Tumor-associated antigens identified early in mouse mammary tumor development can be effective vaccine targets

Sasha E. Stanton, Ekram Gad, Lauren R. Corulli, Hailing Lu, Mary L. Disis
Cancer Vaccine Institute, University of Washington, Seattle WA, 98109, USA

Abstract

Breast cancer vaccines composed of antigens identified by serological analysis of cDNA expression libraries (SEREX) induce antigen specific immune responses in patients but have had disappointing clinical benefits. While many attempts to modify the adjuvants and vaccine method have been tried, one issue not addressed was whether the SEREX tumor-associated antigens identified from late stages of disease were ideal targets. We questioned in the transgenic TgMMTV-neu mouse model whether the antigen repertoire is distinct between early and late stage breast cancer and whether the antigens identified via SEREX from transgenic mice with early or late stage tumors would elicit differential anti-tumor effects to address this question.

Three early stage antigens, Pdhx, Stk39, and Otud6B, were identified from a SEREX screen of mice prior to development of palpable lesions. Formulated into a vaccine, each early antigen inhibited tumor growth (p < 0.0001). The antigens identified from mice with late stage tumors (Swap70, Gsn, and Arhgef2) were unable to inhibit tumor growth when used as vaccines (for example Gsn p = 0.26). Each of the three early stage antigens were essential for tumor survival in syngeneic mouse tumor cells and in human breast cancer cell lines across breast cancer subtypes. Silencing protein expression of the early antigens increased apoptosis (p < 0.0001 for all antigens in mouse and p < 0.05 for all antigens in human triple negative breast cancer) and decreased survival (p < 0.0001 for all antigens in mouse and human triple negative and HER2 positive breast cancer). Overexpression of the early stage antigens in women with breast cancer predicted worse prognosis (p = 0.03) while overexpression of late stage antigens did not impact prognosis (p = 0.09). These data suggest that antigens expressed earlier in breast tumor development and functionally relevant to breast tumor growth may be more effective targets for therapeutic breast cancer vaccines than antigens identified in later disease.

1. Introduction

Tumor-associated antigens have been identified through serological analysis of cDNA expression libraries (SEREX) from autoantibodies present in patients with existing disease as compared to individuals without cancer. Over 2000 breast cancer antigens have been identified by SEREX screening in patients including cancer testis antigens (e.g. MAGE and NY-ESO-1) and overexpressed tumor-associated proteins including HER2, CEA, and NY-BR-62 [1–4]. However, while the cancer vaccines designed from these antigens were immunogenic in early clinical trials, they were not effective at improving clinical outcomes in later phase trials [5–7]. To optimize vaccine efficacy, trials have modified the vaccine design using novel delivery systems and adjuvants [8–10]. Despite improving immunogenicity by changing adjuvants, clinical outcomes were not improved. There has been considerable effort focused on improving vaccine design but little focus on whether the antigens identified in patients with advanced stage disease are the best targets for immune destruction of the tumor [11–13].

The protein repertoire expressed in the tumor changes as a tumor develops through the accumulation of gene activation and inactivation events [14]. Therefore, proteins expressed later in disease may not be associated with common driver pathways as multiple alterations may occur. In evaluating primary component analysis of human breast cancers in metastases and primary tumors, the metastases cluster together and are different from both the primary tumors and genes expressed in normal breast [15]. The genes expressed in metastatic disease that are not found in primary tumors include genes to induce oxidative metabolism,
activate tissue remodeling, and silence the extracellular matrix and may not be essential to cancer survival [16]. In early primary tumors, genes that are overexpressed are associated with proliferation and growth of the tumor and the genes necessary for metastases, genes of invasion and motility, do not give the primary tumor a selection advantage [17,18].

Since the protein repertoire of early and late tumors differs, the antigens exposed to the immune system in early and late tumor development are also different. In previous studies, we had demonstrated that serum autoantibodies were different between development and growth of the tumor and the genes necessary for metastases, genes of invasion and motility, do not give the primary tumor a selection advantage [17,18].

