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TUMOR ANTIGENS AND MARKERS FOR BREAST AND OVARIAN CANCERS

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1. ABSTRACT

Use of vaccines to prevent and treat breast and ovarian cancer is a highly attractive approach because of the expected minimal side effects and the potential to predict individuals likely to benefit from vaccination. To fully harness the capacity of the immune system for this purpose, it is necessary to characterize tumor antigens for these cancers so that purified antigens can be tested for their immunogenicity in individual patients and for their suitability as targets of vaccine-induced immunity. Discovery of novel breast and ovarian tumor antigens is also necessary for developing multi-antigen vaccines composed of multiple tumor antigens. Such vaccines are expected to induce diverse immune responses and minimize emergence of antigen-loss variant tumors that are resistant to vaccine-induced immunity. With the exception of melanomas, for most human cancers including breast and ovarian cancers the repertoire of known tumor antigens remains relatively small. In this review we will discus the importance of characterizing tumor antigens for use in vaccination against cancer and then summarize antigens that have been characterized for human breast and ovarian cancers. We will also emphasize that identification of a novel tumor antigen, while an important first step, needs to be followed by a multi-step process of validation of that antigen. The steps in this validation process are i) to demonstrate that a tumor antigen is over-expressed at a reasonable frequency in primary tumors and in metastases; ii) to demonstrate the immunogenicity of a tumor antigen in an appropriate animal model; iii) to demonstrate its immunogenicity and safety in humans. Additional considerations in this review include: i) discussion of the potential of breast and ovarian tumor antigens as markers for early detection and for monitoring tumor burden in cancer patients; ii) discussion of their potential as prognostic markers of breast and ovarian cancers; and iii) discussion of a unique class of tumor antigens and markers that induce expression of multiple other tumor antigens and markers. Finally, we will discuss the present evidence for potential for autoimmunity that might accompany antitumor vaccination.

2. INTRODUCTION

The history of the field of tumor immunology is punctuated by periods of excitement and somber reflection. These periods, not surprisingly, coincided with new evidence for or against the existence of tumor antigens. The pioneering work and Prehn and Main (1) and of George

Table 1. Level of protective antitumor immunity elicited by L1210 lymphoma tumor clones correlates with the diversity of the T cell response elicited by these clones

Immunogen	Level of protective Immunity elicited against parental L1210 lymphoma of DBA/2 mice	Diversity of the induced T cell response
L1210 clone 3.3	Low, 10-20% of immunized mice protected following challenge with tumorigenic doses of parental L1210 tumor cells.	Low, including Vβ8.2, 15 and 16 subfamilies
L1210 clone 7.15	High, 100% of immunized mice protected following challenge with tumorigenic doses of parental L1210 tumor cells.	High, including Vβ 3, 5.1, 5.2, 6, 7, 8.2, 9, 11, 12, 16 and 18 subfamilies

Klein and his colleagues (2) in the 50's and 60's laid the foundation of the field of tumor immunology. They demonstrated that immunity to chemically-induced tumors in syngeneic and autochthonous host strains of inbred mice was tumor specific, with little cross reaction to normal tissues or to other tumors induced by the same chemical in the same strain of mice (3). This excitement, however, was short lived as data with spontaneous tumors did not support the conclusions derived from studies with chemically-induced tumors. In particular, it was reported that unlike chemically-induced tumors, spontaneously arising tumors failed to induce significant protective antitumor immunity. Such studies with spontaneous tumors presented a major conceptual block to further progress in the field since spontaneously arising tumors were considered more akin to human tumors and the prospects for immunotherapy of cancer appeared dim.

