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TITLE: Identification of Widely Applicable Tumor-Associated Antigens for Breast Cancer Immunotherapy

PRINCIPAL INVESTIGATOR: Jining Bai, Ph.D.

CONTRACTING ORGANIZATION: The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

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Identification of Widely Applicable Tumor-Associated Antigens for Breast Cancer Immunotherapy

Jining Bai, Ph.D.

The Johns Hopkins University School of Medicine
Baltimore, Maryland  21205
E-Mail: jnbai@jhmi.edu

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

This study is the initial phase (the 1st year) of a feasibility study of a novel immunotherapeutic strategy for the treatment of breast cancer. The rationale is based upon recent findings that genes belonging to the pp32 family are differentially and alternatively expressed in most human breast cancers. In general, benign breast tissues express pp32, a tumor suppressor, whereas breast cancers express tumorigenic family members, including pp32r1 and pp32r2. Since pp32r1 and pp32r2 are expressed in nearly all breast cancers, but not in normal adult tissues, they may reasonably serve as targets for antigen-specific immunotherapy. The purpose of the study is to identify tumor-associated antigens (TAA) in pp32r1 and pp32r2, then test their suitability in vitro as immunotherapeutic targets in breast cancer. Currently, the second phase of (in vivo) feasibility is underway. If successful, the results may translate into eventual clinical trials of peptide vaccines or adoptive T cell therapy.
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Introduction:

In the IDEA proposal, we proposed a feasibility study of a novel immunotherapeutic strategy for the treatment of breast cancer. The rationale is based upon recent findings that genes belonging to the pp32 family (Figure 1) are differentially and alternatively expressed in most human breast cancers. In general, benign breast tissues express pp32, a tumor suppressor, whereas breast cancers express tumorigenic family members, including pp32r1 and pp32r2. Since pp32r1 and pp32r2 are expressed in nearly all breast cancers, but not in normal adult tissues, they may reasonably serve as targets for antigen-specific immunotherapy.

Body:

Statement of Works:

Task 1. Identify, synthesize and test candidate peptides that could potentially bind to HLA class I molecules based on the coding sequence of pp32r1 and pp32r2. (Month 1-6)

Task 2. Screen in vitro for candidate pp32r1 & pp32r2 peptides that fulfill the requirements for TAA. (Month 7-12)

Task 3. Evaluate the pp32r1/pp32r2- specific cytotoxicity against a broad range of natural targets (established or primary breast cancer cell lines) to determine range of applicability. (Month 12-20)

Task 4. Evaluate in vivo immunogenicity of pp32r1 and/or pp32r2-derived TAA in human breast cancer animal models. (Month 20-36)

In the first year of this project, we accomplished #1 and #2 Tasks defined by Statement of Work, but also worked to address an interesting suggestion offered by DOD BCRP Scientific Panel.

1) Task #1: Identify, synthesize and test candidate peptides that could potentially bind to HLA class I molecules based on the coding sequence of pp32r1 and pp32r2.

Using Bioinformatics and ImmunoGenetics tools, we analyzed the entire coding region of pp32, pp32r1 and pp32r2 genes for binding affinity with HLA-A*0201 molecule as well as the degradation pattern by proteasomal cleavages. The result of calculation shown (Table 1) that 19 motifs are potentially favorable of binding to HLA-A*0201 molecule with high affinity. To verify the prediction in vitro, HLA-A*0201+ TAP-deficient T2 hybridoma (ATCC) was pulsed with 50ug/ml of each peptide representing the motif (or control) and 5ug/ml of b2-microglobulin for 18hr at 37 C. HLA-A*0201 expression was then measured by flow cytometry using mAb BB7.2 (ATCC) followed by incubation with FITC-conjugated secondary antibody. Fluorescent index of HLA-A*0201 to each peptide can be determined as: (mean fluorescence with peptide - mean fluorescence without peptide) / (mean fluorescence without peptide). The result shown 10 out of 20 motifs is capable of binding to HLA-A*0201 in a concentration dependent manner (Table 1).

2) Task #2: Screen for candidate pp32r1 & pp32r2 peptides that fulfill the requirements for TAA. In order to be qualified as a TAA, a motif has to be able to meet several criteria in addition to the binding to HLA-A*0201. These requirements include (i) the antigen can be naturally processed by tumor cells, (ii) it permits expansion of antigen-specific CTL; (iii) it is presented in a MHC-restricted fashion. CTL assay was carried out to test if the motifs identified in Aim#1 fulfill the requirements for TAA.
In brief, Cr\textsuperscript{51}-labeled target cells (T2 cells pulsed with peptide or cancer cell expressing pp32 family members) were incubated with various numbers of CTL effector cells for 4 hr. Cr\textsuperscript{51}-release assays were performed in triplicate per condition using 5x10\textsuperscript{5} labeled target cells per well in a 96-well plate. Percent specific lysis will be calculated from CPM of (experimental result - spontaneous release)/(maximum release - spontaneous release). The results, summarized in Table 2, indicate that 2 out of 10 motifs fulfilled the above requirement as TAA.

