UNCLASSIFIED

AD NUMBER

ADB266571

NEW LIMITATION CHANGE

TO
Approved for public release, distribution unlimited

FROM
Distribution authorized to U.S. Gov’t. agencies only; Proprietary Info.; Feb 2001. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott St., Fort Detrick, MD 21702-5012.

AUTHORITY


THIS PAGE IS UNCLASSIFIED
Award Number: DAMD17-98-1-8567

TITLE: Identification and Characterization of Internalization Signal of the Prostate Specific Membrane Antigen

PRINCIPAL INVESTIGATOR: Ayyappan Rajasekaran, Ph.D.

CONTRACTING ORGANIZATION: University of California, Los Angeles
Los Angeles, California 90095-1406

REPORT DATE: February 2001

TYPE OF REPORT: Annual Summary

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

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Feb 01). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

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.
NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-98-1-8567
Organization: University of California, Los Angeles
Location of Limited Rights Data (Pages):

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure, or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

[Signature]

[Date: 05/10/01]
Prostate specific membrane antigen is expressed in large amounts in prostate cancer cells and in metastatic deposits. We have identified monoclonal antibodies that are specifically internalized by prostate cancer cells. Characterization of internalization mechanisms by prostate cancer cells is crucial for the improvement of immunotherapeutic approaches for prostate cancer. Internalization and movement of proteins through endosomes and lysosomes are mediated by internalization signals present in the cytoplasmic tail of internalized proteins. Using plasmid constructs that contain various deletions and point mutations in the cytoplasmic tail of PSMA we have characterized a di-leucine motif in the cytoplasmic tail of PSMA as an internalization signal that mediates the internalization of monoclonal antibodies raised against PSMA. We also found that the di-leucine motif of PSMA is sufficient to target PSMA to lysosomes. These findings should provide insights into strategies to improve the efficacy of anti-PSMA antibodies for immunotherapy for prostate cancer.
# Table of Contents

Cover ..................................................................................................................  

SF 298 ..................................................................................................................  2  

Introduction ........................................................................................................  4  

Body ......................................................................................................................  4  

Key Research Accomplishments ................................................................  5  

Reportable Outcomes .......................................................................................  5  

Conclusions ..........................................................................................................  5  

References ...........................................................................................................  6  

Appendices ..........................................................................................................  6
Introduction: Prostate specific membrane antigen (PSMA) is a cell surface glycoprotein expressed almost exclusively in prostate cancer cells. We have identified monoclonal antibodies (mAbs) that are internalized specifically by LNCaP cells (PSMA positive, human prostate cancer cells) (Liu et al., 1997). Internalization and movement of proteins through intracellular pathways (i.e. targeting to endosomes or lysosomes) are mediated by internalization signal/s present in the cytoplasmic domain of internalized proteins (Mukherjee et al., 1997). Although PSMA is efficiently internalized in LNCaP cells via a clathrin dependent endocytic pathway (Liu et al., 1998) the mechanism by which PSMA is internalized in LNCaP cells is not understood. The goal of this proposal was to identify and characterize the internalization signal of PSMA. Characterization of PSMA internalization mechanisms by prostate cancer cells is crucial for the improvement of immunotherapeutic approaches for prostate cancer.

Body: Our negotiated statement of work and the progress accomplished from August 1, 2000 to January 31, 2001 are described below. Two of the subtasks of our approved Statement of Work that remain to be accomplished are: 1.) Generate a PSMA cytoplasmic tail/transmembrane-CAT chimera, transfect LNCaP cells and monitor internalization by biotinylation and confocal microscopic assays. 2). Test the internalization signal in the transmembrane or extracellular domains of PSMA.

