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Expression of cancer testis antigens in human BRCA-associated breast cancers: potential targets for immunoprevention?

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Abstract

Introduction—Novel breast cancer risk-reducing strategies for individuals with germline mutations of the BRCA1 and/or BRCA2 genes are urgently needed. Identification of antigenic targets that are expressed in early cancers, but absent in normal breast epithelium of these high-risk individuals, could provide the basis for the development of effective immunoprophylactic strategies. Cancer testis (CT) antigens are potential candidates because their expression is restricted to tumors, and accumulating data suggest that they play important roles in cellular proliferation, stem cell function, and carcinogenesis. The objective of this study was to examine the expression of CT antigens and their frequency in BRCA-associated breast cancers.

Methods—Archived breast cancer tissues ($n = 26$) as well as morphologically normal breast tissues ($n = 7$) from women carrying deleterious BRCA 1 and/or 2 mutations were obtained for antigen expression analysis by immunohistochemistry. Expression of the following CT antigens was examined: MAGE-A1, MAGE-A3, MAGE-A4, MAGE-C1, CT7, NY-ESO-1, MAGE-C2/CT10, and GAGE.

Results—CT antigens were expressed in 16/26 (61.5%, 95% CI 43–80%) of BRCA-associated cancers, including in situ tumors. Thirteen of twenty-six (50%) breast cancers expressed two or more CT antigens; three cancers expressed all seven CT antigens. MAGE-A was expressed in 13/26 (50%) of cancers, NY-ESO-1 was expressed in 10/26 (38%) of tumors. In contrast, none of the CT antigens were expressed in adjacent or contralateral normal breast epithelium ($P = 0.003$).

Conclusions—We report a high CT antigen expression rate in BRCA-associated breast cancer as well as the lack of expression of these antigens in benign breast tissue of carriers, identifying CT antigens as potential vaccine targets for breast cancer prevention in these high-risk individuals.

Keywords

Cancer testis antigen; NY-ESO-1; MAGE-A; Breast cancer; BRCA1/2; Vaccine; Prevention

Introduction

BRCA1 and BRCA2 are tumor suppressor genes that are involved in DNA repair. Germline mutations of these genes confer a high lifetime risk for a number of malignant tumors, in particular breast and ovarian cancers. More than 300,000 women in the United States are estimated to carry a deleterious mutation in the BRCA1 or BRCA2 genes with a cumulative breast cancer risk at age 70 of 57 and 49%, respectively [1]. Current surveillance recommendations for BRCA mutation carriers include mammography alternating in 6-month intervals with magnetic resonance imaging (MRI) for early detection of breast cancer (<http://www.nccn.org>). Risk reduction can be achieved with prophylactic surgery such as prophylactic bilateral salpingo-oophorectomy (pBSO) and prophylactic bilateral mastectomy (pBM). While surgical risk-reducing approaches reduce cancer mortality [1], they are permanent procedures with profound medical and psychological impact [2–4]. Recently, tools became available to guide women in the decision-making process for risk reduction. While pBM at age 25 plus pBSO at age 40 maximize survival probability, comparable survival may be reached by substituting mammography plus MRI screening for pBM [5]. Women who choose not to undergo pBM may benefit from chemoprevention approaches such as selective estrogen receptor modulators (SERMs) or participation in clinical trials, which focus primarily on endocrine treatments (<http://www.clinicaltrials.gov>). However, endocrine interventions are unlikely to prevent hormone receptor-negative tumors commonly associated with BRCA1 mutations, and data on the efficacy of SERMs for risk reduction in BRCA1 and BRCA2 mutation carriers are limited [6]. Immunologic strategies such as vaccine-based immunotherapeutic approaches could be an ideal modality for primary prevention.

Cancer vaccines have been tested mostly in patients with advanced diseases. In the therapeutic setting, the high disease burden and associated immunosuppressive tumor environment are believed to represent important obstacles limiting the efficacy of the induced immune response. Prophylactic immunization of the host, i.e., before the development of cancer, is considered the most promising setting for cancer vaccines [7–9].

