Cancer testis antigen subfamilies: Attractive targets for therapeutic vaccine (Review)

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Abstract. Cancer-testis antigen (CTA) is a well-accepted optimal target library for cancer diagnosis and treatment. Most CTAs are located on the X chromosome and aggregate into large gene families, such as the melanoma antigen, synovial sarcoma X and G antigen families. Members of the CTA subfamily are usually co-expressed in tumor tissues and share similar structural characteristics and biological functions. As cancer vaccines are recommended to induce specific antitumor responses, CTAs, particularly CTA subfamilies, are widely used in the design of cancer vaccines. To date, DNA, mRNA and peptide vaccines have been commonly used to generate tumor-specific CTAs *in vivo* and induce anticancer effects. Despite promising results in preclinical studies, the antitumor efficacy of CTA-based vaccines is limited in clinical trials, which may be partially attributed to weak immunogenicity, low efficacy of antigen delivery and presentation processes, as well as a suppressive immune microenvironment. Recently, the development of nanomaterials has enhanced the cancer vaccination cascade, improved the antitumor performance and reduced off-target effects. The present study provided an in-depth review of the structural characteristics and biofunctions of the CTA subfamilies, summarised the design and utilisation of CTA-based vaccine platforms and provided recommendations for developing nanomaterial-derived CTA-targeted vaccines.

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Contents

1. Introduction

The cancer testis antigen (CTA) is a large protein family that is exclusively expressed in the testis, placenta and certain types of malignant tumor, and is involved in the regulation of critical processes during tumorigenesis and development (1). Attributed to the blood-testis barrier, CTAs are categorized as immunogenic tumor-associated antigens and deemed optimal targets for the design of therapeutic cancer vaccines (2). To date, >200 CTAs have been identified and documented in the CT database (www.cta.lncc.br), and >100 gene families are highly expressed in malignant tumors. Most genes in the same CTA subfamily are located in adjacent positions on the chromosomes and the encoded proteins generally share similar domains and structural characteristics. In tumor tissues, members of the CTA subfamily are frequently co-expressed and have similar cellular functions. For instance, members of the melanoma antigen family (MAGE) have a highly conserved domain, MAGE homology domain, (MHD), and have essential roles in stress response and cancer progression (3,4). As MAGEs are widely expressed in a wide range of malignancies, vaccines targeting MAGEs have been developed in clinical trials to treat various cancer types, including melanoma and lung cancer (5-7). Therefore, a better understanding of the structural and functional characteristics of the CTA family in malignant tumors is helpful for developing reliable targets for tumor immunotherapy.

Immunotherapeutic strategies targeting CTAs include engineered T-cell receptor T-cell therapy, chimeric antigen receptor T-cell therapy and vaccine-based therapy. Cancer vaccines are and attractive complement or alternative to conventional cancer treatments with great prophylactic

and therapeutic potential (8). Cancer vaccines stimulate tumor-specific immune responses through delivering tumor antigens into antigen-presenting cells (APCs) and induce vigorous antitumor immunity to inhibit tumor growth, recurrence and metastasis (9). Compared with other immunotherapeutic strategies, cancer vaccines provide specific, safe and tolerable control of cancer progression. Furthermore, nanomaterials have been utilized to design vaccine platforms, which improved the efficacy during antigen delivery, processing and presentation to T cells (10). A variety of cancer nanomaterial-based vaccines have been designed to deliver peptide/adjuvant or nucleic acid of CTAs. In addition to commonly used inorganic and organic materials (such as polymers and liposomes), dipeptide-based nanotubes, nucleic acid nanostructures, cell membranes and other biomimetic nanomaterials have also proven effective as methods for delivering vaccine compositions to targeted sites (11-14).

So far, cancer vaccines targeting the CTA family exhibited promising efficacy in tumor control at preclinical and clinical stages (15,16). However, the clinical translation of cancer vaccines is hampered by relatively weak immunogenicity and a suppressive tumor microenvironment (TME). Since members of the CTA subfamily usually share homology in structure and expression patterns, attention should be paid to improving the immunogenicity of CTA vaccines. On the other hand, the application of nanomaterials may also serve as an excellent approach to conquering the suppressive TME and generate a profound antitumor response. In fact, delivery of CTA antigens by nanomaterial-derived systems has been demonstrated, with certain results of inhibiting tumor growth and metastasis (13,17). The present review briefly summarized the structural homology and distinct biological functions of the six CTA subfamilies, providing a systematic understanding of CTA antigens and a comprehensive approach for designing cancer vaccines based on CTA (Table I; Fig. 1). The current status and challenges of cancer vaccines targeting CTAs were also summarized, including the results and adverse events of conventional vaccination (DNA vaccines, mRNA vaccines and peptide vaccines). A proposal was made to develop a nanomaterial-derived cancer vaccine, which holds great promise for overcoming the suppressive immune microenvironment and achieving co-delivery of multiple vaccine components.

2. CTA subfamilies

MAGE family. Since first having been identified as a CTA in 1991, the MAGE family is the largest CTA subfamily consisting of >40 members (18,19). Based on expression pattern and chromosomal location, the human MAGE family is generally divided into type I MAGEs and type II MAGEs (20-22). Type I MAGEs, including MAGE-A, -B and -C subfamily members, are considered CTAs due to their restricted expression pattern in adult testicular germ cells and malignancies. Type II MAGEs, including MAGE-D, -E, -F, -G, -H and -L subfamilies and Necdin, are observed in various tissues, such as embryonic and various adult tissues, such as the brain (20,21,23). Given that type I MAGEs are the most studied CTAs in tumorigenesis and anticancer treatments, the structural features and biofunctions of type I MAGEs will be discussed in this subsection.

Biofunction under normal conditions. Type I MAGEs are located on the X chromosome, including MAGE-As (A1-A12) at q28, MAGE-Bs (B1-18) at Xp21 and Xp22 and MAGE-Cs (C1-3) at Xq27.2 (24,25). Most type I MAGEs are broadly expressed in the testis and placenta under normal physiological conditions, indicating their potential roles in germ cell development (26,27). It has been reported that MAGE-As are involved in embryonic and spermatogenesis development, as well as participation in neuron development (26,28,29,30).

Expression specificity and immunogenicity in malignancies. Aberrant activation of MAGEs has been found in various human cancers with different frequencies. It is noteworthy that numerous MAGEs share co-expression patterns in tumors, including MAGE-A1, -A9 and -A11 in laryngeal squamous cell carcinoma with lymph node metastasis (71.0, 64.5 and 77.4%) (31), MAGE-A9 and -A11 in breast cancer (45 and 66.7%) (32) and MAGE-A1, -A3 and -A11 in glioma (64.1, 51.3 and 57.7%) (33). As far as the regulatory mechanism of expression is concerned, most MAGEs are activated by epigenetic reprogramming in malignancies. DNA hypomethylation and histone modification are thought to be responsible for the extensive expression of MAGEs in tumors (3,34). Treatment with the histone deacetylase inhibitor trichostatin A and the DNA methylase inhibitor 5-aza-2'-deoxycytidine (5-aza-CdR) synergistically activates the expression of MAGE-A1, -A2, -A3 and -A12 in various cancer cells (35). Furthermore, bioinformatics has confirmed that MAGE-A11 and MAGE-A6 were co-expressed in human prostate cancer and formed a protein complex, which enhanced MAGE-A11 stability by inhibiting the ubiquitination of MAGE-A11 (36).