3) Extra Task: Define pp32-related epitopes that is specific for CD4\textsuperscript{+} helper T Cell through MHC Class II.

During DOD BCRP Scientific Review for this Idea Proposal, the Reviewers expressed enthusiasm in whether pp32 family members are capable of activation of CD4\textsuperscript{+} helper T Cell through MHC Class II, in addition to the activation of CD8\textsuperscript{+} T Cell through MHC Class (addressed in task #1 and #2). To carry out this additional, we used several computer algorithms to select pp32r1 and pp32r2 sequences with potential promiscuous HLA-DR binding characteristics. We are currently testing the synthetic peptides corresponding to potential HLA-DR binding sequences for their capacity to stimulate CD4\textsuperscript{+} T in vitro immunization.

Key Research Accomplishments:

We have identified two peptide motifs from pp32 family members, which fulfill the requirement to be TAAs. This study (mostly in vitro) provided bases for further evaluate the widely applicability of these TAAs in breast cancer immunotherapy (Aim #3) and in vivo validation in breast cancer animal models (Aim #4).

Reportable Outcomes:

The result of Specific Aim #1 and #2 were presented at 2002 Era of Hope Department of Defense Breast Cancer Research Program Meeting.


Conclusions:

We demonstrated in vitro that

(i) the oncogenic pp32 family members can be presented by HLA-A*0201,
(ii) the HLA-A*0201 cells bearing these motifs can be recognized and lyzed by pp32r1- or pp32r2- specific CTL in a MHC class I specific manner.
<table>
<thead>
<tr>
<th>1</th>
<th>memgrrihle lrnrtpsdvk elvldnsrsn egklegltde feeleflsti</th>
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<td>kw s a f a k l n</td>
</tr>
<tr>
<td>51</td>
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</tr>
<tr>
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<td>g sd ~ r ~ ~k y</td>
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<tr>
<td>151</td>
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<tr>
<td>200</td>
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</tr>
<tr>
<td>201</td>
<td>------ ------ ------ ------ ------ ------ ------ ------</td>
</tr>
<tr>
<td>249</td>
<td>----- ----- ----- ----- ----- ----- ----- ----- -----</td>
</tr>
</tbody>
</table>

| 249| edvsgeeecd eegyndgevd geedeelge eerggkrkre pedegedd |
| 201| gd g ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ |

**Figure 1. Alignment of pp32, pp32r1 & pp32r2 sequences.**
Differences from the pp32 sequence are indicated underneath. The variant pp32r2 encodes a truncated protein (wavy lines indicate the truncated region).
<table>
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<th>Peptide</th>
<th>BIMAS</th>
<th>LpRep</th>
<th>FPEITHI</th>
<th>T2 Stabilization</th>
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</table>

Table 1. Predicted HLA-A*0201 Binding Motifs and Their Ability to Bind T2 Cells.

Potential motifs was predicted by BIMAS, LpRep, FPEITHI.
The binding of Peptides to Human HLA-A2 was measured by T2 stabilization assay
Positive – calculated fluorescent index greater than 1.0.
Calculated fluorescent index = (Mean fluorescence with peptide - mean fluorescence without peptide)/(mean fluorescence without peptide)
<table>
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<tr>
<th>Peptide</th>
<th>CTL Lysis*</th>
<th>Processing*</th>
<th>MHC I Restriction#</th>
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<td>n/a</td>
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<td>n/a</td>
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</tr>
<tr>
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<td>n/a</td>
</tr>
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<td>+++</td>
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<tr>
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</table>

Table 2. Summary of CTL Assays for Motifs That are Capable of Binding to HLA-A*0201

Cytotoxicity Assay was carried out against Target cells:*

- T2 Cell +/- peptides
- MCF-7 (A2*,pp32r1*,pp32r2*)
- LNCA (A2*,pp32r1,pp32r2)
- MCF-7 (+/- anti-HLA-A2mAb)
Summary of Personnel Partially Supported by This Idea Award:

1) Jining Bai (PI)
   Adetinuke Jagun (Technician)
CURRICULUM VITAE

Name: Jining Bai

Current Appointment: Assistant Professor
Department of Pathology
Johns Hopkins University School of Medicine

