AD_____

Award Number: W81XWH-06-1-0537

TITLE: Identification of Tumor Rejection Antigens for Breast Cancer Using a Mouse Tumor Rejection Model

PRINCIPAL INVESTIGATOR: Mary L. Disis, M.D.

CONTRACTING ORGANIZATION: University of Washington
Seattle, WA 98105

REPORT DATE: May 2007

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-05-2007	Annual	28 Apr 2006 - 27 Apr 2007

4. TITLE AND SUBTITLE
Identification of Tumor Rejection Antigens for Breast Cancer Using a Mouse Tumor Rejection Model

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0537

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Mary L. Disis, M.D.

E-Mail: ndisis@u.washington.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Washington
Seattle, WA 98105

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. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

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

13. SUPPLEMENTARY NOTES

14. ABSTRACT In the proposed study, we aim to identify tumor rejection antigens using mouse tumor rejection models. The study has three specific aims: (1) to determine the antigen repertoire induced by tumor rejection in FVB/N mice; (2) to identify the human homologues of the candidate rejection antigens and determine their immunogenicity; and, (3) to examine the in vivo tumor protection effect of vaccination with plasmids encoding tumor rejection antigens in neu-tg FVB/N mice. In the past year, we have successfully identified the antigen repertoire induced by tumor rejection in FVB/N mice. The human homologues of the candidate rejection antigens have been determined by database mining. We also started in vivo vaccination experiment to examine the tumor protection effect of the candidate antigens. We have made important findings toward each specific aim during last year, and based on our results, we plan to expand our discovery technique to a different tumor model, examine the immunogenicity of the human homologues of the mouse tumor rejection antigens we have identified, and test the in vivo protection effect of plasmid DNA vaccine targeting a combination of antigens.

15. SUBJECT TERMS
Tumor rejection, antigens, mouse model

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	8	USAMRMC

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Introduction

The purpose of this study is to identify tumor rejection antigens using mouse tumor rejection models. In the past, research from our lab has demonstrated that the tumor antigen repertoire in tumor-bearing neu transgenic mice has great similarity to the antigen repertoire in breast cancer patients. Although a number of tumor antigens have been identified in cancer patients, it remains a challenge to identify therapeutically relevant tumor rejection antigens. In the proposed study, we aim to identify tumor rejection antigens using mouse tumor rejection models. The study has three specific aims: (1) to determine the antigen repertoire induced by tumor rejection in FVB/N mice; (2) to identify the human homologues of the candidate rejection antigens and determine their immunogenicity; and, (3) to examine the *in vivo* tumor protection effect of vaccination with plasmids encoding tumor rejection antigens in neu-tg FVB/N mice. In the past year, we have successfully identified the antigen repertoire induced by tumor rejection in FVB/N mice. The human homologues of the candidate rejection antigens have been determined by database mining. We also started in vivo vaccination experiment to examine the tumor protection effect of the candidate antigens. We have made important findings toward each specific aim during last year, as listed in the following.

Key Research Accomplishments

Specific Aim 1: to determine the antigen repertoire induced by tumor rejection in FVB/N mice.

We have established a cohort of mice that rejected tumor (Fig. 1). Serum samples were collected from 20 mice pre-tumor challenge and post-tumor rejection. The serum samples were used for serological screening of cDNA expression library (SEREX) established from syneneic tumors to identify new antibody response developed during tumor rejection. Fig. 2 is a representative immunoblot from SEREX screening showing that antigens that are only recognized by post-tumor rejection serum but not by pre-tumor serum can be identified. Fig. 3 is a Western blot demonstrating that positive results from SEREX can be verified by Western blotting. After extensive SEREX screening, we identified a panel of 10 tumor rejection antigens (Table 1). These include proteins of diverse function, including signal transduction (GPIap1, Tnaaip3), cytoskeletal proteins (Ctnna1, Tln1), chaperon (Hsp40), ion transporter (FxyD3), and a viral protein (Mtv1). The sub-cellular location of the antigens is also diverse, including 3 transmembrane proteins (Tmem57, FxyD3, GPIap1), 2 nuclear proteins (Hnrpl1, Cep290), and 3 cytosol proteins (Ctnna1,Hsp40, and Tln1).

