

Review Article

Cancer-testis antigens: the current status on antigen regulation and potential clinical use

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Abstract: Immunotherapy is theoretically an attractive therapeutic option for patients with hematological malignancies. Various laboratory studies suggested the importance of the choice of tumor antigen for successful immunotherapy. Cancer-testis antigens (CTAs) are potentially suitable molecules for tumor vaccines of hematological malignancies because of their high immunogenicity *in vivo*, even in cancer-bearing patients, and their relatively restricted normal tissue distribution. Tumor cell kill using a CTA-based immunotherapy will, therefore, be more specific and associated with less toxicities when compared to chemotherapy. Many CTAs have been identified in various hematologic malignancies. In this review, we will take the readers through the journey of hopes and the disappointments arisen from the discovery of CTAs. We will describe the features of CTAs and their expression in hematologic malignancies. We will also discuss the mechanisms regulating the expression of these CTAs, from a primary regulatory mechanism involving DNA methylation to secondary controls by cytokines. Finally, we will address the potential obstacles that will prevent the successful use of CTAs as targets for tumor immunotherapy.

Keywords: Cancer-testis antigens, hematological malignancies, DNA methylation, heterogeneity of antigen expression

Tumor immunotherapy was first practiced more than 100 years ago when William Coley, a surgical oncologist, administered Coley's toxin to cancer patients [1]. Since then, with advances in hybridoma technology and the understanding of antigen processing and presentation, a lot of progresses in the area of tumor immunotherapy have been made in the laboratory. They range from engineering of recombinant monoclonal antibodies to the discovery and optimization of antigen presentation. Despite such progresses, except for recombinant monoclonal antibodies such as Rituximab (directed against CD20) and Alemtuzumab (directed against CD52), tumor immunotherapy has not made significant impact into the daily treatment of patients with hematologic malignancies in the clinics. The lack of sufficient clinical impact of tumor immunotherapy arises from the failure to translate the successes observed in the laboratory to the clinic, especially in the area of active tumor immunization, and the inability to increase the applicability of the strategies because of the need for tailor-made vaccines (e.g. idiotypic protein vaccination for B-cell malignancies). Tumor

immunology became a distinct specialty in the early 60s. Both tumor immunology and immunotherapy have since gone through phases of great promises and disappointments. In this paper, we will review the promises that the discovery of Cancer-Testis antigens (CTAs) has brought to tumor vaccine development and the basis for CTA expression, and discuss the subsequent disappointments that have followed.

CTAs and their expression in hematologic malignancies

CTAs are a group of testicular-specific or testicular-predominant proteins that are aberrantly expressed on tumor cells. Although they were initially thought to be encoded only by genes on chromosome X, as the lists of CTAs increased, it became apparent that the gene encoding some of the CTAs were localized in somatic chromosomes. CTAs can, therefore, be divided into two groups: 1). CT-X antigens that are encoded by genes on chromosome X, and 2). Non-X CTA that are encoded by genes on somatic chromosomes [2]. It is estimated that 10% of the genes

Table 1. CTA expression in hematologic malignancies

CTA	Chromosome location	Diseases and frequency of expression (%)	References
SSX	Xp11.23-p11.22	Lymphoma (36%)	[3]
Sp17	11q24.2	MM (26%); DLCL (54.5%)	[10, 15]
Ropporin 1	3q21.1	CLL (43%); AML (18%); MM (37.5%)	[11]
CT45	Xq26.3	HD (58%); DLCL (22%)	[14]
BAGE	21p11.1	MM (14%)	[16]
GAGE 1	Xp11.4-p11.2	MM (41%)	[16]
MAGE-A	xq28	MM (Stage III, 100%; Stage I/II, 33%)	[17]
MAGE C1	Xq26-q27.2	MM (82%)	[17]
NY-ESO-1	Xq28	MM (60%)	[18]
SLLP 1	17q11.2	AML (22%); CML (29%); CLL (29%); MM (35%)	[19]
SPAN-Xb	Xq27.1	MM (20%); CML (60%); CLL (33%); AML (50%)	[20]
SCP1	1p13-p12	MM (10%); CML (23%); AML (5.7%)	[21]
SEMG 1	20q12-q13.2	CML (62%); CLL (42%); CLL (42%); MM (7%)	[22]
HAGE	6q12-q13	CML (57%)	[23]
PRAME	22q11.22	MM (48%); AML (30%); ALL (17%); CML (34%); CLL (28%)	[16, 24, 25]
PASD 1	Xq28	AML (33%); CML (17%)	[26]
Prm 1	16p13.2	CLL (34%)	[27]

