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Abstract

MART-1 (melanoma antigen recognized by T cells) is also known as Melan-A. It has gained wide acceptance as an immunohistochemical marker to diagnose melanoma in biopsy material. MART-1 is not expressed in tissues lacking melanin pigment. Cellular and humoral immune responses against MART-1 have been detected in patients with melanoma and substantial efforts are ongoing to develop MART-1 as a therapeutic target in conjunction with T-cell checkpoint antibodies, cellular immunotherapy and peptide vaccines using a variety of adjuvants.

Keywords

Ipilimumab • Melan-A. *See* Melanoma antigen recognized by T cells (MART-1) • Melanoma antigen recognized by T cells (MART-1) • Animal models • Antigens • Gene • Human immune responses • Immunohistochemical staining panels •

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Immunotherapy • In cancer • Ipilimumab • Malignant melanocytes • Nonamer and decamer peptides • OA1 • Predictive marker • T-cell immune response • Vaccines and cellular therapy

MART-1 (melanoma antigen recognized by T cells) is also known as Melan-A. It is a lineage-specific protein present on melanocytes. The protein consists of 118 amino acids (13 kDa) and has a strongly hydrophobic domain. It is a transmembrane protein and present in the endoplasmic reticulum and *trans*-Golgi network of melanocytes in the skin and retina. It is a useful immunohistochemical marker in the diagnosis of melanoma on biopsy specimens but is also present in benign melanocytes. Due to the absence of MART-1 expression on nonpigmented tissues, there has been significant effort to use it as a target for vaccine and cellular immunotherapy in patients with melanoma.

Biology of the Target

Although the gene for MART-1 was first cloned in (Kawakami et al. 1994a), its function is still unknown. MART-1 protein associates with other melanosomal proteins such as OA1, which are involved with melanosome transport and biogenesis. When MART-1 is inactivated, the OA1-MART-1 complex is destabilized and results in ocular albinism. Based on these findings, MART-1 may serve as an escort protein in the early stages of melanosome formation.

Despite a lack of understanding of its function, the importance of MART-1 in the immune response to melanoma has been recognized since its discovery. The gene for MART-1 was originally determined by examining the cDNA from melanoma cell lines and studying the reactivity of tumor-infiltrating lymphocytes (TIL) obtained from surgical specimens in HLA-A2⁺ patients with metastatic melanoma. When non-MART-1-expressing HLA-A2⁺ cell lines were transfected with MART-1 cDNA, TIL clones were identified that produced interferon- γ when exposed to the MART-1-expressing target cells. MART-1- and HLA-A2-expressing melanoma cells were lysed by the same TIL clones. The genes for the T-cell receptor (TCR) of the TIL were examined and found to have restricted V α and V β gene sequences, confirming the specificity of the TIL for MART-1. The nonamer and decamer peptides of MART-1 recognized by the TCR map to amino acid residues 27–35 and 26–35, respectively. These peptides are part of the transmembrane portion of the protein, yet they have distinctly different conformations. Greater than 90% of TIL derived from melanoma deposits in HLA-A2⁺ patients recognize one or both of these peptides Reviewed in (Romero et al. 2002). The MART-1 27–35 epitope is believed to be the immunodominant peptide for immunological response against melanoma (Kawakami et al. 1994b). An alternative hypothesis has been proposed to account for the observation that a high proportion of melanoma patients (and normal individuals) have T cells that recognize MART-1, yet the melanoma is not eliminated by the T-cell response. The immunogenic peptides of MART-1 in conjunction HLA-A2 bind weakly to the TCR, thus it is likely that tolerance to MART-1 is

induced during thymic processing and selection of T cells. These MART-1-responsive T cells persist after thymic selection because of the weak binding of MART-1 peptide to the TCR. When melanomas express MART-1, the T-cell response may be weak, because MART-1 immunogenic peptides presented to the immune system by the melanoma cells are interpreted as self. As discussed below, there are vaccine and cellular immunotherapies with the potential to break tolerance to MART-1 and mount a more effective immune response to melanoma. Alternatively, malignant melanocytes may develop resistance to attack by cytotoxic T lymphocytes by overexpressing proteins associated with survival and resistance to apoptosis such as NF- κ B and Bcl-2 family members.