Owing to the blood-testis barrier, an immune response to MAGEs has been observed in numerous cancer types, which has been summarized in several excellent reviews (12,37,38). Heterogeneous humoral response against MAGE-A4 and -A10 was detected in patients with melanoma, particularly in stage II patients (39). Antibodies against MAGE-A3 were detected in patients with multiple myeloma (MM) and limited levels of autoantibodies against MAGE-B4 and -C2 were detected in patients with non-small cell lung cancer (NSCLC), both at a frequency of 3% (40,41). In patients with hepatocellular carcinoma (HCC), a specific cellular response against MAGE-A1 and -A3 was observed in 23.4% (11/47) and 32.76% (19/58) of patients, respectively (42). More importantly, researchers have demonstrated a significant correlation between MAGE-A3-specific CD8+ T cells and tumor regression in patients with melanoma (43). In breast cancer, MAGE-A10 was considered the most prevalent CTA, which provoked a CD8+ T-cell response (44).

Biofunction in tumorigenesis and progression. In addition to the co-expression pattern in tumors, most MAGEs also share significant homology in structure and are involved in regulating tumorigenesis and cancer development (45-47). The conserved signature domain shared by MAGEs, which is called the MHD, consists of a stretch of 200 amino acids (48). All human MHDs have 46% protein sequence identity and most of them possess a conserved dileucine motif, particularly in MAGE-As with high conservation at 70% (21,49). The major function of MAGEs is interacting with E3 RING

Table I. Chromosome location and immunogenicity of CTA subfamilies.

CTA	Location	Tumor types	Humoral responses	Cellular responses	(Refs.)
MAGEs	Xq28, Xp21, Xp22, Xq27.2	Melanoma, esophageal squamous cell carcinoma, laryngeal squamous cell carcinoma, breast cancer, glioma, NSCLC	Melanoma, MM, NSCLC	HCC, melanoma, breast cancer	(12,24,25, 31-33,37-44)
SSXs	Xp11.2	Seminoma, melanoma, sarcoma, breast cancer, MM, HCC, gynecological cancer, ovarian cancer	Breast cancer, MM, gynecological cancer, ovarian cancer	HCC, breast cancer, ovarian cancer	(41,42,57, 60,61, 65-67,70-73)
GAGEs	Xp11.23	MM, glioblastoma, HCC, ovarian cancer, HNSCC, colorectal cancer, lung cancer, melanoma	HCC, melanoma	NA	(80,84-93)
XAGEs	Xp11.21-Xp11.3	Lung cancer, HCC, prostate cancer, Ewing's sarcoma, melanoma	Prostate cancer, NSCLC, lung adenocarcinoma	Lung adenocarcinoma, NSCLC	(97-105)
PAGEs	Xp11.2	Prostate cancer, uterus cancer, colorectal cancer, NSCLC	NA	NA	(40,107-111)
NY-ESO-1	Xq28	MLS, SS, osteosarcoma, esophageal cancer, colorectal cancer, breast cancer, thyroid cancer, bladder cancer, lung cancer, adult T cell leukemia, ovarian cancer, HCC, neuroblastoma	Breast cancer, ovarian cancer, melanoma, adult T cell leukemia, lung cancer, thyroid cancer, bladder cancer, esophageal cancer	Breast cancer, HCC, melanoma, neuroblastoma	(38,83,121, 123-138)

CTA, cancer-testis antigen; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer; HNSCC, head and neck squamous cell carcinoma; MM, multiple myeloma; MAGE, melanoma antigen; NA, not available; SSX, synovial sarcoma X; GAGE, G antigen.

ubiquitin ligases to form MAGE-RING ligases (MRLs) and regulate a myriad of processes. Shown by targeted and global proteomics, different MAGEs recognize and bind one specific RING ligase, which impacts ligase activity, specification of novel substrates for ubiquitination and subcellular relocation (22). Specifically, MAGE-A2, -A3, -A6, and -C2 directly bind TRIM28 E3 ubiquitin ligase to reduce p53 and ZNFZ382 protein levels (49,50). In addition to the major role of MRLs, MAGE-As also participate in regulating Cullin-RING ligases (CRLs). MAGE-A11 interacts with S phase kinase-associated protein (Skp2) to modulate substrate specificity of Skp2 and its interaction with cyclin A, regulating cell cycle progression (51). Furthermore, MAGE-B2 serves as a methylation-driven gene facilitating proliferation, migration and invasion of laryngeal cancer cells (52,53).

Furthermore, MAGE-As also impact metabolism via activation of signaling pathways. MAGE-As were proved to sustain cancer cell growth when glycolysis was inhibited (29). Protein kinase AMP activated (AMPK) signaling and autophagy are considered general adaptations of cancer cells in response to metabolic stress during tumor progression and metastasis (54). MAGE-A3/6 was reported to be involved in ubiquitination of AMPK α 1 catalytic subunit through direct interaction, and is also degraded by CRL4-DDB1 and CUL4 associated factor 12 to regulate autophagy and cellular adaptation to nutrition stress (55).

Synovial sarcoma (SS) X (SSX) family. The SSX family consists of nine members (SSX1-9) with high homology (56). According to cytogenetic studies on SS, SSXs were identified as fusion partners of the synaptotagmin (SYT) gene harboring the t(X;18) translocation. SYT-SSX fusion gene expression is observed in nearly all synovial sarcoma tumors and is associated with poor prognosis (57-59).

Biofunction under normal conditions. Mapped at chromosome band Xp11.2, SSX RNAs may be detected in the testis and thyroid at a rather low level, but the proteins are observed only in the testis, particularly in early spermatogenic cells (57,60,61). SSX proteins are distributed in the nucleus and the homology between SSX and Kruppel-associated box (KRAB) domain indicates their role as transcriptional repressors (59,62,63). In addition, SSX proteins are expressed in undifferentiated mesenchymal stem cells but are downregulated after the differentiation of osteocytes and adipocytes, suggesting their involvement in stem cell differentiation (64).

Expression specificity and immunogenicity in malignancies. Members of the SSX family are widely