As their name implies, cancer testis (CT) antigens are expressed in various types of malignant tumors but are absent in normal adult tissues with the exception of testicular germ cells. To date, more than 100 CT antigens and antigen families have been identified [10]. CT antigens can be distinguished in classical CT antigens, mapping to chromosome X, with MAGE-A1 being its prototype, and non-classical CT antigens that map to other chromosomes. Due to their tumor-restricted expression pattern, CT antigens are regarded as valuable targets for the active immunotherapy of cancer, either in the prophylactic or in the therapeutic setting. Several therapeutic cancer vaccine trials of CT antigens such as MAGE-A3 and NY-ESO-1 have demonstrated an excellent safety profile without evidence of clinical auto-immunity as well as the ability to induce cellular and humoral immune responses [11, 12]. The persistence of vaccine-induced B- and T-cell memory responses years after booster immunization has also been demonstrated [12]. While their function is not entirely clear, CT antigens appear to be involved in proliferation [13], stem cell function [14–17], and carcinogenesis of at least some tumors [18]. Consequently, the National Cancer Institute has placed two CT antigens, MAGE-A3 and NY-ESO-1, into the top 10 category of the Project for the Prioritization of Cancer Antigens [19].

While studies have reported varying frequencies of CT antigens in breast cancer [20–29], a high prevalence of CT antigen expression has recently been demonstrated in triple-negative breast cancers [28, 30]. Triple-negative tumors, which lack estrogen, progesterone, and human epidermal growth factor receptor (Her2), are more commonly associated with BRCA1 mutations than other breast cancer phenotypes [31]. Interestingly, recent studies suggest an interaction of BRCA genes/cofactor of BRCA1 (COBRA1) in the regulation of genes located on chromosome X, particularly with those encoding classical CT antigens [32, 33]. However, the presence of CT antigens in BRCA mutation-associated breast cancers has not been studied before. Here, we analyzed the expression of several classical CT antigens in breast cancers from women carrying a deleterious BRCA mutation to confirm antigen expression in a meaningful portion of these high-risk individuals. In order to suggest these antigens as potential vaccine targets for this group of women, we further explored their expression in in situ tumors as well as their absence in morphologically normal breast epithelia of mutation carriers.

Methods

Patients and material

Archived breast tissues from women carrying a BRCA1 and/or BRCA2 mutation were retrieved from the New York University Cancer Institute Tissue Bank and the Department of Pathology for immunohistochemical protein expression analysis. Results of prior commercial BRCA mutation analysis (BRACAnalysis®, Myriad Genetic Laboratories, Salt Lake City, Utah) were recorded for each case. Only tissues from carriers of deleterious mutations were included in this study; mutations with unknown clinical significance were excluded. Patient demographics, tumor stage and grade, or benign histology were recorded. The histological diagnoses were confirmed on hematoxylin–eosin-stained sections by an independent pathologist. Two types of breast tissues were analyzed: (1) breast cancer tissues (obtained from definitive cancer surgeries) and (2) benign breast tissue (obtained from prophylactic mastectomies). Estrogen receptor (ER) and progesterone receptor (PR) status were evaluated by standard immunohistochemistry (IHC) on archived formalin-fixed

paraffin-embedded (FFPE) breast cancer tissue. Staining in 10% of tumor cells was considered a positive result. ER+/PR+, ER+/PR–, and ER–/PR+ breast cancer were grouped as hormone receptor (HR)–positive disease; ER–/PR– constituted the HR-negative group. Her2 status was evaluated on FFPE breast cancer tissue. Her2 positivity was defined as 3+ over-expression by IHC or gene amplification 2.2 by fluorescence in situ hybridization (FISH, Her2 gene copy/chromosome 17 ratio). The research protocol was approved by the New York University Institutional Review Board. Waivers of informed consent and authorization were obtained.

Immunohistochemical analysis

Expression of the CT antigens MAGE-A1, MAGE-A3, MAGE-A4, MAGE-C1, CT7, NY-ESO-1, MAGE-C2/CT10, and GAGE was assessed by IHC on archived FFPE breast tissue. IHC procedures followed standard techniques employing antigen retrieval methods as previously described [23–25]. Briefly, tissue sections were deparaffinized and rehydrated in xylene and a series of graded alcohols. As primary reagents, the following monoclonal antibodies (mAb) to the following CT antigens were used: mAb MA454 (MAGE-A1), mAb M3H67 (MAGE-A3 and probably other MAGE-A antigens, see discussion), mAb 57B (MAGE-A4 and probably other MAGE-A antigens, see discussion), mAb CT7-33 (MAGE-C1, CT7), mAb E978 (NY-ESO-1), mAb CT10#5 (MAGE-C2/CT10), and mAb #26 (GAGE). With the exception of anti-GAGE reagent clone #26, which was acquired commercially (Transduction Labs; Becton–Dickinson, Lexington, KY), all primary antibodies were previously generated by the authors [23, 24, 34–36]. A heat-based antigen retrieval method was employed for all reagents by heating slides in a buffer solution in a household vegetable steamer (95°C, 30 min). Primary antibody incubation was done overnight at 4°C. As a secondary reagent, a biotinylated horse anti-mouse antibody (Vector Labs, Burlingame, CA) followed by an avidin–biotin system (ABC-Elite, Vector) was employed for all primaries except E978, which was detected by the Powervision system (Leica Biosystems, Richmond, IL). Diaminobenzidine served as a chromogen. Counterstaining was performed with Gill's hematoxylin. Testis tissue with preserved spermatogenesis served as a positive control. Immunohistochemical staining was graded by a pathologist blinded to the clinical data based on the percentage of immunopositive tumor cells as follows: negative (no staining), focal (<5% of tumor cells), + (5–25% of tumor cells), ++ (25–50% of tumor cells), +++ (50–75% of tumor cells), and ++++ (>75% of tumor cells).

