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AWARD NUMBER: W81XWH-05-1-0016

TITLE: Antibody-based Drug Carriers for Targeted Prostate Cancer Chemotherapy

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REPORT DATE: May 2006

TYPE OF REPORT: Final

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)*
01-05-2006	Final	31 Oct 2004 – 30 Apr 2006

4. TITLE AND SUBTITLE

Antibody-based Drug Carriers for Targeted Prostate Cancer Chemotherapy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0016

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Texas Health Science Center
Houston, Texas 77030-3900

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

The goal of this project is to generate a library of human antibody Fvs containing prostate tissue homing peptide for specific delivery of chemotherapeutic drug doxorubicin to the site of prostate cancer. We have replaces CDR 3 loop of the antibody VL domain with the sequence encoding prostate tissue homing peptide. Panning of the library with doxorubicin conjugated to BSA yielded a panel of monoclonal doxorubicin specific Fvs that could be used as prostate tissue specific carrier of this drug with increased tissue selectivity and reduced toxicity.

15. SUBJECT TERMS
No subject terms provided.

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

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INTRODUCTION:

The goal of the project was to create a universal, antibody-based drug delivery system for targeted delivery of small chemotherapeutic drugs to the site of prostate cancer. We wanted to explore the ability of the antibodies to reversibly hold small organic molecules (haptens) via non-covalent interactions with six flexible polypeptide loops (CDRs). Binding of this class of molecules including chemotherapeutic drugs usually does not required participation of all six CDR loops. We proposed to use these unutilized CDR loops for targeting such antibody-carrier to the cancer site by replacing it with loop-shaped peptide specific to the cancer site. In the first specific aim we modified the existing human phage display library by introducing a prostate-homing peptide into the 3rd CDR loop of the light chain. The modified library was used to select a group of Fvs specific for a model chemotherapeutic drug, doxorubicin (DOX). Selected Fvs were characterized for their affinity to DOX using spectrofluorometric measurements. In the second specific aim we evaluate the potential of selected Fvs to deliver DOX to the prostate tissue in mice.

BODY.

Task 1. **To generate a panel of DOX-specific Fvs containing prostate homing peptide (Month 1-12):**

a. Introduce a prostate homing sequence, SMSIARL, into human semi-synthetic Fv phage library (Months 1-3). Prostate homing sequence will be introduced into the set of antibody VL-genes by two step PCR. Modified VLs will be cloned into the library in pHEN2 phagemid vector. The efficiency of the cloning will be verified by colony PCR and sequencing. The sequence encoding prostate homing peptide, TCA ATG TCA ACT GCT AGG CTG, was introduced into CDR3 of VL domains (both κ and λ) of Griffin 1 human semi-synthetic Fv library [1] in two PCR steps. The methods described in [2] with certain modification were applied. VH and VL parts of the Fv insert in this library are inserted into pHEN2 vector at separate cloning sites (*Sfi I* and *Apa LI* for VH, and *Xho I* and *Not I* for VL) permitting selective removal of one domain without affecting another. The vector also contains the following sequence elements: a signal peptide, a gene3 structural peptide, a stop codon (amber) between the insert and gene3, a *c-myc* peptide tag, poly(his)$_6$, an IPTG-inducible lac promoter and an ampicillin resistance gene. The amber codon permits secretion of soluble Fv or their expression as p3-fusion proteins on the phage surface, depending on the strain of host *E. coli* (HB2151 cells recognize amber as a stop and TG1 cells recognize amber as Glu). A single back primer containing the sequence of invariable 15-mer Fv linker and *Xho I* restriction site was used in all amplifications. VL genes were obtained by digesting the library with *Apa LI* and *Not I* to preserve the sequence of peptide linker needed for back primer annealing. In the first step VLs were PCR-amplified using 23 V- κ and 23 V-λ forward primers (residues 88-94, Kabat numbering) which represent C-terminal sequence diversity of 26 κ and 31 λ germline genes composing the VL part of the Fv library [1]. Each primer had antisense sequence encoding prostate homing peptide at the 3'-end. In the second PCR step VLs were re-amplified using two forward primers containing antisense sequences encoding prostate homing peptide, conserved FW4 regions of kappa and lambda chains and *Not I* cloning site (See Figure 1). Following completion of the PCR, the amplified DNA bands of the correct size were cut from agarose gels, extracted using Geneclean II (BIO 101) and quantitated by EtBr fluorescence (λ_{em} 590 nm, λ_{ex} 302 nm). Modified VL DNA containing prostate homing sequence was sequentially digested with *Xho I* and *Not I* and inserted into pHEN 2 vector containing the original Fv library digested with the same enzymes. Host cells were transformed by electroporation, clones were selected in ampicillin and the presence of inserts in individual colonies were confirmed by PCR using primers located in the vector upstream and downstream of the insert, yielding EtBr-stained bands at 0.9 kb (See Figure 2). Addition of helper phage (VCSM13) containing a kanamycin resistant gene permits packaging of phage particles from TG1 cell cultures containing both ampicillin and kanamycin. The particles in the supernatant of the culture were precipitated twice with 3% PEG/0.5 M NaCl, yielding phage ready for the selections.