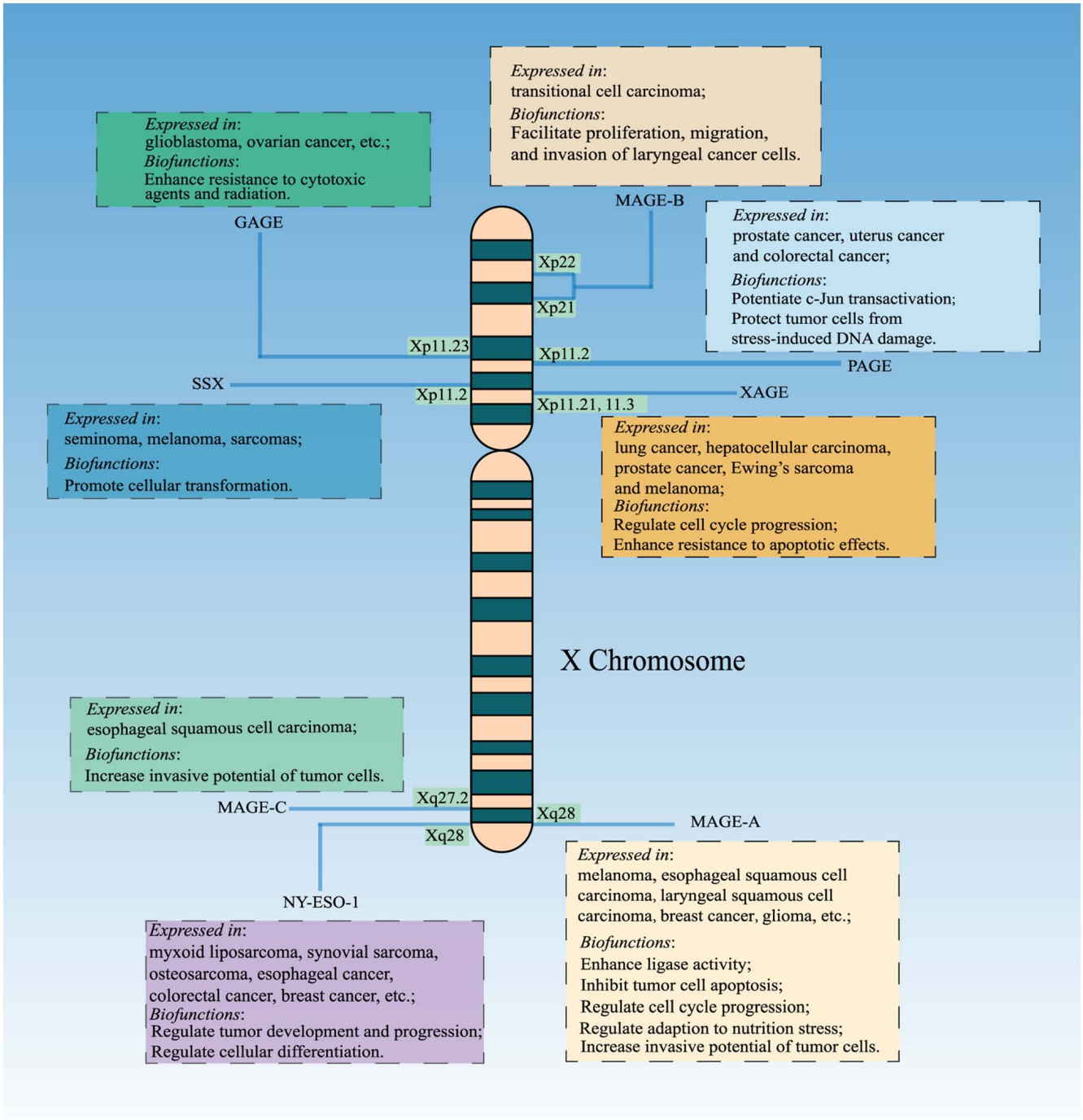

Figure 1. General information of cancer-testis antigen subfamilies: MAGE, SSX, GAGE, XAGE, PAGE and NY-ESO-1. Chromosome localization, expression pattern and biofunction in malignancies were briefly illustrated. MAGE, melanoma antigen; SSX, synovial sarcoma X; GAGE, G antigen.

co-expressed in various tumors, including seminomas (SSX1/2/4, 58%) (65), melanoma (SSX2/3/4, 40%) (66) and sarcomas (11.8-94.1%) (67). It was revealed that SSX proteins are normally expressed in spermatogonia cells, mainly due to genome-wide demethylation (68). Of note, similar demethylation patterns were observed in tumor cell lines and tumor tissues (69).

Immune responses against SSXs have been widely reported in several types of cancer. Recently, T-cell responses to SSX2 were reported in 10.64% of patients with early- or advanced-stage HCC (42). Antibodies against SSX2 have been detected in patients with breast cancer (2%) and MM who have received allogeneic stem cell transplantation (41,70). Furthermore, the peptide epitope of SSX2 was identified to have the potential to react with anti-SSX2 antibodies in the serum of patients with breast cancer and to induce a specific T-cell response *in vitro* (71). In addition to SSX2, immune response against SSX4 is present in gynecological cancer (72). In epithelial ovarian cancer, antibodies against SSX2 and SSX4 have been detected in 2 out 120 patients and specific T-cell response to SSX4 was also identified with SSX4-derived epitopes. These early findings demonstrated the immunogenicity of SSXs and provide SSXs as the primary CTAs used to design cancer vaccines (73).

Biofunction in tumorigenesis and development. Similar to MAGEs, high homology is also observed between SSXs. Two main domains are characterized in SSXs: The N-terminal portion with high homology to KRAB consisting of 75 highly charged amino acids (66) and the SSX repressive domain (SSXRD) formed by 33 amino acids at the C-terminus (62).

Functionally, SSXs have a significant oncogenic role in various tumor types through their KRAB and SSXRD domains. Translocation of the SSXRD domain to the C-terminal end of SYT occurs in SS to form the SYT-SSX fusion oncogene, and the chimeric products of SYT-SSXs exhibit aberrant activity to promote cellular transformation during SS development (74,75). Transactivation of SYT-SSX1/SSX2 proteins leads to transcriptional activation in tumors, whereas unrearranged SSX1/SSX2 proteins have an inhibitory effect due to the repressive KRAB domain at the N-terminus (62). PcG proteins are observed in various types of cancer and associated with pericentromeric heterochromatin region and tumor development (76). Through interaction with various PcG factors via KRAB and SSXRD domains, SS18-SSX and SSX negatively regulate genes involved in multicellular differentiation, stem cell renewal, embryonic development in drosophila and vertebrates, and tumor progression (66,77,78). Researchers have found that SSX genes are co-localized with B cell-specific Moloney murine leukemia virus insertion site 1 (Bmi1), which is a core factor of polycomb repressive complex 1 (PRC1). In addition, there is an intrinsic nucleolar localization signal induced by cellular stress, which consequently leads to dissociation of SSX from Bmi1, resulting in downregulation of SSX protein activity (79).

G family (GAGE). GAGE antigens are typical CTAs frequently expressed in various types of cancer, as well as germ cells in the testis and ovary. The GAGE family comprises at least 16 highly conserved genes.

Biofunction under normal conditions. GAGEs are mapped to chromosome X p11.23 and each of them is located in one of an identical number of highly conserved tandem repeats (80). GAGE proteins are distributed in nuclei and cytoplasm of spermatogonia and primary spermatocytes (81). Beyond the testis, GAGEs expression was also found in primordial germ cells of the gonad primordium, which is maintained until adulthood (82). GAGE proteins were indicated to be expressed in human ectodermal and mesodermal derivatives, implying that they are related to maintaining ground-state pluripotency (83).

Expression specificity and immunogenicity in malignancies. GAGEs are aberrantly activated in 76% glioblastoma and negatively associated with the 2-year overall survival (OS) rate (84). GAGE genes are also highly expressed in head and neck squamous cell carcinoma (81.5%, 22/27) (85). Furthermore, GAGE-1 expression is upregulated in 43.3% of hepatocellular carcinoma tissue (26/60) and GAGE-1/-2 are co-expressed in 26.8% of ovarian cancer tissues (11/41) (86,87). Activation of GAGEs was observed in MM, colorectal cancer, lung cancer and papillary and follicular thyroid cancer (88-91). Like that of most CTAs, GAGE expression is regulated by epigenetics. In breast cancer, for instance, high levels of promoter methylation of GAGE were detected by methylation-specific PCR analysis and enrichment of H3K4me3 was observed to be correlated with different expression levels of GAGEs (92).

Owing to its restricted expression in testis and ovary, antibody against GAGE-1 was reported in patients with HCC (23.33%), liver cirrhosis (13.1%) and hepatitis B (3.3%), as well as normal human individuals (3.4%) (86). Taking serum from patients with melanoma as the specific primary antibody, autoantibodies against GAGE were detected in the serum of 4/72 patients, whereas none were observed in 72 healthy controls (93).

Biofunction in tumorigenesis and development. Unlike MAGEs and SSXs, which have highly conserved protein domains, GAGEs have no distinct secondary or tertiary structure (94), indicating that they are intrinsically disordered proteins despite the homology in their amino acid sequences.

