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Cancer testis antigens as immunogenic and oncogenic targets in breast cancer

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Breast cancer cells frequently express tumor-associated antigens that can elicit immune responses to eradicate cancer. Cancer-testis antigens (CTAs) are a group of tumor-associated antigens that might serve as ideal targets for cancer immunotherapy because of their cancer-restricted expression and robust immunogenicity. Previous clinical studies reported that CTAs are associated with negative hormonal status, aggressive tumor behavior and poor survival. Furthermore, experimental studies have shown the ability of CTAs to induce both cellular and humoral immune responses. They also demonstrated the implication of CTAs in promoting cancer cell growth, inhibiting apoptosis and inducing cancer cell invasion and migration. In the current review, we attempt to address the immunogenic and oncogenic potential of CTAs and their current utilization in therapeutic interventions for breast cancer.

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Breast cancer is the most common cancer affecting women in the USA. In breast cancer, genetic and epigenetic events can generate unique antigens that are expressed in tumors as they progress. These antigens are known as tumor-associated antigens (TAAs), and they either induce an expansion of CD8⁺ cytotoxic T lymphocytes (CTLs) that are capable of rejecting tumor cells or activate B lymphocytes and subsequently elicit a humoral response in the form of tumor-specific antibodies [1]. Breast cancers frequently express TAAs on their surface that may potentially serve as future targets for immunotherapy. TAA-based immunotherapy could be an alternative treatment strategy for patients with hormone receptor-negative breast cancer who lack estrogen receptors, and Her2. The latter are usual targets for therapies such as tamoxifen and trastuzumab, and their absence leaves chemotherapy as the only treatment option for this category of breast cancer patients. These TAAs span a spectrum of immunogenic capacity, TAAs with stronger immunogenic potential is more likely to elicit an adequate immune response. Cancer-testis antigens (CTAs) are a category of TAAs that have received particular attention because of their high immunogenicity [2]. CTA expression is usually restricted to the testis and is otherwise silenced by promoter hypermethylation [3]. In several cancers, however, CTA genes are frequently demethylated and consequently re-expressed, with potentially significant alteration of the host immune response [4]. This cancer-restricted pattern of expression, together with their strong immunogenicity, identifies CTAs as ideal targets for cancer immunotherapy and could prompt the development of CTA-based vaccine treatment that should help patients with limited treatment options such as those with triple-negative breast cancer.

CTA epitopes are presented on the surface of cancer cells in association with human leukocyte antigen (HLA) class I molecules. The antigen-specific T-cell receptor on the surface of CD8⁺ CTLs recognizes the epitope/HLA complex resulting in the killing of the antigen-bearing tumor cells. With active specific immunotherapy, CTAs coupled to appropriate adjuvant can elicit robust antitumor responses. The potential advantages of therapeutic cancer vaccines are that they can augment an established immunogenic response to the tumor, target specific tumor antigens and elicit immunologic memory to prevent recurrence of the tumor.

In addition to immunogenicity, CTAs have oncogenic functions including support of tumor growth (i.e., SSX and CAGE), evasion of apoptosis (i.e., MAGE A/B/C, GAGE and PAGE4), and induction of invasion and metastasis

(i.e., MAGE C2, CAGE, GAGE, XAGE and CT45A1). This novel insight into CTA function will help develop therapeutic strategies that combine immune targeting of oncogenic CTAs with conventional cytotoxic therapies. Accordingly, a significant effort is required to identify optimal therapeutic targets that are highly immunogenic, HLA-presented, cancer-specific and frequently expressed in breast cancer tissues, which make them promising candidates for cancer immunotherapy.

Expression of CTAs in breast cancer

MAGE-A and NY-ESO-1 are the prototypes of CTAs and among the first discovered TAAs that elicit natural cellular immunity in cancers such as melanoma and ovarian, bladder and lung cancer [5,6]. Yet, their expression in breast cancer has been discovered relatively recently. There are more than 100 identified CTAs to date and among those emerging as most relevant to breast cancer are MAGE family (MAGE-A, MAGE-B, MAGE-C), GAGE family (GAGE-A, GAGE-B, PAGE, XAGE), NY-ESO-1, BAGE and BORIS.