Statistical analyses

Descriptive statistics were used to summarize baseline demographic and clinicopathologic characteristics. The frequency of CT antigen expression, defined as any immunopositivity (focal– ++++) by tumor cells, was calculated with its 95% CI. The two-sided chi-square test and/or Fisher's exact test were used to test association between CT antigen expression in invasive and in situ cancers compared with benign breast epithelium. Statistical significance of the test is claimed when its *P* value is less than 5% ($P < 0.05$). Statistical analyses were performed using the statistical software *R*.

Results

Patient characteristics

Archived breast tissue was available from 29 women with deleterious BRCA1 and/or BRCA2 mutations. Cancer specimens were available from 26/29 women, including 4 women from whom paraffin blocks of cancer and contralateral normal breast tissue were available. Morphologically benign breast tissue samples were also available from an additional 3 women without a cancer diagnosis. Patient demographics and BRCA mutation

status are displayed in Table 1. As expected, the median age of patients at cancer diagnosis was below the average for women with sporadic breast cancers.

Of the 26 women with breast cancer, 13 carried a BRCA1 mutation (including one woman with an additional deleterious mutation in BRCA2, reported in the BRCA1 group) and 13 carried a BRCA2 mutation. The three Jewish founder BRCA mutations (BRCA1: 185delAG and 5382insC, BRCA2: 6174delT) were detected in approximately half of the patients (Table 1).

BRCA-associated breast cancers

The tumor characteristics are listed in Table 1. Of 26 BRCA-associated cancers, the majority were early-stage invasive ductal carcinomas (IDC, 17 patients with stage I or II) as well as ductal carcinoma in situ (DCIS, 5 patients). Invasive cancers arising in BRCA1 mutation carriers were predominantly triple-negative (HR and Her2 negative, 66%) and/or poorly differentiated (83%). IDC arising in BRCA2 carriers were also predominantly triple-negative (56%) and/or poorly differentiated (78%), thereby increasing the prevalence of the 'basal-like' phenotype typically associated with BRCA1 in the overall study population. The differentiation status of the invasive tumor was associated with hormone receptor expression as expected, and poorly differentiated cancers were most commonly HR negative ($P = 0.04$, Fisher Exact Test).

CT antigen expression in breast cancers and in morphologically normal breast tissue from BRCA mutation carriers

The frequency of CT antigen expression in BRCA-associated cancers is shown in Table 2. The expression pattern was heterogeneous, ranging from focal to diffuse immunoreactivity. CT antigens were expressed in 16/26 (61.5%) of cancers. CT antigens were expressed across all tumor types and stages including DCIS. Representative IHC stains for selected antigens are shown for BRCA-associated invasive ductal carcinoma (Fig. 1) and DCIS (Fig. 2). MAGE-A was expressed in 13/26 (50%) of cancers, and NY-ESO-1 was expressed in 10/26 (38%) of tumors (Table 3). Thirteen of twenty-six (50%) tumors expressed 2 or more CT antigens, and 10/26 (38%) tumors expressed 3 or more CT antigens. Three tumors (12%) expressed all examined CT antigens.

CT antigen expression was also examined in morphologically normal breast tissue of BRCA1/2 mutation carriers obtained from prophylactic mastectomies ($n = 7$). This included three women without a personal history of breast cancer as well as four women with cancer in the contralateral breast. Morphologically, normal breast tissue did not express any of the examined CT antigens in any of the women (Fig. 3), including those with CT antigen expression in the contralateral tumors. In addition, benign breast tissue adjacent to in situ and invasive carcinoma did not express any of the examined CT antigens.

