

UNCLASSIFIED

AD NUMBER
ADB265796
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies only; Proprietary Info.; Nov 2000. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott St., Fort Detrick, MD 21702-5012.
AUTHORITY
USAMRMC ltr, dtd 10 Jun 2003

THIS PAGE IS UNCLASSIFIED

AD \_\_\_\_\_

Award Number: DAMD17-99-1-9064

TITLE: Heparan sulfate proteoglycans as therapeutic agents  
for breast cancer

PRINCIPAL INVESTIGATOR: Carla Y. Pumphrey, Ph.D.  
Ralph D. Sanderson, Ph.D.

CONTRACTING ORGANIZATION: University of Arkansas for Medical Sciences  
Little Rock, Arkansas 72205

REPORT DATE: November 2000

TYPE OF REPORT: Annual summary training report

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, Nov 00).  
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.

20010507 014

## 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: DAMD17-99-1-9064  
Organization: University of Arkansas for Medical Sciences  
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.

Carole B. Clouston

4-23-01

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE November 2000	3. REPORT TYPE AND DATES COVERED Annual summary, (1 Nov 99 - 31 Oct 00)	
4. TITLE AND SUBTITLE  Heparan sulfate proteoglycans as therapeutic agents for breast cancer			5. FUNDING NUMBERS	
6. AUTHOR(S)  Carla Y. Pumphrey, Ph.D. Ralph D. Sanderson, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  University of Arkansas for Medical Sciences  Little Rock, Arkansas 72205  E-Mail:  pumphreycarlay@exchange.uams.edu			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. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Nov 00). 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.			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) Heparan sulfate proteoglycans (HSPGs) represent a new class of tumor suppressors. The goal of the proposed work is to evaluate the cell growth inhibitory affects and apoptotic potential of HSPG gene therapy <i>in vitro</i> and <i>in vivo</i> . The first task is to develop breast cancer gene therapy and evaluate <i>in vitro</i> . Syndecan-1 expression was evaluated on several breast cancer cell lines. The results demonstrate that each cell line expresses an abundance and similar levels of syndecan-1. Therefore, DNA encoding a c-myc tag was incorporated into the ectodomain of the syndecan-1 gene. In addition, truncated gene cassettes have been constructed to allow for the secretion of syndecan-1. We will next isolate breast cancer cell lines expressing the engineered gene constructs and determine the effects on tumorigenicity. The second task of the proposal is the <i>in vivo</i> analysis of breast cancer gene therapy. MDA-MB-231 breast tumors were established in SCID mice and treated by electroporation with a plasmid containing the full length syndecan-1 gene. The results show no reduction in tumor size however, even the untreated control tumors did not grow. In addition, we were unable to confirm that the syndecan-1 gene was over expressed due to a technical error. We have addressed these problems and are proceeding with the <i>in vivo</i> studies. This project represents the first attempt to use HSPG genes for anti-cancer therapy.				
14. SUBJECT TERMS  heparan sulfate proteoglycans, gene therapy, apoptosis			15. NUMBER OF PAGES 10	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

## Table of Contents

Cover.....	
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	9
Reportable Outcomes.....	9
Conclusions.....	9
References.....	n/a
Appendices.....	n/a

#### **(4). INTRODUCTION**

Heparan sulfate proteoglycans (HSPGs) regulate normal and cancer cell behaviors by binding growth factors, and by mediating cell adhesion and invasion. Current data strongly support the idea that HSPGs are a new class of tumor suppressors. Studies demonstrate the anti-tumor growth properties of three HSPGs, specifically syndecan-1, glypican-1, and betaglycan. Treatment with purified syndecan-1 produces strong growth suppression in multiple myeloma cell lines, a poorly differentiated squamous cell carcinoma cell line, human and murine mammary tumor cell lines, but not normal cell lines. In addition, treatment of multiple myeloma cells with purified syndecan-1 induces apoptosis. Many tumors display an alteration in cell surface HSPG expression. When syndecan-1 is lost from the surface of mammary epithelia, the cells lose epithelial morphology, invade collagen gels and show characteristics of neoplastic growth. When transfected with the syndecan-1 gene, transformed mammary epithelial cells regain morphology and lose neoplastic growth characteristics. *In vivo* experiments demonstrate reduced tumorigenicity of syndecan-1 or glypican-1 expressing multiple myeloma cells and betaglycan expressing breast cancer cells. Therefore, we propose that HSPGs are excellent candidates for gene therapy applications for the treatment and possible eradication of breast cancer. The purpose of the proposed work is to develop HSPG gene therapy and examine HSPGs independently and in combination as mediators of breast cancer cell growth and apoptosis *in vitro* and *in vivo*. This work represents a novel use of HSPGs as anti-cancer therapy.

