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HLA-mediated tumor escape mechanisms that may impair immunotherapy clinical outcomes via T-cell activation (Review)

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Abstract. Although the immune system provides protection from cancer by means of immunosurveillance, which serves a major function in eliminating cancer cells, it may also lead to cancer immunoediting, molding tumor immunogenicity. Cancer cells exploit several molecular mechanisms to thwart immune-mediated death by disabling cellular components of the immune system associated with tumor recognition and rejection. Human leukocyte antigen (HLA) molecules are mandatory for the immune recognition and subsequent killing of neoplastic cells by the immune system, as tumor antigens must be presented in an HLA-restricted manner to be recognized by T-cell receptors. Impaired HLA-I expression prevents the activation of cytotoxic immune mechanisms, whereas impaired HLA-II expression affects the antigen-presenting capability of antigen presenting cells. Aberrant HLA-G expression by cancer cells favors immune escape by inhibiting the activities of virtually all immune cells. The development of cancer therapies based on T-cell activation must consider these HLA-associated immune evasion mechanisms, as alterations in their expression occur early and frequently in the majority of types of cancer, and have an adverse impact on the clinical response to immunotherapy. Herein, the concept of altered HLA expression as a mechanism exploited by tumors to escape immune control and induce an immunosuppressive environment is reviewed. A number of novel clinical immunotherapeutic approaches used for cancer treatment are also reviewed, and strategies for overcoming the limitations of these immunotherapeutic interventions are proposed.

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Abbreviations: APC, antigen-presenting cells; β_2m, β_2 microglobulin; BMDC, bone marrow derived cells; CD, cluster of differentiation; CTL, cytotoxic T-cell; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cells; ESCC, esophageal squamous cell carcinoma; HLA, human leukocyte antigen; Ig, immunoglobulin; IL, interleukin; ILT, immunoglobulin-like transcript; KIR2DL4, killer cell immunoglobulin-like receptor, 2 immunoglobulin domains and long cytoplasmic tail 4; LOH, loss of heterozygosity; mAb, monoclonal antibody; MDSC, myeloid derived suppressor cells; mHLA-G, membrane-bound human leukocyte antigen-G; NACT, neo-adjuvant chemotherapy; NK, natural killer; NKG, natural killer group; PD1, programmed cell death protein 1; PDL1, programmed cell death ligand 1; sHLA-G, soluble human leukocyte antigen-G; sHLA-Gev, extracellular vesicle-associated soluble human leukocyte antigen-G; sHLA-Gfree, free soluble human leukocyte antigen G; TAAs, tumor associated antigens; TAM, tumor-associated macrophages; TCR, T-cell receptor; Th, T helper cells; T-reg, regulatory T-cells

Key words: immunosurveillance, tumor microenvironment, human leukocyte antigens, tumor immune escape, immunotherapy, immune checkpoints

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1. Introduction

Tumors are complex tissues composed not only of tumor cells, but also a repertoire of immune cells that give them special features to allow tumor growth and metastasis. These special features, proposed by Hanahan and Weinberg in 2011, provide the tumor with proliferative signal support, the avoidance of growth suppressors, cell death circumvention, cell immortality, angiogenesis, and invasive and metastatic activation. Two additional features are involved in cancer pathogenesis: The ability to reprogram the cellular metabolism to support neoplastic proliferation, and the ability to evade immune recognition and destruction by T and B lymphocytes, macrophages, and natural killer (NK) cells (1).

Cancer progression typically requires tumor cells to acquire the ability to avoid immune detection and destruction. Thus, understanding the interplay between the tumor, the immune system and the tumor microenvironment is of pivotal importance to providing the rationale for designing therapeutic approaches that trigger specific antitumor immune responses.

Inducing an effective immune response against cancer by immunotherapeutic intervention is a challenge that depends on several factors of the tumor and the immune system functioning together to either eradicate tumors or promote immune evasion. One of the most important factors in the development of antitumor immunotherapies is the constitution of the tumor microenvironment (including immune cell types, cytokine profiles, acidity and oxygenation levels, molecular signatures) as this may positively or negatively impact the arrival and cytotoxic activity of effector cells, thus determining an improved or worse clinical outcome (2). Therefore, to improve immunotherapy outcomes, it is important to alter the tumor microenvironment so that it is permissive for cytotoxic NK and T-cell activity. Thus, depending on the specific disease phenotype of the patient, certain therapeutic approaches must be escalated, while others should be avoided, in order to obtain a desirable clinical response (3).

Altered HLA (human leukocyte antigen)-I expression on the tumor cell surface is an early and frequent event that promotes carcinogenesis, as HLA-I is critical for the immune recognition of tumor cells and signaling between tumor and immune cells (4,5). Several studies reported total or partial loss of classical HLA-I molecule expression in different human tumors (6,7), with at least 50% of multiple HLA allele loss caused by loss of heterozygosity (LOH) events (8). Another HLA-mediated strategy used by tumor cells to avoid recognition by various immune effectors is the aberrant expression of non-classical HLA-I molecules (HLA-E and HLA-G), which function as inhibitor ligands for immune-competent cells, allowing tumor immune escape (9).

As mentioned previously, the complexity of the alterations to HLA expression in carcinogenesis makes selecting a therapeutic target to potentiate antitumor immune responses very difficult. However, correcting these alterations may provide a first step towards improving the currently available cancer immunotherapies.

2. Cancer immune response: Host-protective while tumor promoting

It is possible to separate tumor associated antigens (TAAs) into two main classes: Self and tumor-restricted antigens. Self-antigens include differentiation (including Melan A in melanoma) and overexpressed antigens (including ErbB2 receptor tyrosine kinase 2 in colon, breast and lung cancer), whereas tumor-restricted antigens may be of viral origin (including human papilloma virus in cervical and throat cancer), from the germ line (including NY-ESO-1 in melanoma) or neoantigens (for example mutated antigens, including β-catenin in melanoma) (10). It is possible to induce immunization against tumor self-antigens, potentially generating an effective antitumor T-cell and antibody response (11).

During carcinogenesis, innate and adaptive immunity stimulation occurs. Innate immunity mediates surveillance and tumor lysis in a rapid and non-specific fashion, whereas adaptive immune response is more specific; directed by TAAs that induce T-cell responses and antibody production (12). The main immune effectors of antitumor innate immunity are NK cells, which serve important functions in cancer immune surveillance: These cells express a variety of activating and inhibitory receptors that recognize cellular stress ligands, as well as major histocompatibility complex class I and similar molecules. These interactions mediate their tolerance to healthy self-cells and their cytotoxicity against stressed cells (13-15).

