Cancer Testis Antigens and Immunotherapy: A new Dawn

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Abstract. Immunotherapy for cancer has been recognized as the fourth therapeutic method after surgery, radiotherapy and chemotherapy, which can prevent postoperative metastasis and recurrence and reduce or even eliminate the toxic and side effects of chemoradiotherapy. The development of successful immunotherapy strategies need to use cancer antigens which can be identified by the host’s immune system. This method’s ability in causing antitumor immune response has been fully proved, but it also faces enormous risks and challenges, as finding the highly efficient and specific tumor markers is very difficult. Cancer-testis antigens (CTA) are a special kind of tumor antigens with normal expression restricted to male germ cells in the testis but not in adult somatic tissues. The immune privileged status of CTA gives tumor specificity and makes it an ideal candidate for targeted immunotherapy biomarkers. Here, we briefly review the research history, expression characteristics of CTA, molecular mechanisms of CT gene, and the bright future of immunotherapy in cancer treatment.

1 Introduction

The search for a cure for cancer has been lasting for a long time. Cancer remains by far one of the most threatening diseases to humans. Traditional treatments include surgery, radiation and chemotherapy, which can destroy cancer cells but also do harm to normal tissues. These toxic side effects greatly reduce the quality of life of cancer patients. The fourth modality of immunotherapy has been well documented since 1890 when Coley demonstrated that bacterial products (Coley toxins) had benefits for inoperable cancers and the subsequent application of Bacillus Calmette-Guerin (BCG) and other crude immunostimulants showed benefits that led to regulatory approval of their use in some solid tumors such as bladder cancer. Tumor immunotherapy is simply to use a variety of means to activate the body's anti-tumor immune response, and finally eliminate and kill tumor cells. Scientific research has shown that tumor cells can trick the body's immune system into mistaking them for the body's own normal tissue cells to avoid being attacked and destroyed by the immune system. The goal of tumor immunotherapy is to expose the deception of tumor cells, activate the immune system, recognize, attack and finally kill tumor cells. Although immunotherapy has been shown to be a promising strategy for treating different human cancers, one of the major limitations remains the lack of tumor-associated antigen (TAA) that can be an effective target.

Cancer-testis antigens (CTA) are a category of tumor antigens with normal expression restricted to male germ cells in the testis. In some cases, CTA are also expressed in ovary and in trophoblast. They are important cell surface proteins during reproductive development. In malignancy, this gene regulation is disrupted, resulting in CTA expression in a proportion of tumors of various types. However, their expression in different tumors is heterogeneous and related to the degree of tumor progression. They are expressed wildly and meaningfully in melanoma, liver cancer, bladder cancer and pediatric tumors such as neuroblastoma, etc. So they are ideal biomedical marker to be used in T lymphocytic or antigen-specific and targeted cellular immunotherapy. CTA can be classified as CT-X antigens and non-X CT antigens based on whether their genes are encoded by the X chromosome. CT-X antigens are highly expressed in spermatogonial cells and tend to express multiple CT antigens simultaneously, while non-X CT antigens are distributed throughout the genome and are usually expressed in sperm cells. Up to 10% of the genes on the X chromosome were identified as belonging to the CT-X family.

According to the current information in the CTdatabase (http://www.cta.lncc.br) and GeneBank (https://www.ncbi.nlm.nih.gov/gene), there are now more than 200 gene families, classified into 51 gene families, that are strongly upregulated in cancer where the normal expression is highly biased to the germline. Not all of these have been shown to be capable of...
eliciting immune responses but are collectively termed the CT antigens.

2 History: the past and the present

In 1991, Thierry Boon and his colleagues successfully cloned the first tumor antigen in a groundbreaking study, which significantly changed the field of tumor immunology. MAGE-A genes elicit an HLA class I-dependent cytotoxic response in sensitised lymphocytes against the melanoma cell line MZ2-MEL, and because of that they were discovered firstly 11. One of the most special characteristics of the antigen in question, MAGE-A1, is that it is normally expressed in the germline but not in other normal tissues. But it was not immediately recognized at that time. Then according to subsequent analysis of the pattern of normal tissue expression, all of the cancer antigens initially cloned using cytotoxic T-cell clones and autologous tumor mRNA were found to have testis restricted expression among normal adult tissues. The MAGE-A family is comprised of 12 highly homologous genes clustered at Xq28. The genes are characterized by the presence of a conserved domain (MHD, MAGE Homology Domain). MAGE-A gene expression occurs in the normal human germline, and in a wide range of tumors. MAGE-A1 is expressed in a wide variety of human cancers at the RNA and protein level. Antigen-specific immune responses can be induced by immunization with MAGE-A1.

