Iran J Cancer Prev. 2016 December; 9(6):e4993. doi: 10.17795/ijcp-4993.

Published online 2016 December 6. **Review Article**

Cancer-Testis Antigens: A Novel Group of Tumor Biomarkers in Ovarian Cancers

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Received 2015 December 08; **Revised** 2016 January 11; **Accepted** 2016 December 03.

Abstract

Context: Ovarian cancer is the most fatal gynecological malignancy with no effective screening strategy for early detection. As most cases are being detected in advance stages, conventional therapies are not beneficial for the majority of patients. Cancer-testis antigens (CTAs) are a group of tumor associated antigens with specific expression pattern in cancers which potentiate them for application as cancer biomarkers and targets for immunotherapy.
Evidence Acquisition: We performed a computerized search of the MEDLINE/PUBMED databases with key words: ovarian cancer, cancer-testis antigen, biomarker and immunotherapy.
Results: Thirty five CTAs have been shown to be expressed in ovarian cancer. At least 13 of them have been shown to elicit immune responses in different studies. The pattern of expression for some of them may facilitate molecular classification of different histologic classes of ovarian cancer. In addition, some CTAs such as NY-ESO-1 and MAGE have been used as targets for immunotherapeutic approaches with promising results.
Conclusions: The expression pattern of CTAs in ovarian cancer and the preliminary results of clinical trials indicate that CTAs can be used as targets for immunotherapy of ovarian cancer patients.

Keywords: Cancer-Testis Antigen, Ovarian Cancer, Biomarker, Immunotherapy

1. Context

Ovarian cancer as the most fatal gynecological malignancy has been a subject of screening programs for decades (1, 2). Yet no effective screening strategy has been identified so far. Epithelial, stromal and germ cell tumors are the three main types of ovarian cancer with the first one being the most prevalent. Currently, only about one third of women with early-stage disease can be diagnosed by bimanual examination, CA-125 and transvaginal ultrasonography (3). According to histopathology, immunohistochemistry, and molecular genetic studies, malignant epithelial tumors have been sub-classified to high-grade serous carcinoma, endometrioid carcinoma, clear-cell carcinoma, mucinous carcinoma and low-grade serous carcinoma (4). Numerous genetic and environmental factors have been shown to be implicated in its development including estrogen hormones which have been shown to contribute in tumor progression by enhancing cell proliferation, invasion or cell mobility (5). Even with novel combinatorial chemotherapy regimens and the introduction of intraperitoneal chemotherapy administration, no significant improvement has occurred in the survival of patients. Consequently, novel treatment strategies, such as immunotherapy, are being evaluated for treating ovarian tumors (6). Cancer-testis antigens (CTAs), as a new group of tumor-associated antigens (TAAs) have special characteristics which make them suitable for immunotherapeutic approaches as well as early detection of cancer (7). They are usually absent from normal adult tissues except for the testis but aberrantly upregulated in cancer tissues. Their expression has been evaluated in various cancers of different origins as well as their normal counterparts so far (8-13). Notably, ovarian tissue has an especial situation in this regard. Based on the similarity in biology between testicular and female germ cells, the most outstanding CTA expression has been assumed to occur in the developing ovary. However, various independent studies have shown that adult ovarian tissue is CTA negative (14). As one of the most important characteristics of a putative cancer biomarker is its absence or low level of expression in normal tissue counterpart, we evaluated the data regarding CTA expression in ovarian cancer as well as normal ovary.

2. Evidence Acquisition

In order to gather data about expression of CTAs in ovarian cancer, we performed a computerized search of the MEDLINE/PUBMED databases with key words: ovarian cancer, cancer-testis antigen, biomarker and immunotherapy.

