

Tumor-Associated Antigens in Breast Cancer

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Keywords

Breast cancer · Tumor antigen · Vaccine

Summary

The targets for the immune system are antigens present on cancer cells; however, many are not cancer-specific and may also be found on normal tissues. These antigens are often products of mutated cellular genes, aberrantly expressed normal genes, or genes encoding viral proteins. Vaccines constitute an active and specific immunotherapy designed to stimulate the intrinsic anti-tumor immune response by presenting tumor-associated antigens expressed on normal tissues that are over-expressed on tumor cells.

Introduction

A basic principle of tumor immunology in general and of cancer immunosurveillance in particular is that cancer cells express antigens that differentiate them from their non-transformed counterparts. The targets for the immune system are antigens present on cancer cells; however, many are not cancer-specific and may also be found on normal tissues. These antigens are often products of mutated cellular genes, aberrantly expressed normal genes, or genes encoding viral proteins. The human tumor-associated antigens (TAAs) include differentiation antigens (such as melanocyte differentiation antigens), mutational antigens (such as p53), over-expressed cellular antigens (such as HER2), viral antigens (such as human papillomavirus proteins), and cancer/testis (CT) antigens that are expressed in germ cells of the testis and ovary but are silent in normal somatic cells (such as MAGE and NY-ESO-1).

Vaccines constitute an active and specific immunotherapy designed to stimulate the intrinsic antitumor immune response by presenting TAAs expressed on normal tissues that are overexpressed on tumor cells [1–3]. Malignant cells can

Schlüsselwörter

Mammakarzinom · Tumorantigen · Impfstoff

Zusammenfassung

Die auf Tumorzellen vorhandenen Antigene sind die Angriffspunkte für das Immunsystem. Viele dieser Antigene sind jedoch nicht tumorspezifisch und können auch auf normalen Geweben gefunden werden. Diese Antigene sind oftmals die Produkte mutierter zellulärer Gene, aberrant exprimierter normaler Gene oder virale Proteine kodierender Gene. Impfstoffe stellen eine aktive und spezifische Immuntherapie dar, deren Aufgabe es ist, eine spezifische Antitumor-Immunantwort zu stimulieren, indem sie tumorassoziierte Antigene, die auf normalen Geweben exprimiert und auf Tumorzellen überexprimiert werden, präsentieren.

express both normal self-antigens (expressed at normal or abnormal levels) and specific TAAs that arise from genetic mutations and/or epigenetic changes. These TAAs can show changes recognized by the immune response either through their loss or de novo aberrant expression. Many TAAs have been identified and shown to be specifically recognized by T cells [4–8]. Many tumor antigens used in breast cancer immunotherapy are expressed on normal tissues but are over-expressed or mutated on tumor cells. Some of these antigens are universal tumor antigens, as they are broadly expressed by most tumors. The ideal TAA should fulfill certain criteria [9], the major one being therapeutic function defined as a vaccine-induced clinical response within a controlled trial. The second dominant criterion is immunogenicity, i.e. the ability to elicit T-cell and/or antibody responses. Also, the antigen should be oncogenic, specific, and highly expressed on all cancer cells in patients designated for treatment. Other minor criteria are stem cell expression, number of patients with antigen-positive cancers, number of antigenic epitopes, and cellular location of antigen expression [9]. We will discuss all potential antigens that have been used to construct vaccines for the treatment of breast cancer.