The early stage serum samples were collected from mice every 2 weeks starting at 4 to 6 weeks old until development of a palpable tumor (n = 20) [20]. The late stage serum samples were collected from mice that had developed spontaneous, palpable tumors (n = 10) [19]. The early stage tumor antigens (Pdhx, Stk39, and Otud6B) were autoantibodies identified from a SEREX screen of serum from mice 2 weeks prior to development of a palpable tumor. The late stage tumor antigens (Gsn, Swap70, and Arhgef2) were autoantibodies identified from a SEREX screen of serum of mice with palpable tumors [19].

2.2. Antigens

The early stage serum samples were collected from mice every 2 weeks starting at 4 to 6 weeks old until development of a palpable tumor (n = 20) [20]. The late stage serum samples were collected from mice that had developed spontaneous, palpable tumors (n = 10) [19]. The early stage tumor antigens (Pdhx, Stk39, and Otud6B) were autoantibodies identified from a SEREX screen of serum from mice 2 weeks prior to development of a palpable tumor. The late stage tumor antigens (Gsn, Swap70, and Arhgef2) were autoantibodies identified from a SEREX screen of serum of mice with palpable tumors [19].

2.3. Immunizations and tumor growth

Positive clones, unreactive to control sera from parental mice, were purified to monoclonality and converted to pBK-CMV phagemid by in vivo excision using XLOLR cells and ExAssist helper phage (Stratagene, LaJolla CA). Plasmid DNA was prepared using a Qiagen Mega kit (Qiagen, Valencia CA). The pBK-CMV3 plasmid with or without the indicated antigens was suspended in 50 μL of PBS with CFA/IFA and 50 μg was given by intradermal injection into the ear. For implant studies, five mice per group for the early stage antigens and three mice per group for the late stage tumor antigens were vaccinated three times approximately 14 days apart. A syngeneic MMC mouse carcinoma line derived from a spontaneous tumor in a TgMMTV-neu mouse was implanted 1 week after the last vaccination. The MMC cell line was harvested using a 5-parameter best fit standard graph. The 10 μg/mL lysate dilution for each sample was evaluated. The supernatant was diluted 1:1 with sample buffer and run in duplicate on the plate. The Granzyme B ELISA was performed using the mouse Granzyme B ELISA kit (Ebioscience, Vienna Austria). The assay was performed with the kit instructions using pooled supernatant from the replicates for each ELISPOT. For each sample, the supernatant was diluted 1:1 with sample buffer and run in duplicate on the plate. The 10 μg/mL lysate dilution for each sample was evaluated. The plate was evaluated on a VICTOR V3 plate reader (Perkin Elmer, Waltham MA) and evaluated using SOFT-MAX Pro v5.3 software using a 5-parameter best fit standard graph.
apoptosis control was mouse All Stars positive control and human All Stars positive control (Qiagen, Valencia CA). The negative siRNA control was All Stars universal negative control (Qiagen, Valencia CA). The syngeneic mouse mammary tumor cell line MMC and human cell lines MCF10F (non-malignant breast), HCC1500 (ER positive HER2 negative), HCC70 (ER negative HER2 negative), and SKBR3 (ER negative HER2 positive) were seeded in two 96 well plates (ATCC, Manassas VA). Forty pMol of each individual siRNA and the pooled siRNA were transfected in triplicate (40 pMol of each siRNA and the negative and positive controls and 10 pMol of each siRNA when pooled) (Thermo Scientific, Waltham MA). MMC cells were seeded at a density of 2000 cells/well and transfected using Dharmafect liposomes (Dharmacon, Pittsburgh PA). MCF10F, SKBR3, and HCC70 were seeded at 2000 cells/well and HCC1500 was seeded at 4800 cells/well and transfected using lipofectamine liposomes (Thermo-Fisher, Waltham MA). Transfection conditions, cell number, timing, and reagent concentration was determined by feasibility studies that identified optimal assay conditions using control, positive apoptosis control, and universal negative control siRNA. All transfections were performed in triplicate, were corrected for a no cell background luciferase control, and were normalized to wells transfected with liposomes but no siRNA (control).