3. Results

3.1. Immunotherapy in Ovarian Cancer

The role of immune system in recognition and elimination of ovarian tumor cells has been highlighted by the observations indicating that high numbers of tumor-infiltrating lymphocytes are associated with better progression free and overall survival, whereas the existence of regulatory T cells and expression of T cell inhibitory molecules is linked with a poor prognosis (15). Further evidence for such deduction has been provided by identification of a 126-gene expression signature for predicting overall survival in patients with ovarian cancer. Notably according to this gene signature high-risk ovarian cancer patients are recognized by a significant decrease in expression of immune response related genes, in particular those in the antigen presentation pathway (16). Based on the identification of TILs as important antitumor effectors as well as recognition of potentially immunogenic TAAs in ovarian cancer, immunotherapeutic treatment strategy has been suggested for these patients. The discovery of TAA has been an important step in active immunization of cancer patients using peptide vaccines. The first report describing the cloning of a TAA encoding gene has been published in 1991 and the corresponding gene was subsequently attributed to CTA family and named the melanoma antigen-1 (MAGE-1) (17). In addition to the members of the CTA family (e.g. MAGE-A4 and NY-ESO-1), aberrant upregulation of HER2/neu, folate receptor alpha (FRα), mutated p53 and CA-125 has been demonstrated in tumor tissue and ascite fluid of ovarian cancer patients. These markers have been suggested as putative targets for induction of immune response and subsequent immune-mediated tumor rejection. Therefore, various immunotherapeutic approaches for ovarian cancer including antibodies, immune checkpoint inhibitors, vaccines, and adoptive cell therapy have recently entered clinical testing (6). Bevacizumab and cetuximab are among monoclonal antibodies which showed promising results in clinical trials (18, 19). Immune checkpoints are specific molecules with the ability to inhibit powerful immunologic effector cells. These checkpoints can be used by cancer cells to circumvent immune control and rejection. Consequently, inhibition of these inhibitory pathways with antibodies against cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1) has shown promising results in cancer patients (20). In addition, simultaneous blockade of PD-1and lymphocyte-activation gene 3 (LAG-3) has a synergistic effect on tumor elimination and has been suggested as a combinatorial immunotherapeutic approach (21). Such dual inhibition during T-cell priming has significantly enhanced proliferation and cytokine production by NY-ESO-1-specific CD8+ T-cells in ovarian cancer patients (22). Furthermore, up to now, various peptide vaccines have been designed which can bind to major histocompatibility complex (MHC) molecules and be recognized by T cells. HER2 and MUC1 are among the most studied TAAs in ovarian cancer patients. On the whole, the most promising results of immunotherapy in ovarian cancer have emerged from dendritic cell (DC)-based vaccines. DCs are responsible for processing and presenting antigens and subsequent induction of specific effector and memory T cells. They have been shown to recognize TAAs and have been applied in a small number of clinical trials in ovarian cancer (23). For instance, subcutaneous vaccination with mature DCs pulsed with HLA-A2–restricted HER2 or MUC1 peptides has been shown to result in development of tumor-specific cytotoxic T cells (CTLs) with the ability to restrain HER2 overexpressing cancer cell lines in vitro (24). Another vaccine formulation includes autologous antigen presenting cells (APCs) loaded with HER2 antigen linked to GM-CSF domain and has been administered in metastatic ovarian cancer patients (25). Early clinical results from these studies have indicated a potential to improve the survival of ovarian cancer patients (6). Adoptive T cell therapy is another approach which relies on the presence of adequate numbers of antitumor lymphocytes with proper effector functions to recognize and destroy cancer cells. Such approach would become more successful by appropriate modifications of patients' lymphocytes to generate more tumor specific lymphocytes. T cell receptors (TCRs) are among candidate genes for such modifications. Considering the role of TCRs in recognition of human leukocyte antigen-A2 restricted epitopes from known TAAs including NY-ESO-1, this strategy represent a novel promising modality in ovarian cancer immunotherapy (26).

3.2. Expression of CTAs in Ovarian Cancer

Various studies have aimed at expression analysis of CTAs in ovarian cancer tissues by means of reverse-transcriptase polymerase chain reaction (RT-PCR), western blotting as well as microarray. A recent study has suggested global hypomethylation, and not loss of X chromosome inactivation as the principal mechanism of overexpression of CTAs in ovarian cancer (27).

Table 1 provides a summary of CTAs expression in ovarian cancer as well as their genomic location and biological function. As listed in the table 35, CTAs have been shown to be expressed in ovarian cancer samples. According to patterns of expression, they have been divided to testis-restricted (exclusively expressed in the testis) and testis-selective (expressed in few tissues beside the testis). Spontaneous immune responses have been detected for at least 13 of them in patients.

Some antigens such as SP17 have been previously attributed to CTA family (28). However, recent data demonstrated its expression in a wide array of normal tissues (10). Consequently, it has been omitted from our list of CTAs.

3.2.1. AKAP3 (A-Kinase Anchoring Proteins 3)

AKAP-3 expression has been demonstrated in more than half of the epithelial ovarian cancer specimens at mRNA level. Its expression considerably correlated with increased likelihood of residual tumor and poorer overall survival. Consequently, it has been suggested as an attractive target for antigen-specific immunotherapy in ovarian cancer (29). Another study has indicated a significantly higher frequency of AKAP3 expression in poorly differentiated and advanced stage tumors. In addition, its expression has been shown to be a significant predictor of both overall and progression-free survival in patients with poorly differentiated tumors (30).

3.2.2. AKAP4 (A-Kinase Anchoring Proteins 4)

AKAP4 expression has been shown at both mRNA and protein levels in most of ovarian carcinoma tissue specimens examined in a study. Notably, none of the matched adjacent non-cancerous tissues expressed it. Additionally, more than half of patients have been shown to have circulating antibodies against AKAP4 which maybe of clinical significance in immunotherapeutic approaches (31).

3.2.3. BAGE (B Melanoma Antigen)

BAGE (B melanoma antigen) has been shown to be expressed in both primary and metastatic ovarian cancer lesions with no expression in normal ovary or benign tumors. In addition, a higher expression rate for BAGE has been detected in ovarian cancer patients with ascites as well as patients with serous cystadenocarcinomas. It has been suggested to participate in the occurrence and development of ovarian cancer. The recognition of BAGE antigens by cytotoxic T cells implies that it could have extensive application prospects in cancer immunotherapy (32).