GAGE protein expression was identified in multiple tumors, including neuroblastoma, esophageal carcinoma and stomach cancer (80). Levels of GAGE protein were associated with a poor differentiation level of malignant thyroid diseases, indicating a role in tumorigenesis (91). Cellular levels of the apoptotic regulators interferon regulatory factor 1 and nucleophosmin were regulated by GAGE expression, which contribute to resistance to cytotoxic agents (95). Furthermore, GAGEs are also involved in the development of radiation resistance through the regulation of chromatin accessibility and DNA repair efficiency (96).

X antigen family (XAGE). The XAGE family, consisting of at least 3 homologous clusters (XAGE-1, -2 and -3), was identified after screening the expressed sequence tag database (http://www.ncbi.nlm.nih.gov/ncicgap) for PAGE family member 4 (PAGE-4) homologous genes. Members of the XAGE family are clustered on chromosome X (Xp11.21-Xp11.3), where SSXs, GAGEs and MAGE-D, -H, -I and -J were also mapped (97). XAGEs are mainly expressed in placenta, testis and sarcoma tissues, except XAGE-3, which is expressed only in placenta but not the testis or any tumor lesions (97).

Expression specificity and immunogenicity in malignancies. A total of four transcript variants, XAGE-1a, -1b, -1c, -1d, have been identified in various types of tumor (98), including lung cancer (XAGE-1b and XAGE-1d, 30.6%) (99), hepatocellular carcinoma (XAGE-1b, 64.4%; XAGE-1c, 15.6%; XAGE-1d, 26.0%) (100,101), prostate cancer (XAGE-1, 35,2%) (102) and Ewing's sarcoma (XAGE-1, 33.3%) (97). Of note, XAGE-1b is expressed in almost all melanomas (103).

ELISA of 278 patients with prostate cancer revealed that antibodies against XAGE-1 were detectable in two stage-D2 patients, but not in healthy controls (102). Humoral response to XAGE-1b was also confirmed in patients with NSCLC (104). T-cell response against XAGE-1b was reported in lung adenocarcinoma tissues and T-cell and B-cell epitopes of XAGE-1b protein were identified by Yazdi *et al* (105).

Biofunction in tumorigenesis and development. All XAGE transcripts contain a relatively large secondary open reading frame, which encodes putative proteins in homology with XAGE-1 primary protein (97). Considering strong homology between XAGEs, tumor vaccines targeting multiple XAGEs may become a novel therapeutic strategy for generating efficient antitumor effects.

The association between high levels of XAGE expression and poor outcomes in patients with cancer implies that XAGEs

may have an important role in tumorigenesis and cancer progression. Patients with HCC with positive XAGE-1 mRNA expression had a relatively lower 2-year survival rate (101). In particular, XAGE-1b promoted adenoid cystic carcinoma progression by regulating the cell cycle (shortening the G_0/G_1 and prolonging the G_2/M phase) and enhanced resistance to apoptotic effects induced by tumor necrosis factor-α (106).

PAGE family. The PAGE family is a GAGE-like gene family that was identified by a combination of experimental expression analyses and computerized database mining (107). The PAGE family consists of five members, namely PAGE1-5, which share a significant homology in amino acid sequence (108). Different from the other four members, PAGE-4 is the most well-studied, with significant expression in prostate cancer and therapeutic potential.

Expression specificity and immunogenicity in malignancies. By Northern blot, PAGE-1 RNA expression was revealed in prostate cancer and uterine cancer tissues. Expression of PAGE-2 was detected in the colorectal cancer cell line Caco-2 and PAGE-4 is highly expressed in prostate cancer (109,110). The expression pattern of PAGEs is not as restricted in neoplasms, such as prostate cancer and colorectal cancer, as other CTA families mentioned above (107,111). PAGE-1 mRNA may also be found in certain normal tissues, including testis, prostate, uterus and placenta, which signifies that more attention should be paid to develop PAGE-targeted immunotherapy, avoiding severe side effects (117).

The expression of PAGEs is associated with the demethylation status of CpG residues within regions proximal to the transcription start sites. PAGE-2 expression may also be activated by 5-aza-CdR treatment in colorectal cancer cell lines (111). As to immunogenicity, humoral response against PAGE-3 was reported in 3.8 and 2.9% of patients with NSCLC from two cohorts, but not in patients with benign lung disease (40).

Biofunction in tumorigenesis and development. PAGEs are small proteins containing 102-146 amino acids with high abundance of hydrophilic/charged residues and certain hydrophobic residues, indicating that they are intrinsically disordered proteins (112). As an ensemble of interconverting conformations without rigid 3D structure, these proteins are compared to 'dancing protein cloud' and are inclined to partially form instantaneous secondary structures, which function as potential ligand binding sites in succession (112-115).

PAGEs have important biological roles as cellular transformation promoters and metastasis suppressors in cancer. PAGE-4 is also highly expressed in high-grade prostatic intraepithelial neoplasia and was considered a tumorigenic precursor (116). Consistent with that, upregulated PAGE-4 expression protects cancer cells from oxidative stress through modulating the MAPK signaling pathway (117). PAGE-4 interacts with and potentiates proto-oncogene c-Jun transactivation through conformational changes, indicating a new vulnerability of prostate cancer (118).

New York esophageal squamous cell carcinoma 1 (NY-ESO-1). NY-ESO-1 was cloned from a cDNA library of esophageal cancer using recombinant cDNA library serological analysis technology in 1997 (119). Structurally, it is a 180 amino acid-long protein, containing epitopes of both cellular and humoral responses in the glycine-rich N-terminal and hydrophobic C-terminal regions (120).

Biofunction under normal conditions. According to information from the CTDatabase (www.cta.lncc.br), the NY-ESO-1 family, containing cancer/testis antigen 2 (CTAG2) and cancer/testis antigen 1B (CTAG1B, official name of NY-ESO-1), maps to the Xq28 region of the X chromosome (121). Under normal conditions, the expression of NY-ESO-1 antigen has been documented in human testis, and in germ cells of fetus testis and ovaries at 13-18 weeks; it plateaus at 22-24 weeks, and subsequently, it decreases rapidly (121). NY-ESO-1 was also observed to be upregulated during the differentiation process within developing spermatogonia (122).

Expression specificity and immunogenicity in malignancies. As a well-studied CTA, NY-ESO-1 protein expression has been identified in a variety of cancers, but not in normal adult tissues except immune-privileged organs, such as the testis and placenta (123). NY-ESO-1 has been detected in myxoid liposarcoma (45/64, 70.3%) (124), SS (20/25, 80.0%) (123), osteosarcoma (3/9, 33.3%) (125), esophageal cancer (83/227, 36.6%) (126), colorectal cancer (13/60, 21.7%) and breast cancer (37/97, 38.1%) (127), In addition, upregulated NY-ESO-1 was found in metastatic tumor sites and to be associated with high risk of recurrence and a poor survival rate (128-131). So far, the expression pattern of NY-ESO-1 has been fully illustrated by several excellent reviews (121,132-134). Owing to the specific expression pattern, therapeutic strategies targeting NY-ESO-1 have achieved certain effects with limited off-target events (133), which will be discussed in the next section. Like most CTAs, regulation of NY-ESO-1 expression in tumors is also mediated by several epigenetic events, involving tightly controlled sequential interaction of histone deacetylases, histone methyltransferase, DNA methyltransferases and transcription factors (121). There is a clear correlation between high NY-ESO-1 antigen expression and the hypomethylation status of promoters in various tumor cell types (83).

NY-ESO-1 is one of the most immunogenic CTAs in various cancer types. Humoral response to NY-ESO-1 was reported in several cancers, including breast cancer (73%), ovarian cancer (30%), melanoma (9.4%), adult T-cell leukemia (11.6%), lung cancer (4-12.5%), thyroid cancer (36%), bladder cancer (12.5%) and esophageal cancer (13%) (38,135). In terms of cellular response, NY-ESO-1-specific T-cell response was detected in 10.64% of patients with HCC (42). Patients with melanoma who had NY-ESO-1 antibodies exhibited CD8+ T-cell responses, and NY-ESO-1-specific T cells in patients with neuroblastoma were reported to produce interferon-γ (136-138).

