele.
- Phenotype V: compound phenotype.

The expression of HLA can be lost because of different molecular causes (Fig. 4).

Involvement of alterations in the human leukocyte antigen molecules class I in bladder tumors

Bladder cancer is the most common malignancy of the urinary tract. The global age-standardized rate is 10.1 per 100,000 in men and 2.5 per 100,000 in women.\textsuperscript{22} In Europe, the highest incidence of bladder cancer occurs in Western Europe and in the Southern region, followed by Northern Europe.\textsuperscript{23} The lowest incidence was observed in the regions of Eastern Europe. Although its etiology has not been established, it is generally a multifactorial disease in which both environmental and genetic factors may be involved.\textsuperscript{23}

In Europe, mortality rates for bladder cancer have been reduced in the last decade to 16% in men and 12% in women approximately.\textsuperscript{22}

The expression of the HLA I molecule in bladder cancer has been the most studied in our laboratory,\textsuperscript{13} and we found that 72% of bladder tumors have some loss of HLA I. The frequencies of the various disorders are: 25% loss of all HLA I molecules, 12% loss of locus, and 35% loss of one allele, which can be interpreted as phenotype II or IV. These data show that the loss of expression of HLA I molecules is a common process in bladder tumors.

Analysis of the HLA I negative tumors showed that they have altered antigen processing machinery (APM), responsible for forming the immunoproteasome and binding the peptides to HLA I.\textsuperscript{24,25} We also analyzed the frequency of loss of heterozygosity (LOH), on chromosome 6, which leads to loss of a haplotype (one allele A, one B, and one C).\textsuperscript{26} This irreversible alteration is responsible for phenotype II and it was found in 35% of bladder tumors.

Several studies indicate that bladder cancer in advanced stages has a lower expression of HLA I molecules when compared with early stages.\textsuperscript{15,25,27} It has also been found that the expression of HLA I can be a predictor of the disease. In this context, loss of $\beta$ 2-m has been associated to a greater degree of tumor differentiation (G3) and worse overall survival.\textsuperscript{18} The expression of HLA I molecules in bladder tumors can be used as a prognostic factor for disease-free survival.\textsuperscript{29}

Alterations of human leukocyte antigen class I in prostate tumors

Prostate cancer is the malignancy with the highest incidence in men in the developed world, assuming a significant cost to healthcare systems. Current incidence in Spain is 108 new cases per 100,000 men/year.\textsuperscript{30}

The existing information on the changes in the molecular mechanisms of HLA I in prostate cancer is still limited. Different authors have attempted to explain the basis of these alterations conducting studies both in cell lines and in prostate cancer tissue. We have experimentally observed alterations of the HLA I in 34% of primary adenocarcinomas of the prostate, and in 80% of the metastases in lymph nodes.\textsuperscript{31} It has also been reported that 80% of prostate adenocarcinomas with a Gleason score of 7–8 showed a drastic decrease in HLA I expression. Other studies show 74% of tumors with altered HLA I.\textsuperscript{32} All these studies agree on the high incidence of alterations of HLA I in prostate cancer, especially in advanced stages.

Our laboratory has shown that lack of expression of the HLA A03 allele, in a prostate tumor line, was due to an insertion of a nucleotide, leading to encoding a premature termination codon.\textsuperscript{33} We also managed to recover the HLA expression of the prostate tumor cell line, OECN3, which possesses deletion of the $\beta$ 2-m gene and LOH on chromosome 15. By infecting cells with a non-replicating adenoviral vector carried by the gene of the $\beta$ 2-m, we managed to completely restore the expression of HLA. The histone desaturation, as well as the APM, has also been correlated with loss of HLA expression.\textsuperscript{32}

In prostate cancer, there is also a relation among its clinicopathological features and HLA I. Kitamura et al.\textsuperscript{34} analyzed over 400 cases of prostate cancer and found that the alteration of HLA I correlated with advanced clinical stage of the disease and the low expression of $\beta$ 2-m. Seliger et al.\textsuperscript{33} observed that the low expression of calnexin and HLA were significantly related to a Gleason score greater than or equal to 7, and with early recurrence.

With the current data, we can conclude that there are different conditions that may lead to the loss of HLA I in prostate tumors.

Alterations of human leukocyte antigen class I on renal cell carcinoma

Kidney cancer is the eleventh most common malignancy in men and the fifteenth in women worldwide.\textsuperscript{34} accounting
Tumor cells expressing HLA class I are recognized by T lymphocytes and removed. Tumor cells with altered HLA class I cannot be recognized as foreign by T lymphocytes and can continue growing.

Figure 2 Immune evasion by means of HLA loss. (A) Tumor cells expressing HLA class I are recognized by T lymphocytes and eliminated. (B) Loss of HLA allows tumor cells to avoid the immune response.

Normal HLA phenotype

[Diagram showing normal HLA phenotype]

HLA phenotype in tumors

[Diagram showing altered HLA phenotypes]

Figure 3 Altered phenotypes of HLA class I.

Expression study on surface

Mechanism study

Mechanism type

- HLA - ABC
- Locus A or B
- Alleles

- Defects or genetic mutations in β2m, TAP, LMP
- Regulation mechanisms in β2m, HLA - ABC, TAP, LMP
- Regulation mechanisms of HLA A or B
- LOH (loss of haplotype)
- Genetic mutation recombination

Irreversible
Reversible

Figure 4 Mechanisms of altered HLA class I.
for approximately 3% of all malignant tumors in adults.\textsuperscript{35} The estimated relationship between both sexes is 1:5 men for every woman affected, being more frequent between the fifth and sixth decades of life.\textsuperscript{35}

Kidney cancer is one of the most interesting tumors for HLA expression. There is some controversy about the expression of this molecule in renal cells both normal and tumor. Several authors have reported that renal tumors are positive for HLA I.\textsuperscript{36,37} Conversely, Kitamura et al.\textsuperscript{37} found that 37% of renal tumors lost HLA I.