NK cell cytotoxic activity is either direct or indirect. Direct killing occurs via antibody-dependent cell-mediated cytotoxicity, an adaptive immune cell-killing mechanism mediated by activated NK cells (16), whereas indirect killing occurs through the secretion of cytokines, which exert antitumor effects via the stimulation of immune system regulatory components (17).

Although the main adaptive immune effectors capable of eliminating transformed cells are cytotoxic $CD8^+$ T-cells (18), $CD4^+$ T-cells, through the secretion of a Th1 cytokine profile (19), and B-cells, through the production of antitumor antibodies, also serve important functions in generating a powerful antitumor immune response. However, tumor features, including the nature of tumor antigens (20), immune modulatory factors produced by tumor and host immune cells, and the existence of regulatory cells [including regulatory T-cells (T-regs), myeloid derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs)] favor tumor development and progression (21).

The shift from antitumor innate immunity to a long-lasting adaptive immune response is mediated by lymphoid cells and their products (22). Genetic and epigenetic alterations generate tumor antigens that are recognized by a T-cell's T-cell receptor (TCR) when presented in an HLA-restricted manner. This recognition leads to T-cell priming, activation, proliferation, differentiation and cytokine production, and thus is pivotal for immune response amplitude and quality. Once tumor antigen recognition occurs, CD28 amplifies TCR signaling to completely activate T-cells. These activated effector T-cells leave the lymph nodes in search of tumor cells bearing the cognate HLA-peptide, leading to tumor cell death by T-cell mediated cytotoxicity (23). Under normal physiological conditions, T-cell activation is regulated by a balance between co-stimulatory and inhibitory signals, which ultimately leads to an effective immune response. Immune-suppressing pathway proteins, collectively termed immune checkpoints, are crucial for the maintenance of self-tolerance and for protection from tissue damage caused by the immune response itself (24). Tumors may alter immune homeostasis, suppressing T-cell activation and effector function, driving T-cell tolerance by chronic antigenic stimulation, and simultaneously activating suppressor pathways to prevent T-cell mediated killing. The immune checkpoint molecules associated with these phenomena have been demonstrated to be excellent targets for cancer immune therapy (25).

The best understood co-stimulatory/regulatory pathways are those mediated by CD80, CD86, cytotoxic T-lymphocyte-associated protein (CTLA)-4 and programmed cell death protein (PD)-1. CD80 and CD86 are expressed on the surface of antigen-presenting cells (APCs), bind to CD28 receptors on the T-cell surface and induce interleukin (IL)-2 production to support specific T-cell expansion (26). Once TCR activation occurs, regulatory signals are generated to limit the expansion and activation of TCR-triggered T-cells. CTLA-4 and programmed cell death protein 1 (PD1) are immune

checkpoints capable of limiting T-cell activation in secondary lymphoid organs and activating T, B and myeloid cells (27,28). Upon receptor ligation, T-cells stop clonal expansion and cytokine production (29-31). These regulatory signals compete for ligands and key substrates with co-stimulatory receptors. For example, on activated T-cells, CTLA-4 competes with CD28 molecules for the CD80 and CD86 ligands on APCs to regulate cell cycle proteins and cytokine expression. Another immune check point with pivotal relevance in cancer is HLA-G, a tolerogenic non-classical HLA-I molecule, which binds to CD8 (32), CD160 (33), the inhibitory receptors immunoglobulin (Ig)-like transcript (ILT)-2 and -4, and killer cell Ig-like receptor, 2 Ig domains and long cytoplasmic tail 4 (KIR2DL4) (34,35). Besides ILT-4, all these inhibitory receptors are widely expressed on lymphoid immune cells, whereas myeloid immune cells express CD8, ILT-2 and ILT-4 (36). Thus, immune responses are regulated in order to guarantee an effective immune response while preventing excessive immune activation.

In cancer, tumor cell plasticity may generate tumors with low immunogenicity in response to selective immune pressure exerted by the host immune system, enabling tumor evasion of immune surveillance. This host-protective, tumor-promoting immunity action is known as cancer immune editing, a process that occurs in three sequential phases: i) Elimination; ii) equilibrium and iii) escape (37). Elimination corresponds to the initial phase of cancer immune surveillance, in which the immune system is able to detect and destroy transformed cells, preventing tumor progression. In this phase, immune effector cells, including cytotoxic T-lymphocytes (CTLs) and NK cells, are able to recognize and eliminate tumor cells. Dendritic cells (DC) and $CD4^+$ T-cells are also components of this elimination phase, as they recognize and kill transformed cells long before they become clinically apparent, working as extrinsic tumor suppressors (38). Killing at this phase depends on i) stress ligand expression, including NK group (NKG) 2D; ii) TAA recognition in an HLA-restricted manner and iii) and co-stimulatory signals which completely activate T-cells (39).

Tumor cell variants that survive the elimination phase enter into an equilibrium stage in which the immune system controls tumor outgrowth, but the tumor remains clinically undetectable. Tumor cells with edited immunogenicity eventually continue growing; tumor dormancy is broken, and the edited tumors, which exhibit reduced immunogenicity, grow without immune control and progressively establish an immunosuppressive microenvironment, becoming clinically apparent (40,41). Immuno-editing provides tumor cells with a plethora of molecular tools with which they may control the immune response. The tumor may recruit all immune cells and once inside, they participate in dynamic crosstalk with cancer cells to govern tumor development (Fig. 1). Early eradication or spontaneous tumor regression, as well as tumor promotion and development, depend on the nature of immune cells infiltrating the tumor and on tumor-induced immune factor production (37).

3. Tumor microenvironment: Antitumor and tumor promoting

The adaptive antitumor immune response is not always capable of tumor eradication, potentially due to immune evasion mechanisms including the induction of immunological ignorance and immunological tolerance, or interactions between tumor cells and the host immune response. These phenomena may inhibit T-cell activation and induce tumor resistance against immune attack (37), and are activated by a number of mechanisms that will be described.

At the initial tumor growth stage, tissue damage induces acute Th1 inflammatory responses that favor APC maturation and innate immune cell polarization, promoting the elimination of developing tumors. APC maturation initiates adaptive immune responses mediated by $CD4^+$ and $CD8^+$ T-cells. Simultaneously, the acute activation of B-cells results in the induction of soluble mediators, including antigen-specific Igs capable of complement activation, to coordinate phagocytic or cytotoxic destruction of damaged cells by innate immune cells (42). During inflammation, chemokines control immune cell movement, immune response polarization and T-cell and dendritic cell interactions, while cytokines mediate intercellular communication in the immune system and function as immune regulators (43,44). Chemokine and cytokine expression profiles modulate the functional status of the immune system to negatively impact tumor development and progression.