2.7. Apoptosis assay

Apoptosis was measured with a caspase 3/7 fluorescence assay (Promega, Madison WI). For MMC 50 μL of caspase 3/7 glo reagent was added per well, for MCF10F 100 μL caspase 3/7 reagent was added per well, and for HCC1500, HCC70, and SKBR3 40 μL of caspase 3/7 reagent was added per well. The plates were then incubated for 30 min at 37°C and read by luciferase intensity at 90 min using the Wallac Envision 2104 Multi-label Detector/plate reader with a 96 well aperture (Perkin Elmer, Waltham MA).

2.8. Cell viability assay

Cell viability was measured by ATP quantification using Cell Titer Glo (Promega, Madison WI). For all cell lines, 20 μL of cell titer glow reagent was added per well and then immediately read using the Wallac Envision 2104 Multi-label Detector/plate reader with a 96 well aperture (Perkin Elmer, Waltham MA).

2.9. TCGA expression data

Publically available data from the 817 breast cancer samples published in Cell [24] from the TCGA database were evaluated using the c-portal web-based program [25,26]. Overexpression data was evaluated using both the putative copy number alterations from GISTIC and mRNA expression from RNAseq with z-score of 2.0. The early stage antigens (PDHX, STK39, and OTUD6B) were evaluated separately from the late stage tumor antigens (ARHGEF2, SWAP70, and GSN), The survival data was evaluated with log rank by the c-portal program.

2.10. Statistical analysis

Graphs and statistical comparisons were performed in GraphPad Prism v6.05 software. A students T test was used to compare empty vector and vaccinated mice for each peptide. A two-way ANOVA with Bonferroni’s post-test was used for comparisons between groups for the mouse tumor growth. A one way ANOVA with Dunnett’s comparison was used in rtpCR siRNA knockdown analysis and cell survival/apoptosis analysis. Log-rank analysis was used for survival analysis using TCGA data. Significance was considered at p < 0.05 for all statistical tests.
immune response to 10 μg/mL lysate. The positive control CONA was positive for all samples (data not shown). Vaccination with both the SEREX early and late stage tumor plasmids showed an increased immune response compared to mice vaccinated with empty vector alone. Vaccination with the early stage antigen plasmids increased the mean tumor-specific IFN-γ T cells from 45.7 ± 9.2 in the empty vector vaccinated mice to 131.5 ± 23.9, \( p = 0.03 \) while there was no difference in the mean IFN-γ tumor-specific T cells induced by the splenocyte lysate (\( p = 0.85 \)). Vaccination with the late stage antigen plasmids increased the mean tumor-specific IFN-γ T cells from 45.7 ± 9.2 in the empty vector vaccinated mice to 125.9 ± 26p = 0.05) while there was no difference between the IFN-γ tumor-specific T cell frequency induced by the splenocyte lysates (\( p > 0.79 \)) (Fig. 1D). There was also a significant increase in Granzyme B produced in mice that were vaccinated with the early and late stage antigens demonstrating specific T cells induced by the splenocyte lysate (\( p = 0.85 \)). Vaccination with the late stage antigen plasmids increased the mean tumor-specific IFN-γ T cells from 45.7 ± 9.2 in the empty vector vaccinated mice to 125.9 ± 26p = 0.05) while there was no difference between the IFN-γ tumor-specific T cell frequency induced by the splenocyte lysates (\( p > 0.79 \)) (Fig. 1D). There was also a significant increase in Granzyme B produced in mice that were vaccinated with the early and late stage antigens demonstrating