Previous studies have confirmed the expression of several CTAs in breast cancer tissues. On top of the well-characterized CTAs are MAGE and NY-ESO-1. The mRNA and protein expression of MAGE-A9, -A10 and -A11 was detected in invasive ductal carcinoma samples [7,8], and their expression was significantly higher than the adjacent noncancerous tissues. A study by Bandić *et al.* [4] showed that MAGE-A4 and NY-ESO-1 proteins were expressed in 74 and 40%, respectively, in a cohort of 81 Croatian women with invasive breast cancer. Measuring CTA protein expression in a prospective cohort of 100 German women with primary breast cancer has detected an expression of GAGE in 26%, NY-ESO-1 in 13%, MAGE-3 in 11% and MAGE-4 in only 4% of patients [9]. In this study MAGE and NY-ESO-1 were not the predominantly expressed CTAs. Instead, SCP-1 and SSX-4 were expressed most frequently (both 65%), followed by HOM-TES-85/CT-8 (47%). In a cohort of 82 breast cancer patients, Taylor *et al.* [10] have found that more than two-thirds of tumors expressed, at the mRNA level, at least one of the following CTAs: CTAG1, BAGE1 and MAGEA-10, all of which are known to be potent immunogenic targets of cytotoxic-T-lymphocyte. SPAG, a recently characterized CTA, was reported to be expressed in over 60% of breast cancer cases [11]. Similarly, the expression of several other CTAs were detected in breast cancer tissues such as: LUZP4 [12], ATPase Family, ATAD2 [13,14], RHOXF2, ODF4 [15], SPANX-A/C/D [16], FBXO39, TDRD4 [17] and WBP2NL [18].

An additional aspect to consider, when we discuss CTA expression in breast cancer, is the reliability of the methods used to characterize and measure CTAs in cancer tissues. Quantitative polymerase chain reaction (qRT-PCR) is a simple and sensitive method to characterize CTA profiles of individual tumors especially if tissue material is limited. However, using mRNA as a surrogate parameter of CTA expression assumes that the level of mRNA correlates with the level of the protein it encodes. Unfortunately, this is not true for many CTAs which arise from several steps of processing such as proteasomal degradation and post-translational modifications that take place prior to antigen presentation. Loss of components of the antigen-processing pathway, which has been reported as an immune evasion mechanism in some cancers [19], might contribute to the lack of correlation between mRNA and protein levels of some CTAs such as MAGE-1 [20]. Thus, direct detection and quantification of CTA proteins is expected to be one step closer to the final product that is expressed on cancer cell surface and exert antigenic properties; yet, this approach is not without limitations. One of the biggest challenges that face CTA protein characterization is the less than optimal specificity and sensitivity of most of the commercially available antibodies. Furthermore, post-translational modifications of CTAs, such as protein glycosylation, may modify the ability of the antibodies to recognize and/or bind to their corresponding antigens [20]. In the recent years, mass spectrometry-based methods of proteome analysis have gained wide acceptance in research settings since they are more comprehensive, sensitive and reproducible and hold considerable promise for the discovery of novel TAAs [21,22]. However, it is important to be aware of the limitations of TAA profiling by mass spectrometry which include the need for large amounts of tissue to detect antigens that exist at low concentration. Also, other factors such as sample collection, storage, processing and purification may produce proteomic artifacts that could mask those representing of cancer. Collectively, the large number of CTAs reported by several studies using heterogeneous sets of cancer tissues and cell models, makes it difficult to verify to what extent the inconsistency in the employed methodology has contributed to the existing discrepancy in literature. Therefore, there is a heightened need for integrated genetic and proteomic approaches that are aided by bioinformatic analysis to better characterize the antigenic profiles in breast cancer.