In cancer development, tumor cells and tumor infiltrating immune cells produce antitumor and pro-tumor immune factors, which modulate the tumor immune response. A pro-tumor effect may dominate through various means: Inflamed tumors express high levels of pro-inflammatory innate and adaptive immune signals, as well as several immune-inhibitory factors, including programmed cell death ligand (PDL) 1 and indoleamine-2, 3-dioxygenase. They also recruit forkhead box p3 and T-regs to promote immune escape. Alternatively, non-inflamed tumors express a reduced level of chemokines, resulting in the poor attraction of $CD8^+$ effector T-cells into the tumor mass and poor effector cell trafficking (45). In addition, high levels of vascular markers and high macrophage and fibroblast infiltration also favor tumor growth (Fig. 2) (46,47). TAMs, tolerogenic DCs, regulatory T-cells and MDSCs, which are the main regulatory immune cells recruited by the tumor to create an environment with anti-inflammatory properties, favor tumor growth and survival (45). In addition, the chronic activation of B-cells is deleterious in certain types of cancer, potentially through the production of IL-10 (48). Thus, the nature of immune cells infiltrating the tumor serves a fundamental function in the failure of antitumor immune responses. In breast cancer, immune cell infiltration was previously demonstrated to correlate with an improved prognosis, a reduced tumor diameter and longer recurrence-free survival time (49). In other types of cancer, high $CD4^+$ T-cell infiltration was identified to correlate with tumor progression, potentially because the main tumor infiltrating cells are $CD4^+$ T-reg cells (50).

Alternatively, IL-10 in the tumor microenvironment may generate a neoplastic cell phenotype resistant to CTL-mediated lysis by decreasing transporter associated with antigen processing (TAP)1/2 expression and function, resulting in low peptide translocation into the endoplasmic reticulum, thus affecting HLA-I-mediated antigen presentation (51,52). HLA-I downregulation and non-classical HLA-I molecule neo-expression promote immunosuppression and, therefore,

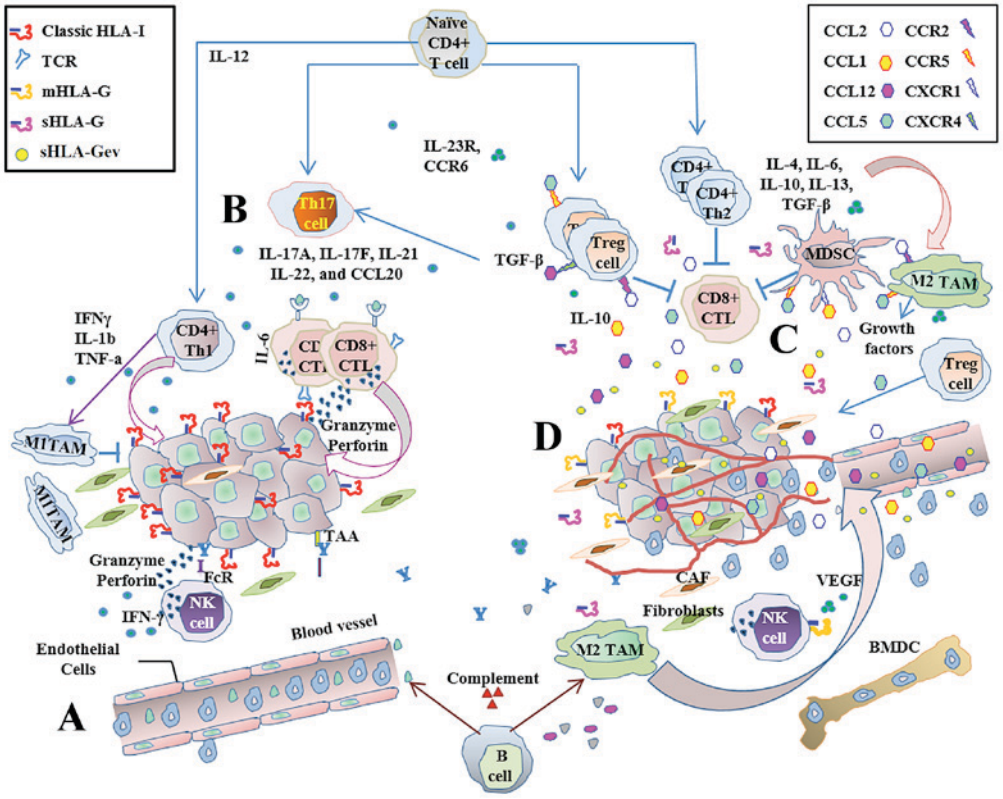

Figure 1. Immune response to cancer: Host-protective while tumor-promoting. The innate and adaptive immune responses are stimulated during carcinogenesis and are capable of surveillance and tumor lysis. (A) The main antitumor immune effectors are NK and CD8$^+$ T-cells, which are capable of responding directly against cancer with cytotoxicity, or by secreting cytokines. Inflammatory cells infiltrate the tumor and exert antitumor immune responses. (B) Cytotoxic CD8$^+$ T-cells are the main adaptive immune effectors. CD4$^+$ T-cells help to improve antitumor immune responses through the secretion of Th1 cytokines. Antitumor immune responses mediated by CTLs are effective and prevent tumor development in HLA-I positive tumor cells, but these immune responses are ultimately insufficient to prevent disease progression. (C) When inflammatory responses become chronic, regulatory cell populations generate a tolerant pro-tumor immune response via cytokine secretion and the production of growth factors. Tumor-promoting activity favors angiogenesis, invasion and metastasis, and is capable of suppressing adaptive immunity. (D) Aberrant expression of classical and non-classical HLA-I contributes to the establishment of an immunosuppressive microenvironment, promoting tumor growth by controlling immune stimulation and suppression signals. NK, natural killer; CD, cluster of differentiation; CTL, cytotoxic T-cell; HLA, human leukocyte antigen; BMDC, bone marrow-derived cells; CAF, cancer-associated fibroblast; CCL, C-C motif chemokine ligand; CCR, C-C motif chemokine receptor; CXCR, C-X-C motif chemokine receptor; FcR, fragment crystallizable receptor; IFN, interferon; IL, interleukin; MDSC, myeloid derived suppressor cells; mHLA-G membrane-bound human leukocyte antigen-G; sHLA-G, soluble human leukocyte antigen-G; sHLA-Gev extracellular vesicle-associated soluble human leukocyte antigen-G; TAAs, tumor associated antigens; TAM, tumor-associated macrophages; TCR, T-cell receptor; TGF-β, transforming growth factor β; Th, T helper cells; T-reg, regulatory T-cells.

tumor immunoescape. A number of studies have demonstrated that HLA-G, HLA-E and IL-10 expression levels in cancer are associated with tumor progression, metastasis and a poor prognosis (53-55), and that the IL-10-positive T-reg cell frequency may be associated with malignant transformation by contributing to immunosuppression in the tumor microenvironment (56). Due to the plethora of possible immunosuppressive features present in a particular tumor entity, it is necessary to personalize the selection of the therapeutic targets for cancer treatment to induce an effective antitumor immune response, thus avoiding the development of tumor chemo-resistance and a subsequent poor outcome.