3.2.4. DPPA2 (Developmental Pluripotency Associated-2)

DPPA2 is a non-X-linked gene which is expressed in pluripotent embryonic cells and is a putative marker of 're-populating' cells with stem cell-like features. It has been shown to be expressed in about one third of epithelial ovarian cancer patients with detectable spontaneous humoral responses in the minority of them. It has been suggested that it has a shared role in embryogenesis and cancer development especially in emergence and/or maintenance of stem cells. In addition, DPPA2 seropositivity has been demonstrated in healthy female donors and patients whose tumors did not express the antigen which implies previous exposure to DPPA2 for instance in pregnancy. On the whole, such data suggest that this antigen can be used as a target in immunotherapy of ovarian cancer patients (33).

3.2.5. GAGE (G Melanoma Antigen) Family

GAGE expression has been shown in a subset of primary and metastatic ovarian cancer patients excluding mucinous and borderline samples (34). GAGE proteins have been shown to be expressed in a subset of oocytes of resting primordial follicles and in maturing oocytes (35) which limits their potential application as cancer biomarkers as well as antigen specific immunotherapy.

3.2.6. LAGE-1 (L Antigen Family Member 1)

LAGE-1 is a CTA with a highly similar amino acid sequence with NY-ESO-1 (13). LAGE-1 expression has been detected in papillary serous, clear cell and mucinous histologic subtypes of ovarian cancer. In addition, antibody to NY-ESO-1/LAGE-1 was present in about one third of patients whose tumors expressed either NY-ESO-1 or LAGE-1. Such antibodies could be detected up to 3 years after initial diagnosis (36).

3.2.7. MAGE (Melanoma Associated Antigen) Family

In a study of CTA expression in ovarian cancer samples, MAGEC1/ CT7 has been the most commonly expressed CTA with positivity in 24.5 % of primary and 35.1 % of recurrent samples. Of note, no mucinous or borderline sample expressed it. Among CTAs expressed in high-grade serous samples, MAGE-C1/CT7 has been the most frequent one. MAGE-C1/CT7 expression has been significantly correlated with grade of endometrioid cancers (34). In the same cohort of patients, MAGE-A4 has been the second most frequently expressed CTA in both primary and recurrent ovarian cancer samples (34). Another study has revealed MAGE-A4 expression in 57% of the serous carcinomas, 9% of the serous tumors of borderline malignancy but not in serous cystadenomas or in the normal ovary. In addition, its expression has been inversely associated with patient survival. Consequently, MAGE-A4 expression has been proposed as a feature of the majority of serous ovarian carcinomas. In addition, a more aggressive regimen

in patients with MAGE-A4 expressing tumors has been suggested (37). MAGE-A3 have also been shown to be expressed in a significant number of ovarian cancer samples in different studies (38). In addition, expression of MAGE-1 and MAGE-3 has been higher in serous cystadenocarcinoma than in other types of ovarian cancer and has been positively associated with tumor differentiation and the clinical stage of the ovarian cancer (32). Furthermore, MAGE3/6 has been among genes which have been detected in exosomes isolated from ovarian cancer patients' plasma. Interestingly, such exosomes were useful for discrimination of ovarian cancer patients from those with benign tumors and healthy controls (39). Another recent study has indicated that expression of MAGE-A1 or -A10 antigens is associated with poor progression free survival (PFS) while MAGE-C1 expression has been correlated with better PFS (40). MAGE-A9 expression has also been demonstrated as a poor prognosis marker (41). Notably, a recent study has shown that MAGE-A8 is among genes whose expression patterns show specific prognostic effects in ovarian cancer patients. It is also among genes which signify an immunoregulatory pattern in tumors. Such pattern is associated with higher stage, lower treatment response, shorter overall survival, and progression free survival (42).

3.2.8. NY-ESO-1 (New York Esophageal Squamous Cell Carcinoma-1)

NY-ESO-1 is perhaps the most studied CTA and has been regarded as the most immunogenic one (68). However, in one study its expression has been shown in a relatively small subset of both primary and recurrent ovarian cancer samples including serous, endometrioid, clear cell and transitional subtypes with no expression in mucinous samples (34). However, another study has shown its expression in mucinous subtypes (36). An independent study has shown its expression in 19% of the serous carcinomas but not in serous tumors of borderline malignancy and cystadenomas (37). Another study has demonstrated its more frequent expression in ovarian cancer samples with no statement about the expression of NY-ESO-1 in distinct histologic subtypes (43). However, the normal epithelium of the fallopian tube has also shown dispersed weak expression of NY-ESO-1 (34). In an immunological survey in ovarian cancer patients, the presence of autoantibodies to NY-ESO-1 has been correlated with increased tumor-infiltrating CD8+, CD4+ and FoxP3+ cells. Consequently, it has been deduced that autoantibodies may collaborate with tumor-infiltrating T cells to influence clinical outcomes in such patients (44).