Discussion

In the present study, we report a high incidence of CT antigen expression in in situ and invasive ductal breast cancers as well as their absence in benign breast tissue of BRCA mutation carriers, thus identifying potential target antigens for preventive cancer vaccines.

In contrast to therapeutic vaccines for cancer, preventive vaccines are given to healthy individuals with a 'normal' immune system, not yet imprinted by cancer. Successful prevention of tumor development and progression has been demonstrated in preclinical transgenic models but requires vaccination at an early stage of cancer formation, preferably targeting precancerous lesions. Several groups for instance have shown that ERBB2-specific vaccination can induce protection against a genetic predisposition to mammary carcinoma in

genetically engineered mice transgenic for rat *ErbB2* gene (summarized in [9]). These results provide a rationale for the development of vaccines to prevent tumors in high-risk individuals. Women with deleterious *BRCA* germline mutations have a high lifetime cancer risk for breast and other cancers and therefore represent a group for whom preventive vaccination may be useful. As the conferred immunity should persist for the individual's life, several requirements for the formulation of preventive vaccines should be considered. Antigens must be administered with adjuvants to induce potent anti-tumor immune responses and immune memory may require repeated booster vaccinations. As suggested by some mechanistic studies in mice, protective immunity may require antigen-specific antibodies, although other studies demonstrated CD8 T cell-mediated protection [9]. The selection of antigens for preventive vaccines is very important. Ideal antigens should fulfill the following requirements: (1) their expression should be restricted to tumors, to avoid normal tissue toxicity; (2) the antigens should be expressed on the cell surface to allow targeting by both cell-mediated and antibody-mediated immune responses; (3) they should play critical roles in tumor development and progression to minimize the risk of immune-mediated selection of escape variants.

While CT antigens may not meet all criteria for an ideal preventative vaccine antigen, their expression is restricted to tumors and data are accumulating that they may be involved in proliferation [13, 27], stem cell function [14–17], and carcinogenesis of at least some tumors [18]. Immunogenicity has been demonstrated for CT antigens such as MAGE-A3 and NY-ESO-1 in clinical trials in the adjuvant setting, including the induction of both cellular and humoral immune responses and the persistence of memory responses years after booster immunization when used with potent adjuvants [11, 12]. Furthermore, clinical evidence is emerging that antigen-specific lymphocytes given by adoptive transfer can induce tumor cell kill as was recently shown for NY-ESO-1-expressing metastatic cancers [37].

While CT antigens are infrequently expressed in unselected breast cancers [23, 25, 26, 29], it has recently become evident that CT antigens such as MAGE-A and NY-ESO-1 are expressed in a substantial portion of triple-negative breast carcinomas [30]. Triple-negative tumors have been defined as a separate group only recently and, therefore, expression of CT antigens in earlier studies may have been biased depending on the number of triple-negative tumors in the analyzed cohorts.

Given that *BRCA*-associated breast cancers are commonly triple negative, we wanted to confirm expression of CT antigens in a meaningful portion of these high-risk individuals. In order to suggest CT antigens as potential vaccine targets for this group of women, we further explored the distribution pattern of expression including pre-neoplastic lesions (DCIS) and sought to confirm the absence of antigens from normal breast tissue in *BRCA* carriers. Since expression of the antigen at the protein level is required for recognition of the cancer cells by anti-tumor immunity, we analyzed protein expression in tumor specimens rather than mRNA. This is of particular importance, as discrepancies have been reported between RT-PCR and immunohistochemical analyses in previous studies of CT antigens [20–22]. We demonstrate a high frequency (61.5%) of CT antigen expression in breast carcinomas from women with deleterious *BRCA* mutations. Encouragingly, almost three quarters of *BRCA1*-associated and more than half of *BRCA2*-associated tumors expressed at least one CT antigen. In accordance with a recent study [30], a substantial portion of triple-negative tumors expressed CT antigens. Since triple-negative tumors carry a poor prognosis despite early detection and are unlikely to be affected by endocrine preventive or therapeutic manipulations, prevention of this phenotype is especially important.

Because the expression of CT antigens in different tumor areas is known to be heterogeneous, we employed full sections of tumor and normal breast tissue rather than

tissue microarray samples. While mAb MA454 is specific for MAGE-A1 [25], the specificity of mAb 57B and M3H67 is less clear and may include other members of the MAGE-A family, such as MAGE-A3 and MAGE-A4 [36, 38–40]. The broader reactivity of these mAbs may be due to recognition of antigenic epitopes that are common to several members of the MAGE-A family [41].