4. HLA-mediated cancer cell escape mechanisms

The malignant transformation of cells is often associated with alterations to gene expression and the antigenic profile. Alterations in HLA expression (including classical and non-classical HLA-I and HLA-II) are frequent and early events during carcinogenesis (4,57). As tumor cells are immunogenic, they must acquire a plethora of molecular mechanisms to avoid destruction by CTLs and NK cells. By downregulating classical HLA-I, they prevent tumor recognition and rejection by CTLs, and by overexpressing non-classical HLA-I molecules they disable all types of immune cell involved in tumor recognition and rejection (including T and B lymphocytes, APCs and NK cells) (58). Conventional changes of HLA expression in malignant cells include total or allele-specific loss of classical HLA-I expression and the induction of non-classical HLA-I and HLA-II expression, potentially due to an immune selection process that enables the initiation of malignant lesions with an HLA-altered phenotype, which will be necessary to consider when designing novel immunotherapies for cancer treatment (59).

HLA expression is crucial for the generation of adaptive immunity, as tumor antigens are presented in an HLA-restricted manner to T-cells, activating them and controlling immune crosstalk (60). Altered HLA expression on the tumor cell surface has been described in a variety of human tumors, with percentages ranging from 60-90% expression in different human tumor types (4,61). These alterations result in different HLA-altered phenotypes, including

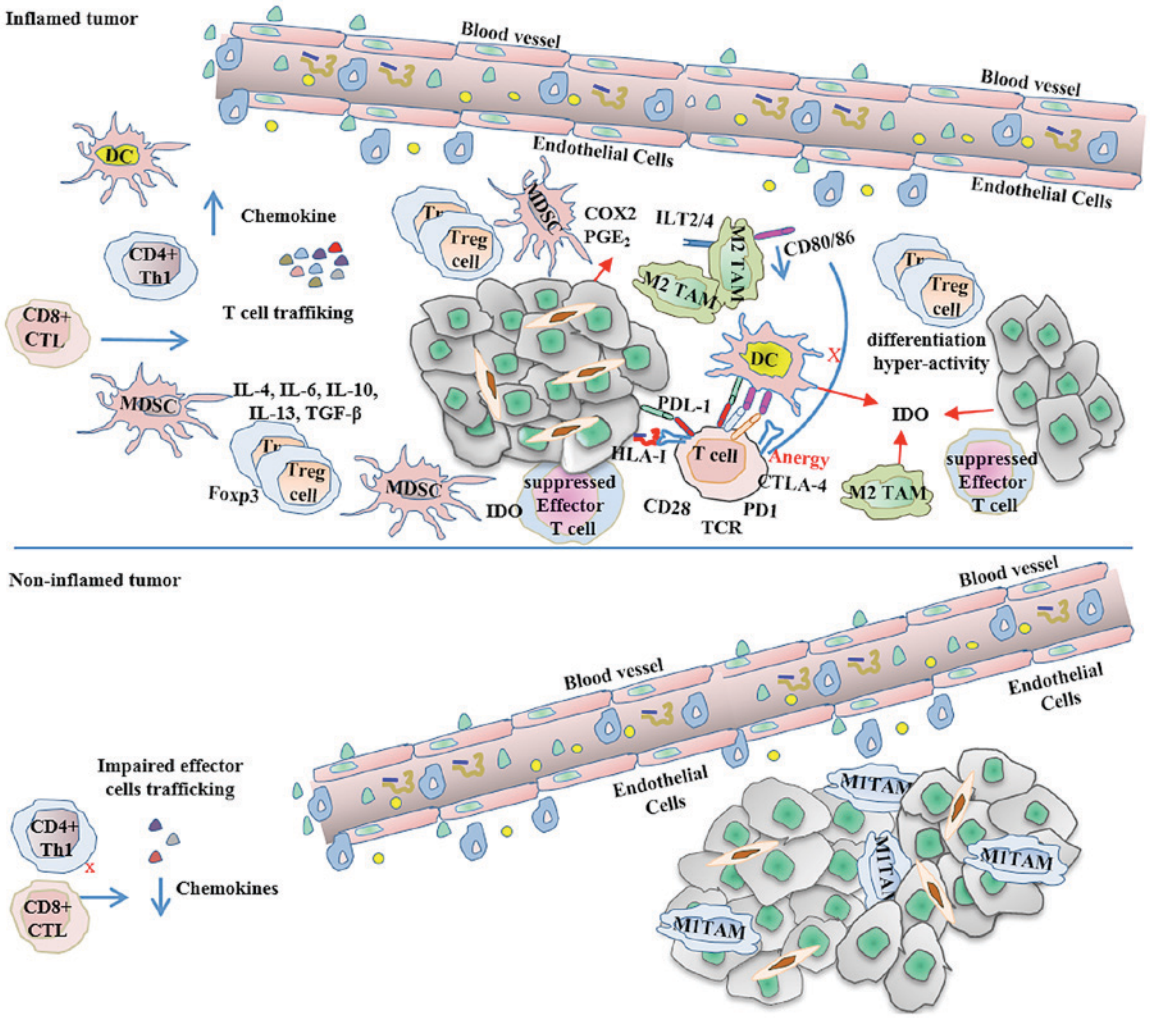

Figure 2. Inflamed and non-inflamed tumors escape immune-mediated destruction. As described by Gajewski *et al* (44), inflamed tumors express high levels of pro-inflammatory innate and adaptive signals, as well as immunoregulatory factors that contribute to the creation of an immunosuppressive environment, in which a dominant effect of negative regulation mediates the tumor escape. In contrast, non-inflamed tumors with poor chemokine production have few effector cells, abundant macrophages and cancer-associated fibroblasts, and express high levels of vascular markers, also allowing tumor escape. CD, cluster of differentiation; COX2, cytochrome c oxidase 2; CTL, cytotoxic T-cell; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cells; Foxp3, forkhead box p3; HLA, human leukocyte antigen; IDO, indoleamine-2, 3-dioxygenase; IL, interleukin; ILT, immunoglobulin-like transcript; MDSC, myeloid derived suppressor cells; PD1, programmed cell death protein 1; PDL1, programmed cell death ligand 1; PGE2, prostaglandin E2; TAM, tumor-associated macrophages; TCR, T-cell receptor; TGF-β, transforming growth factor β; Th, T helper cells; T-reg, regulatory T-cells.