3.2.9. OY-TES-1 (Acrosin-Binding Protein; ACRBP)

Expression of OY-TES-1 has been shown in the majority of ovarian cancer patients examined in a study. However, no correlation was found between antigen expression and stage, grade, histology and survival (45). In addition, a recent study has demonstrated its frequent expression in ovarian cancer tissues as well as their adjacent tissues with the higher immunostaining intensity in the former. No statistically significant correlation has been identified between OY-TES-1 expression and any other clinicopathological characteristic. This data indicate that OY-TES-1 can be an attractive target for immunotherapy for ovarian cancer (46).

3.2.10. PASD1 (PAS Domain Containing 1)

In a study of 191 ovarian cancer tissues, which were mostly stage I (n = 164) and stage II (n = 14) disease, only one stage Ic ovarian cancer patient tissue expressed PASD1a and b at noticeable levels. This may principally be due to the stage I ovarian cancer samples assessed (47).

3.2.11. PIWIL2 (Piwi-Like RNA-Mediated Gene Silencing 2)

PIWIL2 is a germline stem cell gene whose ectopic expression is linked with cancer stem cell development. It has been proposed to be a gatekeeper against DNA damage-mediated carcinogenesis. Additionally, its expression has been associated with increased proliferation and apoptosis inhibition (48). PIWIL2 has been shown to be specifically expressed in epithelial cells (cancerous cells) of ovarian cancer samples in addition to the stromal cells adjacent to tumor cells (49). Another study has shown its higher expression in the primary tumor and metastatic tissues compared with the adjacent normal tissues and has suggested it as a diagnostic biomarker for epithelial ovarian cancer (50). However, its expression in normal lymphocytes has limited its application in immunotherapy (48).

3.2.12. PLAC1 (Placenta Specific 1)

PLAC1 is a human X-linked gene with placenta-specific expression and a putative marker of 'repopulating' cells with stem cell-like features. Its expression has been demonstrated in epithelial ovarian cancer. Such expression has not been correlated with clinicopathological features of patients such as recurrence and survival which may be at least due to selection of advanced stage patients in this study (33).

3.2.13. SCP1 (Synaptonemal Complex Protein 1)

It has been shown to be expressed in a subset of primary ovarian tumors and not in the normal ovarian surface epithelial cell lines. Its expression has been associated

with a higher tumor grade as well as a decrease in survival time. Consequently, it has been suggested as a potential target for vaccine therapy in epithelial ovarian cancer (51).

3.2.14. SGY1 (Soggy-1)

It codes for a secreted protein related to the Dickkopf protein family with inhibitory effects on Wnt pathway during early embryonic development. It is among CTAs whose expressions have been shown to be higher in cancer stem cells than in non-stem cells within cultured cells from lung adenocarcinoma cells, colon adenocarcinoma cells and breast adenocarcinoma cells (52). It has been shown to be expressed in a subset of ovarian cancer samples (53).

3.2.15. SPAG1 (Sperm-Associated Antigen-1)

Its involvement in spermatogenesis has been recognized after the detection of anti-SPAG1 antibodies in the serum of an infertile woman and the consequent sperm agglutination. It has been shown to be expressed in a large proportion of pancreatic ductal adenocarcinomas and promote motility of cancer cells (54). In addition, although it is absent from normal adult ovary, it is expressed in a subset of ovarian cancer samples (54).

3.2.16. SPANX-B (Sperm Protein Associated with the Nucleus on the X Chromosome-B)

SPANX-B has been shown to be expressed in melanoma and carcinomas of lung, ovary, colon and breast. Most importantly, SPANX-B has elicited immune responses in healthy humans. SPANX-B –specific helper CD4+ and cytolytic CD8+ T cells could recognize at least one HLA-DR-restricted Pep-9 epitope and two HLA-A2-restricted Pep-2, and Pep-4 epitopes. The ability of CD8+ T cells to recognize and lyse HLA-A2-expressing tumors has been demonstrated in primary human melanomas (55).

3.2.17. SPANX-N (Sperm Protein Associated with the Nucleus on the X Chromosome-N)

Its protein translation has been shown to occur post-meiotically in human testis. Unexpectedly, a weak expression of SPANX-N has been shown in non gametogenic tissues such as breast, cervix, prostate, lung, ovary, placenta, proximal and distal colon, stomach, and uterus albeit at 50 - 100 times lower levels than that of testis. Although expressed in ovarian cancer, the maximum level of SPANX-N expression similar to that in testis has been only demonstrated in some melanoma cell lines (56).

3.2.18. SSX (Synovial Sarcoma, X) Family

Approximately half of ovarian cancer samples examined in a study have shown the expression of SSX-4 (57). Another study has revealed its expression in a smaller percentage of samples. However, it demonstrated antibodies against SSX4 in a subset of patients which implies that SSX4 could be a potential target for cancer vaccines (58). An independent study has revealed expression of SSX-1, SSX-2, and SSX-4 in a subset of ovarian cancer patients with the latter being the most prevalent. Of note, antibodies against SSX-2 and SSX-4 as well as SSX-4-specific CD4+ T cells have been detected in few patients (59). Historically, the founder member of this family has been identified in a malignant melanoma patient using serological analysis of recombinant tumor cDNA expression libraries (SEREX) (60). Consequently, spontaneous immunological response to this group of CTAs is probable to be seen in cancer patients which facilitates their application in immunotherapy.