#### **(5). BODY**

Task 1. Develop breast cancer gene therapy and evaluate *in vitro*.

- Engineer epitope tag containing human HSPG gene constructs for cell surface expression or secretion.

Ideally, to determine the effect of expression of a transfected gene, the parental cell lines should not actively produce the gene product. This allows for a direct comparison between the behavior of the parental non-expressing cell line and the behavior of the same cell line that expresses the transfected gene.

Syndecan-1 expression has been characterized on the cell surface of several breast cancer cell lines including MCF-7, MDA-MB-231, MDA-MB-435, MDA-MB-436, and Hs578t. The breast cancer cell lines were grown as monolayers in tissue culture flasks, detached by treatment with 1% EDTA only (trypsin cleaves syndecan-1 from the cell surface), stained with the FITC-labeled BB4 monoclonal antibody which is specific for syndecan-1, and analyzed by FACS. Each of these cell lines express significant and in fact abundant levels of syndecan-1 (Figure 1

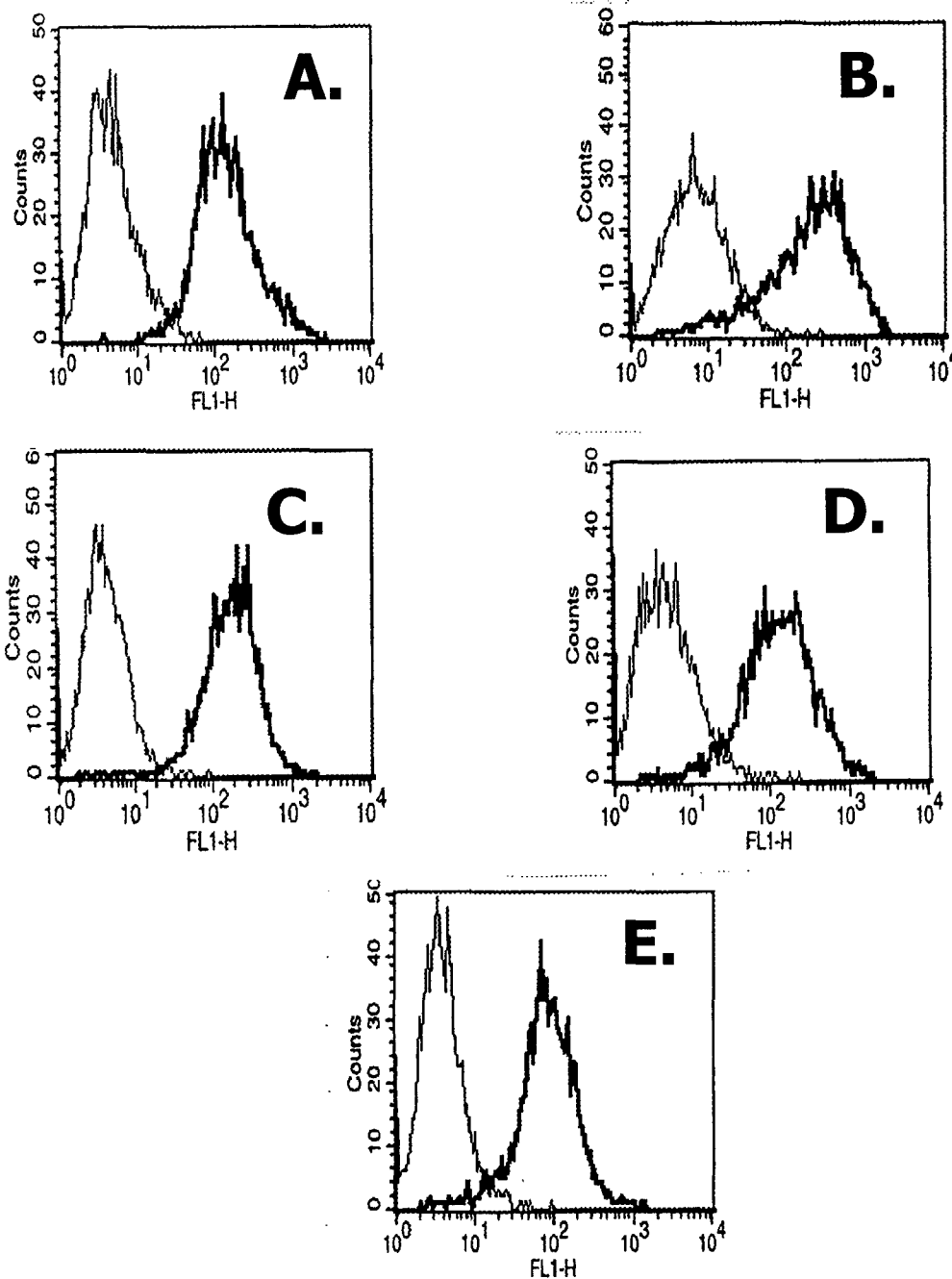
and data not shown.) The mean fluorescence intensity of BB4-FITC staining on each cell line was compared in duplicate experiments and the results indicate that the cell lines express roughly similar amounts of syndecan-1 (Figure 2). Therefore, for the purpose of distinguishing native syndecan-1 from syndecan-1 expressed from transferred gene cassettes, a c-myc tag was incorporated into the human syndecan-1 gene construct. The c-myc tag was inserted 3' to the DNA sequence encoding the amino-terminal signal sequence and 5' to the glycosaminoglycan attachment sites. Primers for the production of three syndecan-1 gene cassettes including a full length cassette and two truncated constructs have been engineered and used to PCR amplify the appropriate DNA fragments (Figure 3). Expression of the truncated cassettes will generate syndecan-1 that will be secreted instead of found on the cell surface. The three syndecan-1 gene constructs have been cloned in plasmid vectors.