Fig. 1. Vaccination with early and late stage tumor antigens recovered by SEREX from TgMMTV-neu mice induce a T cell immune response but only vaccination with early stage antigens inhibit tumor growth. (A) TgMMTV-neu mice received three vaccinations with empty vector and each of the seven early tumor antigens. MMC tumor cells were implanted on day 0. (B) TgMMTV-neu mice received three vaccinations with empty vector and the five late stage tumor antigens that have human homologs. MMC tumor cells were implanted on day 0 ***p < 0.001. (C) Vaccination Schema. (D) MMC syngeneic tumor lysate but not lysate from FVB splenocytes induces an IFN-γ T cell immune response in mice vaccinated with the early and late stage tumor antigens. IFN-γ secreting cells quantified as precursor frequency (y-axis) for FVB mice (n = 5) vaccinated with the plasmids for the early tumor antigens (light gray) or FVB mice (n = 5) vaccinated with the plasmids for the late stage antigens (dark gray) as compared to FVB mice (n = 5) vaccinated with empty control vector plasmid (white). The positive control is concanavalin A (CONA). * p < 0.05 (E) Vaccination with the early and late stage tumor antigens induces a CD8+ T cell immune response. Granzyme B ELISA was performed on the pooled supernatants from the ELISPOT assay. ** p = 0.01 * p < 0.05.

Please cite this article as: S. E. Stanton, E. Gad, L. R. Corulli et al., Tumor-associated antigens identified early in mouse mammary tumor development can be effective vaccine targets, Vaccine, https://doi.org/10.1016/j.vaccine.2019.05.024
increased tumor-specific CD8+ T cells. 10 μg/mL MMC lysate induced 123.8 pg/mL ± 59.4 granzyme B in empty vector vaccinated mice (n = 5) while inducing 392.9 pg/mL ± 86.1 in mice vaccinated with the early stage antigens (n = 5) (p = 0.005 compared to empty vector) and 330.2 pg/mL ± 20.4 granzyme B (p = 0.04 compared to empty vector) in mice vaccinated with the late stage tumor antigens (n = 5). Figure 4) This demonstrates that vaccination with the SEREX plasmids induces both a tumor-specific IFN-γ T cell and CD8+ T cell response and suggests that this type I antigen-specific T cell immune response is responsible for inhibiting tumor growth.

As the early stage antigens were able to delay tumor growth with implanted tumor models, we then evaluated the effect of vaccination on preventing tumors in TgMMTV-neu mice. TgMMTV-neu mice were vaccinated at 6–8 weeks when none of the mice have hyperplasia [32]. Mice vaccinated with the late stage tumor antigens (Swap70, Arhgef2, and Gsn) did not have improved tumor-free survival as compared to empty vector alone (p = 0.23). However, 20% of mice vaccinated with the three early stage antigens (Pdhx, Stk39, and Otud6B) did not develop breast tumors and there was increased tumor free survival even in the animals that did eventually develop tumors (p = 0.02) (Supplemental Figure 4).

Depletion of T cells, not B cells, abrogated the growth inhibition from vaccination with the early stage antigens. For mice vaccinated with Pdhx, Stk39, and Otud6B depletion of CD3+ T cells increased tumor growth (p < 0.0001 compared to empty vector control for all targets). However depletion CD22+ B cells had no impact on vaccine inhibition of tumor growth (p = 0.19 compared to Vaccine + IgG for Pdhx, p = 0.99 for Stk39, and p = 0.68 for Otud6B, Figure 2A). Adoptive transfer of T cells from mice vaccinated with Pdhx, Stk39, or Otud6B, but not T cells from empty vector vaccinated or unvaccinated mice (naïve), was able to inhibit tumor growth in unvaccinated mice with implanted MMC tumors (Supplemental Fig. 3).

3.2. Silencing expression of the “early stage” tumor antigens in breast cancer cells was more likely to impact breast tumor growth than silencing expression of “late stage” antigens.