b. Isolate a panel (8-10 different clones) of DOX-specific Fv-phages (Months 4-9). Clones will be selected by panning the library over DOX-BSA conjugate. DOX-specific clones will be identified by phage ELISA. DOX specificity will be confirmed for these Fvs expressed in soluble form. DOX was conjugated to BSA or ovalbumin via its amino-group by carbodiimide coupling [11]. Phages (10^{12} cfu/ml in PBS, 0.5 M NaCl, 100 ul) were pre-adsorbed on BSA to deplete any BSA binding clones and incubated over Maxisorb Immunotubes coated with DOX-BSA conjugate for 30 min. After several washes with PBS, 0.5 M NaCl, 0.02% Tween-20, bound phages were eluted with 0.1 M Gly-HCl buffer, pH 2.5, immediately neutralized with Tris–base and used to re-infect TG1 or HB2151 cells. After two and five rounds of selection approximately 100 Fv clones expressing soluble Fvs were assayed for DOX binding with DOX-ovalbumin conjugate by ELISA (See Figure 3). 10 DOX-

specific clones were sequenced and analyzed for assignment of class and family according to homologies with sequences in the Kabat database (See Table 1).

Task 2. **To conduct biodistribution studies of free DOX and Dox-Fv complexes (Month 13-18):**

This task was not accomplished because on 11/04/05 PI has resigned his position at UTHSCH.

KEY RESEARCH ACCOMPLISHMENTS.

- Generated a library of human Fvs containing prostate tissue homing peptide in place of VL CDR 3.
- Selected a panel of DOX binding Fv that could be used as a carriers for prostate tissue specific delivery of DOX.

REPORTABLE OUTCOME.

PI was offered a position to lead Antibody Engineering group at Lexicon Genetics Inc. in part based on the experience he obtained while conducting research under this award.

CONCLUSION.

We have generated a panel of prostate tissue homing Fvs that could serve as carriers for tissue specific delivery of chemotherapeutic drug doxorubicin. Their in vivo efficacy still needs to be tested.

REFERENCES.

1. Griffiths AD, Williams SC, Hartley O, Tomlinson IM, Waterhouse P, Crosby WL, Kontermann RE, Jones PT, Low NM, Allison TJ, et al. Isolation of high affinity human antibodies directly from large synthetic repertoires. *EMBO J.* 13:3245-3260. 1994.
2. Paul S, Tramontano A, Gololobov G, Zhou YX, Taguchi H, Karle S, Nishiyama Y, Planque S, George S. Phosphonate ester probes for proteolytic antibodies. *J Biol Chem.* 276:28314-28320, 2001.

APPENDICES.

None

SUPPORTING DATA.

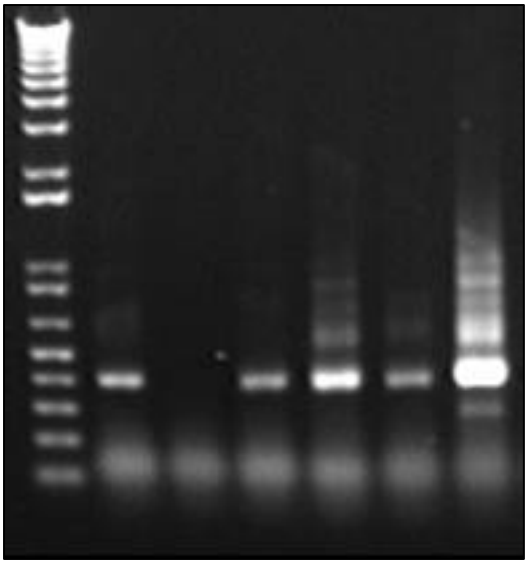

Fig.1 Amplification of VL kappa and lambda domains containing CDR3 replaced with prostate homing peptide

