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Award Number: DAMD17-00-1-0288

TITLE: Population Based Assessment of MHC Class I Antigens Down
Regulation as Markers of Increased Risk for Development
and Progression of Breast Cancer from Benign Breast
Lesions

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REPORT DATE: January 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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20050516 094

REPORT DOCUMENTATION PAGE

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE January 2005	3. REPORT TYPE AND DATES COVERED Annual (1 Jan 2004 – 31 Dec 2004)

4. TITLE AND SUBTITLE
Population Based Assessment of MHC Class I Antigens Down Regulation as Markers of Increased Risk for Development and Progression of Breast Cancer from Benign Breast Lesions

5. FUNDING NUMBERS
DAMD17-00-1-0288

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)
Despite advances in chemotherapy and radiation therapies, advanced breast cancer still carries a high mortality rate. The need for effective therapies is urgent. The overall aim of this research proposal is to recognize early markers of disease and their interaction with other epidemiological risk factors that can serve as risk indicators for subsequent development of breast cancer from precancerous lesions, and as prognostic markers for progression from primary to metastatic disease. The major histocompatibility complex (MHC) class I molecules are found on the cell membrane of all cells in the body and are involved in intercellular communications and in complex interactions with the immune system. Cancer cells with reduced or aberrant MHC molecules have been shown to evade immune surveillance and become selected for cancer progression and spread of disease to distant sites of the body. About half of all breast cancers have complete loss or reduced level of MHC class I molecules and this finding has been associated with increased tumor invasiveness and more aggressive cancers with poorer outcome. The outlined studies are expected to better define the clinical significance of abnormal MHC class I molecules in precancerous and invasive breast lesions as markers of immunological events that could affect survival, selection, and outgrowth of precancerous cells, and their subsequent progression to breast cancer. These MHC losses could also mark more aggressive tumors and thus contribute to selection of appropriate treatments in individual cases.

14. SUBJECT TERMS
Major histocompatibility complex (MHC) class I molecules, disease progression markers, survival, breast cancer

15. NUMBER OF PAGES
9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

INTRODUCTION

It has been known for some time that malignant transformation of cells is frequently associated with abnormalities in the expression of MHC class I antigens (1). These abnormalities appear to play a role in the clinical course of the disease (1) and to have a negative effect on the outcome of T cell-based immunotherapy for malignant diseases (2, 3). In breast lesions examined for expression of MHC class I, approximately half (51%) of carcinomas had an abnormally low content of HLA-A, -B, and -C determinants (4). Down regulation of HLA class I antigens in breast carcinomas may be more frequent than previously reported suggesting that alterations of HLA class I could represent an important step associated with tumor invasion providing tumor cells with the ability to escape recognition by T-lymphocytes (5). The overall aim of our research is to better define the role of MHC class I antigen loss and its interaction with other histo-pathologic and epidemiological factors that can serve as risk indicators in the progression of primary breast cancer to metastatic disease.

BODY: Statement of Work
TASK 1:

In women with primary and metastatic lesions of the breast to determine whether HLA Class I antigen loss and down regulation is greater in those with late stage and metastatic disease than in women with early stage disease (months 1-48); to determine whether among women with concurrent preneoplastic lesions and breast tumors HLA Class I antigen loss or down regulation is more frequent in the tumor than in the pre-neoplastic lesion (1-54); association with histopathologic characteristics of the lesions, including estrogen and progesterone receptor status (months 1-54); and disease survival (1-58).

     a: Begin construction of the breast cancer cohort (3000 cases). The Pathologist Dr.Raju and the P.I will begin screening breast cancer cases for delineation into Stage1-IV, and for the presence of concurrent lesions of benign proliferative and cancer lesions, together with normal breast tissue. We will design appropriate forms to record histopatholgical and clinical data based on our current NIH project forms and instruments (Instruments section in original grant)

     b: Retrieval of H & E slides for cases

     c: Review of slides

     d: selection of tumor blocks and sectioning of tissue for immunohistochemistry assays

     e: begin HLA class I immunoassays, as slides become available

     f: Continue construction of the breast cancer cohort, the concurrent lesion cohort and the histopathologic data gathering. See Pathology Review Form (PRF) (Instruments Section) for histopathologic parameters.

     g: continue HLA class I immunoassays as additional cases are entered into the cohort

     h: Annual reports will be written

     i: Initial manuscripts on the PBD cohort will be written

PROGRESS (January 1, 2004- December 31, 2004):

1. The finalized Pathology Review Form (PRF) has been converted into the Teleform version for electronic data entry. Electronic data entry has been accomplished for 287 cases. Thus far over 5300 pathology reports have been obtained. A finalized data base of HFHS breast cancer tumor Registry patients has been completed totaling 6338 cases.
2. H& E slides have been retrieved from the pathology archives for an additional 150 cases, for a total of 1440 cases.
3. 100 additional cases have been reviewed by the pathologist on PRF forms for a total of 1150 cases
4. Selection of tumor blocks completed for an additional 100 cases bringing the total cases to 880 thus far.
5. Sectioning of tissue for immunohistochemistry assays: completed for 800 cases.
6. HLA class I immunoassays: completed for 380 cases
7. The revised finalized HLA Immunohistochemistry Form has been converted into the Teleform version for electronic data entry Appendix #1).
8. Medical abstraction form: completed in 470 cases