3.2.19. TAG Family

The TAG-1, TAG-2a, TAG-2b, and TAG-2c genes have been shown to be expressed in different epithelial cancers including ovarian cancer. Notably, cytotoxic T lymphocytes specific for two HLA-A2-restricted epitopes from these antigens have recognized tumor cells expressing both the corresponding class I MHC encoded molecule and the TAG genes. Consequently, TAG-derived peptides have been suggested as appropriate therapeutic vaccine components against epithelial cell-derived malignancies (61).

3.2.20. TPX1 (Testis-Specific Protein 1)

It codes for an integral protein of the outer dense fibers and the acrosome of spermatids. Its expression has been shown in ovarian cancer. However, a very weak and inconsistent expression of it has been detected in endometrium (53).

3.2.21. TSGA10 (Testis-Specific Gene 10)

The TSGA10 gene has been initially demonstrated by differential mRNA display to be expressed only in adult testis (62). Afterwards, its expression has been shown in various cancers (8, 63, 64), including ovarian cancer (65). Although it has been shown to elicit humoral responses in hepatocellular carcinoma and malignant melanoma patients, there is no evidence for its immunogenicity in ovarian cancer patients (65).

3.2.22. TSPY1 (Testis Specific Protein, Y-Linked 1)

TSPY1 has been reported to participate in the control of cell cycle progression, cell proliferation and tumorigenesis. It is a germ cell specific marker with no expression in fetal ovary. Its protein has been detected in a significant number of dysgerminoma and gonadoblastoma ovarian tumors (66).

3.2.23. TTK (TTK Protein Kinase)

TTK codes for a protein kinase which is a regulator of the mitotic spindle-assembly checkpoint. Its expression has been shown to be associated with cell proliferation. It has been shown to be expressed in ovarian cancer cell lines as well as patients who had malignant ovarian cancer effusions in the peritoneal cavity (67). Although not assessed in ovarian cancer patients yet, specific T-cell responses to epitope peptides originated from TTK were repeatedly induced in patients with esophageal squamous cell carcinoma (68) which implies its suitability for active immunotherapy of other cancer patients.

3.2.24. XAGE1 (X Antigen Family Member 1)

Although attributed to CTA family, it has been shown to be expressed in lung and peripheral blood lymphocytes at mRNA level. In addition to ovarian cancer, it has been shown to be expressed in Ewing's sarcomas, alveolar rhabdomyosarcomas and breast, lung, and prostate cancers. Western immunoblot analysis has shown its expression in nuclear, cytoplasmic and membrane fractions of cancer cells. In addition, from two identified transcript, XAGE-1b has been shown to be the dominant transcript (69). Although another study has confirmed the predominant expression of the mentioned transcript in the testis and tumors, it failed to detect XAGE-1b protein expression in 8 ovarian cancer samples (70).

3.3. Immunogenicity of CTAs in Ovarian Cancer

As revealed by previous studies, identification of proteins which are involved in the immune response would provide a better recognition of the early stage immune response to cancer in addition to key knowledge about antigens that may be appropriate for immunotherapy (71). Some CTAs have been shown to elicit humoral and/ or cellular immune responses in ovarian cancer patients. Possibly the most immunogenic one has been NY-ESO-1 (72). For instance, the NY-ESO-1 epitope 157 - 170 has been shown to stimulate both Th1 and Th2 type CD4+ T cell responses in epithelial ovarian cancer patients (73). As stated before, its limited expression in some histologic subtypes may limit its potential application in immunotherapy. However, DNA methyltransferase inhibitors have been shown to enhance its expression and increase the presence of circulating antibodies to NY-ESO-1 (74). Other CTAs such as MAGE, BAGE and GAGE have been shown to elicit immune responses as well. Besides, specific SPAG9 antibodies have been detected in majority of epithelial ovarian cancer patients which implies its immunogenicity in these patients as well as its potential for early diagnosis (75). Some other CTAs such as OY-TES-1 have been shown to elicit humoral responses in a smaller percentage of ovarian cancer patients (45).