(CTA = Cancer-Testis antigen; MM = multiple myeloma; AML = Acute myeloid leukemia; ALL = Acute lymphoblastic leukemia; CML = Chronic myeloid leukemia; CLL = chronic lymphocytic leukemia; HD = Hodgkin's disease; DLCL = Diffuse large cell lymphoma).

on the chromosome X encode CTAs [2]. In general, CT-X genes tend to be expressed in the spermatogonia and non-X CT genes in the later stage of germ-cell differentiation, such as spermatocytes [2-6].

The first group of CTAs, the MAGE family, were identified in 1991 using cytotoxic T-cell clones from a patient with malignant melanoma against an expression cDNA library [7]. This work was groundbreaking in tumor antigen identification and, at that time, together with advances made in the understanding of antigen processing and presentation the MHC molecules, brought great promises to tumor immunology and immunotherapy. This approach, however, is extremely labor-intensive. Since then, many other simpler approaches have been employed to isolate novel CTAs, ranging from Serological Screening of Expression cDNA library (SEREX) [8] to gene data mining [9], bioinformatics [10] and the use of a known CTA as the bait in a yeast two-hybrid system [11]. Many research groups concentrated in the last ten years in the isolation of novel CTAs in the belief if an ideal molecule can be identified, tumor immunotherapy might play an important role in

the treatment of various malignancies. Despite advances in antigen identification and the availability of a long list of candidate molecules that satisfy the criteria to be CTAs, the development of most of these molecules have not progressed beyond the stage of antigen characterization. Therefore, clinical tumor immunotherapy remains highly investigational even with the discovery of many new tumor antigens and the ability to improve antigen presentation using dendritic cells.

CTAs are theoretically ideal targets for tumor immunotherapy. Unlike most auto-antigens, CTAs are highly immunogenic, even in the autologous cancer-bearing patients. Furthermore, because of their very restricted normal tissue expression, immunotherapy targeting CTAs is expected to be more specific and less toxic. These two theoretical properties of CTAs have arisen from the belief that, because they are testicular-specific, they are normally only expressed in the immune privileged testicles where there is an apparent lack of human leukocyte antigen (HLA) class I molecules on the surface of germ cells [12], and the presence of the blood-testis barrier that protects the expo-

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sure of the antigens to the immune system [13]. Therefore, CTA-reactive T-cells are not negatively selected from the immune repertoire of the host, preserving the integrity of high affinity CTA-reactive precursor T cells that may be necessary for successful tumor immunotherapy. This may, however, not necessarily be the case in those CTAs that are expressed in spermatogonia or spermatocytes localized in the non-protected side of the blood-testis barrier, the basal compartment of the seminiferous epithelium [13].

Although most of the initial works were primarily on solid tumors, many CTAs have, in the last ten years, been identified to be expressed in hematologic malignancies. **Table 1** shows the CTAs expressed in these diseases [3, 10, 11, 14-27]. Probably a reflection of the poor prognosis of the disease and so an increased effort to investigate novel approaches that include immunotherapy, CTA expression in multiple myeloma is the most characterized of all the hematologic malignancies. Overall, CTA expression in multiple myeloma increases as the disease progresses [16]. CTAs that have been reported to be expressed by myeloma cells include MAGE-A

[17], MAGE C1 [17], Sp17 [10], NY-ESO-1 [18], SLLP1 [19], SPAN-Xb [20], SCP 1 [21] and SEMG 1 [22].