Further progress in this field stemmed from studies that showed that apparently nonimmunogenic spontaneous tumors are actually weakly immunogenic and can elicit specific protective immunity under appropriate conditions, including use of adjuvants like BCG (4) or mutagenization (5). These studies re-affirmed the existence of antigens on spontaneous tumors and set the stage for the pursuit of the discovery of tumor antigens. The first breakthrough in these efforts came from the Boon lab that reported the isolation of the first human melanoma tumor antigen recognized by cytotoxic T cells (6). Since then, continued efforts primarily from the Boon and the Rosenberg labs (7) led to the identification of a large number of antigens expressed in human melanomas (8,9). These pioneering studies established an important paradigm that many tumor antigens are non-mutated self-proteins that are over-expressed in the tumor tissue and that show restricted expression in normal human tissues. This understanding engendered renewed interest in identifying tumor antigens in cancers other than melanoma. Interestingly, a number of novel approaches have been employed for characterizing tumor antigens expressed in these tumors since the tumor specific CTL lines have been difficult to generate in these non-melanoma tumors. This review article will focus on the status of research on breast and ovarian tumor antigens. Specifically, we will first summarize evidence on the potential for tumor vaccines in breast and ovarian cancers, emphasize the need to isolate novel human tumor antigens and then describe the antigens currently known for breast and ovarian cancers. Following that, we will discus other potential applications of these antigens including as surrogate and prognostic markers of breast and ovarian cancers.

3. VACCINE APPROACH CAN BE EFFECTIVE IN THE PREVENTION AND TREATMENT OF BREAST AND OVARIAN CANCERS

Independent reports have shown that vaccination with Her2/neu cDNA or protein can prevent mammary tumor development in neu transgenic mice (10-12). These studies coupled with reports of correlation between existent cellular immunity and long term survival of breast cancer patients (13-15) suggest that deliberate vaccination may prevent breast cancer development in healthy women and lead to long-term cures in breast cancer patients harboring minimal residual disease. Indeed, recent clinical trials with antigen-derived and anti-idiotype vaccines in breast cancer patients support these ideas (16-17). Similarly, a recent report using anti-idiotypic mimic of CA125 antigen as vaccine showed considerable promise for a vaccine approach against ovarian cancer as well (18). To further enhance and fully evaluate the potential of vaccine approach to immunoprevention and immunotherapy of breast and ovarian cancers, we believe that some of the deficiencies outlined below must be addressed.

4. MULTI-ANTIGEN VACCINES ARE NEEDED FOR ELICITING PROTECTIVE ANTITUMOR IMMUNE RESPONSES

Most tumor antigens are self-proteins, and due to mechanisms of self-tolerance generally elicit weak immune responses. Such weak immune responses can sometimes exhibit antitumor activity (19) but generally require further enhancement to observe significant antitumor effects (20-21). To further bolster the antitumor efficacy of tumor vaccines, vaccination with multiple tumor antigens is most desirable. Such multi-antigen vaccines are expected to increase the overall level and diversity of antitumor immune responses thereby limiting tumor growth and minimizing emergence of antigen-loss tumor variants that are resistant to vaccine-induced immunity. These ideas are supported by published reports that show correlation between clinical responses in melanoma patients and the induction of immune response against multiple melanoma tumor antigens (22,23). Similarly, in our own work with L1210 lymphoma tumor model of DBA/2 mice we found that the protective antitumor immunity correlates with the induction of a diverse T cell response, presumably, against multiple tumor antigens (24,25 and data summarized in Table 1.

5. ISOLATED TUMOR ANTIGENS HAVE MANY ADVANTAGES

Pioneering work with tumor lysate/tumor RNA-pulsed DCs (26, 27), and tumor-derived heat shock proteins (28) have demonstrated that strong antitumor immune responses can be elicited by using such polyvalent vaccines. To further improve upon these advances it is necessary to use isolated tumor antigens. There are many advantages of vaccination with isolated tumor antigens that justify efforts to identify these antigens. Thus, the polymorphism of MHC antigens in the out-bread human population ensures that the individual cancer patients respond very differently to specific tumor antigens. This suggests the need to select antigens that are immunogenic in individual patient for use in the vaccination of that patient. Furthermore, availability of isolated tumor antigens eliminates the need for autologous tumor as the source of tumor antigen preparation. This is particularly relevant for breast cancer since, due to screening mammography, size of the breast tumors at diagnosis is usually small and probably not enough for repeated vaccination. Moreover, isolated tumor antigens can be tested for their suitability as vaccine targets since different tumor antigens may differ substantially as tumor rejection antigens depending on their function in tumor growth and/or progression. Thus, a tumor antigen that is part of a pathway critical to tumor growth and /or progression is likely to be a better tumor rejection target as it is less likely to be lost from tumor cells. Perhaps, the most compelling reason for using isolated tumor antigens is in the prophylactic setting when vaccination is used to prevent cancer development in healthy individuals at risk for developing cancer.