Task 2. *In vivo* analysis of breast cancer gene therapy.

- Establish tumors in the mammary fat pads of NUDE mice. Treat tumors with therapeutic HSPG delivered by retrovirus or liposomes. Evaluate HSPG gene localization, expression, tumor burden, and induction of apoptosis.

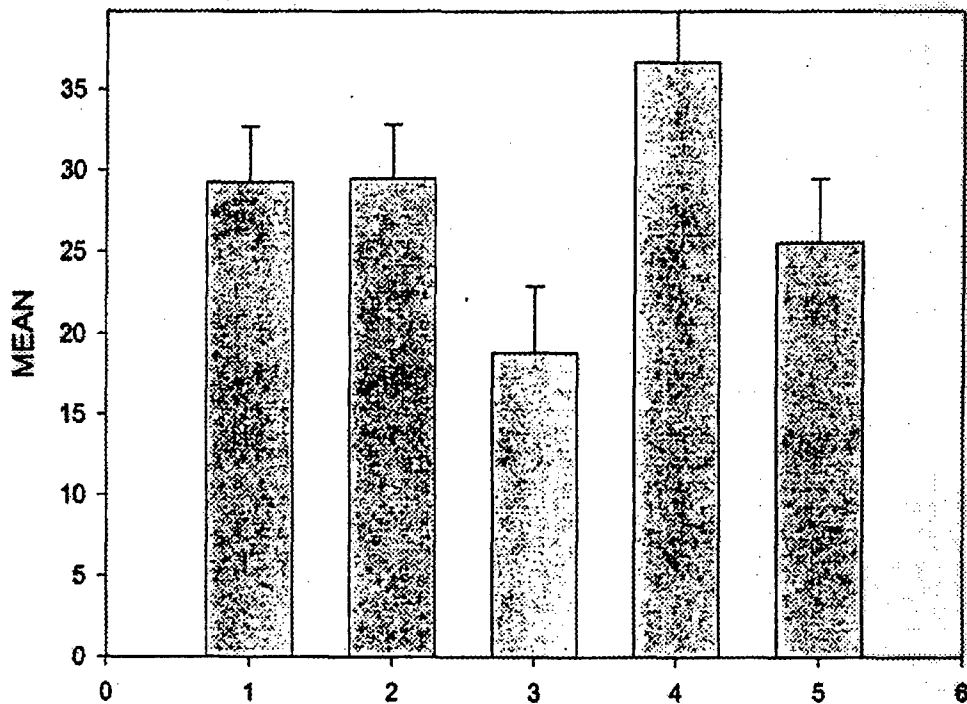
*In vivo* experiments have begun to test the effect of increased syndecan-1 expression on tumor growth. MDA-MB-231 breast cancer cells were injected into the mammary fat pad of SCID mice and tumors were established. Plasmid DNA only or plasmid DNA containing the full length human syndecan-1 gene cassette was electroporated into tumors two times per week for several weeks. Tumor growth was measured every other day and at the end of the experiment the animals were sacrificed and the tumors excised and stained for syndecan-1 expression. The results showed no reduction in tumor size however, the tumors were initially very small and did not grow appreciably regardless of treatment. The data from the tumor staining was inconclusive due to technical error. Therefore, we are not sure that syndecan-1 was effectively expressed.