Leukemia cells have also been found to express CTAs. HAGE [23], SPAN-Xb [20] and SEMG 1 [22] have been found in up to 60% of patients with chronic myeloid leukemia. Other CTAs expressed in CML cells include PRAME [16, 24, 25] and PASD 1 [26] that showed lower expression frequencies. In CLL, the CTAs that have been reported to be expressed include SEMG 1 [22], SLLP 1 [19], Prm 1 [27], Ropporin 1 [11] and SPAN-Xb [20]. These results, therefore, suggest that there are indeed already an abundance of CTAs that may be used for tumor immunotherapy in hematologic malignancies.

Hematologic malignancies as a target for immunotherapy

Immunotherapy is an ideal approach for patients with hematologic malignancies. Experience from allogeneic hematopoietic stem cell transplant demonstrating the concept of graft-versus-tumor effect strongly suggests the susceptibility of the tumor cells from hematologic

malignancies to the killing effect of cytotoxic T lymphocytes. Therefore, provided that suitable tumor antigens are targeted in the optimal clinical setting, immunotherapy should be effective and yet non-toxic. Advance of molecular biology now provides us with the opportunity and ability to closely monitor patients with various hematologic malignancies for minimal residual disease. Therefore, one could envisage the application of immunotherapy to patients with minimal but detectable residual hematologic malignancies so that the efficacy of the approach in patients with minimal residual disease, where successes are most likely, could be studied. Application of immunotherapy in such a setting will theoretically result in higher *in vivo* effector: target ratio to maximize tumor cell kill by the cytotoxic T lymphocytes. However, immunotherapy has so far been applied primarily to patients who have already been heavily pre-treated and failed chemotherapy. The immune repertoire of these patients, unfortunately, is already severely compromised prior to immunotherapy. The lack of successes from immunotherapy is, therefore, not surprising.

Regulation of CTA expression

The expression of most genes is regulated through complex interactions between DNA sequences, histones and specific transcription factors/repressors. DNA methylation and modifications of amino acid residues in histone tails work together to regulate gene expression. These processes result in the modification of the structure of chromatin so that the chromatin is transformed from a configuration in which DNA transcription occurs to a configuration in which transcription is repressed [28]. The methylation and acetylation of specific histone residues provides a histone code that signals whether the gene is in a transcriptionally active state.

In normal cells, most of the CpG dinucleotides at gene promoter regions are unmethylated, whereas CpG islands found at other portions of the genome are methylated. The methylation of CpG islands prevents the transcription of the gene. There are, however, exceptions to this. Many of the CpG islands of imprinted genes and X-linked genes are methylated [29, 30]. Some genes also exhibit CpG island DNA methylation in a cell type-specific manner; for example, the CpG island of the gene may be methylated in mesenchymal cells but not in epithelial cells

[31, 32].

DNA methylation is carried out by one of three DNA methyltransferase enzymes (DNMTs), using S-adenosyl-methionine as the methyl donor [33]. The activities of all three DNMTs are blocked by DNA hypomethylating agents such as azacytidine. These agents are incorporated into the DNA of dividing cells, where they irreversibly inhibit the activity of DNMTs and prevent hypermethylation of CpG island. DNMTs also appear to act as platforms for a number of other proteins that maintain histones in configurations that regulate the transcriptional capability of chromatin [28]. These proteins include histone deacetylase enzymes (HDACs) and methyl-CpG binding proteins (MBD1, MBD2).

Decreased levels of overall genomic methylation are common findings in tumorigenesis of most cancers [34]. The decrease in global methylation begins early and before the development of frank tumor formation [35, 36]. Promoter hypomethylation, therefore, accounts for the aberrant expression of many tumor-associated antigens, including CTAs. As the cancer progresses and hypomethylation becomes more widespread, these tumor-associated antigens are more likely to be expressed.