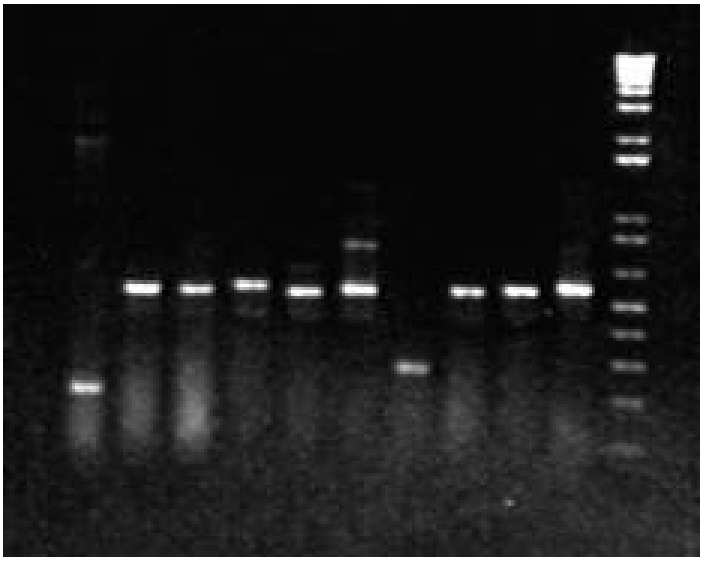

Fig.2 Colony PCR confirming presence of Fv-inserts in the Fv phage display library containing prostate tissue homing peptide.

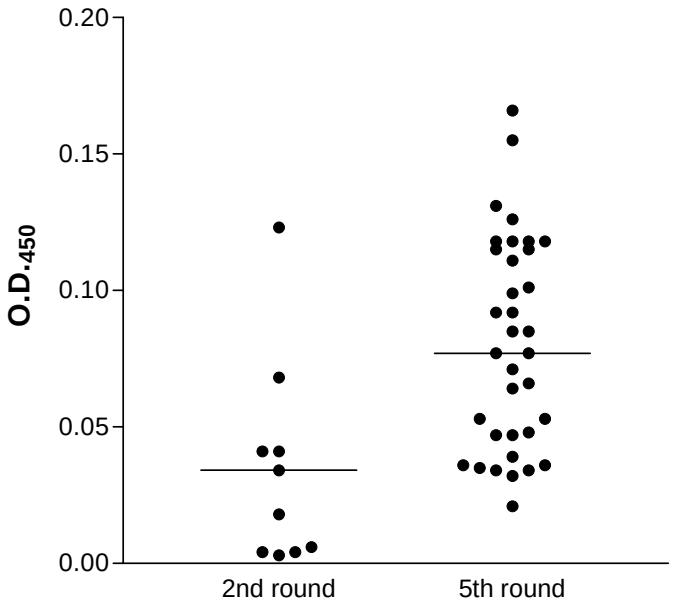

Fig. 3 Enrichment of the library with DOX-binding clones after second and fifth rounds of panning with DOX-BSA conjugates determined by ELISA with soluble monoclonal Fvs.

Table 1: Germline gene assignment for selected DOX-binding Fv clones:

Clone	Heavy chain	Light chain
A2	VH3-23 519 e-150 VH3-53 422 e-121 VH3-66 398 e-113	V1-16 565 e-163 V1-17 525 e-152 V1-11 422 e-121
A3	VH3-23 559 e-162 VH3-53 412 e-118 VH3-48 408 e-116	V2-13 553 e-160
A4	VH3-23 551 e-159 VH3-53 404 e-115 VH3-48 400 e-114	V2-13 553 e-160
A5	VH3-23 549 e-159 VH3-53 402 e-115 VH3-48 398 e-113	V2-13 559 e-162
A6	VH3-20 511 e-147 VH3-9 391 e-111 VH3-23 337 4e-95	V1-16 500 e-144 V1-17 460 e-132 V1-11 357 e-101
A7	VH6-1 595 e-172	V2-13 571 e-165
A8	VH1-45 543 e-157 VH1-2 313 6e-88 VH1-46 305 1e-85	V2-13 561 e-162
A9	VH1-69 567 e-164 VH1-18 337 4e-95 VH1-8 329 1e-92	V2-13 553 e-160