The biological role of CTAs in breast cancer development and progression remains poorly understood. However, a growing experimental evidence indicates that their expression could have a role in tumorigenesis via regulating

cancer cell proliferation and apoptosis as well as their invasive and metastatic priorities. Thus, studying the immunogenicity of these CTAs may facilitate targeting them via immunotherapy, which will benefit a broad group of breast cancer patients especially those with negative hormonal tumors who are not eligible for hormonal treatment. Furthermore, identifying the oncogenic properties for CTAs may help develop a robust, synergistic approach that combines immunotherapy with conventional cytotoxic therapies. In the following sections, we will discuss the immunogenic and oncogenic roles of CTAs in breast cancer.

CTAs as immunogenic targets in breast cancer

CTAs are excellent targets for cancer immunotherapy such as cancer vaccination, adoptive T-cell transfer with a chimeric T-cell receptor or as an adjuvant treatment with the conventional cancer cytotoxic drugs. CTA-based immunotherapy represents a highly synergistic approach that has a significant potential of improving patient's survival because they are more specific, lower in toxicity and have potential to eradicate residual disease and metastases and the immunological memory induced by cancer vaccines can prevent tumor recurrence [23,24]. CTA-based vaccination has yielded favorable immunological and clinical responses in some neoplastic lesions such as melanoma and ovarian, bladder and lung cancer (reviewed in Gjerstorff *et al.* [25] and Fratta *et al.* [26]). It was recently reported that in MAGRIT Phase III Trial, MAGE-A3-based vaccine did not increase disease-free survival in patients with non-small-cell lung cancer (NSCLC) compared with the placebo-receiving patients [27]. However, it is worth mentioning that the aggressive nature of the NSCLC and accordingly, the short survival of patients with NSCLC might constitute a great obstacle in assessing the long-term efficacy of CTA-based vaccines. Furthermore, the strongly immunosuppressive tumoral environment in NSCLC and the use of a vaccine that is based on one CTA isoform (MAGE-3A) might have contributed to the lack of clinical benefits in this trial.

MAGE-A and NY-ESO-1 are on the top of the list of CTAs that have been tested in a variety of clinical settings [28] and are currently being investigated in many cancer vaccination trials, some of which have provided encouraging outcomes [29,30]. For example, patients with melanoma and ovarian cancer showed significant induction of both humoral and cellular immune responses and increased survival in response to NY-ESO-1 and MAGE-A vaccines and adoptive T-cell transfer [25,31]. Dendritic cell-based, tumor-associated antigen peptide-based, and whole cell-based vaccines are currently tested approaches of cancer immunotherapy [32].

Despite these promising results of CTA-based immunotherapy in many cancers, the reported vaccination trials in breast cancer patients are very few which might indicate either an actual lack of conducting CTA-based vaccination trials in breast cancer or null results that were not published. After thorough research, we came across two trials. The first by Janosky *et al.* reported two cases of metastatic breast cancer with immune response and local remission of skin metastasis after *in situ* vaccination with MAGE-specific T-cells [33]. Another clinical trial (Phase I) that was published as an abstract in the ASCO meeting, 2012 by Fujita *et al.* [34] demonstrated that combining five kinds of CTA peptide vaccination (CDCA1, URLC10, KIF20A, DEPDC1 and MPHOSPH1) in nine patients with metastatic and advanced breast cancer induced local immunological response at site of injection in 44% and systemic increase in cytotoxic T-cells in 78 and 60% of patients who completed at least two cycles of the treatment demonstrated significant tumor regression. Currently, there are few ongoing clinical trials that investigate the effect of MAGE-A or NY-ESO-1-based vaccines on patients with primary or metastatic cancers that express these antigens including breast cancer [35,36].