T cells that recognize these peptides can also be isolated and expanded from the peripheral blood of patients with melanoma. Although most of the immunobiology of MART-1 has been studied in patients with the HLA-A2 haplotype, the same MART-1 epitopes are recognized by T cells in patients expressing HLA-B44 and HLA-B45. Surprisingly, approximately 70% of healthy HLA-A2⁺ individuals with no history of melanoma have CD8⁺ T cells that recognize a MART-1 peptide.

Knowledge of the amino acid sequence and protein chemistry of the dominant antigenic epitopes of MART-1 has been useful in developing synthetic peptides for therapeutic vaccine trials (reviewed below) and synthetic tetramers that can be used as reagents for immunological monitoring.

Target Assessment

MART-1 is not a prognostic factor and is not measurable in the peripheral blood. MART-1 is commonly used in immunohistochemical staining panels to diagnose melanoma in conjunction with S-100 and HMB-45. MART-1 is an excellent target for therapeutic development since it is present in a high percentage of melanomas and immune responses (albeit ineffective in controlling the tumor) are already present in many patients at baseline and because it is expressed only in pigment-making cells. The T-cell immune response to MART-1 can be measured with tetramer assays. Although assessment of immune response is important in developing any immunotherapy, the correlation of immune response to MART-1 with regression of melanoma is inconsistent in most human clinical trials, a finding similar to many other assays for melanoma antigens.

Role of the Target in Cancer

Rank: 8

MART-1 is already established as widely used immunohistochemical diagnostic test in surgical pathology to analyze specimens suspected to be melanoma. There is no direct correspondence between MART-1 expression and prognosis in melanoma; however, it can be used in conjunction to other markers to detect circulating tumor

cells and melanoma micrometastases, which have prognostic significance. MART-1 has significant potential as a therapeutic target for vaccines and cellular therapy.

High-Level Overview

Diagnostic, Prognostic, and Predictive Uses

MART-1 is not a useful predictive marker in melanoma. As described above, it is useful in the immunohistochemical analysis of biopsy specimens suspected of being melanoma in conjunction with other markers such as gp100 and HMB-45. T-cell responses to MART-1 can be assessed with tetramer and are commonly used in analyzing immune responses in clinical trials that have been performed using MART-1-targeted therapy.

Therapeutics

MART-1 has been used as a target for inducing antitumor immune responses in patients with melanoma since 1994, when the specificity of MART-1-specific TIL clones was recognized in HLA-A2⁺ patients (Cole et al. 1994). There have been numerous MART-1 clinical trials involving peptide vaccines, irradiated MART-1-expressing melanoma cell line vaccines, T cells transduced with a TCR for MART-1, TIL, peptide-loaded dendritic cells (DC), tumor-cell-loaded DC (Palucka et al. 2006) and combinations including peptide vaccines with a variety of adjuvants, and DC vaccines plus anti-CTLA-4 therapy (Ribas et al. 2009). Tumor regressions, some of which are durable, have been observed with each of these immunotherapy platforms. Objective response rates for MART-1-based immunotherapy range from 3% for peptide vaccines to over 50% for TIL-based approaches. This broad range of response is comparable to immunotherapy directed at other known melanoma antigens (e.g., gp-100, MAGE-A3, NY-ESO-1, and others), but MART-1-directed therapy does not appear more effective than other targeted approaches in melanoma. The more central issue with antigen-specific immunotherapy is that even though good targets can be defined, other aspects of immune response include breaking tolerance, sustaining cytolytic responses, developing effective immunological memory, and overcoming the inhibition of immune response mediated through regulatory T cells.