Consistent with previous data in breast cancer and other malignancies, CT antigen expression was heterogeneous in the breast cancer samples analyzed [23, 25]. While a homogeneous expression pattern may be advantageous for tumor destruction by the adaptive immune system, recent data suggest that CT antigens may be predominantly expressed on cancer stem cells [14–17]. Therefore, elimination of CT antigen-positive cells may be effective at eradicating the tumor.

The absence of CT antigens in morphologically normal breast epithelia of mutation carriers is another important finding of the present study. It is consistent with prior observations that CT antigens are not expressed at the protein level in morphologically normal tissues or nonmalignant tumors [23–25]. Although one study reported expression of CT antigens in benign prostatic hyperplasia, expression was also unusually high (up to 80%) in prostate carcinomas, raising the possibility of non-specific staining [42]. Our study demonstrates that morphologically normal breast epithelium in the contralateral breast as well as adjacent to in situ or invasive cancers does not express CT antigens. This finding suggests that CT antigen protein expression occurs during neoplastic transformation of breast tissue. The observation that CT antigens were also expressed in early in situ tumors (DCIS), as shown in a prior study for NY-ESO-1 [26], underlines the potential value of these antigens for cancer prevention. In addition, CT antigens are frequently expressed in other cancers that develop in BRCA mutation carriers, such as ovarian carcinoma and melanoma [43, 44], which may be useful when using these targets in immunoprevention.

It must, however, be stressed that the process of immunoediting, while not yet demonstrated in the clinical setting for CT antigens, could be an obstacle to effective prevention with CT antigen vaccines. Under the selective pressure exerted by the vaccine-induced immune response developing tumors may downmodulate the antigen, unless required for tumor cell survival (oncoantigens), resulting in antigen-loss variants. As CT antigens are only expressed intracellularly and rely on antigen processing and presentation by MHC molecules for immune recognition, tumor downmodulation of MHC-I or the peptide-processing machinery could also avert effective immunoprevention. Results from an ongoing Phase III therapeutic vaccine trial involving a single CT antigen in the minimal residual disease setting [45] may provide useful information about occurrence of antigen downmodulation in recurring tumors.

Conclusions

We demonstrate a high incidence of CT antigen expression in human BRCA1/2-associated breast cancers, including in situ carcinoma, suggesting that these antigens should be further studied to develop immunoprevention approaches for reducing the high risk of cancer development in mutation carriers.