Biofunction in tumorigenesis and development. NY-ESO-1 expression in cancer tissues was indicated to be associated with lymph node metastasis, higher differentiation grade and advanced clinical stage, indicating an important role in regulating tumor development and progression (121,134). In MM, NY-ESO-1 knockdown caused impaired growth of MM cell lines and reduced osteolytic lesions, and it upregulated the expression of E-cadherin, p21 and p53 *in vivo* (139). Furthermore, NY-ESO-1 expression in mesenchymal stem cells was downregulated after differentiation, suggesting a role

in cell differentiation (38). Furthermore, a positive correlation between NY-ESO-1 and forkhead box P3 levels was reported in the TME of NSCLC (140).

3. Design and application of CTA-based tumor vaccines

Due to their restricted expression pattern in tumors, CTAs are promising targets for therapeutic vaccines. Certain CTA subfamilies, such as MAGEs, are more attractive candidates for the development of cancer vaccines. Thus far, numerous CTA-based tumor vaccines have been developed and are able to induce antitumor response through direct administration as a DNA vaccine, RNA vaccine or protein vaccine (Table I). In addition, nanomaterial-derived delivery systems have caught the attention of researchers, with higher delivery efficiency and induced robust immune response. These vaccine platforms are presented in the following sections (Fig. 2).

Design of CTA-based cancer vaccines

Targeted antigens of cancer vaccines. As mentioned previously, numerous CTAs are expressed in various tumor tissues at different frequencies. However, not all of them have been used to construct cancer vaccines due to the irregular expression frequencies and relatively low immunogenicity (141). MAGE, NY-ESO-1, SSX, cancer-testis SP-1 (CTSP-1), TTK protein kinase (TTK1), insulin-like growth factor II mRNA-binding protein 3 (IMP-3), sperm lysozyme-like protein 1 (SLLP1), placenta enriched 1 (PLAC1), lactate dehydrogenase C, sperm autoantigenic protein 17 (sp17) and PRAME nuclear receptor transcriptional regulator (PRAME) are the most widely-used CTAs in tumor vaccines at present, which have demonstrated promising results in preclinical research (15,142-146). However, vaccines targeting SSX, CTSP-1, SLLP1 and PLAC1 are barely used in clinical trials due to limitations regarding safety, stability and effectiveness (147). According to data from www.clinicaltrials.gov, NY-ESO-1 and MAGEs are the most widely-used CTAs to treat malignancies with high immunogenicity (accounting for 37 and 36% of all clinical trials, respectively). They may stimulate both cellular and humoral immune responses with considerable safety and antigenicity (148). Vaccines targeting LY6K (also known as URLC10), TTK1, IMP-3, PRAME and sp17 are also used in several clinical trials to control tumor progression (www.clinicaltrials.gov).

Melanoma, lung cancer and ovarian cancer have been categorized as CTA-high-expressing malignancies, breast cancer and prostate cancer as CTA-moderately-expressing malignancies, and colorectal cancer, kidney cancer and pancreatic cancer as CTA-low-expressing malignancies (83). Consistently, most CTA-based vaccine trials were performed in patients with melanoma (72.36%), particularly those targeting MAGEs and NY-ESO-1. According to data from clinical trials, lung cancer is the second-most common malignancy treated with CTA vaccines and therapeutic targets are LY6K, TTK and IMP-3 (149). There have been fewer clinical trials on the use of CTA vaccines for the treatment of ovarian cancer than lung cancer and the commonly used target is NY-ESO-1, similarly to the role of NY-ESO-1 in melanoma (Fig. 3).

DNA vaccines. In addition to gene targets, the form of antigen delivery also has an important impact on vaccination efficacy, which includes DNA vaccines, RNA vaccines and peptide vaccines. Among them, DNA vaccine is a well-studied type of vaccination with the longest history. DNA vaccines may induce both cellular and humoral responses and have several advantages, such as low incidence of side effects, high stability, simplicity and repeated administration (150). Innate immune response may also be stimulated by DNA vaccine due to the presence of CpG motifs and the double strand nucleotide (151). Taking melanoma as an example, studies using multiple melanoma-associated antigens are ongoing, combining them with molecular adjuvants to enhance the antitumor effect (152).

RNA vaccines. mRNA vaccine represents a promising immunotherapy approach with rapid development, safe administration and relatively low-cost manufacture (153). Compared to DNA vaccine, advantages of mRNA vaccine are as follows: i) Protein expression rate and magnitude of mRNA are higher than for DNA vaccines; ii) unlike DNA vaccines, there is no insertional mutagenesis for mRNA vaccines, which do not integrate into the genome (8); and iii) production of mRNA vaccines is less time-consuming and they are less comprehensive to manufacture than plasmid DNA (154,155). In general, mRNA vaccine has attracted widespread interest for the treatment of both infectious disease and malignancies (156).

Peptide vaccines. Peptide vaccines are characterized by better safety and tolerance without any serious adverse events in comparison to traditional anti-tumor therapies and are considered a promising vaccination approach, which directly delivers synthetic or natural tumor-specific, -associated peptide to induce antitumor effects (157,158). There are several advantages of peptide-based therapeutic cancer vaccines, including convenient production, low carcinogenic potential, cost-effective manufacture, high chemical stability and insusceptibility to pathogen contamination (156). Peptide-based vaccine has become a major focus of cancer vaccine study with promising clinical possibilities. Peptides used for cancer vaccines usually consist of small peptides (generally 7-14 amino acids) with immunogenicity expressed on target cells. Peptide vaccines have been tested in clinical trials for multiple cancers, including esophageal cancer (159,160), melanoma (161), lung cancer (162,163), head and neck squamous cell carcinoma (164) and pancreatic cancer (165).

Application and challenges of CTA-based cancer vaccines

DNA vaccines. SSXs and MAGE-As are commonly used targets of DNA vaccines to generate antigen-specific CD4+ and CD8+ T-cell responses (166,167). Taking advantage of the homology between MAGE-As, researchers designed and optimized a consensus MAGE-A DNA vaccine to treat melanoma, which was able to cross-react with numerous MAGE-A isoforms. Immunization with the MAGE-A vaccine in mice induced robust CD8+ T-cell responses against multiple isoforms (14/15), exhibited cytotoxic effects to significantly inhibit tumor growth and prolonged mouse survival to a median of 50 days, which was 2-fold of that of the control group (168). SSXs are also widely used in cancer vaccines to induce humoral and cellular responses (15,176,169,170). In a previous report, DNA vaccine encoding SSX2 was reported to induce enhanced peptide-specific immune responses and cytotoxic T cells were detectable in mice immunized with modified