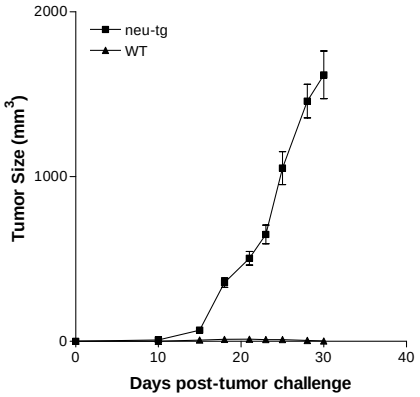

Figure 1. FVB/N tumor rejection model. One million MMC tumor cells were injected subcutaneously into wild type (WT) or neu transgenic (neu-tg) FVB/N mice. Shown are tumor size in neu-tg mice (■), and parental mice (▲).

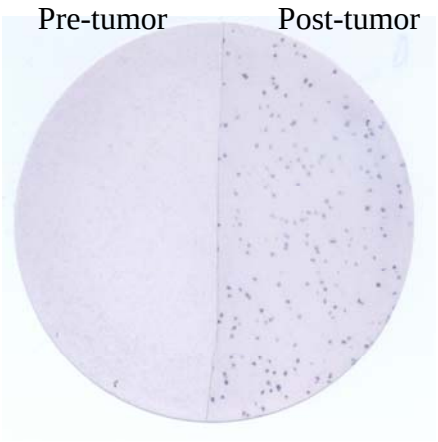

Figure 2. SEREX can be used to identify tumor rejection antigens. Shown is a representative SEREX blot showing that Mtv1, one of the identified antigens, is only reactive to post-rejection serum.

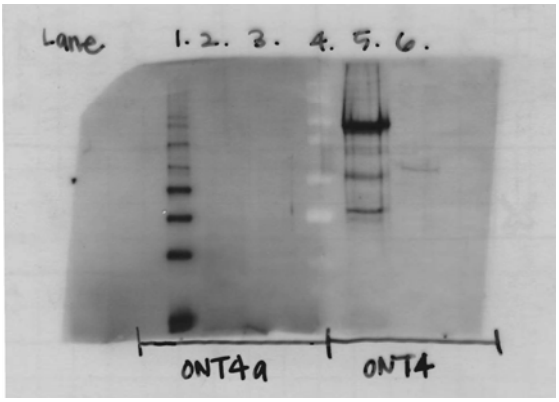

Figure 3. Western blot verifying the antibody response identified in SEREX. ONT4a is pre-tumor serum from a neu-tg mouse, and ONT4 is the post-tumor rejection serum from the same mouse.

The expression profile of these antigens were examined using real time RT-PCR. RNA was extracted from 3 normal mammary glands, 7 spontaneous tumors, and a panel of normal tissues, including brain, lung, liver, spleen, et al. As shown in Fig. 4, some antigens are expressed in normal tissue as well as tumors (Fig 4A-D). FxyD3 and Mtv1 have a restricted expression pattern with little expression in normal tissues (Fig. 4E-F).