Preclinical Summary

There is an extensive preclinical literature of using and assessing MART-1-targeted therapy in animal models and ex vivo analysis of human immune responses to this antigen. Recent work has focused on understanding why immune response to this commonly expressed target is suboptimal in controlling melanoma. For instance, Li et al. showed that TIL after undergoing rapid expansion protocols had markedly

reduced CD28 expression, decreased responsiveness to restimulation with MART-1 peptide and increased apoptosis (Li et al. 2009). These problems could be overcome by growing the TIL in IL-15 and IL-21. This work has potential for improving and maintaining T-cell responses to other antigens and other tumor types.

Clinical Summary

MART-1 is a useful and commonly used component of the immunohistochemical staining panels to confirm the diagnosis of melanoma in routine pathology assessment. The clinical targeting of MART-1 to treat established melanoma remains experimental, although it has been studied for over 15 years. The best strategy for inducing consistent and durable immune responses against MART-1 is unknown, but this criticism can be applied to all other tumor antigens that have been tested in clinical trials thus far.

Although vaccination to achieve clinically significant antitumor effects in humans remains a work in progress, a recent observation in patients with melanoma treated with ipilimumab described by Klein et al. helps to affirm the importance of immune responses to MART-1. Patients who achieved regression of melanoma after ipilimumab immunotherapy had infiltration of regressing tumor nodules with MART-1-specific CD8+ T cells (Klein et al. 2009). Some individuals displayed a 30-fold increase of MART-1-specific T cells in the peripheral blood and in skin biopsies taken from areas of erythematous rash induced by ipilimumab. This finding suggests that MART-1 is central to an effective immune response in melanoma and may also be linked to some of the autoimmune toxicities induced by ipilimumab. There is another important aspect to the ipilimumab work that is applicable to MART-1 and other tumor antigens, namely, that effective immune responses to cancer require T cells with enhanced effector and memory function. The main pathways that influence T-cell survival, effector function, and memory after exposure to antigen are CTLA-4, PD-1, OX40, and 4-1BB. Antagonists to CTLA-4 and PD-1 and agonists to OX40 and 4-1BB used in conjunction with vaccines to MART-1 and other tumor-specific antigens have great potential for therapeutic development in melanoma.

Immune responses to other melanoma antigens such as gp-100, NY-ESO-1, and MAGE-A3 in conjunction with MART-1 may result in more robust clinical responses. There are many clinical trials studying antigen combinations using multivalent peptide vaccines, ex vivo antigen presentation with adoptive transfer of “educated” cytotoxic T cells, and adoptive transfer of T cells with engineered TCRs to recognize MART-1 and other relevant melanoma antigens. These more advanced antigen presentation platforms could be used in conjunction with cytokines like IL-15 or IL-21 to increase central memory T cells without increasing the number or activity of regulatory T cells, which can dampen immune response as is known to occur after IL-2 administration.

A deeper understanding of the signals that promote T-cell survival and activity after antigen exposure and reagents to modify those signals are likely to result in

more consistently effective immunotherapy for melanoma and other malignancies. Targeting MART-1 and other tumor antigens administered with biological agents to influence T-cell behavior shows great promise in unlocking the potential of vaccines for melanoma and other solid tumors.

Anticipated High-Impact Results

- DC pulsed with peptides for MART-1/gp100/Tyrosinase/NY-ESO-1/MAGE-3 in conjunction with lymphodepletion and autologous lymphocyte infusion, Weber et al.
- Phase II trial of extended dose anti-CTLA-4 antibody ipilimumab (formerly MDX-010) with a multi-peptide vaccine for resected stages IIIC and IV melanoma, Weber et al.
- Phase II Randomized Study of a Lymphodepleting Conditioning Regimen Comprising Cyclophosphamide, Fludarabine Phosphate, and Total-Body Irradiation Followed by Anti-MART-1 and Anti-gp100 T-Cell Receptor Gene-Engineered Autologous Peripheral Blood Lymphocytes, High-Dose Aldesleukin, and gp100:154–162 or MART-1:26–35 (27L) Peptide Vaccination in Patients With Metastatic Melanoma, Rosenberg, et al.

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