In our laboratory, we carried out a study in order to clarify this controversy.\textsuperscript{38} We performed a immunohistochemical analysis to analyze the expression at the surface of different HLA proteins. The study was completed with the laser microdissection of kidney cells both tumor and normal and quantification of the expression of messenger RNA (mRNA) of the heavy chain and the $\beta_2$-m by means of the quantitative PCR. The results showed that 97.5% of clear cell renal tumors, 100% of chromophobe cells, and 77.8% of papillary cells expressed HLA-ABC and $\beta_2$-m. Surprisingly, we found that in renal tubular cells, only 12.9% of the normal tissue samples were positive for HLA I. In order to confirm these results, we quantified the mRNA levels. We observed that there was a greater level of mRNA of HLA-ABC and $\beta_2$-m in tumor cells than in normal tubular cells, confirming the results obtained by immunohistochemistry.\textsuperscript{38}

We studied the frequency of LOH in the 6p21 region of chromosome 6, in clear and chromophobe cells of renal carcinoma, finding a low frequency of HLA haplotype loss (6.6%) in clear cells. By contrast, the frequency of LOH in chromophobe cells was 10 times higher (3 of the 5 cases analyzed). These results show that the LOH in 6p21 is not a common mechanism leading to altered HLA class I in clear cell carcinomas but it is very common in chromophobe cell tumors. We have also demonstrated that the various types of kidney tumors differ not only in histopathological criteria, but also in the HLA involvement in the immune system escape.

Although the data show that kidney tumors do not lose HLA so often, as in other urological tumors, there does exist a relationship between the loss of HLA and the most advanced stages. The tumors that lose HLA have a worse prognosis. The 5-year survival of patients with positive HLA kidney tumors was 95%, while those with negative HLA tumors decreased to 61%.\textsuperscript{39} Kidney tumors with aggressive features, such as greater size, a more advanced stage, and higher frequency of metastases, have also been associated with a lower expression of HLA I.\textsuperscript{39} In our laboratory, we also compared the transcription of the heavy chain and the $\beta_2$-m, both in primary and metastatic renal tumors, and we found that there is greater expression of these molecules in primary tumors than in normal tissue, but that the metastatic tumors were characterized by a lower expression of MHC.\textsuperscript{40}

This low frequency of alterations of the HLA I molecule may be because this tumor develops another type of mechanism of tumor escape, and it may explain why kidney tumors are of those with better response to immunotherapy.

**Human leukocyte antigen and immunotherapy**

The use of immunotherapy aims to activate the immune system to make it effective against cancer and facilitate its destruction. Although some patients manage to eliminate the tumor, there is a high number of patients who do not respond to the treatment.\textsuperscript{41} One of the most successful immunotherapies in bladder cancer, to date, is the use of Bacillus Calmette-Guerin (BCG). The superiority of BCG after TUR has been confirmed compared with TUR alone or TUR and chemotherapy in preventing recurrences of Ta-T1 tumors.\textsuperscript{42} BCG therapy reduces the recurrence to 40%, significantly better than the 70% recurrence shown by mitomycin therapy.\textsuperscript{43} For optimal effectiveness, BCG should be given on a maintenance schedule.\textsuperscript{44} Because of the essential role of the MHC on the recognition of tumor cells and, therefore, on the success of immunotherapy, the relationship between these molecules and the prognosis of immunotherapy has also been studied.

Kitamura et al.\textsuperscript{45} studied 30 patients treated with BCG. They found a significant relationship between the alteration of HLA I and worse survival. The patients who were positive for HLA I had a 5-year survival of 55.7% while those who were negative only 19.1% ($p = 0.019$). Videira et al.\textsuperscript{46} analyzed the expression of different antigen-presenting molecules and cytokines in a group of 59 patients treated with BCG. They found that the relapse-free group showed significantly greater HLA I expression in normal tissue. Carretero et al.\textsuperscript{45} have compared primary tumors and recurrences of tumors treated with BCG or mitomycin. They found increased HLA I expression in tumors with better prognosis after BCG instillation. At the same time, they found that the tumors in which the BCG failed had a much higher percentage of genetic alterations than recurrence-free tumors. They finally found that the recurrences of the tumors treated with BCG showed greater losses of HLA I than their respective primary tumors. Yasukawa et al.\textsuperscript{46} studied the recurrences of kidney cancer after immunotherapy consisting of vaccination with hTERT and found that these recurrences were negative for HLA I.

**Conclusions**

We can conclude, from this study, that HLA I molecules play a fundamental role in tumor escape from the immune system. Both in bladder and prostate tumors, the percentage of loss of HLA is very high. Kidney tumors, in early stages, show no loss of HLA I; however, those more advanced stages and metastases do so frequently. These data may help us, in the future, to predict the degree of tumor response to a specific treatment.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**References**

1. Rosenberg SA, Dudley ME. Cancer regression in patients with metastatic melanoma after the transfer of autologous antitu-
2. Romero JM, Jiménez P, Cabrera T, Cózar JM, Pedrinaci S, Tallada M, et al. Coordinated downregulation of the antigen presenta-
machine and HLA class I/beta2-microglobulin complex is
Involvement of HLA class I molecules in the immune escape of urologic tumors