4. CTA-Based Immunotherapy in Ovarian Cancer

The relatively low survival rate and high relapse rate of patients with ovarian cancer necessitate the search for novel treatment modalities such as immunotherapy. With the assumption that most of patients have micrometastases, even after complete response to frontline surgery and chemotherapy, immunotherapy can be considered for patients (36). As ovarian cancer is one of cancers with frequent expression of CTAs, many patients would benefit from CTA-based immunotherapy even after failure of first- and second-line therapies (34). The most widely used CTAs in clinical trials of cancer patients including ovarian cancer is NY-ESO-1 (72). Perhaps the most promising example of NY-ESO-1- based immunotherapy has been a phase II trial of treatment of 22 recurrent ovarian cancer patients with an NY-ESO-1 vaccine containing recombinant vaccinia and fowlpox vectors resulting in improvement of median overall survival to 48 months in patients having immune activation compared to 15 months for patients that lacked immune activation (76). MAGE-A3 is another CTA whose expression pattern and immunogenicity suggest its suitability for immunotherapy. A recent study has shown that co-culture of autologous T lymphocytes with MAGE-A3-expressing DCs would result in production of CTLs with the ability to secret IFN-γ and kill MAGE-A3+ epithelial ovarian cancer cells. Consequently, this form of DC immunotherapy has been suggested for management of epithelial ovarian cancer (77). As revealed by some studies, CTAs tend to be coexpressed in cancer samples (34). This pattern of expression implies the presence of a unique mechanism for aberrant expression and facilitates design of polyvalent vaccines. At present, the most frequent use of MAGE, BAGE and GAGE antigens in the ovarian cancer vaccine is the peptide vaccine and DC vaccine (32). However, the heterogeneity of antigen expression between various histologic tumor types and even between cells of a tumor mass impedes selection of certain antigen for immunotherapy (32). One possible solution for this problem is application of polyvalent vaccines. Such strategy is also regarded as a solution for downregulation of certain CTAs in recurrent tumors due to immune escape (28). Such phenomenon has been observed in a patient who experienced a NY-ESO-1-negative tumor recurrence after complete objective response to NY-ESO-1 peptide vaccine (78). Table 2 provides a list of clinical trials with CTAs-based vaccines conducted in ovarian cancer.

Table 2. Selected Clinical Trials in Ovarian Cancer Patients Expressing Cancer-Testis Antigens

Reference	Immunological Response/Trial Status	Number of Patients	Phase	Study Year	Vaccine / Adjuvant
(79)	Completed	26-56	I	July 2000	MAGE-12 peptide vaccine/ Montanide ISA-51
(80)	Completed	Not provided	I	May 2003 - July 2006	NY-ESO-1b peptide vaccine/ Montanide ISA-51
(81)	Completed	9	I	July 2005 - August 2008	Cholesterol-Bearing Hydrophobized Pullulan HER2 Protein 146 (CHP-HER2) and / NY-ESO-1 Protein (CHP-/ NY-ESO-1)
(82)	Completed/Humoral and cellular immune responses	18 ovarian cancer patients after chemotherapy for primary or recurrent disease with or without residual disease	I	2007	NY-ESO-1 short peptide/ incomplete Freund's adjuvant
(83, 84)	Completed/Cellular immune response	26	I	August2008 - June 2011	NY-ESO-1 Overlapping Peptides (OLP4) with or without Immunoadjuvants Montanide and Poly-ICLC
(85)	Completed/Cellular immune response	9 with or without residual or recurrent disease after primary therapy	I	2008	Subcutaneous & intradermal multi peptide vaccine (FBP, Her-2/Neu & MAGE-A)/ Montanide ISA-51, GM-CSF
(86)	Compeleted/Humoral and cellular immune responses	9 ovarian cancer patients with complete clinical response to primary therapy	I	2008	Short NY-ESO-1 peptide/ Montanide ISA-51
(87)	Completed	6	I	November 2008- June 2011	ALVAC(2)-NY-ESO-1(M)/TRICOM vaccine
(88)	Completed	18	I	April 2009- June 2013	5-aza-2'-deoxycytidine (decitabine) in combination with immunization with NY-ESO-1 protein/ Montanide and GM-CSF
(89)	Active, not recruiting	30	I	March 2012- September 2016	DEC-205/NY-ESO-1 Fusion Protein CDX-1401
(90, 91)	This study is ongoing, but not recruiting participants. Humoral and cellular immune responses	7	I	August 2012	ALVAC(2)- NY-ESO-1 (M)/TRICOM Vaccine/ mTOR Inhibition With Sirolimus
(92)	Active, not recruiting	30	I	November 2013	IDC-G305 NY-ESO-1 recombinant protein/ GLA-SE
(93)	This study is currently recruiting participants.	12	I	January 2015	Autologous NY-ESO-1-specific CD8-positive T cells/ palliative radiation therapy
(94)	This study is currently recruiting participants.	12	I	March 2015-March 2017	NY-ESO-1 Specific TCR Gene Transferred T Lymphocytes
(95)	This study is currently recruiting participants.	36	I	April 2015- December 2019	NY-ESO-1 TCR-transduced T cells/ Drug: Cyclophosphamide- Fludarabine
(78)	Completed Humoral and cellular immune responses	19 ovarian cancer patients without evidence of disease after primary therapy	I/II	2007	Intradermal recombinant virus carrying NY-ESO-1
(96)	The recruitment status of this study is unknown.	15	I/II	July 2012	NY-ESO-1 in Combination With the Adjuvant MPLA of Bordetella Pertussis.
(97)	This study is currently recruiting participants.	10	I/IIa	June 2013- July 2019	Cytoreductive chemotherapy followed by infusion with NY-ESO-1 (C259) transduced autologous T cells
(98)	This study has been withdrawn prior to enrollment.	0	I/IIb	December 2013- September 2017	Recombinant ALVAC(2) - NY-ESO-1 (M)/TRICOM in Combination With INCB024360
(99)	This study is currently recruiting participants.	98	I/IIb	August 2014- February 2018	DEC-205/ NY-ESO-1 Fusion Protein CDX-1401, Poly ICLC, and IDO1 Inhibitor INCB024360
(100)	Completed	22	II	July 2004- January 2008	Recombinant Vaccinia- NY-ESO-1 (rF- NY-ESO-1) and Recombinant Fowlpox- NY-ESO-1 (rF- NY-ESO-1)
(85)	Completed/Cellular immune responses	4 epithelial ovarian cancer patients after primary debulking surgery	II	2006	NY-ESO-1short peptide/ incomplete Freund's adjuvant
(101)	This study is ongoing, but not recruiting participants.	28 patients undergo primary optimal cytoreductive surgery	II	April 2006 - August 2016	MAGE-A1, Her-2/neu, FBP peptides vaccine / synthetic tetanus toxoid helper peptide emulsified in Montanide ISA-51 / Drug: carboplatin - paclitaxel