The therapeutic potential of breast cancer CTAs has also been shown in a variety of *in vitro* and mechanistic studies. A study by Liu *et al.* [37] have identified two PLAC1 peptides (p28 and p31) and their synthetic analogs (p28-1Y9V, p31-1Y2L) as high-affinity peptides, with strong binding affinity and stability to the HLA-A*0201 molecule. These peptides induced a potent cytotoxic T-cell response that could lyse MCF-7 cells. Another peptide (p101-111) that was derived from SXX2 antigen showed dual immunogenicity for both helper and cytotoxic T-cells resulting in both humoral and cellular-mediated immune responses *in vitro* [38].

There is growing evidence that breast cancer is immunogenic. However, it appears that the spontaneous immune responses are unable to control tumor growth and, thus, further research is needed to enhance the host immune responses against breast cancer by therapeutic interventions. To improve the chance of women with breast cancer to benefit from the emerging immunotherapeutic strategies, it is necessary to understand the pattern of expression of CTAs in breast cancer, identify the most prevalent and immunogenic subtypes and exploring their function and relation to breast cancer outcomes.

The family of CTAs is expanding rapidly, and new members are continuously added to the list. The expression of these CTAs varies widely among different studies; however, a common and consistent finding is that no individual

Figure 1. Schematic illustration of the immunogenic versus oncogenic functions of cancer-testis antigens and their potential clinical applications.

CTAs that are expressed on the surface of breast cancer cells may have immunogenic and oncogenic properties. The strongly immunogenic CTAs may induce an expansion of CD8⁺ T cells that are capable of rejecting tumor cells (cellular immunity) or activate B cells that elicit a humoral response in the form of tumor-specific antibodies. At the same time, CTAs may demonstrate oncogenic capabilities in breast cancer. They have been shown to be involved in steps of tumorigenesis that include induction of tumor growth and angiogenesis, evasion of apoptosis, enhancement of tumor migration (invasion and metastasis) and generation of genomic instability. Accordingly, CTAs are excellent candidates for synergistic approaches that combine both immunotherapies with conventional cytotoxic therapies.

CTA: Cancer-testis antigen.

CTA is expressed in all breast cancers and that CTAs are always expressed in heterogeneous combinations in different individuals. This heterogeneity is expected to minimize the efficacy of standardized immunotherapy regimens which entails introducing the next generation of immunotherapy approaches that take into consideration patient's CTA repertoire. Also, this indicates the importance of developing a polyvalent vaccine that covers a large number of the most common and immunogenic CTAs in breast cancer tissues; a strategy that is already in clinical development for the treatment of melanoma [39].

T-cell responses, particularly the one mediated via the CD8⁺ CTL, are believed to be the most important element of immune responses that target CTAs in breast cancers. Previous studies have identified CTL responses against the most prevalent three CTAs: CTAG1, BAGE1 and MAGEA10 in breast cancers [40–42]. A vaccine that targets these three antigens would cover more than two-thirds of breast tumors. HLA-presented peptide epitopes of these CTAs provide potential targets for a clinically relevant cytotoxic T-cell response. Therefore, therapeutic vaccines based on the naturally presented HLA-CTA peptide repertoire might induce tangible effects on tumor eradication and patient survival. CTAG1 is the most studied of the three CTAs, and its peptides are presented by several HLA molecules including HLA A2, HLA A31, HLA B7, HLA B35, HLA B51, HLA Cw3 and HLA Cw6, which are common HLA alleles in the general population [43]. MAGEA10 is also a well-studied CTA, and its peptides are presented by HLA A2 and HLA B53 [44]. BAGE1, which is expressed in more than 10% of breast

Table 1. Expression and function of cancer-testis antigens in breast cancer.