Human Epidermal Growth Factor Receptor 2

The human epidermal growth factor receptor 2 (HER2) is a 185-kDa protein receptor with tyrosine kinase activity and extensive homology to the epidermal growth factor receptor. HER2 is expressed in many epithelial tumors and overexpressed in approximately 25% of all primary breast carcinomas. Overexpression of HER2 is associated with poor prognosis. HER2 is a suitable target because it involves an extracellular domain (ECD) that can be targeted by antibodies produced by B cells. These antibodies could act either via a functional pathway (i.e. blocking of the HER2 signaling pathway) or an immune mechanism such as antibody-dependent cell-mediated cytotoxicity. Spontaneous T and B cell responses have been observed in patients with HER2-positive tumors, confirming the immunogenicity of HER2 [10]. The use of HER2 peptides as a potential target for breast cancer immunotherapy arose from experimental evidence in the rat showing that immunization with a mixture of peptides derived from the ECD and intracellular domain (ICD) of human HER2, but not the whole protein, elicited a delayed hypersensitivity [11]. Based on these findings, clinical trials with HER2 peptides were conducted. These studies were associated with minimal toxicity, suggesting that it is possible to generate anti-HER2 responses also in humans without major signs of autoimmunity. Vaccination with HLA class I peptides likely requires additional antigen-specific or non-specific helper activity to generate long-lived immunity [12]. A major goal of these studies was to overcome the problems associated with using class I epitopes alone, i.e. antigen instability or aggregation because of the short length of the peptide. Interestingly, in a first study in the adjuvant setting, 29 patients with no evidence of disease after surgery for HER2-positive breast or ovarian cancer received 3 level doses of a HER2 ICD protein vaccine. The vaccine was administered intradermally, monthly for 6 months, with granulocyte-macrophage colony-stimulating factor as an adjuvant. The vaccine was well tolerated. The majority of patients developed HER2 ICD-specific T-cell immunity. The dose of vaccine did not predict the magnitude of the T-cell response. The majority of patients also developed HER2-specific immunoglobulin G antibody immunity. Vaccine dose did not predict magnitude or avidity of the HER2-specific humoral immune response. Although the dose of vaccine did not impact the magnitude of T-cell or antibody immunity elicited, patients receiving the highest dose developed HER2-specific immunity more rapidly than those who received the lowest dose [13]. Another study in the adjuvant setting used a vaccine formulation containing a truncated recombinant HER2 protein (dHER2) combined with a new potent immunological adjuvant. dHER2 includes the ECD and a part of the ICD of the HER2 protein. The trial's objective was to evaluate safety and immunogenicity. The vaccine was well tolerated overall and showed minimal toxicity. No symptomatic cardiac dysfunction was observed. Antibody

responses against dHER2, ICD, and ECD were elicited. Antibodies to the ECD and the ICD are induced in a dose-dependent manner, suggesting that the higher-dose vaccine may be required for future phase II and III studies. These studies suggest that in order to have a maximal induction of the immune response against HER2 in terms of antibody and T-cell responses, both the ECD and ICD of the protein should be included in the vaccine formulation. This should guarantee also the induction of a long-lasting immunological memory.

Mucin 1

Mucin 1 (MUC-1) is a membrane-associated glycoprotein expressed by many types of ductal epithelia, including pancreas, breast, lung, and gastrointestinal tract. It is overexpressed and aberrantly glycosylated in malignant cells. It is a multifunctional protein involved in the protection of mucous membranes, signal transduction, and modulation of the immune system. More than 70% of cancers overexpress MUC-1, making this antigen a potential target for immunotherapy [14]. MUC-1 is sufficiently immunogenic to elicit strong antitumor immunity as a TAA [15]. Preclinical studies using tumor cells expressing MUC-1 protein or peptide antigen concluded that MUC-1 could induce humoral response without inducing cellular response [16–19]. It seems complex generating cytotoxic T lymphocytes (CTL) effectors *in vivo* maybe because of the induction of T-cell anergy by tumor-derived MUC-1 [20]. In the attempt to optimize MUC-1 presentation by antigen-presenting cells (APCs), several experimental studies in xenografts have targeted the mannose receptor on APCs by using antigens that have been linked to mannose [21–23]. The MUC-1 peptide-based vaccines have been able to elicit a humoral response but not a cell-mediated response [24]. These studies suggest that it is possible to elicit a CTL response against MUC-1 antigen, but the antibody response is unpredictable. Moreover, it is important to emphasize that the MUC-1 peptides used for vaccination do not resemble the form of antigen found on tumor cells. These cells in fact bear a molecule that is often underglycosylated as compared to their normal counterparts and could not be detected by antibodies elicited towards a non-glycosylated peptide.