To address these issues, we have amended our protocols. First we have treated the MDA-MB-231 cells in culture with an antibiotic for mycoplasma which should increase tumor growth *in vivo*. In addition, we have switched to the NUDE mouse model to eliminate any possible interference by the immune system. We have found that with these two amendments we can establish and grow tumors. Therefore, this task is moving forward.

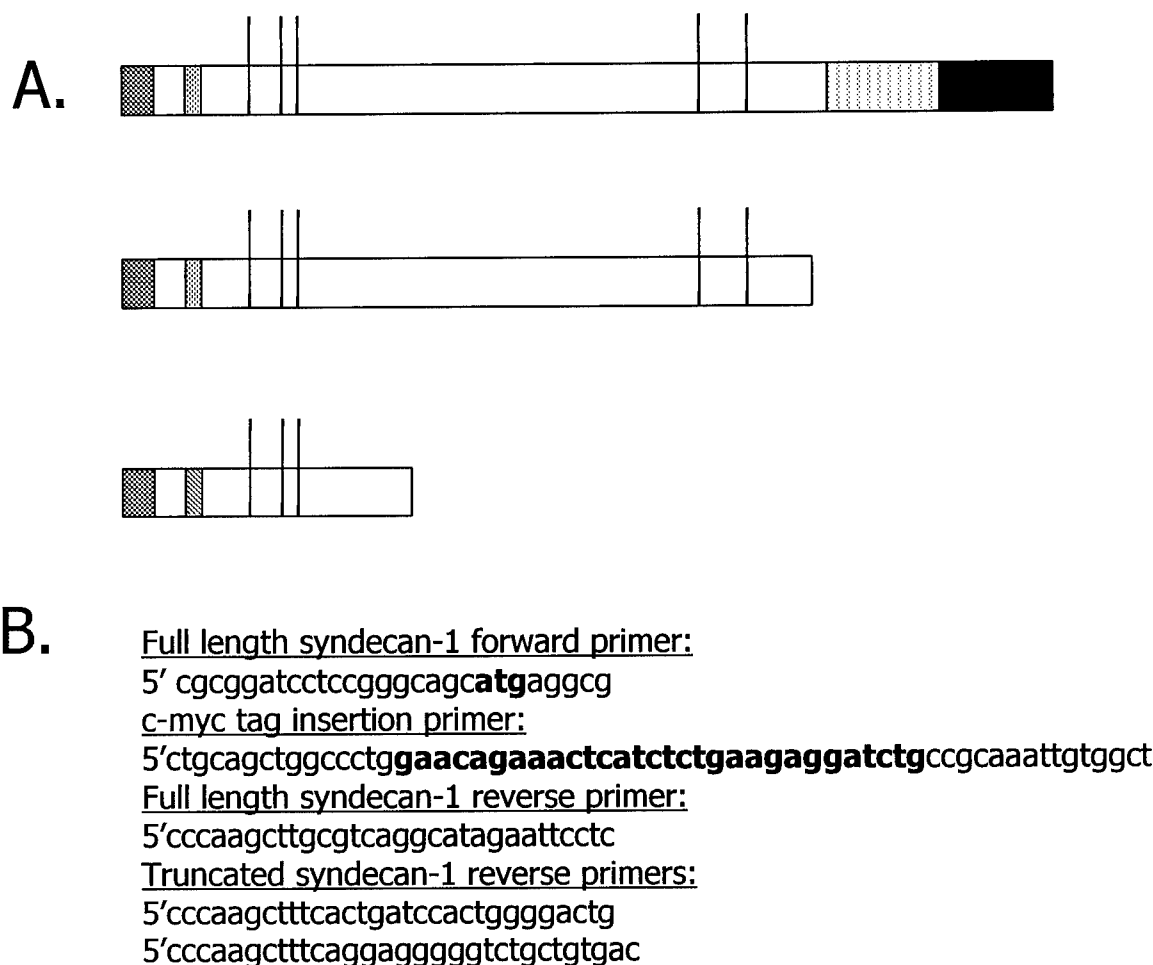


**Figure 1. Breast cancer cell lines express abundant syndecan-1 on the cell surface.** HBL-100 (A), MCF-7 (B), MDA-MB-435 (C), MDA-MB-436 (D), and MDA-MB-231 (E) breast cancer cell lines were stained with BB4-FITC monoclonal antibody to syndecan-1 or an isotype matched control FITC-labeled antibody. FACS analysis reveals a shift in fluorescence when cells were stained with BB4 (dark line) versus control antibody (lighter line) indicating extensive amounts of cell surface syndecan-1 on each breast cancer cell line.





**Figure 2. Breast cancer cell lines express similar amounts of syndecan-1 on the cell surface.** Breast cancer cell lines were stained with BB4-FITC and analyzed by FACS in duplicate experiments. The mean fluorescence intensity, which indicates the amount of cell surface syndecan-1 present on each cell line was compared. Error bars reflect standard deviation. Lane 1., HBL-100, lane 2., MCF-7, lane 3., MDA-MB-231, lane 4., MDA-MB-435, and lane 5., MDA-MB-436.



**Figure 3. Full length and truncated syndecan-1 protein structure and primers for gene construction.** Panel A. illustrates the c-Myc tagged full length syndecan-1 and two truncated forms. The full length syndecan-1 is composed of an N-terminal signal sequence (gray box), an ectodomain (open box) containing glycosaminoglycan attachment sites (lines), a transmembrane domain (hatched box), and a cytoplasmic domain (black box). The c-Myc tag (dotted box) was inserted in the ectodomain between the signal sequence and glycosaminoglycan attachment sites. One truncated form of syndecan-1 lacks the transmembrane and cytoplasmic domains while the other lacks these domains along with most of the ectodomain. Panel B. list the primers used to insert the c-myc tag into the syndecan-1 gene and the primers used for the production of full length and truncated syndecan-1 gene cassettes. The syndecan-1 start codon and the c-myc tag DNA sequences are in bold.