6. THE NEED TO DEFINE NOVEL TUMOR ANTIGENS FOR HUMAN BREAST CANCERS

With the exception of melanoma, for most human solid tumors only a few tumor antigens have been identified. For breast cancer tumor these include mucin-1 (MUC-1), carcinoembryonic antigen (CEA) and Her2/neu (29-31). In addition, some cancer/testis antigens (NY-ESO-1, SCP-1 and CT-7) originally identified in melanomas and other cancers were subsequently also shown to be expressed in about 20 to 30% of breast tumors (32,33). Mammaglobin, a breast tissue specific protein, is over-expressed in about 23% breast tumors (34) and is a potential breast tumor antigen. Thus, majority of the presently known breast tumor antigens including Her-2/neu, NY-ESO-1, SCP-1, CT-7 and mammaglobin are over-expressed in relatively small fraction (20 to 30%) of breast tumors. At this level of over-expression frequency one needs more than 10 antigens to provide coverage to >95% of the patient population. The CEA and MUC-1 are expressed in 50% and virtually 100% of breast tumors respectively, the latter however, is also expressed in a number of normal human tissues including lung, colon, pancreas and kidney (35).

The use of SEREX (serologic analysis recombinant cDNA expression libraries) approach has recently uncovered three additional interesting candidate breast tumor antigens designated as NY-BR-1(36), NY-BR-62 and NY-BR-85 (33). NY-BR-1 appears to be a breast specific transcription factor. It is expressed in high percentage (84%) of breast tumors and shows restricted expression in normal human tissues including normal breast and testis (36). However, expression in tumors does not seem to be increased significantly in comparison to normal breast, and whether this will discourage its use as an antigen especially in the prophylactic setting for prevention of breast cancer development in women at risk for developing this cancer must await further work. In this regard, it is critical to determine how the expression of the NY-BR-1 protein in breast tumors compares with that in normal breast tissue.

NY-BR-62 and NY-BR-85 on the other hand are over-expressed in human breast tumors. Of these, NY-BR-62 appears quite interesting as it shows restricted expression in normal human tissues including normal testis and thymus (33). NY-BR-85, in contrast, shows significant expression in several normal human adult tissues including colon, spleen, testis and thymus. Thus, overall, among the putative breast tumor associated antigens identified by the SEREX method, NY-BR-1 and NY-BR-62 look promising. However, their over expression in tumors needs to be validated at the protein level using large panels of primary breast tumors and metastases.

Our lab has also been interested in identifying novel breast tumor-associated cDNAs with a potential as tumor antigens against this cancer. Using the Digital Differential Display (DDD) method we found that prostate epithelium-derived Ets transcription factor (PDEF) cDNA occurs at relatively high frequencies in the cDNA libraries from normal human breast tissue and human breast tumors. In contrast, this cDNA is either undetectable or present at low frequencies in the cDNA libraries from other normal human tissues. RT/PCR expression analysis of PDEF confirmed the DDD results and showed PDEF to be over expressed in 14 out of 20 primary human breast tumors and in one metastases tested. Also, consistent with the DDD data, RT/PCR analysis of PDEF expression showed highly restricted expression in normal human tissues (37 and see below). These results suggest that PDEF is a breast tumor associated cDNA and should be further evaluated for its potential as a breast tumor antigen. In particular, it is important to demonstrate that PDEF protein is frequently over-expressed in primary breast tumors as well as in breast tumor metastases. We are working to generate PDEF specific antibody reagents for this purpose.

