Award Number: DAMD17-01-1-0538

TITLE: Oncogenic Members of pp32 Family, the Widely Applicable Targets for Immunotherapy in Human Breast Cancer

PRINCIPAL INVESTIGATOR: Jining Bai, Ph.D.

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

REPORT DATE: June 2002

TYPE OF REPORT: Final

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
**Title and Subtitle**

Oncogetic Members of pp32 Family, the Widely Applicable Targets for Immunotherapy in Human Breast Cancer

**Author(s)**

Jining Bai, Ph.D.

**Performing Organization Name(s) and Address(es)**

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

**Sponsoring/Monitoring Agency Name(s) and Address(es)**

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

**Supplementary Notes**

Approved for Public Release; Distribution Unlimited

**Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)**

This study is the initial phase 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 this 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.

**Subject Terms**

TAA, Immunotherapy

**Security Classification of Report**

Unclassified

**Security Classification of This Page**

Unclassified

**Security Classification of Abstract**

Unclassified

**Number of Pages**

12

**Price Code**

Unlimited
Table of Contents

Cover.................................................................................................................1
SF 298.................................................................................................................2
Introduction........................................................................................................4
Body...................................................................................................................4
Key Research Accomplishments.................................................................5
Reportable Outcomes......................................................................................5
Conclusions....................................................................................................5

References.....................................................................................................n/a

Appendices....................................................................................................6
  1) Table............................................................................................................6
  2) Personnel....................................................................................................7
  3) CV...............................................................................................................8

3
Introduction:

In this concept 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 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:

Since Statement of Work is not available for Concept Proposal, the list of Specific Aims will be used to report the progress of this project.

1) Specific Aim #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 50μg/ml of each peptide representing the motif (or control) and 5μg/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 19 motifs is capable of binding to HLA-A*0201 in a concentration dependent manner (Table 1).

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, Cr51-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. Cr51-release assays were performed in triplicate per condition using 5x105 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) Evaluate whether the pp32r1- or pp32r2- specific CTL can recognize a broad range of breast tumors (optional). As an optional study proposed in the Concept Project, this study is currently under way in the PI's Lab to evaluate the widely applicability of the identified candidate TAA.
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 in vivo validation in breast cancer animal models.

Reportable Outcomes:

After the completion of the 3rd Specific Aim (Optional), we are expected to apply for U.S. Patent provision and to publish the results supported by this Concept Award.

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 lysed by pp32r1- or pp32r2- specific CTL in a MHC class I specific manner.
<table>
<thead>
<tr>
<th>Peptide</th>
<th>BIMAS Score</th>
<th>LpRep Score</th>
<th>FPEITHI Score</th>
<th>Binding to T2 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0801-01</td>
<td>3499.535</td>
<td>3.37</td>
<td>26</td>
<td>+</td>
</tr>
<tr>
<td>0801-02</td>
<td>1591.602</td>
<td>2.46</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>0801-03</td>
<td>805.719</td>
<td>5.76</td>
<td>17</td>
<td>+</td>
</tr>
<tr>
<td>0801-04</td>
<td>681.542</td>
<td>3.54</td>
<td>28</td>
<td>+</td>
</tr>
<tr>
<td>0801-05</td>
<td>636.316</td>
<td>4.19</td>
<td>25</td>
<td>+</td>
</tr>
<tr>
<td>0801-06</td>
<td>445.216</td>
<td>6.90</td>
<td>27</td>
<td>-</td>
</tr>
<tr>
<td>0801-07</td>
<td>481.542</td>
<td>3.13</td>
<td>26</td>
<td>+</td>
</tr>
<tr>
<td>0801-08</td>
<td>432.319</td>
<td>4.87</td>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td>0801-09</td>
<td>399.682</td>
<td>7.69</td>
<td>23</td>
<td>+</td>
</tr>
<tr>
<td>0801-10</td>
<td>379.216</td>
<td>5.81</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>0801-11</td>
<td>301.331</td>
<td>3.12</td>
<td>27</td>
<td>+</td>
</tr>
<tr>
<td>0801-12</td>
<td>281.542</td>
<td>3.47</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>0801-13</td>
<td>264.498</td>
<td>6.72</td>
<td>24</td>
<td>+</td>
</tr>
<tr>
<td>0801-14</td>
<td>226.014</td>
<td>3.54</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>0801-15</td>
<td>212.775</td>
<td>6.43</td>
<td>19</td>
<td>+</td>
</tr>
<tr>
<td>0801-16</td>
<td>172.752</td>
<td>6.81</td>
<td>21</td>
<td>+</td>
</tr>
<tr>
<td>0801-17</td>
<td>148.896</td>
<td>5.87</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>0801-18</td>
<td>139.730</td>
<td>6.72</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td>0801-19</td>
<td>105.719</td>
<td>7.99</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>MGA1 (P.Ctrl)</td>
<td>734.189</td>
<td>4.86</td>
<td>26</td>
<td>+</td>
</tr>
<tr>
<td>ID9 (N.Ctrl)</td>
<td>0.000</td>
<td>n/a</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Peptide</th>
<th>CTL Lysis</th>
<th>Processing</th>
<th>CTL Expansion</th>
<th>MHC I Restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0801-01</td>
<td>+</td>
<td>n/a</td>
<td>Yes</td>
<td>n/a</td>
</tr>
<tr>
<td>0801-03</td>
<td>+++</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>0801-04</td>
<td>+</td>
<td>n/a</td>
<td>Yes</td>
<td>n/a</td>
</tr>
<tr>
<td>0801-05</td>
<td>+</td>
<td>n/a</td>
<td>Yes</td>
<td>n/a</td>
</tr>
<tr>
<td>0801-07</td>
<td>+++</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>0801-09</td>
<td>+</td>
<td>n/a</td>
<td>Yes</td>
<td>n/a</td>
</tr>
<tr>
<td>0801-11</td>
<td>-</td>
<td>n/a</td>
<td>No</td>
<td>n/a</td>
</tr>
<tr>
<td>0801-13</td>
<td>+</td>
<td>n/a</td>
<td>Yes</td>
<td>n/a</td>
</tr>
<tr>
<td>0801-15</td>
<td>-</td>
<td>n/a</td>
<td>No</td>
<td>n/a</td>
</tr>
<tr>
<td>0801-16</td>
<td>-</td>
<td>n/a</td>
<td>No</td>
<td>n/a</td>
</tr>
<tr>
<td>MGA1 (P.Ctrl)</td>
<td>+++</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>ID9 (N.Ctrl)</td>
<td>-</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 2. Summary of CTL Assays for Motifs That are Capable of Binding to HLA-A*0201
Summary of Personnel Partially Supported by This Concept Award:

1) Jining Bai (PI)
2) 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: jnbai@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

1986-1988  
Research assistant, Institute of Material Sci. & Tech.,  
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:**

10
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 SPOROE 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:

- 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