the neo-expression of non-classical HLA-I molecules like HLA-G, which primarily function as inhibitor ligands for immune-competent cells (6,7), and HLA-E, which together with HLA-G and IL-10, is associated with the evasion and progression capacities in tumor entities including lip squamous cell carcinoma (62). HLA-G and HLA-E exhibit limited polymorphism, low cell surface expression and restricted tissue distribution (63). They exert several immune regulatory functions: HLA-G has immuno-tolerogenic properties and inhibits CTL and NK cell lytic functions (64), whereas HLA-E may act as an immuno-tolerogenic or immuno-activating molecule depending on the NK cell receptor it is attached to. HLA-G inhibits immune cells from binding to ILT2, ILT4 and KIR2DL4 receptors (65,66), whereas HLA-E is the major ligand required for the inhibitory NK cell receptors CD94/NKG2A and CD94/NKG2B expressed in NKs and CTLs to produce immune tolerance, but also for the CD94/NKG2C activating receptor expressed on NK cells and cytotoxic T-cells to support their cytotoxic activity (67,68). Thus, due to the pivotal immune function of HLA molecules, alterations in their expression may be the most common evasion mechanism used by tumor cells to avoid immune responses (39).

It is possible to classify HLA-altered tumor cell phenotypes into two main groups: Reversible regulatory or irreversible structural defects. Reversible HLA class I regulatory defects may occur at any step of synthesis, assembly, transport and/or molecular surface expression, and are caused by genetic, epigenetic, transcriptional or post-transcriptional events, resulting in regulatory abnormalities that it is possible to recover with cytokine treatment. In contrast, structural defects caused by mutation events that disrupt HLA-I heavy chain and β_2 microglobulin (β_2m) genes are irreversible (69).

In cancer, HLA class I loss occurs frequently and is predominantly caused by genetic aberrations in chromosomes 6p21.3 and 15q21 (70). It has been reported that at least 50% of

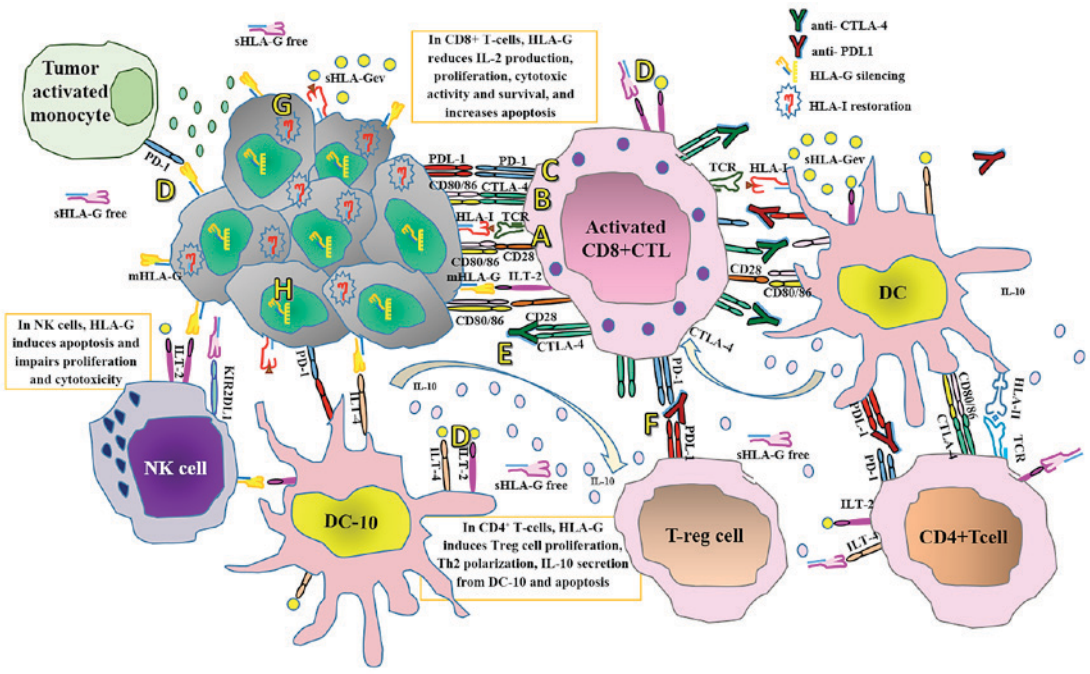

Figure 3. Current immune checkpoint blockade therapies and proposed adjuvant therapies for personalized cancer treatment. (A) T-cells are activated when TCRs bind antigens in a major histocompatibility complex-restricted manner on antigen presenting cells, in concert with CD28-CD80/CD86 mediated co-stimulation. (B) At the tumor site following T-cell activation, CTLA-4 is translocated on the T-cell surface and competes with CD28 for binding the CD80/CD86 ligands. This interaction delivers an inhibitory signal, which abrogates T-cell activation and proliferation. (C) Tumor cells express PDL1 and when this interacts with PD1 expressed by T-cells and other immune cells, it interferes with several T-cell signaling pathways that promote the induction of T-cell anergy, impairing the lytic capacity of T-cells on tumor cells at the HLA-I antigen-presenting stage. However, PD1 and CTLA-4 expression depend on T-cell activation that, in turn, depends on antigen recognition in an HLA-I-restricted manner. (D) On the other hand, interactions of membrane-bound and soluble HLA-G isoforms with their specific inhibitory receptors expressed by immune cells, including ILT-2, ILT-4 and KIR2DL4, impairs virtually all antitumor immune responses. In contrast to PD1 and CTLA-4, HLA-G expression does not require T-cell activation. (E) Thus, although therapy with anti-CTLA-4 monoclonal antibodies impairs the immunosuppressive CTLA-4 signal, promoting interactions between CD80/CD86 and CD28 and keeping T-cells activated, and (F) anti-PDL1 therapy may restore the activity of antitumor T-cells that have become quiescent, (G) tumor cells bearing defective HLA-I expression may be refractory to these therapeutic approaches. Targeting the aberrant HLA-I expression at the tumor cell surface may improve the clinical efficacy of these approaches, and (H) silencing HLA-G expression or blocking the inhibitory HLA-G receptors on immune cells may prevent inhibitory signaling and restore the effector antitumor capacity of immune cells. TCR, T-cell receptor; CD, cluster of differentiation; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PDL1, programmed cell death ligand 1; PD1, programmed cell death protein 1; HLA, human leukocyte antigen; ILT, immunoglobulin-like transcript; KIRD2L4, killer cell immunoglobulin-like receptor, 2 immunoglobulin domains and long cytoplasmic tail 4; DC-10, interleukin-10-secreting dendritic cells; DC, dendritic cells; IL, interleukin; mHLA-G, membrane-bound human leukocyte antigen-G; NK, natural killer; sHLA-Gev, extracellular vesicle-associated soluble human leukocyte antigen-G; sHLA-Gfree, free soluble human leukocyte antigen G; Th, T helper cells; T-reg, regulatory T-cells.