Among normal tissues, PDEF showed significant expression in normal prostate as described previously (38) and in trachea as reported by us (37). Thus, in the female body trachea seems to be the only tissue with significant expression of PDEF. It should be stressed that with the exception of certain cancer/testis antigens described above, other known and candidate breast tumor antigens including MUC1, Her-2/neu, CEA and NY-BR-1, NY-BR-62 and NY-BR-85 show significant expression in one or more normal human tissues. Based on this observation, the PDEF expression characteristics in normal human tissues and in breast tumors make PDEF an attractive candidate for evaluation as a breast tumor antigen.

We have compared the relative frequencies of NY-BR-1, NY-BR-62, NY-BR-85 and PDEF cDNAs in the

Table 2. Relative frequencies of selected breast tumor associated cDNAs in the cDNA libraries from normal breast tissue and breast tumors

cDNA	Relative frequency in cDNA libraries from normal breast tissue	Relative frequency in cDNA libraries from breast tumors
NY-BR-1	0.0000275	0.0000972
NY-BR-62	0.0000000	0.0000194
NY-BR-85	0.0000000	0.0000000
PDEF	0.0002399	0.0050766

cDNA libraries from normal human breast tissue and breast tumors using the Virtual Northern blot method available at http://cgap.nci.nih.gov/CGAP/Tissues/VirtualNorthern?ORG=Hs&CID=, the NCI-CGAP web site. The data is shown above in Table 2.

From the above table, the relative expression levels of PDEF are 8.7-fold higher in comparison to NY-BR-1 cDNA in the cDNA libraries from the normal breast tissue. Similarly, relative frequency of PDEF in the cDNA libraries from breast tumors is 52-fold higher than NY-BR-1 and about 261-fold higher than NY-BR-62. This comparison suggests that PDEF is highly over expressed in breast tumors in comparison to NY-BR-1 and NY-BR-62 cDNAs. We recognize that the relative expression data shown in Table 2 using a given set of cDNA libraries can be flawed depending on the chance expression of specific cDNAs in these libraries and would requires independent verification at the protein level. Nonetheless, the apparent low level representation of NY-BR cDNAs in the available cDNA libraries may be the reason for our failure to identify these cDNAs by the DDD method that lead to the identification of PDEF as a breast tumor associated cDNA (37).

Interestingly, in Table 2 above, NY-BR-85 cDNA was not found in the cDNA libraries from normal breast tissue and breast tumors. This suggests relatively low level expression of this cDNA in the normal and malignant breast tissue. This observation when considered with the reported expression of NY-BR-85 in several normal human tissues may render NY-BR-85 a less interesting candidate as a breast tumor antigen.

In summary, a number of breast tumor antigens have been defined to date, however, majority of these are over-expressed in relatively small fractions of breast tumors. Although, a few breast tumor-associated cDNAs including NY-BR-1, NY-BR-62 and PDEF appear promising candidates for novel breast tumor antigens, their further validation in large panels of primary breast tumors and breast tumor metastases is critical and remains to be demonstrated. As a result, the feasibility of a multi-antigen vaccine approach against breast cancer remains questionable at present.

7. NEED TO IDENTIFY NOVEL OVARIAN TUMOR ANTIGENS

Like breast cancer, in ovarian cancer as well there is a paucity of known tumor antigens. Some of the known ovarian tumor antigens including Mucin-1 (35) and Her-2/neu (40) were initially described as breast tumor antigens. The CA125, long used as a serum marker for monitoring tumor burden in ovarian cancer patients and also shown to be a target of antiidiotypic vaccine against this cancer was recently cloned (41). On the basis this molecular evidence, CA-125 is the previously described MUC16 antigen. The restricted expression of CA-125 in normal human tissues including normal breast and lung and frequent over-expression in ovarian tumors (42) makes CA-125 an attractive target of vaccine development against this cancer. Recently, folate binding protein was shown to be frequently over expressed in ovarian tumors and appears to be a useful target for vaccine development (43), however, a concern remains that like mucin 1 this protein is also expressed in several normal human tissues including choroid plexus, kidney, thyroid and lung (44).

HOXB7 is another candidate antigen in ovarian cancer (45). Using the SEREX method antibodies to HOXB7 were found in about one third of ovarian cancer patients. In addition, HOXB7 was expressed at much higher levels in ovarian tumors in comparison to normal ovaries. Among the other normal human tissues, HOXB7 is reportedly expressed in normal kidney and colon (46). On the basis on this restricted expression in normal human tissues and frequent over-expression in ovarian tumors, HOXB7 appears to be an attractive ovarian tumor antigen.