CTAs	Breast cancer clinical specimens/cell lines	Breast cancer molecular subtypes	CTA oncogenic Function	Ref.
ACTL8	Breast cancer tissues	TNBC-Basal-like	Survival and evasion of apoptosis	[62]
ALDH1A1	Breast cancer tissues, MDA-MB-468 and SUM159	NS	Metastasis, invasion and stem cell proliferation	[66–68]
ATAD2	Breast cancer tissues	ER positive	Growth, invasion and metastasis	[13,69]
BAGE	Breast cancer tissues	TNBC	Invasion and metastasis	[10]
BORIS (CTCFL)	Breast cancer tissues MDA-MB-231, and MCF7	ER and PR positive	Survival, transcriptional regulation and epigenetic modifications	[70,71]
CAGE	Breast cancer tissues	NS	Growth, survival and angiogenesis	[72]
CDCA1	MCF7, MDA-MB-231, MDA-MB-453, ZR-75-1 and HTB-19	NS	Proliferation and mitosis	[73]
CT45A1	Breast cancer tissues and MCF-7	NS	Modulation of cell morphology, adherence and motility, metastasis and invasion	[52,74]
CTAG1	Breast cancer tissues and SUM159	TNBC	Invasion and metastasis	[10,16]
FBXO39	Breast cancer tissues, MCF-7 and MDA-MB-231	NS	Tumorigenesis	[17]
GAGE	Breast cancer tissues	TNBC-Basal-like	Survival, invasion, metastasis, epigenetic modifications and stem cell proliferation	[75]
GPAT2	MDA-MB-231	NS	Proliferation, survival, anchorage independent growth and migration	[49]
HORMAD1	MDA-MB-468	TNBC-Basal-like	Genomic instability and hormonal resistance	[62,76]
KIF20A	Breast cancer tissues and MCF-7	ER positive	Growth, survival and hormonal resistance	[77]
LUZP4	Breast cancer tissues	Luminal A, Luminal B and Basal-like	Codifies a leucine zipper protein that is involved in mRNA exporting in cancer cells	[12]
MAGE-A	Breast cancer tissues, MCF-7 and MDA-MB-231	TNBC	Survival, invasion, metastasis and epigenetic modifications	[7,8,75]
NY-ESO-1	Breast cancer tissues	TNBC	Growth and metastasis	[56,59]
ODF4	Breast cancer tissues and MDA-MB-231	Her2-enriched	Proliferation and transcriptional activity	[15]
PAGE4	Breast cancer tissues	NS	Survival and evasion of apoptosis	[78]
PIWIL2	Breast cancer tissues and MDA-MB-231	NS	Survival and evasion of apoptosis	[79]
PLAC1	Breast cancer tissues, MCF-7 and BT-549	NS	Motility, migration, invasion and G1-S cell cycle progression	[80]
PRAME	Breast cancer tissues	TNBC-Basal-like	Proliferation, differentiation and Dominant repressor of retinoic acid Signaling	[62,81]
RHOXF2	Breast cancer tissues, MCF-7 and MDA-MB-231	Her2-enriched	Proliferation and transcriptional activity	[15]
SPAG	Breast cancer tissues and MDA-MB-231	TNBC	Growth, metastasis and invasion	[11,82]
SPANX-A/C/D	Breast cancer tissues and SUM159	TNBC	Invasion and metastasis	[16,75]
SSX1/2/4	Breast cancer tissues and MCF-7	NS	Proliferation and genomic instability	[83]
TDRD4	Breast cancer tissues and MDA-MB-231	NS	Tumorigenesis	[17]
TSP50	Breast cancer tissues and MDA-MB-231	NS	Threonine protease that performs a critical role in tumorigenesis	[84]
WBP2NL	Breast cancer tissues, MCF-7 and MDA-MB-231	NS	Proliferation and tumorigenesis	[18]
XAGE	Breast cancer tissues and MCF-7	NS	Invasion and metastasis	[75,78]

CTA: Cancer-testis antigen; NS: Not specified; TNBC: Triple negative breast cancer.

cancers, is presented by HLA Cw16, however, bioinformatic models identified potential high-affinity of BAGE peptide epitopes for several other HLA molecules, and even a larger number of potential high-affinity epitopes were reported for MAGEA10 [41,45].