5. Conclusions

Ovarian cancer has been regarded as a cancer with frequent and high expression of CTAs. Most studied CTAs have been shown to be absent from adult ovarian tissues. Consequently, expression of CTAs in fetal ovary does not limit application of CTAs as cancer biomarkers or immunotherapy targets. Evaluation of CTA expression in distinct pathologic cancer subtypes may provide clues for molecular classification of ovarian cancers. However, this field is still in its infancy and future researches are needed. Furthermore, these antigens have a potential for specific diagnosis of malignancy both in the tumor specimen and in malignant ascites as revealed for BAGE, GAGE-1/2, MAGE-1, and MAGE-3 (102).

Footnotes

Authors' Contribution: Zahra Taherian-Esfahani, Atieh Abedin-Do, Elahe Nikpayam, Behnoosh Tasharofi and Akram Ghahghaei Nezamabadi contributed in electronic search and designing tables; Soudeh Ghafouri-Fard designed the study and wrote the manuscript; All authors read and approved the final manuscript; Zahra Taherian-Esfahani and Atieh Abedin-Do equally contributed in the study.

Financial Disclosure: None Declared.

Conflict of Interests: None Declared.

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Table 1. Expression of Cancer-Testis Antigens in Ovarian Cancer

Reference	Expression Pattern Among-normal Tissues	Gene Locus	Function	Expression in Histologic Subtypes of Ovarian Cancer	Expression in Ovarian Tumor Cell Line	Expression in Other Cancers	Immunologic Response	Cancer-Testis Antigen
(45)	Testis selective	12p13.31	Binds the acrosome protein proacrosin and is involved in packaging the acrosin zymogen into the acrosomal matrix	Papillary serous, clear cell, endometroid, mucinous, undifferentiated, transitional and mixed carcinosarcoma	SK-OV-3	Bladder, colon, prostate, liver	CD8+ T cell response	ACRBP (OY-TES-1)
(29)	Testis selective	12p13.3	Participate in protein-protein interactions with the R-subunit of the protein kinase A as well as sperm-associated proteins	Serous, endometroid, clear cell, mixed	IOSE, HOSE, SVOV3, SKOV3, OV432	Breast, lung , cervical cancers	Unknown	AKAP3
(31)	Testis selective	Xp11.2	Signal transduction via targeting cyclic adenosine monophosphate-dependent protein kinase-A	Epithelial ovarian cancer , serous adenocarcinoma, serous papillary carcinoma	-	Non small lung cancer, breast	Humoral responses	AKAP4
(32)	Testis re-stricted	21p11.1	Unknown	Metastatic lesions of ovarian cancer, primary ovarian cancer tissues	Not ex-pressed in SKOV3, COC1, A2780	Expressed in a wide range of tumors		BAGE
(34, 37, 103, 104)	Testis re-stricted	Xq28	-	Serous, clear cell , endometroid	-	Expressed in a wide range of tumors	Humoral responses	CTAG1B (NY-ESO-1)
(53)	Testis re-stricted	19q13.33	Secreted antagonist of Wnt signal transduction, may be involved in acrosome assembly or function	Not mentioned	-	Breast, gyneco-logical, lung-melanocyte	-	DKKl3 (SGY)
(105)	Testis re-stricted	3q13.13	-	Epithelial ovarian cancer	-	Lung cancer , colon and rectum cancer, melanocyte malig-nancies	Humoral responses	DPPA-2
(34)	Testis re-stricted	Xp11.23	Chromatin regulation	Serous, clear cell, endometroid	-	Expressed in a wide range of tumors	Some GAGE epitopes are recognized by T cells.	GAGE
(106)	Testis re-stricted	Xp11.23	Chromatin regulation	Serous, mucinous	A2780	Expressed in a wide range of tumors		GAGE 1/2
(106)	Testis re-stricted	Xp11.23	Chromatin regulation	Mucinous	A2780	Expressed in a wide range of tumors	-	GAGE3/6
(36)	Testis selective	Xq28	-	Papillary serous, clear cell, mucinous, undifferentiated	-	Melanoma, lung, esophageal cancer	Humoral responses	LAGE (CTAG-2)