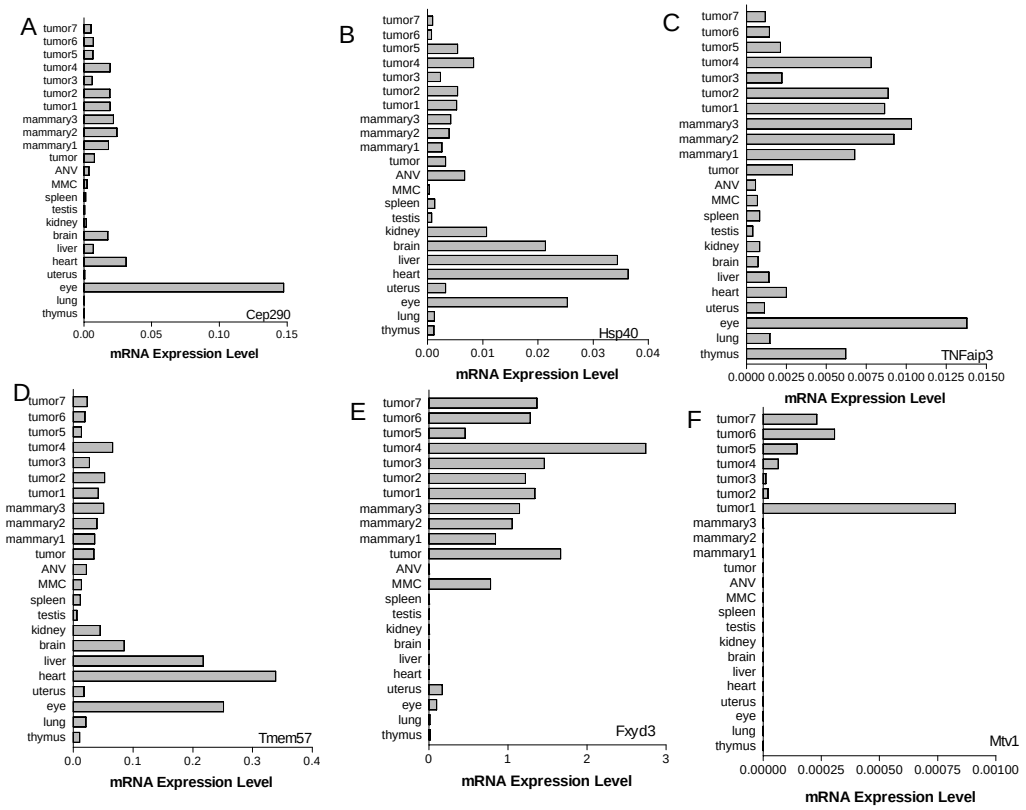

Figure 4. SEREX-identified tumor rejection antigens (TRA) have different expression patterns. **A-D**: Four of the TRAs are expressed in normal tissue in addition to tumor tissues. **E**: Fxyd3 has a restricted expression in mammary tissue and tumor. **F**: Mtv1 is exclusively expressed in tumor tissue.

Specific Aim 2: to identify the human homologues of the candidate rejection antigens and determine their immunogenicity.

The immunogenicity of the mouse tumor rejection antigens was evaluated by searching information on PubMed and the Human Cancer Immunome Databse (www2.licr.org/CancerImmunomeDB/). Six out of the ten antigens have been shown to have immunogenic human homologues deposited in the human SEREX database, as listed in Table 2.

The immunogenicity of the human homologues will be further examined using patient samples from our repository. However, this analysis will only be performed on the antigens that show therapeutic value in vaccination experiments.

Specific Aim 3: to examine the *in vivo* tumor protection effect of vaccination with plasmids encoding tumor rejection antigens in neu-tg FVB/N mice.

We have started vaccination experiments using plasmid DNA encoding the tumor antigens. Mice were given three vaccines (50ug plasmid DNA per mouse), two weeks apart. Tumor challenge using a syngeneic tumor cell line was give at two weeks after the third vaccine. The tumor growth was measured twice a week using venier calipers. Mice receiving adjuvant alone were included as negative control; mice receiving a plasmid encoding the intracellular domain of human Her2/neu (hICD) were included as positive control. As shown in Fig. 5, vaccination using Mtv1, FxyD3, and Cep290 had tumor protection effect. As a control, vaccination targeting the antigens we previously identified from tumor-bearing mice, such as Swap70 and Rock1, did not have tumor protection effect (Fig. 6). This suggests that antigen discovery using tumor rejection mice rather than tumor bearing mice is more likely to yield therapeutically relevant targets.

We recognize that tumor implant model is not optimal in testing the effect of vaccine. Due to the long latency (7-10 months) it takes to develop spontaneous tumors in these mice, it is impractical to test every candidate antigen in spontaneous tumor model. Therefore, we used the implant tumor model as a quick screening method. In the future, we will test the best candidate antigens in the spontaneous tumor setting. We will also test the combination of different antigens for potential synergistic effect.

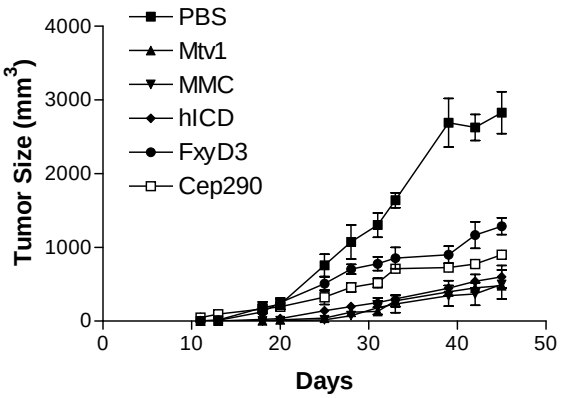

Figure 5. Vaccination targeting tumor rejection antigens, Mtv1, FxyD3, or Cep290, resulted in tumor protection. Mice (3 per goup) were vaccinated with plasmid DNA encoding Mtv1, FxyD3, Cep290, or hICD, or irradiated whole tumor cells at day -42, -28, and -14. Live MMC cells were given subcutaneously on day 0.