We have also evaluated PDEF as a potential ovarian tumor associated antigen and have tested 22 primary ovarian tumors for expression of PDEF. It was found that 13 out of 22 (59%) ovarian tumors over-express PDEF in comparison to normal ovarian tissue (our unpublished data). This data when considered in conjunction with highly restricted expression of PDEF in normal human tissues suggest that PDEF is also an ovarian tumor-associated cDNA and an attractive candidate for an ovarian tumor antigen.

8. VALIDATION OF BREAST AND OVARIAN TUMOR ANTIGENS

Identification of a novel tumor antigen is an important first step in the multi-step process of validation of that antigen as a vaccine target. The next steps in this process are i) to demonstrate that a tumor antigen is over-expressed at a reasonable frequency in primary tumors and in metastases; ii) to demonstrate the immunogenicity of a tumor antigen in an appropriate animal model; iii) to demonstrate its immunogenicity and safety in humans**.** Below, we will first outline the importance of the various steps in the validation process and then discus the status of the various candidate breast and ovarian tumor antigens

with respect to their progression along this validation process.

9. CANCER VACCINES FOR PREVENTION OF PRIMARY TUMOR DEVELOPMENT AND FOR TREATMENT OF METASTASES

Cancer vaccines can be used to control cancer at two levels. These include i) prevention of primary tumor development, and ii) treatment of metastases to prevent tumor recurrence and progression. Therefore, for an antigen to be a target of vaccine at each of these levels, it is critical to demonstrate that the antigen is expressed in the primary tumors as well as in the metastases. Her-2/neu is an interesting example since it is reported to be over-expressed in about 60% of pre-invasive breast tumors (47), but only in 20 to 30% of invasive primary breast cancers that seed metastases. Thus, although presently Her-2/neu-derived vaccines are proposed for treatment of residual disease in breast cancer patients, it is quite conceivable that in future a lot more attention will be given for testing Her-2/neu-derived vaccines to prevent tumor development in women at high risk for developing this cancer.

Among the various breast and ovarian tumor antigens described above, information on their expression in primary and metastatic tumors is available for Her-2/neu and some of the other antigens including MUC1, CEA and CA-125. In contrast, for other recently described candidate tumor antigens including NY-BR-1, NY-BR-62, PDEF and HOXB7 the data on their expression frequencies in large panels of primary tumors and metastases is not available. In the absence of this data, any estimation of the potential use of these antigens as vaccine targets remains speculative.

10. DEMONSTRATING IMMUNOGENICITY OF A TUMOR ANTIGEN

Once a candidate tumor antigen is identified based on its expression characteristics, it is necessary to demonstrate its immunogenicity since mere expression does not guarantee that the protein is adequately processed to provide the relevant peptides for binding to MHC and presentation to T cells. More than 400 allelic variants of HLA-A and B locus antigens have been described (48). While most of these antigens occur at low frequencies in the human populations, exceptions are the major HLA alleles of the HLA-A locus. These include HLA-A1, -A2 and -A3, -A11, -A24, -A28 and -A30 antigens. Identification of peptides restricted by these seven HLA-antigens is most desirable since these antigens provide vaccine coverage to >80% of the Asian, Black and Caucasian populations (49). Of these seven antigens, HLA-A2 is most prevalent in the human population and serves as a model antigen for determining the presence of immunogenic peptides in a given protein.

Two approaches have been used for determining the immunogenicity of a tumor antigen in association with HLA-A2 antigen. One involves the use of HLA-A2/K^b transgenic mice as tools for assessing immunogenicity *in vivo*, and the second involves use of an *in vitro* assay using human T cells and APCs from HLA-A2 positive individuals. Some useful features of these two approaches are outlined below.