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References

1. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. *J Clin Oncol.* 2007; 25(11):1329–1333. [PubMed: 17416853]
2. Frost MH, et al. Long-term satisfaction and psychological and social function following bilateral prophylactic mastectomy. *JAMA.* 2000; 284(3):319–324. [PubMed: 10891963]
3. van Oostrom I, et al. Long-term psychological impact of carrying a BRCA1/2 mutation and prophylactic surgery: a 5-year follow-up study. *J Clin Oncol.* 2003; 21(20):3867–3874. [PubMed: 14551306]
4. Michelsen TM, Dorum A, Dahl AA. A controlled study of mental distress and somatic complaints after risk-reducing salpingo-oophorectomy in women at risk for hereditary breast ovarian cancer. *Gynecol Oncol.* 2009; 113(1):128–133. [PubMed: 19178933]
5. Kurian AW, Sigal BM, Plevritis SK. Survival analysis of cancer risk reduction strategies for BRCA1/2 mutation carriers. *J Clin Oncol.* 2010; 28(2):222–231. [PubMed: 19996031]
6. Visvanathan K, et al. American society of clinical oncology clinical practice guideline update on the use of pharmacologic interventions including tamoxifen, raloxifene, and aromatase inhibition for breast cancer risk reduction. *J Clin Oncol.* 2009; 27(19):3235–3258. [PubMed: 19470930]
7. Finn OJ. Cancer immunology. *N Engl J Med.* 2008; 358(25):2704–2715. [PubMed: 18565863]
8. Disis ML. The ultimate in cancer chemoprevention: cancer vaccines. *Cancer Prev Res (Phila).* 2010; 3(4):406–409. [PubMed: 20332303]
9. Lollini PL, et al. Vaccines for tumour prevention. *Nat Rev Cancer.* 2006; 6(3):204–216. [PubMed: 16498443]
10. Simpson AJ, et al. Cancer/testis antigens, gametogenesis and cancer. *Nat Rev Cancer.* 2005; 5(8): 615–625. [PubMed: 16034368]
11. Valmori D, et al. Vaccination with NY-ESO-1 protein and CpG in Montanide induces integrated antibody/Th1 responses and CD8 T cells through cross-priming. *Proc Natl Acad Sci USA.* 2007; 104(21):8947–8952. [PubMed: 17517626]
12. Atanackovic D, et al. Booster vaccination of cancer patients with MAGE-A3 protein reveals long-term immunological memory or tolerance depending on priming. *Proc Natl Acad Sci USA.* 2008; 105(5):1650–1655. [PubMed: 18216244]
13. Jungbluth AA, et al. The cancer-testis antigens CT7 (MAGE-C1) and MAGE-A3/6 are commonly expressed in multiple myeloma and correlate with plasma-cell proliferation. *Blood.* 2005; 106(1): 167–174. [PubMed: 15761016]
14. Cronwright G, et al. Cancer/testis antigen expression in human mesenchymal stem cells: down-regulation of SSX impairs cell migration and matrix metalloproteinase 2 expression. *Cancer Res.* 2005; 65(6):2207–2215. [PubMed: 15781633]
15. Akers SN, Odunsi K, Karpf AR. Regulation of cancer germline antigen gene expression: implications for cancer immunotherapy. *Future Oncol.* 2010; 6(5):717–732. [PubMed: 20465387]
16. Yawata T, et al. Enhanced expression of cancer testis antigen genes in glioma stem cells. *Mol Carcinog.* 2010; 49(6):532–544. [PubMed: 20082319]
17. Gedye C, et al. Cancer/testis antigens can be immunological targets in clonogenic CD133 + melanoma cells. *Cancer Immunol Immunother.* 2009; 58(10):1635–1646. [PubMed: 19221743]
18. Ajiro M, et al. Involvement of RQCD1 overexpression, a novel cancer-testis antigen, in the Akt pathway in breast cancer cells. *Int J Oncol.* 2009; 35(4):673–681. [PubMed: 19724902]
19. Cheever MA, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res.* 2009; 15(17):5323–5337. [PubMed: 19723653]
20. Sugita Y, et al. NY-ESO-1 expression and immunogenicity in malignant and benign breast tumors. *Cancer Res.* 2004; 64(6):2199–2204. [PubMed: 15026363]
21. Taylor M, et al. Breast cancer is a promising target for vaccination using cancer-testis antigens known to elicit immune responses. *Breast Cancer Res.* 2007; 9(4):R46. [PubMed: 17650306]
22. Mischo A, et al. Prospective study on the expression of cancer testis genes and antibody responses in 100 consecutive patients with primary breast cancer. *Int J Cancer.* 2006; 118(3):696–703. [PubMed: 16094643]