Based on these findings, other tumor antigens encoded by testicular restriction genes, including BAGE and GAGE, were also found using cytotoxic T cells isolated from the same patient who discovered MAGE-A1. In the same way, SYCP1 antigens were also isolated.

The genetic methods of cloning MAGE, BAGE and GAGE were technically challenging because they required the establishment of autologous CTL lines and tumor cell lines from the same patient, which was not easy to achieve for most epithelial tumor types. To overcome these limitations, a technique called SEREX was designed to isolate tumor antigens that elicit high titer immunoglobulin G responses in human hosts, known as SEREX for recombinant tumor cDNA expression libraries.

Then these initial discoveries were soon extended through the identification of antigens recognized by antibodies in the sera of cancer patients. One of the first antigens discovered by this approach was NY-ESO-1 that appears to be the most immunogenic of the CT antigens and has become the major focus of experimental therapeutic vaccine development within the Ludwig Institute. NY-ESO-1 antibodies are present in the serum of a significant number of cancer patients. Up to 50% of patients bearing NY-ESO-1-expressing tumors spontaneously develop specific antibody responses during the course of their disease. Serum antibody frequencies to NY-ESO-1 appear related to gene expression in the tumor, and may increase with higher stage or grade. However, it is reported in one of the recent researches that NY-ESO-1 induces strong immune responses in cancer patients but has limited objective clinical responses to NY-ESO-1 expressing tumors due to effect of competitive negative signaling from immune-checkpoints and immune-suppressive tumor microenvironment. The major aim of researchers now is to counter potential roadblocks in therapeutic pathways encompassing NY-ESO-1. This can potentially be achieved via combination therapy to increase the efficacy of NY-ESO-1-specific immunotherapeutic interventions.

SEREX clones of recombinant human melanoma cDNA from human melanoma using autologous serum were serologically analyzed and several neomelanoma antigens with immunogenicity in autologous hosts were identified. A variety of antigens have been detected in subsequent work, such as CT7/ MAGE-C1, SCP-1, HOM-TES-85, cTAGE-1, OY-TES-1, CAGE and so on.

Emerging data suggest that they may play a key role in many important cellular processes, such as signal transduction, transcriptional regulation, presumed proto-oncogenes and cell growth, but there are few research achievements on the function of CT antigens, and people only have a relatively clear understanding of the function of several CT antigens. For example, CAGE-1 is predominantly expressed during postmeiotic stages and CAGE-1 is a novel component of the acrosome of mammalian spermatids and spermatozoa. SCP-1 is an important component of the synaptic complex and is closely related to the pairing, exchange and separation of chromosomes. SPANXN2 regulates TGCT cell migration via EMT- and AKT-related proteins although its role in the occurrence and development of TGCT remains to be fully elucidated.

In addition to immunogenicity, CTAs have oncogenic functions including support of tumor growth, evasion of apoptosis and metastasis. For example, CAGE-1, SCP-1, OY-TES-1, CTAGE-1, HOM-TES-85, cTAGE-1, MAGE-C1, CAGE and CT45A1 were expressed more frequently in different types of tumors. For example, CAGE-1 is predominantly expressed during postmeiotic stages and CAGE-1 is a novel component of the acrosome of mammalian spermatids and spermatozoa. SCP-1 is an important component of the synaptic complex and is closely related to the pairing, exchange and separation of chromosomes. SPANXN2 regulates TGCT cell migration via EMT- and AKT-related proteins although its role in the occurrence and development of TGCT remains to be fully elucidated.

3 Expression: heterogeneity among different tumors

The expression of CT-X antigens vary greatly among different types of tumors. For example, CT-X antigens are frequently expressed in bladder, lung, ovarian and hepatocellular carcinomas, and melanoma, according to reverse transcription polymerase chain reaction (RT-PCR) analysis, but are rarely observed in the kidney or colon, gastric cancer, leukemia and lymphoma cells.

Specific CTA is expressed at different frequencies in different types and subtypes of tumors. Melanoma was most frequently expressed, followed by sarcoma, lung cancer, and breast cancer. In general, the expression of antigens in tumor tissues has the following three characteristics.

Firstly, the same CT antigen is expressed differently in different tumor tissues. For example, NY-TLU-57, GAGE1, SAGE1 were expressed more frequently in tumor samples than in healthy tissues. But SAGE1 were only expressed in 3-5% of ER-negative and 0-2%
of ER-positive cancers 26. Surprisingly, it is detected that no SAGE1 is expressed in the 67 lung cancers (mainly non-small lung cancer), while MAGE-A proteins were present in 15% and 7-16% of these tumor types, respectively 27.