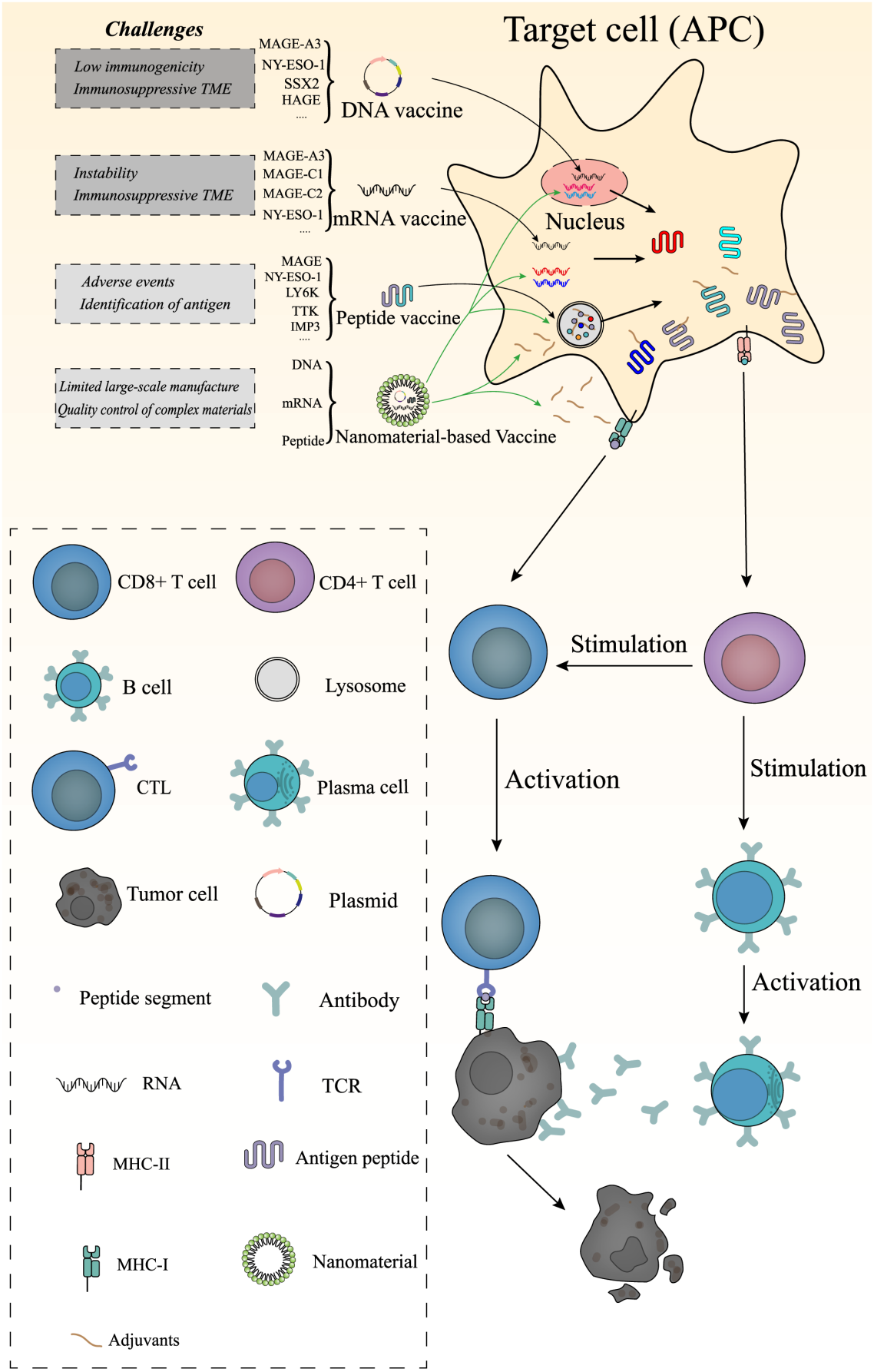

Figure 2. Schematic illustration of cancer-testis antigen-based cancer vaccine therapy. The general process of different types of cancer vaccine exerting their anti-tumor effects. Challenges of DNA vaccine, mRNA vaccine, peptide vaccine and nano vaccine were also indicated. APC, antigen-presenting cells; CTL, cytotoxic T lymphocyte; TCR, T-cell receptor; MHC, major histocompatibility complex; TME, tumor microenvironment; MAGE, melanoma antigen; SSX, synovial sarcoma X; HAGE, H antigen; IMP3, insulin-like growth factor II mRNA-binding protein 3; TTK, TTK protein kinase.

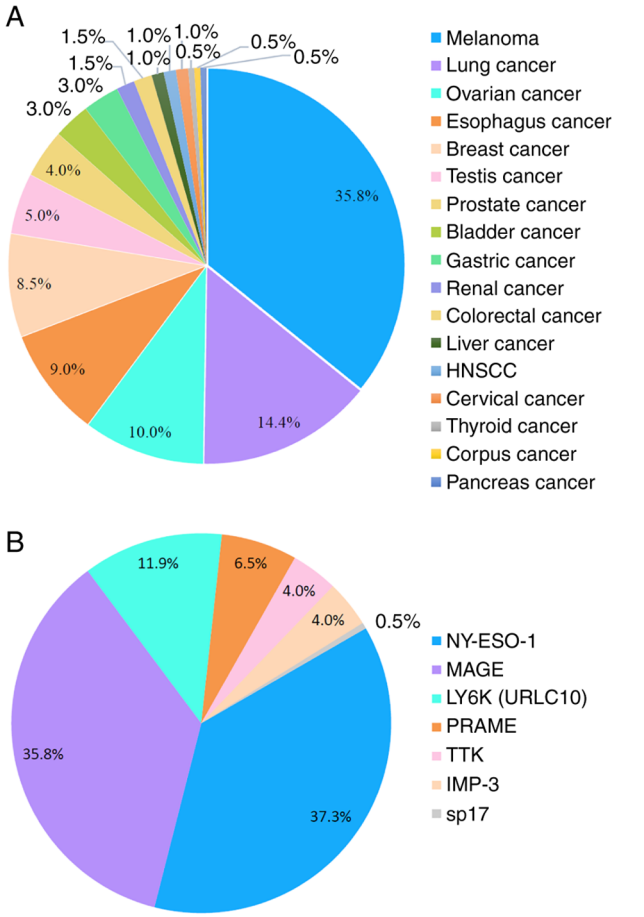

Figure 3. Pie charts of (A) cancers treated with CTA-based vaccines in clinical trials and (B) CTAs utilized to design cancer vaccines in clinical trials. HNSCC, head and neck squamous cell carcinoma; MAGE, melanoma antigen; CTA, cancer-testis antigen; LY6K, lymphocyte antigen 6 complex locus K; URLC10, up regulating lung cancer 10 gene; PRAME, PRAME nuclear receptor transcriptional regulator; TTK, TTK protein kinase; IMP-3, insulin-like growth factor II mRNA-binding protein 3; sp17, sperm protein-17.

SSX2 plasmid DNA vaccine (176). In addition to single-gene DNA vaccines, fusion-gene DNA vaccines are being studied and have demonstrated higher immunogenicity. In preclinical research, both SSX2-MAGEA3 and MAGEA3-SSX2 DNA vaccines achieved improved antitumor effects in the treatment of esophageal cancer compared to either MAGEA3 or SSX2 DNA vaccine (15).

Safety concerns regarding DNA vaccines are usually hypersensitivity reaction and mutation risk. In a phase I/II clinical trial for prostate cancer, 50% (13/26) of patients exhibited a delayed-type hypersensitivity reaction after treatment with naked DNA of proteasome 20S subunit alpha and CD86 (171). In another phase I trial of erb-b2 receptor tyrosine kinase 2-postive breast cancer, mild to moderate complications were reported in 82% of patients with injection site reactions, 36% with fatigue and 33% with flu-like syndrome (172). Furthermore, DNA vaccines may integrate into the host genome and increase the risk of genomic alteration; the production of anti-DNA autoantibodies may also limit their application (173).