Using alanine scan mutagenesis we have identified that a di-leucine motif in the cytoplasmic tail of PSMA functions as an internalization signal. To further confirm that the di-leucine motif of PSMA functions as the internalization signal we transferred the first five amino acids containing the di-leucine motif (LLDWM) to a non-internalized protein, the interleukin-2 receptor α-chain (Tac). Tac is a transmembrane protein and like PSMA contains a small cytoplasmic tail (Dittrich et al., 1996). If the amino acid sequence LLDWM of PSMA is sufficient for PSMA internalization then the Tac containing the LLDWM amino acid sequence (Tac-LLDWM) in the cytoplasmic tail should internalize. Internalization of Tac was monitored by the uptake of a mAb 7G7, which is raised against the extracellular domain of Tac. In COS cells expressing a wild type Tac (Tacwt) construct, Tac was distinctly localized to the plasma membrane. As expected, incubation of these cells with mAb 7G7 did not result in the internalization of this antibody confirming that Tacwt is not internalized as reported earlier (Dittrich et al., 1996). By contrast, Tac-LLDWM internalized the mAb 7G7 and the internalized antibody clearly co-distributed with the internalized FITC-transferrin. These results indicate that the di-leucine signal of PSMA is sufficient for its internalization and confers ability to internalize for a non-internalized protein. Experiments are in progress to test whether mutation of the di-leucine motif to di-alanine (Tac-AADWM) will prevent the internalization of monoclonal antibody 7G7. These results reveal that the internalization of PSMA requires primarily the cytoplasmic domain and the extracellular or the transmembrane domain may not be necessary for its internalization. To further confirm that the extracellular domain is not involved in the internalization of PSMA we deleted most of the extracellular domain and generated a plasmid construct which primarily contain the cytoplasmic tail and the transmembrane domains fused to a V5-epitope. Internalization of this construct can be monitored by its ability to internalize an antibody against V5-epitope. Experiments are in progress to test whether cells expressing this construct will internalize. These experiments will conclusively prove that the
cytoplasmic tail di-leucine motif of PSMA is involved in the internalization of antibodies against PSMA.

During the course of these studies we consistently noticed that internalized PSMA antibodies were localized to lysosomes in LNCaP cells or in COS cells. A cytoplasmic di-leucine motif has been shown to be involved in the lysosomal targeting of several membrane proteins (Dittrich et al., 1996; Letourneur and Klausner, 1992). Therefore, we tested whether the di-leucine motif of PSMA also functions as a lysosomal targeting signal. For this purpose, COS cells transfected with wild type PSMA, and PSMA with a mutated di-leucine signal to di-alanine (PSMA-L4A/L5A), were incubated with mAb J591. Cells were then immunofluorescently double labeled for PSMA and a lysosomal marker LAMP-1 and visualized by confocal microscopy. Cells expressing PSMA_{wt} showed co-localization with LAMP-1 containing lysosomal vesicles. In contrast, cells expressing the PSMA-L4A/L5A construct showed PSMA staining predominantly on the plasma membrane and no lysosomal staining was detected. From these results we conclude that the di-leucine motif of PSMA functions not only as internalization signal but also mediates its transport to lysosomes. Currently, we are testing whether Tac-LLDWM is targeted to the lysosomes. We are also testing whether mutation of the di-leucine motif to di-alanine (Tac-AADWM) will prevent its targeting to lysosomes. Collectively, these studies will establish that the di-leucine signal in the cytoplasmic tail of PSMA is sufficient for its internalization and lysosomal targeting.

Key Research Accomplishments:

- Identified an internalization signal in the cytoplasmic tail of PSMA.
- Identified and characterized the di-leucine motif in the cytoplasmic tail as PSMA internalization signal.
- Characterized that the di-leucine motif alone is sufficient for PSMA internalization.
- Identified that the di-leucine motif of PSMA is also a lysosomal targeting signal.

Reportable outcomes: Two manuscripts are in preparation:


Conclusions: We have identified that a di-leucine motif in the cytoplasmic tail of PSMA functions as an internalization and lysosomal targeting signal. Identification of the di-leucine signal of PSMA as a lysosomal targeting signal has important implications for the immunotherapy of prostate cancer. Lysosomal targeting of PSMA indicates that the internalized PSMA antibodies will be targeted to the lysosomes and eventually be degraded. This might reduce the half-life of anti-PSMA antibodies used for the immunotherapy of prostate cancer. Therefore, these findings suggest that drugs that inhibit lysosomal targeting may improve the efficacy of anti-PSMA antibodies for treatment of prostate cancer.
References:


Appendices: None
MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for grants. Request the limited distribution statements for the Accession Document Numbers listed at enclosure be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

[Signature]

PHYLIS M. RINEHART
Deputy Chief of Staff for Information Management

Enclosure