The expression of each of the early stage tumor antigens Pdhx, Stk39, and Otud6B was essential for survival of the syngeneic TgMMTV-neu mouse cell line MMC. Silencing Pdhx reduced tumor cell survival by 58% (p < 0.0001) and increased apoptosis by 90% (p < 0.0001), silencing expression of Stk39 reduced MMC survival by 49% (p < 0.0001) and increased apoptosis by 70% (p < 0.0001), and silencing Otud6B decreased MMC survival by 44% (p < 0.0001) and increased apoptosis 80% (p < 0.0001) (Figure 3A and B). Of the late stage tumor antigens silencing Gsn decreased survival by 78% (p < 0.0001) and increased apoptosis by 120% (p < 0.0001) but silencing Arhgef2 and Swap70 did not significantly decrease survival (p = 0.08 for Arhgef2) or increase apoptosis (p = 0.99 and p = 0.07, respectively).

The early stage tumor antigens Pdhx, STK39, and OTUD6B are overexpressed in both ductal carcinoma in situ (DCIS) and breast cancer [20]. We demonstrated that Pdhx, STK39, and OTUD6B were necessary for tumor survival in human breast cancer cell lines across breast cancer subtypes. In the hormone receptor positive cell line HCC1500 (ER positive) silencing STK39 decreased survival (p = 0.03) and silencing OTUD6B increased apoptosis (p = 0.02) (Figure 4A and B). Silencing all three of the early stage antigens decreased survival (p < 0.0001 for PDHX, STK39, and OTUD6B) and increased apoptosis (PDHX p = 0.03, STK39 p = 0.02, and OTUD6B p = 0.05) in the triple negative breast cancer cell line HCC70. In the HER2 positive cell line SKBR3, silencing all three early stage antigens decreased survival (PDHX and STK39 p < 0.0001 and OTUD6B p = 0.05) and both STK39 and OTUD6B increased apoptosis (STK39 p = 0.0002 and OTUD6B p < 0.0001). These responses were specific because they were not seen in cells transfected with liposome (control), cells transfected with a non-specific siRNA control (negative control), or untransfected cells (untransfected, white bars). Each siRNA specifically decreased the expression of the mRNA of the target of interest by RT PCR (Supplemental Figs. 1 and 5).

3.3. Over expression of the “early stage” antigens have a prognostic impact in patients with breast cancer

Publically available data from the TCGA database were evaluated using the c-bioportal web-based program [25,26]. Overexpression of the early stage antigens Pdhx, STK39, and OTUD6B are associated with increased tumor volume (p < 0.0001 and 0.99 for Stk39, and p = 0.07 for Otud6B, Figure 5). The expression of each of the early stage tumor antigens Pdhx, STK39, and OTUD6B was essential for survival of the syngeneic TgMMTV-neu mouse cell line MMC. Silencing Pdhx reduced tumor cell survival by 58% (p < 0.0001) and increased apoptosis by 90% (p < 0.0001), silencing expression of STK39 reduced MMC survival by 49% (p < 0.0001) and increased apoptosis by 70% (p < 0.0001), and silencing OTUD6B decreased MMC survival by 44% (p < 0.0001) and increased apoptosis 80% (p < 0.0001) (Figure 3A and B). Of the late stage tumor antigens silencing Gsn decreased survival by 78% (p < 0.0001) and increased apoptosis by 120% (p < 0.0001) but silencing Arhgef2 and Swap70 did not significantly decrease survival (p = 0.08 for Arhgef2) or increase apoptosis (p = 0.99 and p = 0.07, respectively).