23. Jungbluth AA, et al. CT7 (MAGE-C1) antigen expression in normal and neoplastic tissues. *Int J Cancer*. 2002; 99(6):839–845. [PubMed: 12115486]
24. Jungbluth AA, et al. Immunohistochemical analysis of NY-ESO-1 antigen expression in normal and malignant human tissues. *Int J Cancer*. 2001; 92(6):856–860. [PubMed: 11351307]
25. Jungbluth AA, et al. Expression of MAGE-antigens in normal tissues and cancer. *Int J Cancer*. 2000; 85(4):460–465. [PubMed: 10699915]
26. Theurillat JP, et al. NY-ESO-1 protein expression in primary breast carcinoma and metastases: correlation with CD8 + T-cell and CD79a + plasmacytic/B-cell infiltration. *Int J Cancer*. 2007; 120(11):2411–2417. [PubMed: 17294444]
27. Otte M, et al. MAGE-A gene expression pattern in primary breast cancer. *Cancer Res*. 2001; 61(18):6682–6687. [PubMed: 11559535]
28. Grigoriadis A, et al. CT-X antigen expression in human breast cancer. *Proc Natl Acad Sci USA*. 2009; 106(32):13493–13498. [PubMed: 19651608]
29. Kavalari R, et al. Expression of MAGE tumour-associated antigens is inversely correlated with tumour differentiation in invasive ductal breast cancers: an immunohistochemical study. *Virchows Arch*. 2001; 439(2):127–131. [PubMed: 11561752]
30. Curigliano G, et al. Cancer-testis antigen expression in triple-negative breast cancer. *Ann Oncol*. 2011; 22(1):98–103. [PubMed: 20610479]
31. Anders CK, Carey LA. Biology, metastatic patterns, and treatment of patients with triple-negative breast cancer. *Clin Breast Cancer*. 2009; 9(Suppl 2):S73–S81. [PubMed: 19596646]
32. Aiyar SE, et al. Regulation of clustered gene expression by cofactor of BRCA1 (COBRA1) in breast cancer cells. *Oncogene*. 2007; 26(18):2543–2553. [PubMed: 17043641]
33. Jazaeri AA, et al. BRCA1-mediated repression of select X chromosome genes. *J Transl Med*. 2004; 2(1):32. [PubMed: 15383145]
34. Jungbluth AA, et al. Monoclonal antibody MA454 reveals a heterogeneous expression pattern of MAGE-1 antigen in formalin-fixed paraffin embedded lung tumours. *Br J Cancer*. 2000; 83(4):493–497. [PubMed: 10945497]
35. Zhuang R, et al. Generation of monoclonal antibodies to cancer/testis (CT) antigen CT10/MAGE-C2. *Cancer Immun*. 2006; 6:7. [PubMed: 16594646]
36. Kocher T, et al. Identification and intracellular location of MAGE-3 gene product. *Cancer Res*. 1995; 55(11):2236–2239. [PubMed: 7757970]
37. Robbins PF, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J Clin Oncol*. 2011
38. Dhodapkar MV, et al. Expression of cancer/testis (CT) antigens MAGE-A1, MAGE-A3, MAGE-A4, CT-7, and NY-ESO-1 in malignant gammopathies is heterogeneous and correlates with site, stage and risk status of disease. *Cancer Immun*. 2003; 3:9. [PubMed: 12875607]
39. Rimoldi D, et al. Anti-MAGE-3 antibody 57B and anti-MAGE-1 antibody 6C1 can be used to study different proteins of the MAGE-A family. *Int J Cancer*. 2000; 86(5):749–751. [PubMed: 10797302]
40. Landry C, et al. Monoclonal antibody 57B stains tumor tissues that express gene MAGE-A4. *Int J Cancer*. 2000; 86(6):835–841. [PubMed: 10842198]
41. De Plaen E, et al. Structure, chromosomal localization, and expression of 12 genes of the MAGE family. *Immunogenetics*. 1994; 40(5):360–369. [PubMed: 7927540]
42. Hudolin T, et al. Immunohistochemical expression of tumor antigens MAGE-A1, MAGE-A3/4, and NY-ESO-1 in cancerous and benign prostatic tissue. *Prostate*. 2006; 66(1):13–18. [PubMed: 16114059]
43. Velazquez EF, et al. Expression of the cancer/testis antigen NY-ESO-1 in primary and metastatic malignant melanoma (MM)–correlation with prognostic factors. *Cancer Immun*. 2007; 7:11. [PubMed: 17625806]
44. Odunsi K, et al. NY-ESO-1 and LAGE-1 cancer-testis antigens are potential targets for immunotherapy in epithelial ovarian cancer. *Cancer Res*. 2003; 63(18):6076–6083. [PubMed: 14522938]

45. Brichard VG, Lejeune D. Cancer immunotherapy targeting tumour-specific antigens: towards a new therapy for minimal residual disease. *Expert Opin Biol Ther.* 2008; 8(7):951–968. [PubMed: 18549325]

Fig. 1. Immunohistochemical staining of invasive ductal breast carcinoma for CT antigens in BRCA-1-positive patient (Case 108, $\times 10$): **a** HE staining displaying area of invasive ductal carcinoma of the mammary gland, **b–f** serial sections of corresponding breast tissue immunohistochemically stained with mAb MA454 (**b**, focal positive), mAb M3H67 (**c**, ++ positive), mAb 57B (**d**, ++ positive), mAb E978 (**e**, + positive), and mAb #26/GAGE (**f**, ++ + positive)

Fig. 2. Immunohistochemical staining of DCIS for CT antigens in BRCA-2-positive patient (Case 211): **a** overview of several extended ducts displaying areas of DCIS (HE, $\times 4$); **b–f** higher magnification ($\times 10$) of DCIS lesions immunohistochemically stained with mAb MA454 (**b**, focal positive), mAb M3H67 (**c**, focal positive), mAb 57B (**d**, focal positive), mAb CT7-33 (**e**, focal positive), and mAb CT10#5 (**f**, focal positive)