Despite the efficacy and safety demonstrated in clinical trials, clinical translation of DNA vaccines targeting CTAs remains limited, mainly by two factors: Immunosuppressive TME and low immunogenicity profiles in human studies. According to a previous report, optimized SSX2 DNA vaccination led to increased expression of programmed cell death 1 (PD-1) and PD-1 ligand 1 on CD8+ T cells and tumor cells, respectively, signifying the importance of combined treatment with chemotherapy, radiation therapy and immune checkpoint blockade (2,150,174). Conversely, strategies to improve immunogenicity have been categorized into several aspects, including antigen selection, vaccine construct optimization and delivery method diversity (167). For antigen selection, homology between CTA subfamily members should be considered to avoid safety events and personalized antigens are universally recommended. Vaccine construct design is also responsible for DNA vaccine efficacy. Future design should pay more attention to codon optimization, promoter selection and plasmid vector backbone, as well as adjuvant selection (167).

Effective delivery methods have an important role in DNA vaccination. In general, it is more complex to deliver DNA vaccine than RNA vaccine due to its larger dimension and the necessity for nuclear localization. Plasmids containing target sequences are usually administered directly into the tumor site to produce specific antigens, as well as by mucosal delivery and intramuscular injection (171). Electroporation and intradermal needle-free delivery system were recently developed to enhance vaccination efficacy (175). Furthermore, rapid advancements in biomaterials have facilitated improvements in the efficacy of DNA vaccines (176). Nanoparticles (liposomes or polymeric particles) are recommended to deliver DNA vaccines to target cells, which significantly enhanced encapsulation efficiency and stability, improved cellular uptake and avoided toxicity (176,177). However, the hallmarks of DNA vaccines are their ability to present native conformational immunogens and prime both humoral and cellular immune responses (167). DNA vaccines remain a well-accepted strategy with their stability, scalability and low cost for manufacture, and it is worthwhile to make efforts to develop and investigate methods with improved delivery efficacy (176).

mRNA vaccines. A phase I/IIa clinical trial applied RNA vaccine encoding five tumor-associated antigens (NY-ESO-1, MAGE-C1, MAGE-C2, survivin and a trophoblast glycoprotein named CV9201) to treat 46 patients with NSCLC. Administrated intradermally, CV9201 generated antigen-specific immune responses in 63% of patients, and the median progression-free survival (PFS) and OS were prolonged to 5.0 [95% confidence interval (CI), 1.8-6.3] months and 10.8 (95% CI, 8.1-16.7) months, respectively. The two- and three-year survival rates were 26.7 and 20.7%, respectively. Furthermore, only mild to moderate adverse events were observed in most patients (16). Similar to DNA vaccines, a combined treatment strategy was recommended to conquer the immunosuppressive TME and to improve outcomes. mRNA-based vaccine encoding six NSCLC antigens (NY-ESO-1, MAGE-C1, MAGE-C2, 5T4, survivin and MUC-1) was utilized in a phase Ib clinical trial in combination with local radiation. Monitoring data revealed increased antigen-specific humoral immunity and cellular immunity in 80 and 40% of patients, respectively. This vaccination has

achieved significantly prolonged PFS and OS at 2.87 (95% CI 1.43-4.27) months and 13.95 (95% CI 8.93-20.87) months, respectively (178). Furthermore, researchers recently reported an intravenous-administrated liposomal RNA vaccine (RNA-LPX), which encoded four non-mutated, tumor-associated antigens, including NY-ESO-1, MAGE-A3, tyrosinase and transmembrane phosphatase with tensin homology. For 89 patients with melanoma with treatment-refractory tumors (previous checkpoint inhibitor treatment), vaccination induced a durable antigen-specific cytotoxic T-cell response and a higher tumor regression rate was achieved at 35% after combined treatment with anti-PD-1 (179).

In general, adverse events of mRNA vaccination are mild to moderate, such as flu-like syndrome and injection site reaction. Recently, safety concerns about mRNA vaccines were raised, as a higher occurrence of adverse effects was observed, particularly grade 3 adverse reactions, including anaphylactic shock, myocarditis and pericarditis, cytokine release syndrome and cerebral venous thrombosis (180,181). Furthermore, modified mRNA may combine with serum proteins and form a vascular occlusion, which has potential toxicity (182). In a phase I/II clinical study (NCT03639714), patients with advanced metastatic cancers were treated with mRNA vaccine encoding neoantigen. Most of them were well-tolerated but one patient experienced pyrexia, duodenitis and increased transaminases and hyperthyroidism (183). mRNA vaccine may be administered by various methods, such as intradermal, intranasal, subcutaneous, intranodal, intratumoral, intramuscular and intravenous injection (184). Thereafter, delivery to target cells is achieved usually by lipid-based nanoparticles to protect mRNA from degradation and may significantly improve the delivery efficiency of mRNA vaccines (180). Viruses may also be designed to deliver mRNA encoding peptides that are later displayed by tumor cells and/or other cells (185). Furthermore, peptide vectors and polymer vectors may also facilitate the delivery of mRNA vaccines (186).

However, there are still several points to be fully elucidated concerning the specific mechanism of mRNA vaccine delivered to the immune system, and strategies to overcome the instability of mRNA, as well as to improve the effectiveness of most vaccines, require to be developed (148). Furthermore, the immunosuppressive TME remains the most significant hurdle for mRNA vaccines to induce a robust antitumor effect (8). Future investigations should pay more attention to improving antigen expression efficacy and duration, as well as to promoting antigen presentation efficiency.

Peptide vaccines. Similar to DNA vaccines, MAGEs and NY-ESO-1 are also the most common targets of peptide anticancer vaccines. A previous clinical trial reported significant tumor regression observed in 28% of patients with melanoma (7/25) who received peptide vaccine treatment targeting MAGE-3.A1, but no cytotoxic T-lymphocyte (CTL) responses were detected. In particular, complete tumor regression was observed in two patients who survived >2 years after the treatment (5). Vaccination with human leukocyte antigen (HLA)-A2-binding NY-ESO-1 peptides generated detectable specific antibody in 41.7% (5/12) of patients with metastatic tumors expressing NY-ESO-1, and peptide-specific CD8+ T-cell reactions were detected in 4 of 7 NY-ESO-1 antibody-negative patients (136). In a phase II clinical trial using peptide vaccine targeting three CTAs (TTK, LY6K, IMP-3), LY6K-, IMP3- and TTK-specific CD8+ T-cell responses were observed in 63, 60 and 45% of patients with esophageal cancer, respectively. Of note, the median survival time of HLA-A*2402-positive groups were improved to 4.6 and 2.6 months, respectively (160). On the other hand, adjuvants are usually applied to induce more efficient T-cell responses in combination with CTA peptides. For instance, the immunostimulant AS15 was administered with recombinant MAGE-A3 protein to enhance the antitumor effect in 25 patients with resected stage IIB-IV melanoma (NCT01425749), and durable antibody responses were observed in all patients, as well as T-cell response in sentinel immunized nodes. Either injected intramuscularly (Group A, n=13) or intradermally/subcutaneously (Group B, n=12), MAGE-A3/AS15 vaccination achieved multifunctional CD4+ T-cell responses to MAGE-A3 in 64% of patients (16/24) and prolonged OS to two years in 90% of patients (187).

In terms of adverse events of peptide vaccines, erythema is the most frequently encountered, while rare events include nausea, increased aspartate aminotransaminase, diarrhoea, myalgia and fatigue. Peptides given alone do not elicit strong immune responses *in vivo* due to quick degradation, absence of danger signals required for APC activation and a lack of costimulatory ability (188). These limitations may be overcome by appropriate formulations. Antigen peptides, adjuvants, as well as targeting sequences, may be encapsulated into a single package that generates a strong T cell-mediated response (188). Poly (lactic-co-glycolic acid) (PLGA) and liposomes are the most widely used drug delivery applications and have been studied for numerous years with great biosafety and biodegradability (189). In a recent preclinical study, PLGA nanoparticles were designed to deliver an immunogenic peptide to enhance antigen delivery and presentation, and generated a robust CD8+ CTL response against multiple myeloma in comparison with free peptide (189).