multiple HLA allele loss is caused by LOH, which is a frequent mechanism for HLA haplotype loss in various types of human tumor (71). Irreversible total HLA-I loss frequently occurs due to the coincidence of two molecular events: Mutation of one β_2m gene, and the loss of the second copy by LOH. This alteration has been described in various types of malignancy (72).

In cervical cancer, HLA-I downregulation occurs early in tumor development and is associated with HLA-G upregulation. The majority of HLA-G$^+$ tumors also expresses IL-10, thus suggesting the involvement of IL-10 in the generation of an immunosuppressive environment, by downregulating classical HLA-I and upregulating HLA-G expression (73). The HLA-G primary transcript generates seven different protein isoforms by alternative splicing, including four membrane-bound isoforms, HLA-G1, G2, G3 and G4, and three soluble (s)HLA-G5, G6 and G7 isoforms (64). The soluble forms are secreted as free soluble HLA-G molecules (sHLA-Gfree) or in extracellular vesicles (sHLA-Gev), enabling tumors to inhibit virtually all immune cells (Fig. 3) (74,75).

HLA-G expression by the tumor prevents immune responses by a variety of strategies, including the prevention of cell lysis by CTLs and NK cells, the induction of tolerant myeloid DCs, and the induction of anergic or immunosuppressive CD4$^+$ and CD8$^+$ T-cells (76). It has also been demonstrated that NK cells may acquire an immune-suppressive phenotype through HLA-G$^+$ tumor cell trogocytosis (77) and that sHLA-G exerts immunosuppressive functions by inducing apoptosis mediated by Fas cell surface death receptor/Fas ligand in circulating antigen specific T-cells (32). Furthermore, different sHLA-G subcomponents exhibit different prognostic impacts on the clinical outcome of patients with breast cancer treated with neo-adjuvant chemotherapy (NACT): High levels of sHLA-Gev prior to NACT were associated with disease progression and stem cell-like circulating tumor cells, whereas high sHLAGfree levels were associated with improved clinical outcome. However, total sHLA-G levels, without considering sHLA-Gfree and sHLA-Gev subcomponents, were not associated with clinical parameters (65,78). HLA-G1 and HLA-G5 are the full-length membrane-bound and soluble isoforms, respectively, and require peptide association for their correct expression, whereas the other membrane-bound and soluble isoforms have low stability and have different *in vivo* functional activities (9,79).

HLA-II alterations also function in immune escape by impairing the antigen-presenting capability of peripheral blood monocytes in patients with acute leukemia (80). An association between HLA-II variants and breast cancer susceptibility has been suggested in Chinese breast cancer patients. In this population, HLA-II variants may be associated with prognosis: The expression of HLA-DQB1 may indicate a poor prognosis, whereas HLA-DRB5 may be associated with a good prognosis (81). In addition, aberrant expression of HLA-DRB1 and HLA-DQB1, which may occur due to aberrant gene methylation, serves key functions in the pathogenesis of esophageal squamous cell carcinoma (ESCC), by influencing immune response to specific tumor epitopes and by promoting ESCC occurrence and progression (82). Furthermore, in the population of Guangdong, China, the occurrence of certain HLA-II alleles, including DPB1*1301, DPB1*0202, DQB1*030302, and DQB1*050301, occurred with higher frequency in patients with cervical cancer than in controls, suggesting that they may confer susceptibility to cervical cancer. On the other hand, the occurrence of the DRB1*13-DQB1*06 haplotype was significantly lower in patients with cervical cancer compared with controls, suggesting that this haplotype may confer a decreased risk of cervical cancer within this population (83).

5. Novel immunotherapeutic approaches against cancer

Current therapies against cancer include chemotherapy (84), radiation therapy (85), immunotherapies (86), biological therapies and targeted therapies. Therapeutic schemes currently in clinical trials include cryosurgery, hyperthermia and cancer vaccines designed to prevent (prophylactic) or treat (therapeutic) cancer (84). A large volume of research is being produced concerning strategies to induce antitumor immunity, including via innate and adaptive effector mechanisms. The blockade of immune checkpoints may trigger the antitumor immune response, while co-stimulatory receptor agonists and inhibitory signals antagonists may induce antigen-specific T-cell response amplification, potentially transforming human cancer therapeutics (24). Currently, a range of therapeutic agents that exploit this mechanism are in clinical trials (Table I).

Although novel immunotherapeutic approaches with a number of different molecular targets and modes of action are currently in development, obstacles including difficulties in immunological monitoring, poor clinical trial design and the absence of cancer vaccine regulation make it difficult to achieve an adequate evaluation of effective immune responses following antitumor therapy. It is possible to overcome these obstacles by improving patient selection, using combined therapies to impact several immune signaling pathways simultaneously, identifying novel biomarkers to evaluate clinical responses and coordinating immunological monitoring for clinical trials. These improvements must be achieved prior to successful clinical translation (87). Combined therapies use checkpoint inhibitors as immunological adjuvants to boost cancer immunotherapy and vaccines. The inhibition of signaling pathways, including vascular endothelial growth factor to inhibit angiogenesis, epidermal growth factor receptor to inhibit proliferating signals or telomerase to interfere with replicative immortality enablement, are examples of targeting the hallmarks of cancer (1). Cytokines have potential therapeutic and preventive applications, but the associated systemic toxicity limits their use in treating cancer. To overcome this problem, novel recombinant antibody-cytokine fusion proteins have been designed to maximize cytokine therapy efficacy by exploiting the specific tumor-targeting ability of monoclonal antibodies (mAb) and the immune stimulatory ability of cytokines, to induce antitumor immune responses while preventing systemic toxicities of cytokine therapy alone (88).