Carcinoembryonic Antigen

Carcinoembryonic antigen (CEA) is a 180-kDa glycoprotein that is overexpressed in a wide range of carcinomas, including colorectal, gastric, pancreatic, non-small cell lung, and breast carcinomas. It is an adhesion molecule, and its overexpression in cancer cells promotes adhesion and metastasis. CEA is one of several oncofetal antigens that may serve as a target for active anticancer-specific immunotherapy. However, as CEA

is normally expressed, the immune system commonly becomes tolerant to it. Therefore CEA peptide-based vaccines firstly must break this tolerance. Clinical trials using a CEA-based vaccines for the treatment of different types of human cancers showed overall safety and efficacy [25–29]. Among the different CEA-based cancer vaccines, dendritic cell (DC)- and recombinant virus-based vaccines seem the most valid [25, 26]. However, although vaccination improved time to progression (TTP) and, sometimes, survival by inducing a strong immune response, it failed to eradicate the disease maybe because of the negative effect of the tumor microenvironment on the immune response. Hence, in order to develop more efficient and effective cancer vaccines, new clinical trials combining them with chemotherapy, radiotherapy, and drugs targeting those factors responsible for immunosuppression are warranted.

Human Telomerase Reverse Transcriptase

As a potential molecular therapeutic target for cancer, the human telomerase reverse transcriptase (hTERT) has been extensively evaluated because of its wide expression in human cancer cells and critical functional role in tumor growth and development. One proposed clinical strategy is hTERT-directed immunotherapy, supported by the identification of immunogenic hTERT epitopes that triggered tumorlytic T cells in preclinical studies [30]. Telomerase maintains chromosomal integrity by protecting telomeric DNA that would otherwise be lost during successive rounds of cell division in rapidly dividing cells such as tumor cells. hTERT is a common and immunogenic tumor antigen expressed in about 85% of all cancers. hTERT peptides or DC-based vaccines elicit an immune response in various tumors [30, 31]. A phase I clinical trial was performed to evaluate the clinical and immunological impact of vaccinating advanced cancer patients with the HLA-A2-restricted hTERT I540 peptide presented with keyhole limpet hemocyanin (KLH) by *ex vivo* generated autologous DCs. As measured by peptide/MHC tetramer, enzyme-linked immunospot, and cytotoxicity assays, hTERT-specific T lymphocytes were induced in 4 out of 7 patients with advanced breast or prostate carcinoma after vaccination with DCs pulsed with hTERT peptide. Tetramer-guided high-speed sorting and polyclonal expansion achieved highly enriched populations of hTERT-specific cells that killed tumor cells in a MHC-restricted way. Despite concerns of telomerase activity in rare normal cells, no significant toxicity was observed. Partial tumor regression in 1 patient was associated with the induction of CD8⁺ tumor-infiltrating lymphocytes. These results demonstrate the immunological feasibility of vaccinating patients against telomerase, and provide the rationale for targeting self-antigens with critical roles in oncogenesis [32].

Sialyl-Tn

The Tn, TF, and sialyl-Tn (STn) antigens represent the immature glycosylation products of serine and threonine of the protein core and are naturally masked by the complete glycosylate chain. All 3 epitopes are strongly expressed on cancer cells, and may be associated with disease progression and metastasis. STn is a core-region carbohydrate antigen constituted by the premature 2–6 sialylation of N-acetylgalactosamine, whose expression has been associated with some human tumors. In a randomized phase II trial, 23 patients with metastatic breast cancer were randomized to receive 100 mg STn linked to KLH with DETOX-B adjuvant with or without low-dose cyclophosphamide (intravenous or oral). All patients developed IgG and IgM responses to STn. 2 patients reported a minor response, while 5 patients experienced stable disease. Patients pretreated with cyclophosphamide had a higher antibody titer and longer survival [33, 34]. Out of 40 patients, 33 with high-risk or metastatic breast cancer received the Theratope[®] STn-KLH vaccine (Biomira Inc., Edmonton, Alberta, Canada) following high-dose chemotherapy and stem cell rescue. Out of 26 evaluable patients, the authors described a positive enzyme-linked immunospot assay for IFN- γ in 11 patients. The vaccine was well tolerated. Patients with highly specific lytic activity of peripheral blood lymphocytes had a longer remission compared to patients who displayed less specific immune activity [35, 36]. In a large phase III trial, 1,028 patients with metastatic breast cancer and no evidence of progressive disease after a first-line chemotherapy, were randomized to Theratope or control during concomitant endocrine therapy. Patients with evidence of immune response had a survival benefit; patients included in the Theratope arm had an improvement in progression-free survival. A randomized, double-blind, phase III trial in 1,030 women with metastatic breast cancer failed to demonstrate a significant improvement in TTP or overall survival (OS). Analysis of a pre-stratified subset of patients receiving endocrine therapy showed a difference in OS.