CTAs as oncogenic targets in breast cancer

Several studies reported the activation of CTAs in breast cancer cells demonstrating their role in cell cycle regulation, proliferation, apoptosis and promoting invasion in breast cancer. MAGE, a well-characterized CTA, has been shown to play a significant role in breast cancer cell survival via inhibiting P53, an important tumor suppressor gene,

thereby increasing cell proliferation and reducing apoptosis [46]. It has been suggested that MAGE-A inhibits P53 by binding to the P53 core domain and regulating its transcriptional activity or by modulating p53 acetylation through the recruitment of HDAC3 [47]. In addition to regulating p53 directly, MAGE-A can modify the function of MDM2 and MDM4 which are important negative regulators of the p53 gene [48]. GPAT2 is another example of CTAs which mechanism of action has been studied in breast cancer cells (i.e., MDA-MB-231 and MCF7). Silencing of GPAT2 reduced MDA-MB-231 cell proliferation, anchorage-independent growth, migration and tumorigenicity [49]. Similarly, *in vitro* and *in vivo* xenograft studies showed that silencing of the SPAG9 reduced growth and invasive potential of breast cancer cells via upregulating p21 along with other pro-apoptotic molecules and epithelial markers and downregulating signaling molecules that are involved in cell cycle progression and epithelial–mesenchymal transition (EMT) [50]. TSP50, a newly discovered TSA, has also been shown to be involved in breast cancer cell proliferation and cell cycle progression via inhibiting P53 signaling, inducing cyclin D and cyclin E, and promoting G1-S transition [51].

Other CTAs have been shown to promote breast cancer cell invasion and metastasis and their expression in breast cancer tissues correlated with a higher risk of organ metastasis. The ability of these CTAs to initiate EMT in breast cancer cells provides a plausible explanation for their association with enhanced invasion and metastasis. On the top of this category of CTAs are CT45A1, TSP50, MAGEC2, SPANX-A/C/D and CTAG2. Shang *et al.* [52] have shown that CT45A1 overexpression in MCF7 breast cancer cells is associated with upregulation of several essential EMT genes such as TWIST1, ALDH1A1 and the tyrosine-protein kinase KIT. MAGE-C2 is also capable of inducing EMT phenotype in MCF7 breast cancer cells by binding to a transcriptional repressor, KAP1, which plays a critical role in promoting cadherin switching and acquiring mesenchymal cell molecular patterns [53,54]. Similarly, TSP50 has been shown to induce EMT in breast cancer cells, yet it is also capable of activating a group of matrix metalloproteinases that degrade the extracellular matrix and facilitate tumor cell migration and invasion [55]. SPANX-A/C/D and CTAG2 are also involved in breast cancer cell migration and metastatic potential, and the underlying mechanism is thought to be a consequence of CTA-induced modifications in Lamin A/C which subsequently results in remodeling of extracellular matrix, reduction in E-cadherin and loss of cell adherence [16].

