Vaccine targeting EMT/Stem cell antigen for the prevention of breast cancer metastasis

Overall goals of the project (BIG PICTURE):
The overall goal of the project is to develop a multi-antigen breast cancer vaccine that targets proteins involved in breast cancer progression and metastatic development. These proteins are also important in the pathogenesis of triple negative breast cancer (TNBC), a subtype of breast cancer where metastases occur early in the course of disease and survival is limited. It has been described that different breast cancer cells will undergo epithelial to mesynchymal transformation (EMT) which is an important step in the development of anchorage independent aggressive disease. Moreover, it has been noted that breast cancer stem cells have also upregulated proteins that have been associated with EMT. Our group has shown that upregulation of normal self proteins can make them immunogenic. We hypothesize that upregulated proteins that are (1) associated with EMT, (2) associated with breast cancer stem cells, and (3) shown to be poor prognostic indicators in both univariate and multivariate analysis will make excellent candidates for a breast cancer vaccine if they are immunogenic. Such a vaccine could be given to all patients with breast cancer, after optimal treatment in the adjuvant setting, to prevent the development of metastatic disease. Such a vaccine would also be particularly useful in the prevention of relapse in the triple negative breast cancer subtype.

Specific aims:

(1) To identify candidate proteins that are (1) upregulated in EMT, (2) upregulated in the breast cancer stem cell, and (3) are an independent predictor of poor prognosis in breast cancer.
(2) To determine whether those proteins are immunogenic by assessing whether candidate protein specific IgG antibodies can be detected in volunteer donors and breast cancer patients. .
(3) To determine whether pan-DR binding peptide epitopes, derived from the defined antigens, can be identified and whether peptide specific T cells raised can recognize native proteins.
(4) To assess whether a vaccine targeting EMT/Stem cell antigens can prevent or inhibit the growth of triple negative tumors in a transgenic mouse model of basal cell breast Ca.

SUMMARY

<u>YEAR 1-2</u>

<u>1. To identify candidate proteins that are (1) upregulated in EMT, (2) upregulated in the breast cancer stem cell, and (3) are an independent predictor of poor prognosis in breast cancer</u>.

- Complete literature search on candidate proteins
- Make sure the search is annotated, may serve as basis for review article
- Build article file and keep updated
- Decide on approximately 10 candidates to pursue. Ideally the proteins would have different mechanisms of action so that the vaccine could potentially target many biologic pathways.
- Start collecting breast cancer samples

<u>RESOURCE PEOPLE</u>
Dan Herenden
Nora Disis
Jennifer Childs (regulatory)
Doreen Higgins (clinical sample collections)

<u>2. To determine whether those proteins are immunogenic by assessing whether candidate protein specific IgG antibodies can be detected in volunteer donors and breast cancer patients</u>.

- Determine whether recombinant proteins and MoAb are available for candidates
- If so, develop indirect ELISAs
- Screen with donors from Puget Sound Blood Bank
- Screen with sera from TNBC patients
- If recombinant proteins are not available, will need to develop His tag ELISAs
- Validate positive with Western blot
- Identify the incidence and specificity of Ab responses to cancer vs. controls.
- Identify candidate proteins as antigen or not via IgG Ab response

RESOURCE PEOPLE
Mei Wu (ELISA)
Liz Broussard (ELISA)
Yi Yang (His tag-if needed)

3. To determine whether pan-DR binding peptide epitopes, derived from the defined antigens, can be identified and whether peptide specific T cells raised can recognize native proteins.

- Using computer modeling, identify pan DR binding epitopes for each candidate
- Construct peptides
- Screen peptides via ELISOPT in 20 cases and controls for immunogenicity
- Generate T cell lines form responding peptides and evaluate for the ability of peptide specific T cells to respond to proteins
- Phenotype lines via flow cytometry and cytokine secretion
- Determine the avidity of the T cell lines
- Identify top candidate epitopes for inclusion in a vaccine

RESOURCE PEOPLE
Dan Herenden (epitope mapping)
Dan/Meredith Slota (ELISPOT)
Yushe Dang (T cell culture and analysis)

4. To assess whether a vaccine targeting EMT/Stem cell antigens can prevent or inhibit the growth of triple negative tumors in a transgenic mouse model of basal cell breast Ca.
End of Year 2-into 3

FIRST 12 MONTH WORK PLAN

1. To identify candidate proteins that are (1) upregulated in EMT, (2) upregulated in the breast cancer stem cell, and (3) are an independent predictor of poor prognosis in breast cancer

Months 1-2

- Complete standard training
- Identify candidate proteins
- Develop article file
- Identify which protein have recombinant available as well as monoclonal antibodies
- Begin to set up systems to collect sera and T cells from TNBC patients
- Collect and store human specimens

2. *To determine whether those proteins are immunogenic by assessing whether candidate protein specific IgG antibodies can be detected in volunteer donors and breast cancer patients*

Months 3-10

- Develop indirect ELISAs- define performance characteristics
- Screen normal volunteers and cancer patients
- Validate positive and negatives by Western blot analysis
- Determine incidence and level of response
- Identify proteins as antigens or not

3. *To determine whether pan-DR binding peptide epitopes, derived from the defined antigens, can be identified and whether peptide specific T cells raised can recognize native proteins*

Months 5-12

- Predict epitopes from antigens
- Construct peptides
- Screen by ELISPOT
- Generate T cell lines, assess response to protein and phenotype
- Expect at least 3 candidate antigen to be fully characterized by the end of the first year

4. *To assess whether a vaccine targeting EMT/Stem cell antigens can prevent or inhibit the growth of triple negative tumors in a transgenic mouse model of basal cell breast Ca*.

Months 11-12

- Determine protein expression of candidate antigens in cell line started from C3T
- Determine protein expression of candidate antigens in spontaneous tumors started from C3T