Secondly, the expression frequency of different CT antigens in the same tumor tissue is also different. In a former research, scientists analysed the expression pattern of OCT2, SSX2-4, and SAGE1 in 36 SS cases and four intratubular SS (ISS) as well as a series of normal testis samples throughout development and it is concluded that OCT2, SSX and SAGE1 reveal the phenotypic heterogeneity of spermatocytic seminoma reflecting distinct subpopulations of spermatogonia 28.

Thirdly, CTA expression tends to be aggregated, that is, it is found that some tumor specimens express multiple CT antigens simultaneously, while others will be completely free of CT antigens. The highest frequency of expression was found in esophageal squamous cell carcinomas: Positive staining for MAGEA, CT45, CT7, SAGE1, GAGE, NXF2, NY-ESO-1, and CT10 was observed in 57%, 38%, 33%, 33%, 33%, 29%, 29%, 19%, and 14% of squamous cell carcinomas, respectively, suggesting a potential diagnostic role for cancer-testis antigens in the evaluation of premalignant squamous lesions 29.

3.1 Melanoma

Melanoma is the first malignant tumor with testicular antigen (CTAs) expression and immunogenicity documented. Some cancer testis antigens with strong metastatic potential have been shown to be expressed in melanoma samples 30. A kind of CTA called preexpressively expressed melanoma antigen (PRAME), is expressed in many primary and metastatic melanomas and is co-expressed with CLASS HLA I in many of these melanomas. In addition, PRAME-specific T cells have been shown to recognize PRAME-positive melanoma cell lines, making them targets for immunotherapy 31. Some CTAs’ expression has been shown to correlate with disease progression or clinical outcomes. For example, expression of MAGE-A1, MAGE-A4 and NY-ESO-1 was negatively associated with recursion-free survival in a large number of melanoma patients 32.

3.2 Esophageal cancer

In esophageal cancer, although the relationship between the expression and prognosis of MAGE-A, NY-ESO-1, TTK and LAGE-1 remains controversial, the MAGE-A, NY-ESO-1, LAGE-1 and TTK are highly expressed, which can induce specific CTLs for tumor cells to produce special effect. Some clinical trials have shown that immune treatment in patients with esophageal cancer is safe and effective, and provides a new treatment strategy for the treatment of esophageal cancer 33.

3.3 Non-small cell lung carcinoma

The MAGE-A3 cancer/testis antigen is frequently expressed in non-small cell lung cancer (NSCLC) and vaccination with MAGE-A3 in patients with MAGE-A3-positive NSCLC has shown promising results. One of results shows that the CT antigens GAGE, NY-ESO-1 and SP17 are expressed in a considerable proportion of NSCLC and may therefore serve as candidate targets for immunotherapeutic treatments of this disease. Furthermore, GAGE and NY-ESO-1 were present in more than 50% of the tumor cells in 63.6% (28/44) and 70% (14/20) of the positive cases, respectively 34.

3.4 Ovarian cancer

Based on multiple independent large databases, including Genotype-Tissue Expression (GTEx), and human proteomic and TCGA data, one group recently systematically explored the molecular landscape of CT genes in 19 cancer types. CTA family members in ovarian cancer reported to date include MAGE genes, NY-ESO-1, SSX, and CT45, which are categorized as CT-X antigens, and BORIS, PRAME, PIWIL, and AKAP3/4, which are categorized as non-X CTAs 35.

3.5 Adenoid cystic carcinoma of the head and neck

Adenoid cystic carcinoma of the head and neck is a rare but highly malignant tumor. According to a former research, eighty-four cases of ACC were identified, and Expression of NY-ESO-1 was found in 48/84 (57.1%) and of pan-MAGE in 28/84 (31.2%) 36. As such a significant fraction of ACC patients show expression of the cancer testis antigens NY-ESO-1 and pan-MAGE, subgroup of patients with a poor prognosis carries antigens targetable by specific immunotherapy 37.

3.6 Breast cancer

Breast cancer is still the most common cancer affecting women in the whole world, posing a serious threat to women's lives and health. It has been indicated that CT antigens expression is closely related to the ER status 38. Other CTAs such as HORMAD1, CXorf61, ACTL8 and PRAME were found to be highly expressed in the breast cancers that have basal cell properties 39. Furthermore, there are many other CTAs that predict invasion and metastasis of breast cancer such as ATAD2 and GPAT2 40.

There are also personalized CTA expressions in other tumors, which will not be repeated here.