11. USE OF HLA-A2/K^6 TRANSGENIC MICE TO DEMONSTRATE IMMUNOGENICITY OF TUMOR ANTIGENS *IN VIVO*

HLA-A2/K^b transgenic mice have emerged as an important tool for demonstration of the immunogenicity of human tumor antigens. These mice express a hybrid α chain in which the first and second extracellular domains of the H-2K^b α chain are replaced with those of HLA-A2 α chain. This replacement ensures optimal interaction of the hybrid molecule with HLA-A2 binding peptides and with the mouse CD8 molecule on T cells of the transgenic mice. The latter interaction is required for optimum T cell selection and priming. Pioneering work from several laboratories with these transgenic mice showed that the antigen processing machinery of mouse and man are functionally equivalent since the same HLA-A2 restricted ovalbumin and influenza virus encoded peptide epitopes were selected for presentation to T cells in both humans and in HLA-transgenic mice (50-52). Moreover, the CTL responses against a large panel of HLA-A2 binding peptide epitopes derived from human pathogens were found to be concordant in HLA-A2 positive human volunteers and HLA-A2/K^b transgenic mice for 71% of the tested peptides (53). Such substantial similarities in the antigen processing function and the extensive overlap in T cell repertoire of mouse and man make HLA-A2/K^b transgenic mice very attractive tools for identifying HLA-A2 restricted immunogenic peptides from human tumor antigens. A very significant advantage of using HLA-A2/K^b transgenic mice in such research is that these mice provide an easily accessible in *vivo* system for testing the immunogenicity of human tumor antigens. Others have previously used these mice to study HLA-A2 restricted CTL responses to MUC-1 and CEA tumor antigens (54,55).

Majority of the studies with HLA-transgenic mice used peptides derived from putative tumor antigens for assessing their immunogenicity. These peptides are predicted by using computer algorithms (56,57), and typically a large number of peptides are screened to identify a few immunogenic peptides in the sequence a given antigen. This process has now become considerably more efficient with the development of more sophisticated algorithms and with the use of more than one algorithms (58,59). The peptide specific CTLs are then tested for their capacity to lyse HLA-A2 positive tumor cells that endogeneously express the tumor antigen.

The use of HLA-transgenic mice for the purpose of demonstrating immunogenicity of human tumor antigens may be criticized on the grounds that these mice express endogenous mouse homolog of a tumor antigen that may differ significantly in the primary amino acid sequence from the human tumor antigen. Hence, certain peptide epitopes that are found to be immunogenic in HLA-transgenic may not have been subject to mechanisms of tolerance due to their absence in the mouse homologue.

While, this is a valid criticism, studies in mice transgenic for human tumor antigens have shown that tolerance to self-antigens is not absolute. Thus, high avidity T cells specific to self peptide-MHC complexes are deleted from the periphery, whereas low avidity T cells escape deletion and are present in the periphery (60,61). Importantly, activation of low avidity T cells was shown to be adequate for protection against the tumor challenge (19). Thus, the primary reason for using HLA-transgenic mice for determination of the immunogenicity of tumor antigens is the convenience of their availability. Clearly, these *in vivo* studies in HLA transgenic mice must also be supported by *in vitro* experiments using autologous human APCs and T cells and these are briefly outlined below.

12. AUTOLOGOUS HUMAN APCS AND T CELLS TO DEMONSTRATE IMMUNOGENICITY OF HUMAN TUMOR ANTIGENS *IN VITRO*

An alternative method frequently used to the demonstrate immunogenicity of tumor antigens uses computer algorithms to predict HLA binding peptides in the sequences of human tumor antigens followed by testing of the peptides for activation of CTL responses *in vitro.* The *in vitro* induced CTLs are then tested for their capacity to lyse target tumor cells expressing the relevant tumor antigen and the HLA-A2 antigen. Ability to induce CTL responses in this assay suggests potential immunogenicity *in vivo*. Over the years many laboratories have used variations of this assay for the demonstration of immunogenic CTL epitopes in Her-2/neu, CEA and MUC1 and NY-ESO-1 antigens (62-65). A representative protocol uses peptide-pulsed autologous dendritic cells and T cells from patients or normal individuals that express HLA-A2 antigen. More recently, pulsing of DCs with protein in place of peptides was demonstrated as a viable method for identifying immunogenic peptides (66). Similarly, analysis of *in vivo* vaccine-induced immune response against multiple melanoma antigens also was used to demonstrate the immunogenicity of tumor antigens and the identification HLA-restricted epitopes in these antigens (67). In summary, over the years, there has been considerable progress in developing assays for demonstrating the immunogenicity of tumor antigens. These assays should be used to demonstrate the immunogenicity of novel candidate breast and ovarian tumor antigens.