The expression of CTAs varied widely among breast cancer patients and some studies reported a trend toward having higher risk features such as advanced histological grade and clinical stage, lymph node metastasis and hormonal receptor negative status among cases with high CTA expression [10,56–58]. Also, CTA expression correlated with distinct molecular patterns which might indicate their relevance as prognostic biomarkers in breast cancer. For example, NY-ESO-1 expression that was measured in a cohort of 1234 breast cancer patients correlated with reduced disease-free survival. NY-ESO-1 expression was also associated with a higher level of tumor-infiltrating lymphocytes which suggests that it is a strong immunogenic CTA that could serve as an ideal immunotherapeutic target [59]. Similarly, MAGE expression in breast cancer tissues correlated with advanced tumor stage, lymph node metastasis, larger tumor size, tumor recurrence and poor overall survival. Also, MAGE was significantly associated with ER- and PR-negative status. These studies indicated that MAGE is a potential novel prognostic marker in breast cancer. A study conducted at Ludwig Institute for Cancer Research, NY reported higher levels of MAGE-A and NY-ESO-1 in ER-negative (47%) than ER-positive (8%) breast cancer cases and in brain metastasis (66%) than primary tumors [57]. Lee *et al.* [59] have shown that NY-ESO-1 was mainly expressed in triple negative breast cancer samples (TNBC). Curigliano *et al.* have confirmed immunohistochemically that both MAGE-A and NY-ESO-1 are highly expressed in TNBC compared with ER-positive tumors in a small cohort of 100 invasive breast cancer samples [60]. In another study by Chen *et al.* [58], analyzed the protein expression of several CTAs including MAGE-A, NY-ESO-1, CT45, GAGE and SAGE1 in 454 invasive ductal carcinomas. They reported more frequent expression of CTAs in ER-negative than ER positive cancers; however, HER2 status had no consistent effect on CTAs expression. In this study, MAGE-A and NY-ESO-1 were also highly expressed in TNBC, showing similar results to what has been reported by Curigliano *et al.* [60]. Similarly, an earlier study by Grigoriadis *et al.* [61] demonstrated a higher expression frequency of MAGEA and NY-ESO-1 as well as the CTAs that are located on the X chromosome (CTA-X) in ER- and PR-negative breast cancer cell lines and primary breast carcinomas. Collectively, these findings indicate that CT antigens expression is closely related to the ER status. Other CTAs such as HORMAD1, CXorf61, ACTL8 and PRAME were found to be highly expressed in the breast cancers that have basal cell properties [62]. Furthermore, there are many other CTAs that predict invasion and metastasis of breast cancer such as ATAD2 [13], GPAT2 [49], NY-BR-1 [63] and BORIS [64] (Table 1 and Figure 1).

Future perspective

This different subtypes and frequencies of CTA expression in breast cancer could be a major source of discouragement of vaccination trials in this type of cancer. In general, heterogeneity in CTA expression and immunogenicity and the inherent immune evasion ability of some cancers are the most common challenges that face the development of an effective immunotherapy. However, these obstacles should not stand in the way of developing effective breast cancer vaccines or immunotherapies that might be the only hope for some women with hormone-resistant breast cancers. New strategies to circumvent these challenges in the broad field of immunotherapy are being currently investigated. Recently, it has been shown that designing vaccines that contain peptides from several CTAs is a successful strategy to overcome the problem of CTA heterogeneity among individuals and prevent immune evasion that might occur through selective loss of single target antigens [65]. Also, there is a need for the formulation of single patient-tailored vaccines which would comprise a significant part of the individual patient's CTA repertoire, including strongly immunogenic unique tumor antigens. More research is needed for a better understanding of the involvement of CTAs in the mechanisms of antitumor immune responses that mediate the clinical outcome of breast cancer. It will help to comprehend the molecular basis of immune recognition and tumor immunogenicity as well as to reveal the molecular characterization and functional heterogeneity of tumor-associated immune cells. Also, a significant effort is required to elucidate disparities in the biology, response to immunotherapy and outcomes seen in women with breast cancer from different racial/ethnic groups. Thus, future studies should focus on regimens integrating observational studies of CTA expression and immune responses in clinical samples, correlational studies with outcomes such as recurrence and survival and finally mechanistic studies that investigate CTA function and immunogenicity.

Executive summary

- The cancer-restricted pattern of expression of cancer-testis antigens (CTAs) and their strong immunogenicity identifies them as promising candidates for cancer immunotherapy.
- Previous studies reported the expression of CTAs in breast cancer tissues mainly MAGE and NY-ESO-1.
- Theoretically, more than two-thirds of breast cancer patient would benefit from CTA-based vaccines, however, results from CTA-based vaccination trials were not as aspired.
- In addition to their immunogenic nature, CTAs have demonstrated a role in cancer cell growth and invasive properties.

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