The early stage tumor antigens Pdhx, STK39, and OTUD6B are overexpressed in both ductal carcinoma in situ (DCIS) and breast cancer [20]. We demonstrated that Pdhx, STK39, and OTUD6B were necessary for tumor survival in human breast cancer cell lines across breast cancer subtypes. In the hormone receptor positive cell line HCC1500 (ER positive) silencing STK39 decreased survival (p = 0.03) and silencing OTUD6B increased apoptosis (p = 0.02) (Figure 4A and B). Silencing all three of the early stage antigens decreased survival (p < 0.0001 for PDHX, STK39, and OTUD6B) and increased apoptosis (PDHX p = 0.03, STK39 p = 0.02, and OTUD6B p = 0.05) in the triple negative breast cancer cell line HCC70. In the HER2 positive cell line SKBR3, silencing all three early stage antigens decreased survival (PDHX and STK39 p < 0.0001 and OTUD6B p = 0.05) and both STK39 and OTUD6B increased apoptosis (STK39 p = 0.0002 and OTUD6B p < 0.0001). These responses were specific because they were not seen in cells transfected with liposome (control), cells transfected with a non-specific siRNA control (negative control), or untransfected cells (untransfected, white bars). Each siRNA specifically decreased the expression of the mRNA of the target of interest by RT PCR (Supplemental Figs. 1 and 5).
pression of the early stage antigens predicted worse prognosis in large breast cancer patient cohorts while overexpression of the late stage tumor proteins did not similarly predict worse prognosis [24,33]. From the Cancer Genome Atlas study of 463 patients using microarray data, there was decreased median overall survival of 90.8 months in patients that had overexpression of OTUD6B, STK39, or PDHX (n = 147) as compared to patients without overexpression (n = 316) whose survival was over 50% at the time of evaluation (p = 0.005, data not shown). Overexpression of ARHGEF2, SWAP70, and GSN did not predict worse survival with patients that overexpressed the late stage tumor antigens (n = 125) having a mean overall survival of 129.6 months and patients without overexpression of the late stage tumor antigens (n = 338) having a median survival of 113.7 months (p = 0.99) [33]. Similarly, in a study of 807 lobular breast cancer patients, patients with overexpression of the early stage antigens (n = 343) had median survival of 113.7 months while patients without overexpression of the early stage antigens (n = 464) had median survival of 146.4 months (p = 0.03, Fig. 5A). Patients that have overexpression of the late stage tumor antigens (n = 262) had a median survival of 60% at 240 months while patients that do not have overexpression of the late stage tumor antigens (n = 545) had a median survival of 113.7 months (p = 0.09) (Fig. 5B) [24].

4. Discussion:

Not all proteins overexpressed in breast cancer are important for breast tumor development and survival [34,35]. In this study of proteins identified from the TgMMTV-neu mouse, we demonstrated that tumor-associated antigens expressed early in the course of breast tumor development were necessary for tumor survival and more effectively inhibited tumor growth as vaccines than tumor-associated antigens in advanced disease. This suggests that these early stage tumor antigens (PDHX, STK39, and OTUD6B) may be more effective vaccine targets than antigens identified in later stage disease (SWAP70, GSN, and ARHGEF2) because of a greater functional importance.

To improve tumor immune destruction, a T cell immune response should be developed to cells that are important for the growth of the tumor [36,37]. Identifying targets that are necessary...
for breast tumor survival, particularly across breast cancer subtypes could provide a vaccine response that is widely applicable across breast cancer patients [38]. The expression of the three early stage tumor antigens was essential for breast cancer survival both in the syngeneic mouse tumor cell line and across human breast cancer cell lines of different subtypes. These three proteins have been shown in the literature to be important in enhancing cell growth. PDHX is an ubiquitin E3 ligase that converts pyruvate to acetyl-co-A and regulates glucose metabolism in colon cancer [39,40]. STK39 is a kinase involved in cell survival and is overexpressed in many cancers including non-small cell lung cancer [27,41–43]. OTUD6B is a deubiquitase important in cell survival and proliferation in non-small cell lung cancer [44]. The roles of the late stage tumor antigen proteins are associated with motility and metastases. Keratin 2–8 is involved in breast cancer invasion, Swap70 is associated with malignant transformation of breast cells, and Rock1 and Arhgef2 induce breast cancer metastases and invasion through the RhoA pathway. [29,30,45] Gsn is been associated with increased TNF-b which enhances metastatic activity by inducing stromal invasion and has been associated with increased metastases in the lungs in estrogen negative breast cancer [31,46]. The three early stage tumor antigens are affect tumor
Fig. 5. Overexpression of the early stage antigens is associated with worse survival in patients with breast cancer. mRNA expression and copy number alterations were evaluated from patients with invasive breast cancer (n = 807 patients), cases without the target overexpression in blue and cases with the target overexpression in red (A) PDHX, OTUD6B, and STK39 (without overexpression n = 467 and with overexpression n = 340). Log rank p = 0.02 (B) GSN, SWAP70, and ARHGEF2 (without overexpression n = 550 and with overexpression n = 257). Log rank p = 0.09. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
growth across the subtypes, suggesting that they affect common cancer growth pathways. The expression of the three early stage tumor proteins are associated with metastases in breast cancer suggesting they are involved in invasion. This study suggests vaccination against early stage antigen targets that are associated with tumor growth rather than tumor invasion are targeting earlier stages in tumor development and may be the only effective antigens in this study.