TASK 2
Final analysis and report writing (months 56-60)

a: Final analysis of epidemiological risk factor data, histopathological and clinical data and HLA expression results will be performed.

b: A final report and additional manuscripts on the breast cancer cohort will be prepared

Progress: PENDING

KEY RESEARCH ACCOMPLISHMENTS
- The Henry Ford Health System Tumor Registry data, which is the source of our study cohort starting from 1981 through 2000 was validated and verified for vital patient information against the SEER data (2003 progress item). We have so far acquired a total patient database of 4,900 validated and verified breast cancer cases. The verification and validation of our constructed breast cancer cohort, completed as part of our 2003 progress goal has been followed up with a complete database construction of all pathology report numbers (surgical path numbers) numbering 5139. This a critical task as the HFHS Tumor Registry does not indicate the pathology report numbers. The latter is imperative for any tissue analysis which requires information of the path number and the specific tissue block. A major accomplishment has been the careful retrieval, and data entry of pathology reports for an updated database of 6338 patients.

- Manuscript Publications/Submissions:
     See Reportable Outcomes below.
- Completed a total of 380 cases for HLA assessment

5

REPORTABLE OUTCOMES

1. MANUSCRIPTS/REPORTS

A: DOWNREGULATION OF HLA-A AND BW6, BUT NOT BW4, ALLOSPECIFICITIES IN LEUKEMIC CELLS. AN ESCAPE MECHANISM FROM CTL AND NK ATTACK?
Demanet C, Mulder A, Deneys V, Worsham MJ, Maes P, Claas FH, Ferrone S. Down-regulation of HLA-A and HLA-Bw6, but not HLA-Bw4, allospecificities in leukemic cells: an escape mechanism from CTL and NK attack? Blood. 2004;103(8):3122-30.

B: HLA ANTIGEN EXPRESSION IN BREAST CANCER: A MULTICENTRIC STUDY UTILIZING FORMALIN-FIXED PARAFFINIZED TISSUES. M J. Worsham[1], R. Nanavati[1], U. Raju[1], S.R. Wolman[2], T. Cabrera[3], F. Garrido[3], E. A. Repasky[4], B. Hylander[4], M. Feenstra[5], M.Verdaasdonk[5], M.Schipper[5], M.Tilanus[5], S.Ferrone[4]. [1] Cancer Genetics Research, Department of Pathology, Henry Ford Health Systems, Detroit, MI, 48202, USA [2] Uniformed Services Univ., of the Health Sciences, Bethesda, MD 20814, USA, Hosp., Univ., Virgen de las Nieves, Granada, Spain, [4] Roswell Park Cancer Institute, Buffalo, NY 14263, [5] Univ., Hosp., Utrecht, The Netherlands.
Submitted to: Pending submission to *Tumor Antigens*

C: EXPRESSION OF MHC CLASS I AND II EXPRESSION SIGNIFICANTLY DISCRIMINATES AMONG BENIGN, CARCINOMA IN SITU, AND MALIGNANT LESIONS OF THE BREAST. Submitted abstract to 2005 Era of Hope DOD Meeting (Appendix # 1)

CONCLUSIONS:
A: DOWNREGULATION OF HLA-A AND BW6, BUT NOT BW4, ALLOSPECIFICITIES IN LEUKEMIC CELLS. AN ESCAPE MECHANISM FROM CTL AND NK ATTACK?

HLA class I antigen defects may have a negative impact on the growing application of T cell based immunotherapeutic strategies for treatment of leukemia. Therefore in the present study taking advantage of a large panel of HLA class I allele-specific human monoclonal antibodies we have compared HLA class I antigen expression on leukemic cells with that on autologous and allogeneic normal cells. Downregulation of HLA-A and/or -B allospecificities was present in the majority of the patients studied. However, downregulation did not affect all HLA class I alleles uniformly, but was almost exclusively restricted to HLA-A allospecificities and to HLA-B allospecificities which belong to the HLA-Bw6 group. The latter allospecificities, at variance from those which belong to the HLA-Bw4 group, do not modulate the interactions of leukemic cells with NK cells. Therefore our results suggest that the selective downregulation of HLA-A and HLA-Bw6 allospecificities associated with HLA-Bw4 preservation provides leukemic cells with an escape mechanism not only from CTL, but also from NK cells. As a result T cell-based immunotherapeutic strategies for leukemia should utilize HLA-Bw4 alloantigens as restricting elements since a selective HLA-Bw4 allele loss would provide leukemic cells with an escape mechanism

B: HLA ANTIGEN EXPRESSION IN BREAST CANCER: A MULTICENTER STUDY UTILIZING FORMALIN-FIXED PARAFFINIZED TISSUES (Appendix # 3).