Studies involving the MAGE family of CTAs in solid tumors suggested that DNA hypomethylation plays a key element in the regulation of CTA expression. These studies demonstrated the correlation between global DNA hypomethylation and the expression of CTAs [37]. Other works showed the direct link between hypomethylation of the CTA promoter genes and expression of the CTAs, so that treatment of the tumor cells with the DNA hypomethylating agent, 5-azacytidine, induced DNA hypomethylation and the subsequent expression of CTAs [38-40]. Gene regulation via the hypomethylated promoter gene appeared to be predominantly through the interaction between the hypomethylated promoter sequence and the MeCP 2 protein [41]. Despite the very tight relationship between DNA hypomethylation and gene expression, it is clear that other factors also play a part in regulating CTA expression because in some tumor cells, e.g. colon cancer cell, although the DNA is universally hypomethylated, the expression of CTA is not common.

The observation that the genome in colon cancer cells are highly hypomethylated and yet ex-

pression of CTAs in colon cancer is not common suggests the presence of secondary regulatory mechanisms controlling the expression of some CTAs. For SPAN-Xb, it was found that both IL-7 and GM-CSF upregulated SPAN-Xb expression in myeloma cells [40]. The action of IL-7 and GM-CSF was clearly dependent on the presence of a hypomethylated SPAN-Xb promoter gene since SPAN-Xb-negative myeloma cells did not respond to IL-7 and/or GM-CSF. However, if SPAN-Xb promoter gene in SPAN-Xb-negative myeloma cells was first hypomethylated with 5-azacytidine, SPAN-Xb expression could be further upregulated using these two cytokines that appeared to exert their functions in promoting SPAN-Xb expression via the promoter gene. Similarly, SEMG 1 expression in CLL cells could be further upregulated by IL-4 and IL-6 once the SEMG 1-negative CLL cells were treated with 5-azacytidine [42].

CTAs as targets for immunotherapy

Although the highly immunogenic nature and the very restricted normal tissue expression of CTAs make them ideal targets for immunotherapy, one major obstacle to their successful use in the clinical setting is the heterogeneous expression of most of the CTAs, even within individual patients. Most studies have concentrated on the expression frequencies of these CTAs without addressing the degrees of heterogeneity of expression of the antigens. This feature of CTAs hampers their successful use as targets for immunotherapy in the clinics. One could envisage the successful eradication of CTA-expressing tumor cells with a CTA-based immunotherapy, only to be faced with the selection for CTA-negative tumor variants within individual patients.

Although it is possible to upregulate the expression of these CTAs using DNA hypomethylating agents and also certain cytokines, it remains to be determined if the DNA hypomethylating agents would also induce the expression of CTAs in other somatic cells, hence increasing the risk for multi-organ toxicities. Until this problem of CTAs can be overcome, it is likely that CTA-based tumor immunotherapy will remain within the laboratory.

Despite the long list of CTAs in hematologic malignancies, so far there have only been two patients with hematologic malignancies reported

to have been treated with CTA-based immunotherapy. Both patients had multiple myeloma. One patient relapsed after allogeneic stem cell transplant and he received Sp17-pulsed dendritic cells followed by low dose interleukin-2 [43]. He achieved a transient drop of 90% of his serum paraproteinemia. Another patient received a syngeneic stem cell transplant for her multiple myeloma. However, prior to stem cell donation, the donor was immunized with the MAGE-A2 protein, formulated in AS02B [44]. MAGE-A3-specific immune responses were detected more than one year after the transplant and the patient remained in remission of the myeloma, 2.5 years after the transplant. While very limited conclusion regarding the efficacy of CTA-based tumor immunotherapy can be made from these two cases, one could nevertheless deduce that CTA-based tumor immunotherapy is safe.

Conclusions

Ideal tumor antigens are protein molecules that are specifically, abundantly, and stably expressed by all the tumor cells and uniformly among tumor cells within individual patients. They should also be highly immunogenic, absent from normal tissues and are crucial for the survival of the tumor cells. Unfortunately there is yet a tumor antigen that satisfies all these criteria. Until then, despite potential advantages of immunotherapy and unique features of CTAs, the journey for the advance of clinical tumor immunotherapy will likely continue to be very slow and bumpy.

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