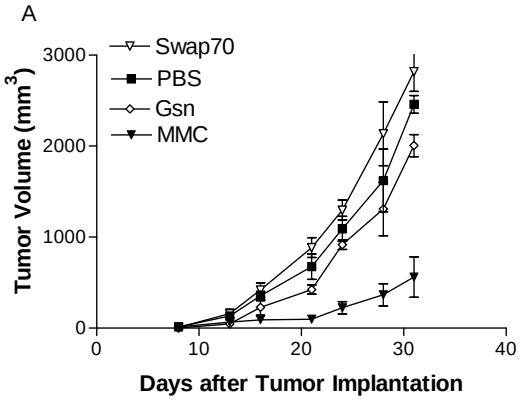

Figure 6. Vaccination targeting tumor antigens identified from tumor-bearing mice, Swap70 and Gsn, did not have tumor protection effect. Mice (3 per goup) were vaccinated with plasmid DNA encoding Swap70 or Gsn, or irradiated whole tumor cells at day -42, -28, and -14. Live MMC cells were given subcutaneously on day 0.

Table 1. SEREX-identified antigens identified from tumor rejection mice

Antigen	No. of clones	Gene Symbol	Function	Chromosome location	Sub-cellular location
Mouse mammary tumor virus	6	Mtv1	Viral protein		
Catenin (cadherin-associated protein) alpha 1	4	Ctnna1	Actin and cadherin binding, tight juction	18B1	cytosol
Heat shock protein 40	2	Hsp40	Protein binding, chaperon	4B1	cytosol
Transmembrane protein 57	2	Tmem57		4D3	membrane
Centrosomal protein 290 kDa	1	Cep290	Protein binding, transcription activator	10D1	nucleus
FXYD domain containing ion transport regulator 3	1	Fxyd3	Ion transport, tumor metastasis		membrane
Talin 1	1	Tln1	Actin binding, focal adhesion	4B1	cytosol
Heterogenous nuclear ribonuclear protein L-like	1	Hnrpl	RNA binding, RNA and mRNA processing	7B1	nucleus
GPI-anchored membrane protein 1	1	GPIap1	Cell cycle control	2E2	membrane
TNF alpha-induced protein 3	1	Tnfaip3	Zinc finger protein A20, DNA and protein binding, anti-apoptotic	10A3	cytosol/ nucleus

Table 2. Mouse tumor rejection antigens have immunogenic human homologues.

Antigen	Gene Symbol	Immunogenic Human Homologue	Human SEREX clone
Catenin (cadherin-associated protein) alpha 1	Ctnna1	yes	NY-REN-13
Heat shock protein 40	Hsp40	yes	MO-BC-1001
Centrosomal protein 290 kDa	Cep290	yes	NY-TLU-66 HOM-Ov1-214
Talin 1	Tln1	yes	NY-BR-88
Heterogenous nuclear ribonuclear protein L-like	Hnrpl1	yes	MO-OVA-131
TNF alpha-induced protein 3	Tnaaip3	yes	MO-OVA-200

Reportable Outcomes

Publications:

Lu H, Knutson KL, Gad E, Disis ML. The tumor antigen repertoire identified in tumor-bearing Neu transgenic mice predicts human tumor antigens. Cancer Res 2006; 66: 9754-61.

Hailing Lu, Ekram Gad, Amy Chang, Kristin Seymour, and Mary L. Disis. The identification of tumor rejection antigens in murine models that are associated with human homologues. Poster presentation at AACR, 2006

Hailing Lu, Amy Chang, Emily Larson, Ekram Gad, and Mary L. Disis. Identification of an immunological signature of tumor rejection in the neu transgenic mouse. Oral presentation at AACR, 2007

Conclusion and Future Directions

Studies during the last funding year have clearly shown that potential tumor rejection antigens can be identified from tumor rejection mice. Preliminary in vivo vaccination data has suggested that antigens identified from tumor rejection mice but not tumor bearing mice have therapeutic values. Based on these results, we have set up the following goals for the studies in the next two years: 1) continue identify tumor rejection antigens using mouse tumor rejection model. We will create a different tumor rejection model and compare the antigen repertoire in each model, which will lead to the identification of critical targets that show up in different models; 2) examine the immunogenicity of the human homologues of the mouse tumor rejection antigens. In addition to the database search as we have done previously, we will use patient serum and PBMC to examine the antibody and T cell response to the human homologues of the mouse antigens. This comprehensive evaluation will only be performed to the antigens that show tumor protection effect in mice; 3) test the in vivo protection effect of plasmid DNA vaccine targeting a combination of antigens. Studies in the last year have shown that vaccination with plasmid DNA coding Mtv1, FxyD3, or Cep290 have tumor protection effect. For the next step, we will test the combination of these plasmids to determine if there is any synergistic effect. These antigens will also be tested in combination with HER2 vaccination, which is known to have an anti-tumor effect in these mice. We will also test the effect of vaccination in preventing the development of spontaneous tumors.