Fig. 3. Immunohistochemical staining of breast tissue for CT antigens in BRCA-1-positive patient (Case 302, $\times 10$): **a** HE staining displaying normal lobule and mammary gland, **b–f** serial sections of corresponding breast tissue immunohistochemically stained with mAb MA454 (**b**, negative), mAb M3H67 (**c**, negative), mAb 57B (**d**, negative), mAb CT10#5 (**e**, negative), and mAb #26/GAGE (**f**, negative)

Table 1

Patient demographics, BRCA mutation status, and tumor characteristics

	BRCA1 mutation-associated cancers (<i>n</i> = 13)	BRCA2 mutation-associated cancers (<i>n</i> = 13)	Normal breast tissue from BRCA mutation carriers (<i>n</i> = 7)
Age			
Median	43	46	50
Range	36–60	26–70	31–60
Ethnicity			
Caucasian Ashkenazi Jewish	5/13 (38.5%)	8/13 (61.5%)	4/7 (57.1%)
Caucasian, non-Jewish	7/13 (53.8%)	2/13 (15.4%)	2/7 (28.6%)
Asian	1/13 (7.7%)	2/13 (15.4%)	1/7 (14.3%)
Hispanic	0	1/13 (7.7%)	1/26 (3.8%)
BRCA1/2 mutation			
del185AG	5/13 (38.5%)	0	4/7 (57.1%)
5385insC	1/13 (7.7%)	0	0
6174delT	0	6/13 (46.2%)	0
Other	7 [*] /13 (53.8%)	7 ^{**} /13 (53.8%)	3 ^{***} /7 (42.9%)
In situ carcinoma	1/13 (7.7%)	4/13 (30.8%)	0
Invasive carcinoma	12/13 (92.3%)	9/13 (69.2%)	0
AJCC stage			
I	7/12 (58.4%)	3/9 (33.3%)	0
II	4/12 (33.3%)	3/9 (33.3%)	0
III	1/12 (8.3%)	3/9 (33.3%)	0
HR status			
HR negative	8/12 (66.7%)	5/9 (55.5%)	0
HR positive	4/12 (33.3%)	4/9 (44.4%)	0
Her2 status			
Her2 positive	1/12 (8.3%)	1/9 (11.1%)	0
Her2 negative	11/12 (91.7%)	8/9 (88.9%)	0
Tumor grade			
I/II	2/12 (16.7%)	2/9 (22.2%)	0
III	10/12 (83.3%)	7/9 (77.8%)	0
Benign	0	0	7/7 (100%)

AJCC American joint commission on cancer, *HR* hormone receptor, *Her2* human epidermal growth factor receptor, *DCIS* ductal carcinoma in situ

^{*} 1135insA, 4184del4, C44F, 3731delA, exon13ins6 kb, R1751X, 1406insA (and R2494X in BRCA2)

^{**} R2336P, 7297delCT, 504insA, R3128X, S2378X(7361C > G), E1857X(5797G > T), 5301insA

^{***} C44F (BRCA1), 8867del5 (BRCA2), K2013X (BRCA2)

Table 2

Frequency of any CT antigen expression in BRCA1/2-associated breast cancers (by subtype) and benign breast epithelium

	CT antigen expression	95% CI (%)	P value
DCIS	3/5 (60%)	17–100	ns
Invasive cancers	13/21 (62%)	41–83	
Triple-negative IDC (all poorly differentiated)	9/13 (69%)	44–94	
Poorly differentiated IDC	12/18 (67%)	45–84	
Well/moderately differentiated IDC	1/3 (33%)	0–88	
DCIS/IDC	16/26 (61.5%)	43–80	0.003
Histologically normal breast epithelium	0/7 (0%)	0–37	

DCIS ductal carcinoma in situ, *IDC* invasive ductal carcinoma

Table 3

Frequency of MAGE-A and NY-ESO-1 antigen expression in BRCA1/2-associated breast cancers (by subtype and staining)

	Immunopositivity	MAGE-A	NY-ESO-1
Poorly differentiated IDC		10/18 (56%)	7/18 (39%)
	++/+++ /++++	7	4
	Focal/+	3	3
Well/moderately differentiated IDC		0/3 (0%)	1/3 (33%)
	++/+++ /++++	0	0
	Focal/+	0	1
DCIS		3/5 (60%)	2/5 (40%)
	++/+++ /++++	0	0
	Focal/+	3	2

DCIS ductal carcinoma in situ, *IDC* invasive ductal carcinoma