The blockade of immune checkpoints using human immune-modulatory mAbs are in preclinical and clinical development. These mAbs target immune system components rather than the tumor itself, thus resulting in different responses to anti-CTLA-4 therapy (89), compared with conventional antitumor mAbs, chemotherapies and immunotherapies (including vaccines and cytokines) in terms of the pattern of response; such as the response time to the therapy, duration of response and adverse event profile (90). Another benefit of anti-immune checkpoint mAb therapy is that it is possible to use it to treat a variety of malignancies (91), including hepatocellular carcinoma, which constitutes a significant challenge for conventional cancer immunotherapy, as the unique immune response in the liver favors immune tolerance, impairing the therapeutic action of immunotherapy (92). The clinical use of these drugs induces immune-related adverse events, including rashes, colitis, thyroiditis and hepatitis, and clinical management for these symptoms typically consists of treatment discontinuation or symptomatic management with steroids or other immunosuppressive agents (93).

Activation of the immune system through CTLA-4, PD1 and programmed cell death ligand 1 (PDL1) immune checkpoint blocking is a promising cancer therapy strategy (91,94). As the clinical success of targeting PD1/PDL1 or CTLA-4 depends on blocking the regulatory activities of the receptor-ligand interactions, it is important to consider that CTLA-4, PD1 and PDL1 expression depend on TCR activation, and that HLA-I expression on cancer cell surfaces is a prerequisite for a successful T-cell activation. Thus, lack of HLA-I expression by tumor cells has a major effect on tumor recognition and the further activation of T-cells, which remain unstimulated and incapable of recognizing cancer cells. In this scenario, anti-CTLA-4, -PD1 and -PDL1 therapies would not work (Fig. 3) (95). Thus, HLA status on the tumor cell surface must first be assured to determine the suitability of an immunotherapy treatment based on T-cell activation following TAA recognition.

Cancer immunotherapy strategies to activate T-cell-mediated antitumor responses include the use of antibodies to target inhibitory molecules that impair T-cell cytotoxicity (25), adoptive cell transfer with tumor infiltrating lymphocytes expanded *in vitro* (96), or genetically modified cytotoxic T-cells (97,98). However, $CD8^+$ T-cells have been established to recognize and destroy HLA-I positive tumor cells. As human cancers are frequently characterized by alterations in HLA-I expression, attempts to treat cancer by increasing the $CD8^+$ T-cell response will be unsuccessful in patients harboring tumors with negative or deficient HLA-I expression. Thus, a requirement for achieving successful clinical responses following administration of T-cell activation based immunotherapy is, again, to verify whether these important molecules for T-cell cytotoxicity are correctly

Table I. Molecular therapies targeting immune regulation in cancer.

Therapy	Mode of action	Limitations	Target/reagent type	Indications	PMID
Antibodies					
Trastuzumab	Highly selective agonism or blockade of extracellular protein-protein immune pathways.	Expensive and time-consuming manufacturing and development costs; challenges in achieving high tumor exposure	HER2	HER2-positive breast cancer, HER2-positive advanced gastric cancer	27526299 10211534 16328600
Bevacizumab	Long half-life, non-immunogenic, includes human or humanized vaccine agonists (targets include gp100, mucin 1 and MAGE family member A)		Vascular endothelial growth factor	Non-small-cell lung cancer, colorectal cancer, breast cancer	18565863 26257518
Cetuximab			EGFR	Colorectal cancer and head and neck cancer	27446583 27511844 27465221
Panitumumab			EGFR	Colorectal cancer	27438067 27354619
Rituximab			CD20 B-cell surface antigen	Primary mediastinal B-cell lymphoma, non-Hodgkin's B-cell lymphoma	27477167 27479818 27497027
Ibritumomab tiuxetan					
Alemtuzumab			CD52 lymphocyte surface antigen	Refractory chronic lymphocytic leukemia, T-cell lymphoma	26489498 26201283
Gemtuzumab ozogamicin			CD33 leukemic-cell surface antigen linked to calicheamicin	Acute myeloid leukemia	11970767
CT-011 (humanized immunoglobulin G1)			PD1	Advanced hematologic malignancies	18483370
Tositumomab			CD20 B-cell surface antigen	Non-Hodgkin’s B-cell lymphoma, diffuse large B-cell lymphoma	26832194 26257518
Ipilimumab Tremelimumab (CP-675,206)			CTLA-4	Metastatic melanoma Metastatic melanoma, mesothelioma renal cell carcinoma, breast cancer.	18838703 19052265 27042127

Table I. Continued.

Therapy	Mode of action	Limitations	Target/reagent type	Indications	PMID
Nivolumab			PD1	Advanced melanoma	27093328 27013881 27099755
Pembrolizumab				Advanced melanoma, metastatic renal carcinoma	
Recombinant cytokines					
Denileukin diftitox	Agonism or blockade of protein-protein immune pathways. (granulocyte-macrophage colony stimulating factor, IL-7, -12, -15, -18 and -21)	Antigenicity, poor pharmacokinetics, high toxicity	Recombinant IL-2 and fragments of diphtheria toxin (binds CD25R on T-cells)	Cutaneous T-cell lymphoma	26240767
Aldesleukin			IL-2	Melanoma, renal-cell carcinoma	27471714 25424850
Interferon α-2a and b			Recombinant interferon	Hairy-cell leukemia, chronic lymphocytic leukemia, Kaposi's sarcoma, melanoma, non-Hodgkin's lymphoma, multiple myeloma, renal cancer	14965794 26601863 7680399
Small molecules					
Imiquimod	Uniquely suited for intracellular targets, but equally applicable to cell surface or extracellular targets	Off-target activities, dose-limiting toxicities, ineffective at blocking protein-protein interactions, require daily doses	Toll-like receptor 7 agonist	Basal-cell carcinoma	26450707
Imatinib, nilotinib or dasatinib			Abl proto-oncogene, PDGFR, KIT proto-oncogene	Chronic myeloid leukemia, gastrointestinal stromal tumors, metastatic chordoma, chemoresistant Kaposi's sarcoma	26180502 27231512 17032555 26628884 26796903
Gefitinib			EGFR	Non-small cell lung cancer	27212579
Erlotinib			EGFR	Non-small cell lung cancer, advanced pancreatic cancer	12882624 27401642

Table I. Continued.