Addresses:
Office: Division of Molecular Pathology
Department of Pathology
Johns Hopkins School of Medicine
Room B-302
418 N. Bond Street
Baltimore, MD 21205
Phone: (410) 955-6920
Fax: (410) 502-5158
E-mail: jnbei@jhmi.edu

Home: 8641 Willow Oak Road
Baltimore, MD 21234
Phone: (410) 882-4638

Education & Training:

1983-1988 B. Eng., Department of Engineering Physics,
Tsinghua University, Beijing, P. R. China

1990-1996 Ph.D. / Graduate studies, Department of Biophysics,
Johns Hopkins University Baltimore, MD

1996-1999 Post-doctoral Fellow, Division of Molecular Pathology
Department of Pathology, Johns Hopkins Medical Institutions,
Baltimore, MD
**Professional Experiences:**

1985-1986  Instructor, Computer programming  
School of Professional Studies  
Tsinghua University, Beijing, P. R. China

Tsinghua University, Beijing, P. R. China

1988-1990  Graduate studies, Department of Biol. Sci. & Tech.,  
Tsinghua University, Beijing, P. R. China

1989-1990  Teaching assistant, Biology Lab, Department of Biol. Sci.  
Tsinghua University, Beijing, P. R. China

1991-1995  Pre-doctoral Fellow, Department of Embryology  
Carnegie Institution of Washington, Baltimore, MD

1992-1993  Teaching Assistant, Reproductive Physiology  
Johns Hopkins University.

1996-2000  Research Fellow, Division of Molecular Pathology  
Department of Pathology, Johns Hopkins Medical Institutions,  
Baltimore, MD

2000-2001  Research Associate, Division of Molecular Pathology  
Department of Pathology, Johns Hopkins Medical Institutions  
Baltimore, MD

2001-2002  Instructor, Division of Molecular Pathology  
Department of Pathology, Johns Hopkins Medical Institutions  
Baltimore, MD

2002-  Assistant Professor, Division of Molecular Pathology  
Department of Pathology, Johns Hopkins Medical Institutions  
Baltimore, MD

**Bibliography:**

**Refereed Publications**


**Abstracts**


**Invention & Patents:**

Grants & Contracts:

Current:

1) National Research Award  Komen Foundation  Principal Investigator
   Active  (12/99-06/02)  $100,000 (annual direct)
   Development of Novel Therapeutic Target and Approach for Breast Cancer
   – Repairing Common Defects in Breast Cancer by Restoration of pp32.

2) IRG  JHMI  Principal Investigator
   Active  (05/01-05/03)  $20,000 (annual direct)
   Development of a Novel Transgenic Mouse Model for Human Prostate Cancer

3) Idea Award  DOD/CDMRP  Principal Investigator
   Active  (10/01-10/04)  $100,000 (annual direct)
   Identification of Widely applicable Tumor-Associated Antigens for Breast Cancer ImmunoTherapy.

4) Pilot Award  Breast Cancer SPORE/oncology  Principal Investigator
   Active  (04/02-04/03)  $40,000 (annual direct)
   HOXB7, Widely Applicable Targets for Immunotherapy against Breast Cancer.

Pending:

1) RO1  NIH  Principal Investigator
   Pending  (01/03-12/06)  $225,000 (annual direct)
   Localization and Molecular Interaction of pp32 Family Members

Complete:

1) Concept Award  DOD/CDMRP  Principal Investigator
   Active  (05/01-06/02)  $50,000 (annual direct)
   Oncogenic Members of pp32 gene family, Widely Applicable Targets for Immunotherapy against Breast Cancer.

Honors & Awards:

Honored Student, Tsinghua University (1983-1988)
Outstanding College Graduate Award, National Education Commission of China (1988)
Winner of Natural Philosophy Competition, Tsinghua University (1990)
Travel Award, European Symposium in Signal Transduction (1991)
Dean’s Fellowship, Johns Hopkins University (1990-1995)
Pathology Fellowship, Johns Hopkins Medical Institution (1996-1999)
National Research Award, Susan G. Komen Breast Cancer Foundation (1999-2001)
Concept Award, Congressionally Directed Medical Research (2000-2001)
Idea Award, Congressionally Directed Medical Research (2001-2004)
Invited Lectures:

1) Alterations in pp32 Gene Family – A Novel Molecular Targets in Breast Cancer Therapy.  
The 4th National Mission Conference for Breast Cancer  
Washington D.C.  
September, 2000

2) pp32 Gene Family, Potential Therapeutic Targets for Breast Cancer and Prostate Cancer.  
National Cancer Institute  
Beijing, P.R. China  
October, 2000

3) pp32 Gene Family at the Crossroad of Oncogenesis and Tumor Suppression.  
The Cancer Congress 2000  
Beijing, P.R.China, October, 2000