(106) (107)	Testis restricted	Xq28	MAGE family proteins bind to and activate RING E3 ubiquitin ligases. MAGE-A proteins interact with p53 proteins and may block the association of p53 with its cognate sites in chromatin.	Serous cystadenocarcinomas, mucinous, granulosa cell tumors, of Krukenberg tumors, metastatic Surface-epithelial-stromal tumors, serous, transitional-cell, yolk-sac tumors, fibrosarcoma	-	Expressed in a wide range of tumors		MAGE- A1
(107)	Testis restricted	Xq28		Surface-epithelial-stromal tumors, serous, Yolk-sac tumors	-	Expressed in a wide range of tumors	-	MAGE-A2
(107)	Testis restricted	Xq28		Surface-epithelial-stromal tumors, serous, mucinous, endometrioid	-	Expressed in a wide range of tumors	-	MAGE-A3/A6
(37) (107)	Testis restricted	Xq28		Serous, serous tumors of borderline malignancy Surface-epithelial-stromal tumors, serous adenocarcinoma, endometrioid	-	Expressed in a wide range of tumors	-	MAGE-A4 MAGEA4/B4
(41)	Testis restricted	Xq28		Benign ovarian tumor, borderline ovarian tumor	-	Hepatobiliary and lung cancers	-	MAGE-A9
(40)	Testis restricted	Xq28		Not mentioned	-	Hepatobiliary and lung cancers	Humoral responses	MAGE-A10
(40) (34)	Testis restricted	Xq26		Not mentioned Primary and recurrent ovarian cancer	-	Head and neck cancer, squamous cell lung carcinoma	-	MAGE-C1
(53)	Testis selective	6p21-qter	May regulate ion channel activity and is relevant for sperm-oocyte interaction	Not mentioned	-	Breast, melanocytic lesion, prostate cancer	-	TPX1 (CRISP2)
(108)	Testis restricted	8p21.3	Anti-apoptosis and promote proliferation in tumor cells	Not mentioned	-	Breast, prostate and testicular cancers	-	PIWIL2
(54)	Testis restricted	8q22.2	A signal transduction protein during fertilization	Not mentioned	-	Expressed in a wide range of tumors	-	SPAG1
(56)	Testis restricted	Xq27.1	Unknown	Not mentioned	OVCAR8, SKOV3, MDAH2774, SKOV3, ES-2	Melanocytic and testicular cancers	-	SPANX-A1
(55)	Testis restricted	Xq27.1	Unknown	Not mentioned	BG-1, OVCAR3, OVCAR4, IGROV1	Hematologic cancers	Humoral and cellular responses	SPANX-B1

(58, 59)	Testis restricted	Xp11.23-p11.22	Transcriptional regulator	Epithelial ovarian cancers	Various cell lines	Hematologic malignancies, brain, hepatobiliary cancer, lung cancer, melanocytic lesion	Cellular responses	SSX1
(58, 59)	Testis restricted	Xp11.23-p11.22	Acts as a transcriptional regulator	Epithelial ovarian cancers	Various cell lines	Expressed in a wide range of tumors	Humoral and cellular responses	SSX2
(57, 59)	Testis restricted	Xp11.23-p11.22	Transcriptional regulator	Epithelial ovarian cancers	Various cell lines	Expressed in a wide range of tumors	Humoral immune responses	SSX4
(57)	Unknown	Xp11.23-p11.22	-	Not mentioned	-	-	unknown	SSX5
(51)	Testis restricted	1p12-p13	Interacts with SYCE1 and CESC1 as part of the assembly of the synaptonemal complexes and with RAD51	Papillary serous	Various cell lines	Expressed in a wide range of tumors	Humoral and cellular responses	SYCP1 (SCP1)
(61)	Testis restricted	5p13	-	Not mentioned	Various cell lines	Melanocytic cancers		TAG
(65)	Testis restricted	2q11.2	Involved in cell division, differentiation and migration.	Not mentioned	-	Expressed in a wide range of tumors	-	TSGA10
(66)	Testis restricted	Yp11.2	Participates in the control of cell cycle progression, cell proliferation and tumorigenesis	Dysgerminoma, dysgerminomas with gonadoblastoma, gonadoblastoma	-	Hepatobiliary and testicular cancers	-	TSPY1
(67)	Testis restricted	6q13-q21	Regulator of the mitotic spindle-assembly checkpoint	Not mentioned	HEY, OCCl, OCC5, ARO, SW1736, NPA	Myeloma, gastric, lung, esophageal	Cellular immune response	TTK