Therapy	Mode of action	Limitations	Target/reagent type	Indications	PMID
Sunitinib			VEGFR, PDGFR, FLT3	Gastrointestinal stromal tumors, renal cell carcinoma, pancreatic cancer	15639298 27374084
Sorafenib			VEGFR, PDGFR, FLT3	Clear renal cell carcinoma, hepatocellular carcinoma	16425993 27487101

PMID, PubMed identifier; HER2, ErbB2 receptor tyrosine kinase 2; CD, cluster of differentiation; PD1, programmed cell death protein 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; IL, interleukin; EGFR, epidermal growth factor receptor; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; FLT3, Fms-related tyrosine kinase 3.

expressed by cancer cells. Such expression would be an appropriate predictive biomarker to determine which patients should enter into these treatment schemes (84). Other patients may first require a neo-adjuvant scheme to restore normal HLA expression on cancer cells prior to the utilization of T-cell activation or immune-checkpoint blockade-based immunotherapies.

A range of immunotherapeutic schemes have been designed to modify the tumor microenvironment to improve the response to therapy in patients with cancer; however, restoration of normal HLA-I expression in cancer cells is of pivotal importance to ensure the immunogenicity of these schemes. Current strategies to restore normal HLA-I expression work well only when the molecular mechanism mediating HLA-I downregulation is reversible, as when HLA-I downregulation is due to heavy chain structural defects its expression is difficult to correct. Adenovirus-mediated gene transfer may be a powerful strategy to correct HLA expression, as human β_2m gene transfer to tumor cells negative for HLA-I following β_2m structural alteration has been demonstrated to restore HLA-I expression on tumor cell surface (99). The restoration of HLA expression reestablishes tumor cell immunogenicity, thereby inducing T-cell activation in a peptide-specific, HLA-restricted manner, suggesting that gene transfer of the β_2m gene may be a suitable neo-adjuvant therapy prior to T-cell activation-based immunotherapy in the patients harboring tumors negative for HLA-I due to β_2m structural alteration (100). Table I further summarizes the main molecular therapies targeting immune regulation in cancer.

6. Conclusions

The benefit of conventional therapies is often limited by collateral damage to normal tissues. Radiotherapy induces massive cell death and chemotherapy toxicity is directed against all actively proliferating cells. During massive cell death, $CD8^+$ T-cells specific for tumor antigens undergo repeated TCR stimulation due to the persistence of TAAs. Chronically stimulated T-cells gradually lose their ability to secrete IL-2, tumor necrosis factor-α and interferon-γ, and are finally eliminated by apoptosis in a process known as T-cell exhaustion, which is characterized by the overexpression of inhibitory receptors (101). PD1, CTLA-4, lymphocyte-activation gene 3, T-cell Ig and mucin domain-3 and T-cell immunoreceptor with Ig and ITIM domains, among others (102), dampen the stimulation of an effective antitumor immune response by immunotherapeutic drugs. However, in patients with an exhausted immune system, blocking of these receptors leads to T-cell activation, suggesting that the restoration of a non-exhausted immune context may improve immune activation. Previous results have indicated that T-cell exhaustion is reversible, which may have profound implications for cancer treatment (103).

Novel immune-based therapies for cancer include adoptive cell therapy, tumor vaccines, cytokines or the inhibition of immune suppressive mechanisms including with immune checkpoint inhibitors, and the depletion of T-regs or MDSCs. The search for targets for the design and improvement of novel therapies should include the search for biomarkers to measure therapeutic activity and evaluate potential synergy among different immune-therapeutic modalities (104). However, as a large number of signaling pathways are typically associated with carcinogenesis, it is probable that a single therapeutic

agent inhibiting one molecular target in a given tumor will not be sufficient to eradicate the entire tumor mass. Advances in knowledge of antitumor immune responses have been facilitated by the development of targeted therapies for cancer control, including anti-CTLA-4 antibodies, the therapeutic success of which suggests that immunotherapy may achieve long-lasting and durable antitumor immune responses in patients with cancer (105).

Blocking immune checkpoints may restore immune function in certain scenarios, depending on the HLA phenotype. In tumors with normal HLA-I expression, inhibitors of PD1 or anti-CTLA-4 mAbs function as PD1 and CTLA-4 expression depend on T-cell activation that, in turn, is dependent on HLA-restricted antigen recognition. Therefore, tumors bearing defective HLA-I expression may be refractory to these therapies due to their inability to present TAAs to CTLs. Reestablishment of normal HLA expression on the tumor cell surface by gene therapy may improve the clinical impact of anti-CTLA-4 and PD1 immunotherapies and restoring HLA-I expression may be an adjuvant therapy not only for TCR-stimulation-based immunotherapies, but also therapy based on checkpoint blocking. The combination of immunotherapy with conventional therapy, for example chemotherapy, has been demonstrated to produce a significant increase in the clinical response of patients with cancer, despite the toxicity caused by chemotherapy to immune system cells (106-108).

Another important factor associated with HLA is the aberrant expression of HLA-G, as most tumors neo-express HLA-G at various stages of their evolution and HLA-G neo-expression deactivates all antitumor immune responses. As plasmatic (free or vesicular) and membrane-bound (m) HLA-G expression is significantly increased in most cancer types and associated with poor prognosis, it is possible to use the levels of membrane-bound human leukocyte antigen-G on tumor and immune cells and/or sHLA-G (free or as part of extracellular vesicles) in plasma as diagnostic and prognostic tools in cancer patients. Furthermore, HLA-G may also serve as a therapeutic target for blocking mAbs or interfering RNAs (109).

HLA-G expression, in contrast with CTLA-4 and PD1, does not depend on T-cell activation and is capable of blocking antitumor immune responses by inhibiting all immune effectors, from APC activation to effector priming, as well as blocking activated CTL and NK cell function. Taking into account all the scientific evidence concerning the function of HLA loss of expression, it is possible to speculate that a cancer therapy targeting aberrant HLA-I expression would restore T-cell recognition of tumor cells and thus improve the clinical response to immunotherapies based on CTLA-4, PD1 and PDL1 expression. In addition, silencing of HLA-G expression or blocking the inhibitory ILT-2/4 receptors on immune cells may prevent inhibitory signaling and restore the antitumor effector capacity of immune cells. It may be possible to extend HLA-based clinical applications to the design of promising tools not only for diagnostic application to improve immunotherapeutic management of the disease, but also as prognostic markers for the clinical outcome of therapies, including NACT. A variety of studies focused on targeted therapies to modify the immunoregulatory nature of the tumor microenvironment are underway, and perhaps in the future, early diagnosis will allow early immunotherapeutic treatment, thus leading to improved survival rates.

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