4 Molecular mechanisms of CT gene expression

CT genes share a common mechanism of regulation at the transcriptional level 42. A lot of researches have indicated that epigenetic regulation is a key mechanism in the transcriptional regulation of CT genes 43. The
mechanism of CTA activation includes the following several categories.

4.1 DNA hypomethylation in CT gene activation

DNA methylation plays an important role in the epigenetic hierarchy of CT gene expression. DNA hypomethylation harmonizes CT gene promoters in EOC and is associated with advanced disease.

Initial evidence suggests that activation of MAGE-A1 in cancer cells is caused by hypomethylation of promoter DNA 44. It is reported that in ovarian cancer, DNA hypomethylation drives expression of BORIS, which is partly determined by the global DNA methylation status 45. And the same was subsequently found in NY-ESO-1, MAGE genes and some other CT genes.

4.2 Histone modification and modulation

Histone deacetylation and DNA methylation play a central role in the control of tumor gene expression, including transcriptional inhibition of tumor suppressor genes and genes involved in chemotherapy sensitivity 46. In a former research, it is reported that repression of NY-ESO-1, MAGE-A1, and MAGE-A3 coincided with DNA hypermethylation, recruitment and binding of polycomb group proteins, and histone heterochromatin modifications within the promoters of these genes 47. However, up to now, studies on the effect of histone modification on CT gene expression are limited, and most of the current studies usually adopt pharmacological methods.

5 Roles in cancer immunotherapy

CT antigens can be combined with exosomes for antigen presentation. Several CT antigens are expressed in exosomes and corresponding tumors. For example, MAGE-B4 gene expression in urinary exosomes of bladder cancer patients was higher than normal control samples 48. And testicular specific lactate dehydrogenase C4 was up-regulated in serum exosomes of breast cancer patients 49. Because immunogenic CT antigens are selectively expressed in tumor tissues and exosomes, they have the potential to be used as antigen-specific cancer vaccines or diagnostic markers 50.

CTAs are promising targets for cancer immunotherapy due to their expression in cancers and their rarity in normal tissues. With the identification of CTA peptides and clinical trials of CTA multipeptide vaccines, establishing personalized CTA peptide vaccines has become possible. Challenges remaining include the search for promising targets, identification of additional immunogenic CTA peptides, choice of suitable clinical settings, and development of feasible combination therapy 51. Humoral immune responses and cellular immune responses against NY-ESO-1 and MAGE-A3 have been detected 52, and the restricted epitopes have been identified as the recognition sites for CD8+ cytotoxic T lymphocytes (CTLs) 53.

To date, five tumor immunotherapy pathways based on CT genes/antigens have been identified:

A. Antigen-presenting cell approach: use CT antigen peptide to incubate autoantigen presenting cells, and then make vaccine into tumor cells expressing CT proteins and corresponding HLA-i molecules to induce specific CTL response, which can be enhanced by IL-2;

B. Melanoma cell inoculation path, namely, melanoma cells expressing CT protein are made into vaccine for tumor patients;

C. Gene transfection pathway, using the virus as the vector, carrying THE CT gene to make a vaccine, to the tumor patients inoculation;

D. Dendritic cell inoculation approach, that is, using CT antigen peptide to incubate monocyte derived dendritic cells, and then inoculated into the tumor patients;

E. The method of antigen peptide inoculation is CT antigen peptide directly inoculated into the body of tumor patients, mainly MAGE and NY-ESO-1.

In summary, CTA is an excellent target for anticancer drug discovery, tumor therapy and diagnostic biomarkers, and is also valuable in immunotherapy, tumor genesis and malignant progression research.

6 Conclusion

Cancer testis antigens are normal testicular antigens that are abnormally expressed in tumor cells. Because CT antigens are widely expressed in a variety of tumors, they are ideal molecules for immunotargeting and for the development of cancer vaccines for use in T lymphocytic or antigen-specific and targeted cellular immunotherapy. Many outstanding scientists have made great contributions to the field of CTA, and the discoveries of this field have opened up new ideas and enticements for cancer treatment and a bright future.

However, the researches in this field are still not thorough enough. At present, we do not have a clear understanding of the role of CT genes in human normal tissues and the expression characteristics of different CTA in different tumors. Current research focuses on the development and study of CTA functional cell pathways, the establishment of different tumor specific CTA expression and other aspects. In addition, in the actual experiment, a reliable evaluation mechanism must be established to determine whether a CTA gene plays a role in the upstream. The method of cell inoculation can be adopted to determine the true role of the gene through the survival performance of the cell.

Despite the promise of using CTA-driven strategies to treat cancer patients, the complexity of human malignancies requires new standards to be established for all of the scientists working in the field to verify the practical applicability of CTAS.
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