It is noteworthy that cell surface expression is not necessary for a tumor antigen to be a target of vaccine-induced immunity since antitumor immunity is primarily T cell-mediated and cytotoxic T cells recognize peptides derived from cellular proteins in complex with MHC class I antigens on the tumor cell surface. From this understanding, an intracellular antigen is likely to be as good a source of MHC-binding immunogenic peptides as another membrane protein of comparable size.

Of the various putative known and candidate breast and ovarian tumor antigens described previously, only a few have been evaluated rigorously as vaccines in humans. Thus, mucin-derived carbohydrate and anti-idiotype vaccines appear to prolong survival in breast cancer patients who have been vaccinated following high dose chemotherapy followed by bone marrow transplantation, a setting with minimal residual disease (16,17). Similarly, anti-idiotypic anti CA-125 antibody vaccination showed significantly prolonged survival for ovarian cancer patients in comparison to control non-vaccinated patients (18). Clinical trials with Her-2/neu-derived peptide vaccines are currently underway (68). Apart from these limited studies, vast majority of the putative breast and ovarian cancer antigens remain untested with regard to their antitumor activity in patients.

13. TUMOR ANTIGENS AS MARKERS FOR EARLY DETECTION AND FOR MONITORING OF TUMOR BURDEN IN BREAST AND OVARIAN CANCER PATIENTS

Tumor markers are typically used for early detection of cancer occurrence/recurrence and for monitoring the tumor burden in cancer patients. For most human solid tumors tumor markers are likely to represent molecules that are secreted or shed from the tumor cell into body fluids. In this respect certain tumor antigens that are expressed on the tumor cell surface or secreted from the tumor cells are likely candidates as tumor markers. Indeed for human breast cancer, CA15-3 a MUC1 specific marker is in routine use for monitoring cancer recurrence and tumor burden in breast cancer patients (69). CEA has also been similarly used in the clinics for monitoring breast cancer patients, however, recent studies show that it is a less desirable marker than CA15-3 (70). A desirable characteristic of a tumor marker is that it is expressed in most or all breast cancers. For this reason, most of the cancer/testis antigens described above that are expressed in small fractions of breast tumors are unlikely candidates for novel breast tumor markers. Among the novel candidate breast tumor antigens reported to be frequently over-expressed in human breast tumors including NY-BR-1, NY-BR-62 and PDEF; NY-BR-1 and PDEF are putative transcription factors with intracytoplasmic or nuclear localization hence unlikely to be available in body fluids in significant quantities. Similarly, NY-BR-62 apparently also is a nuclear proteins (33), hence unlikely candidates as conventional tumor markers. On the other hand, there is a recent trend in tumor marker research to develop sensitive PCR-based assays for detection of circulating tumor cells in cancer patients (71,72). Due to their limited tissue distribution and frequent over expression in breast tumors, we believe that NY-BR-1, NY-BR-62 and PDEF should be evaluated as potential markers of circulating breast tumor cells using PCR-based assays (37).

Among ovarian tumor antigens, CA-125 is a cell surface glycoprotein and has been in use for monitoring ovarian cancer patients for detection of cancer recurrence and tumor burden (73). In contrast HOXB7 and PDEF belongs to families of transcription factors with intracytoplasmic and nuclear localization, hence unlikely candidates as conventional tumor markers. On the other hand, HOXB7 and PDEF should be evaluated as markers for detection of circulating ovarian tumor cells using PCR-

based assays. Clearly, there is a need to continue the search for novel markers of ovarian cancer especially since >75% of the newly diagnosed ovarian cancer patients present with advancer disease.