While both the early and late stage tumor antigens induced both a Th1 CD4+ T cell and CD8+ T cell immune response to the tumor, the early stage antigens were more effective in inhibiting tumor growth. However, this study has potential limitations including that the targets recovered were from one mouse mammary tumor model TgMMTV-neu that is a model of luminal B disease. Based on the siRNA knockdown evaluation in human breast cancer cell lines show that these genes are essential for tumor cell survival across subtypes, but evaluation of the protein expression of these targets in tumors across breast cancer subtypes will be important to ensure that they are common breast cancer targets. Another limitation for a therapeutic vaccine is that, while we have shown the early stage antigens are essential for breast cancer survival, we have not demonstrated their efficacy on TgMMTV-neu mice bearing spontaneous tumors and these studies would need to be performed for developing a therapeutic vaccine. A third limitation of the SEREX assay is that we may not have discovered all of the early or late stage antigens and may be missing important targets.

This study is to demonstrate proof of principal that the antigens identified earlier in tumor development are more effective vaccine targets but are not an exhaustive panel of early antigens.

This study has demonstrated that three early tumor-associated antigens (Pdhx, Stk39, and Otud6B) identified by a SEREX screen in mouse mammary tumor model TgMMTV-neu prior to palpable tumor development, are superior antigen targets for the inhibition of tumor growth than antigens identified from mice with late stage tumors. This study suggests that transgenic mouse mammary tumor models are powerful tools to identify early antigens that, due to detection limitations, we are currently unable to identify in women and suggests that consideration of the functional relevance of these antigens is important in vaccine design.

Declaration of Competing Interest

Lauren R. Corulli, Hailing Lu, and Ekram Gad have no disclosures to declare. Sasha E. Stanton receives research funding from Pfizer, Genentech, Seattle Genetics, Ephithany, EMD Serono, Precigen, and Jansen

Acknowledgement

The research was funded by 5R01CA129417–4 United States, For Mary Disis the Athena Distinguished Professor of Breast cancer Research is United States, The American Cancer Society is United States, The U01CA141539 is United States, And for Sasha Stanton Both the NIH T32 and ITHS NIH KL2 are United States.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.05.024.

References

[21] Guy CT, Cardiff RD, Muller WJ. Induction of mammary tumors by expression of mammary tumor model TgMMTV-neu mice bearing spontaneous tumors and these studies would need to be performed for developing a therapeutic vaccine. A third limitation of the SEREX assay is that we may not have discovered all of the early or late stage antigens and may be missing important targets.
[22] This study suggests that transgenic mouse mammary tumor models are powerful tools to identify early antigens that, due to detection limitations, we are currently unable to identify in women and suggests that consideration of the functional relevance of these antigens is important in vaccine design.

Declaration of Competing Interest

Lauren R. Corulli, Hailing Lu, and Ekram Gad have no disclosures to declare. Sasha E. Stanton receives research funding from Pfizer, Genentech, Seattle Genetics, Ephithany, EMD Serono, Precigen, and Jansen

Acknowledgement

The research was funded by 5R01CA129417–4 United States, For Mary Disis the Athena Distinguished Professor of Breast cancer Research is United States, The American Cancer Society is United States, The U01CA141539 is United States, And for Sasha Stanton Both the NIH T32 and ITHS NIH KL2 are United States.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.05.024.

References