Peptide vaccines are promising therapeutic approaches with safety, good tolerance and effective immunization (157). A general concern of peptide-based vaccines is the relatively low frequency of CD8+ T and CD4+ T-cell responses (190,191). Thus, challenges are still to be overcome to develop peptide vaccines in the future, which include identification of immunogenetic and neoepitopes, and stimulation of more effective T-cell responses (158).

Nanomaterial-derived vaccine. In spite of the frustrating results of tumor vaccines in clinical trials, attempts never ceased to improve the efficacy of vaccination cascades, including antigen identification, antigen encapsulation, antigen delivery, antigen release and antigen presentation (10). Nanomaterials have attracted increasing attention due to their potential to enhance the cancer vaccination cascade and facilitate antitumor effect with less off-target events. Widely used nanomaterials for developing tumor vaccines include polymeric nanomaterials, endogenous nanocarriers, lipid-based nanoparticles and biomimetic cell membrane-derived nanosystems (10,192). In general, nano vaccines are produced via electrostatic interaction, covalent linking and hydrophobic interaction to ensure efficient co-encapsulation of antigens as well as adjuvants (193). The antigens and adjuvants released from nano vaccine systems may effectively stimulate the

maturation of dendritic cells, which in turn induce the effector T-cell response via cross-presentation and cytokine secretion. Furthermore, cross-presentation, mediated by antibody-antigen immune complex uptake via Fcγ receptors on APCs, also holds significant potential to stimulate long-term antitumor cellular immunity (194). Compared with conventional vaccines, nanovaccines share advantages of increased immunogenicity and co-delivery of multiple antigens, prolonged biological activity, enhanced bioavailability, controlled antigen release and protection of antigens from degradation (195,196).

Due to improved delivery efficiency, nanovaccines have markedly expanded targeted antigens and facilitated the development of individualized vaccines. However, CTA-targeted nanovaccines are still to be fully investigated despite these advantages mentioned above. In a preclinical trial, nanovaccines loaded with MAGE-3 peptides have demonstrated great anti-tumor activity in mice with transplanted gastric cancer and the tumor inhibition rate was as high as 37.81% after treatment (197). In this research, peptide/chitosan was conjugated with deoxycholic acid nanoparticles to encapsulate MAGE-3 peptide, and vaccination resulted in the generation of MAGE-3-specific CTLs and achieved significant tumor regression. Nanoparticle assembled from pyruvate dehydrogenase E2 subunit effectively delivered NY-ESO-1 and MAGE-A3 and achieved an additive effect to induce a specific cell-mediated response resulting in 15-fold and 9-fold increases in cytotoxicity targeting cancer cells, respectively (17). Recently, Verma *et al* (13) designed a self-assembled peptide-based nanotube entrapping a MAGE-3-derived peptide (F-ΔF-M3) to increase the stability and cellular uptake of M3. After immunization, CTL responses were provoked in mice and led to a remarkable inhibition ratio of tumor growth at 41% (13). In another recently published phase I clinical trial, NY-ESO-1 expression in dendritic cells was induced after delivery via a modified lentivirus-based vector LV305 to treat 39 patients with sarcoma and other types of solid tumor (melanoma, non-small cell lung cancer, ovarian cancer and breast cancer). After intradermal injection, disease control was achieved in 56.4% of all patients, and of note, in 62.5% of patients with sarcoma. NY-ESO-1-specific CD4+ and/or CD8+ T cells were generated in 52% of all patients (57% of sarcoma patients), and median PFS reached both 4.6 months for all patients (95% CI, 2.7-11.7 months) and patients with sarcoma (95% CI, 2.5-8.6 months) (198).

Due to repeat administration of cancer vaccines and slow degradability of delivery materials, they may accumulate and cause toxicity in the liver (199). While membrane vesicles are usually considered ideal vectors to cargo vaccine components, the complex contents in those vesicles may cause impaired glucose tolerance and fasting hyperglycemia, and prolong *in vivo* residence due to difficult metabolism (200). Genetically modified membrane vesicles may also cause symptoms such as fatigue and fever, or even continuous tumor development, which probably resulted from cargo in membrane nanovesicles (201,202). Mesoporous silica nanoparticles have been considered a classic delivery platform for cancer vaccines with multiple advantages, but the inert Si-O-Si framework may prevent degradation and lead to long-term biosafety issues (203). Furthermore, the multicomponent hybrid nanomaterials may cause complex biodegradation and excretion problems, and potential toxicity is another concern of metal ions and organic nanomaterials (204).

It has been well accepted that nanovaccines represent a novel anticancer strategy. Given the broad activation of CTAs in various cancer types and homology among subfamilies, nanovaccine targeting one or more CTAs is expected to facilitate antitumoral effects. However, nanovaccines have several drawbacks, including fast clearance, and the mechanisms by which nanoparticles are excreted from organisms after cellular uptake/targeting remain largely elusive (179). In addition, the effect of the physical properties of nanoparticles on the biological interaction between the material and the human body requires to be further studied to ensure the stability and operability of the nanovaccine design process.

4. Perspective and conclusion

CTAs are a large protein family expressed in malignant tumors and male testicular tissues, possessing certain immunogenicity due to the blood-testis barrier, and may stimulate humoral and cellular immunity in patients with malignancies. Members of the same CTA subfamily usually share a similar structure, co-expression pattern and biofunctions in tumors. Exploration of the structural characteristics, biological functions and immunogenicity of CTA families is helpful for developing antitumor treatment strategies. The MAGE, SSX, GAGE, XAGE and PAGE families are all X chromosome-linked CTA families and are activated in various malignant tumors. Co-expression pattern and structural homology shared between subfamily members render them optimal targets for tumor diagnosis and treatment. So far, therapeutic strategies targeting CTA have gained increasing attention to treat tumors either used independently or in combination with other treatments (148). CTAs are usually used as cancer vaccine targets, yet clinical translation is still limited in spite of promising results achieved at the preclinical stage. The underlying reasons may be attributed to the heterogenous expression in tumors and restricted expression of certain CTAs in normal tissues. Thus, the co-expression pattern and structural homology of CTA subfamily members should be taken into account when designing CTA-based therapies.

Cancer vaccines represent a promising therapeutic modality with several advantages. DNA, mRNA and peptide-based cancer vaccines are commonly-used vaccination forms with their own merits and drawbacks. During vaccination, low immunogenicity is generally observed, leading to limited anticancer efficacy. Furthermore, the immunosuppressive TME also hinders the immune response induced by a specific antigen. In view of this, nanomaterial-derived delivery systems may be applied to realize co-delivery of multiple antigens, as well as immune modulators to reverse the immunosuppressive TME. Since antigen identification has long been attributed to be the main reason for the low efficiency of the cancer vaccination cascade, resulting in poor performance in clinical trials, CTA subfamilies should be considered optimal candidates for designing cancer vaccines. In particular, CTA-based nanovaccines will become an attractive strategy for enhancing the antitumor effects of cancer vaccines in the future. At the same

time, attention should also be paid to maintaining the balance between the complexity and composition of the nanostructure and the therapeutic effect, to minimize the toxicity of nanomaterials and maximize the therapeutic efficacy.

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Authors' contributions

Conceptualization, FFC; methodology, SNR and ZYZ; analysis and interpretation of data, MYL and DRW; writing-original draft preparation, SNR and ZYZ; writing-review and editing, SNR; visualization, RJG; design and funding acquisition, XDF and FFC. All authors have read and agreed to the published version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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