Wilms' Tumor Gene

The Wilms' tumor gene (WT1) was initially identified in sporadic and hereditary cases of Wilms' tumor as being either mutated or overexpressed [37]. WT1 is involved in cell growth regulation or differentiation. As a result of the alternative use of 2 promoters, alternative splicing, alternative use of translation initiation sites, and RNA editing, the WT1 gene can encode as many as 24 different isoforms. The WT1 protein is able to bind DNA by means of 4 zinc fingers but also interacts with several other proteins and can shuttle between the nucleus and cytoplasm. Some isoforms are transcriptional activators or repressors, but others can act as RNA-processing factors [38]. WT1 is expressed in normal adult tissues of

different mammalian species. This expression is limited to particular cell types, including glomerular podocytes, ovarian granulosa, testicular Sertoli cells, mammary duct and lobule cells, and splenic parenchyma [39, 40]. CD34+ pluripotent hematopoietic stem cells produce WT1 transiently during maturation [41–43]. One rat model study reports induction of WT1 expression in the vasculature of the heart in response to local ischemia or hypoxia. This is thought to reflect the protein's role in neoangiogenesis, which is in line with the documented expression of WT1 in the vascular endothelium of tumors. WT1 protein is expressed in blood vessels but not in cardiomyocytes of normal human heart samples. However, in cases of chronic ischemic disease, WT1 protein was detected in cardiomyocytes as well. Hypoxia can also induce WT1 protein expression in renal tubular cells [44, 45]. In contrast to the restricted expression in adult tissues, WT1 is widely expressed in a number of cancers and appears to act as an oncogene as interference with WT1 function inhibits proliferation and induces apoptosis, making WT1 a target for cancer immunotherapy.

Several WT1 antigenic HLA class I restricted peptides have been identified [46]. CTLs raised in vitro against some of these epitopes could lyse WT1-expressing cancer cells in an HLA-restricted manner. WT1-specific helper epitopes are able to elicit CD4+ helper T-cell responses [47, 48]. Peptide- or DNA-based WT1 immunotherapeutics have been tested in mice. These were able to induce WT1-specific CTLs, resulting in the rejection of challenges by WT1-expressing tumor cells. Of concern was whether the expression of WT1 by a subset of CD34+ marrow cells would give rise to marrow toxicity when confronted with a vaccine-induced immune response to WT1. WT1-specific CTLs, capable of inhibiting leukemia progenitor cell colony growth, did not inhibit progenitor cell colony growth from normal marrow. Also, the induced immune response did not cause any damage to other tissues expressing

WT1 at physiological levels [49, 50]. Results of phase I/II trials mostly evaluating HLA class I-restricted peptide vaccines in patients with WT1-positive tumors describe the results obtained after treatment of approximately 116 patients with different types of cancer [51, 52]. Half of the treated patients displayed signs of clinical activity, with a few cases of drastic tumor regression. In one of the clinical trials targeting WT1, tumor regression was observed in 2 metastatic breast cancer patients who received WT1 peptide. In 1 patient, this was associated with an increase in the numbers of WT1 tetramer-positive T cells in the peripheral blood. No damage to physiologically WT1-expressing normal tissues was reported, and the treatment was well tolerated overall [53].

Conclusion

Antigens present on cancer cells are the targets for the immune system; however, many are not truly cancer-specific as they may also be found on normal tissues. Antigens that are found and studied in breast cancer include CEA, HER2, MUC-1 which is hypoglycosylated in adenocarcinomas, carbohydrate antigens (Tn, TF, STn), p53 – a tumor suppressor gene mutated in cancers, TERT, and WT1. Therapeutic efficacy and immunogenicity are the most important characteristics that have to be taken into account when selecting TAAs as immunotherapeutic targets. The choice of specific TAAs is paramount in order to translate the most promising TAAs into vaccines for cancer treatment or prevention.

Disclosure Statement

The author has no conflicts of interest to disclose.

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