14. TUMOR ANTIGENS AS PROGNOSTIC MARKERS OF BREAST AND OVARIAN CANCERS

Since tumor antigens are preferentially over-expressed in the tumor tissue, an expectation is that they may have a role in the biology of tumor growth and/or progression and thus serve as prognostic markers. This expectation is generally not borne out by the available evidence. Thus, among the known breast and ovarian tumor antigens, Her-2/neu is recognized as an independent marker of prognosis in these cancers (74) and is in use in the clinics. Additionally, it seems that MUC1 and CA 125 appear to have some predictive value for poor prognosis, but are not being used routinely for this purpose in the clinics. Whether any of the other candidate breast and ovarian tumor antigens including NY-BR-1, NY-BR-62, PDEF and HOXB7 turn out to be prognostic markers of breast or ovarian cancer remains to be shown. Our preliminary data with PDEF suggests that PDEF expression in ovarian tumors correlates with better prognosis for ovarian cancer patients (Odunsi et al, our unpublished data).

15. TUMOR ANTIGENS AND MARKERS THAT INDUCE EXPRESSION OF MULTIPLE OTHER TUMOR ANTIGENS AND MARKERS

A unique class of tumor antigens are those that simultaneously induce the expression of multiple other tumor antigens in tumor cells. In this way, a diverse immune response may be elicited against multiple tumor antigens following chemotherapy-induced tumor cell death and cross priming, resulting in the control of tumor growth and spread. Such significant impact on the inhibition of tumor progression may in turn translate into better prognosis for cancer patients. Among the breast tumor antigens, NY-BR-1 and PDEF are potential members of this class of tumor antigens since as transcription factors they have the potential to induce the expression of other proteins as additional breast tumor antigens. Similarly, in ovarian cancers, PDEF and HOXB7 have the potential to induce a host of novel tumor antigens that in turn may elicit a diverse immune response against these tumors and affect the course of tumor progression. In this scenario, it should be noted that NY-BR-1, PDEF and HOXB7 themselves need not be as immunogenic as the antigens induced by these proteins. Similar comment applies to their role as tumor markers, i.e. some of the proteins induced by transcription factors may be secreted or shed into the circulation and serve as better tumor markers for early detection of tumor occurrence/recurrence and for monitoring of tumor burden in breast and ovarian cancer patients.

16. TUMOR IMMUNITY VERSUS AUTOIMMUNITY

As it has become clear that vast majority of human tumor antigens are non-mutated self-proteins, a natural question is what are the consequences of inducing strong immune responses against such antigens i.e. is autoimmunity a significant consequence of antitumor immunity? The experimental evidence addressing this issue to date is equivocal. The clinical trials data with MUC-1, CEA and CA-125 derived vaccines have not uncovered a serious threat of autoimmunity in the vaccinated patients. On the other hand, a recent report suggests that a strong immune response when elicited by repeated immunization with a single antigen shared between tumor and normal tissues can lead to adverse autoimmune reaction (75). In yet another setting, studies show that there exists a window of opportunity whereby immunization with self-tumor antigens can lead to tumor eradication while sparing the normal tissue expressing the self-antigen (19). One explanation for such discrimination between normal and tumor tissues may be related to the level of antigen expression. Since tumor cells generally over-express tumor antigens in comparison to normal tissues, tumor cells are better targets of cytotoxic T cells in comparison normal tissues, especially, since antitumor T cells are generally of lower avidity. However, multiple vaccinations may expand residual higher avidity T cells that escaped elimination during tolerance induction, and this may reduce discrimination between tumor cells and normal cells as targets, leading to autoimmunity. One way to reduce chances of developing autoimmune reaction against normal tissues is to use multiple tumor antigens in vaccination. This way, the overall strength of the immune response against the tumor can be very high, whereas, only a subset of this response is directed against individual normal tissues since tumor antigens can be chosen that are expressed in a non-overlapping fashion in individual normal tissues. This could reduce the need for repeated vaccinations. Clearly, one needs to proceed with caution. The hope is that any adverse autoimmune reactions will be manageable once cancer is eliminated.

17. ACKNOWLEDGMENTS

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