Despite the possible clinical significance and potential for T-cell based immunotherapy, evaluation of malignant lesions for HLA class I antigen expression is not performed routinely, even for patients who are candidates for such therapy. This reflects, at least in part, reluctance by pathologists to utilize frozen tissue sections in IHC assays. Little information is available about the usefulness of formalin-fixed paraffin-embedded tissues (FFPT) as substrates in IHC assays to evaluate tissue expression of HLA antigens. We therefore undertook a multicenter study to develop and standardize an IHC protocol using FFPTs and anti-HLA mAbs. To determine if loss of expression of MHC Class I molecules at the protein level reflect alterations at the gene level, DNA from microdissected normal and tumor tissue were evaluated with microsatellites at the MHC class I 6p21.3 locus (HLA-A, B, C determinants) and at the 15q21 beta 2 microglobulin locus for concordance of expression. HLA class I antigen down-regulation in conjunction with cellular heterogeneity of expression in three breast carcinoma cases was concordantly reported by the four participating laboratories with the anti-HLA class I antibody HC-10 and with the anti-beta 2 microglobulin L368. Furthermore, no staining of normal and malignant mammary cells was detected by the four laboratories in the lesions stained with the anti-HLA class II LGII. In contrast, infiltrating lymphocytes were strongly stained by LGII. Downregulation of class was reflected by LOH in cases 1 and 3 for the 15q21 locus and in case 1 at the 6p21 locus. The results indicate that FFPTs represent a useful substrate upon which to monitor HLA antigen expression in malignant lesions, especially when appropriate markers are used to differentiate malignant cells from lymphocytes and dendritic cells.

The manuscript is pending submission to "*Tumor Antigens*"

C: EXPRESSION OF MHC CLASS I AND II EXPRESSION SIGNIFICANTLY DISCRIMINATES AMONG BENIGN, CARCINOMA IN SITU, AND MALIGNANT LESIONS OF THE BREAST. Maria J. Worsham, James J. Yang , Xia Sheng, Roberta Sheffer, Jingfang Cheng, Usha Raju (Appendix # 1)

Malignant transformation of cells is frequently associated with abnormalities in the expression of MHC class I antigens. These abnormalities appear to play a role in the clinical course of the disease and to have a negative effect on the outcome of T cell-based immunotherapy for malignant diseases. Approximately half (51%) of breast carcinomas have altered regulation of HLA class I, which may represent an important step associated with tumor invasion providing tumor cells with the ability to escape recognition by T-lymphocytes. We report on the expression behavior of MHC class I (anti-HLA class I heavy chain mAb HC-10) and class II (anti-HLA-DR, DQ, DP mAb LGII-612.14) in formalin-fixed paraffin-embedded tissue from 189 invasive breast cancer cases. Tumor areas concurrent with benign proliferative lesions, normal breast epithelium, normal "other" (skin, lymph node), lymphocytes in lymphocytic infiltrates, and in situ lesions (DCIS, LCIS) within a 5 micron section were marked by the study pathologist as part o the pathology review process and immunohistochemically evaluated with the anti-HLA class I heavy chain mAb HC-10, and the anti-HLA-DR, DQ, DP mAb LGII-612.14. The lymphocyte specific anti-CD45 antibody marked the presence of infiltrating lymphocytes and dendritic cells, and served as in internal control, particularly when normal breast epithelium was absent. Three staining categories were defined as follows: absent expression: 0-<25%, heterogeneous: >25% <50%; positive staining: >50%. Of the 189 cases, 149 had multiple records, yielding a total of 338 records, where each record is derived from either a different tissue block (of the same biopsy) or a subsequent biopsy.

7

To assess differences in expression of HC-10 and LG-II in the normal breast epithelium (N), benign proliferative (BPL), carcinoma in situ (CIS), and tumor (T), log-linear models were employed. For LGII, the uniform associated model (UA) model was an excellent fit underscoring lack of expression of LGII in epithelial lesions. The odds ratio analyses (for N, BPL, CIS, T) however, indicate that when LGII is expressed (presence of staining of >25%), it is significantly more likely to occur in the tumor as compared to BBD and CIS lesions, using normal epithelium as a reference, with CIS having a higher likelihood than BBD, and BBD a higher likelihood than normal epithelium. For HC10, although goodness-of–fit for the UA model was weaker suggesting a more variable expression of HC10 among the four lesion types, the HC10 expression trend was similar to LGII.

Thus, there was a significant correlation of expression levels of HC10 and LGII and breast lesion type pointing in the direction of upregulation in tumor cells, rather than downregulation as commonly reported. The implications of the latter with respect to immune surveillance status surrogates of HC10 and LGII and patient outcome are underway.

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APPENDIX

1:      Abstract to the 2005 DOD ERA of Hope Meeting

EXPRESSION OF MHC CLASS I AND II EXPRESSION SIGNIFICANTLY DISCRIMINATES AMONG BENIGN, CARCINOMA IN SITU, AND MALIGNANT LESIONS OF THE BREAST

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