## **(7). 1). KEY RESEARCH ACCOPLISHMENTS**

### Task 1.

- Syndecan-1 expression profile was determined on several breast cancer cell lines including MCF-7, MDA-MB-231, MDA-MB-435, MDA-MB-436, and Hs578t. Each of these cell lines express abundant amounts of syndecan-1 on the cell surface.
- A c-myc tag has been added 3' to the amino-terminal signal sequence of the syndecan-1 gene construct.
- Two truncated syndecan-1 gene constructs have been produced by PCR amplification and cloned in plasmid vectors.

### Task 2.

- Treatment of breast cancer tumors in SCID mice with the full length human syndecan-1 gene was inconclusive.

## **CONCLUSIONS**

The objective of the proposal is to transfer HSPG gene constructs into breast cancer cells lines and tumors growing in mice to test the ability of these tumor suppressor genes to slow growth and possibly eradicate tumors. Several different syndecan-1 gene cassettes have been produced during this year and the full length syndecan-1 gene has been electroporated into established breast cancer tumors in SCID mice. While the results of the *in vivo* study were inconclusive, we are confident that we have worked out the technical difficulties and will address this important question. This work represents the first attempt to use HSPG genes for the purpose of anti-cancer therapy.



DEPARTMENT OF THE ARMY  
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND  
504 SCOTT STREET  
FORT DETRICK, MD 21702-5012

REPLY TO  
ATTENTION OF

MCMR-RMI-S (70-1y)

10 Jun 03

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 this Command. Request the limited distribution statement for the enclosed accession numbers 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. Kristin Morrow at DSN 343-7327 or by e-mail at [Kristin.Morrow@det.amedd.army.mil](mailto:Kristin.Morrow@det.amedd.army.mil).

FOR THE COMMANDER:

A handwritten signature in black ink, appearing to read "Phyllis M. Rinehart", is written over the typed name and title.

PHYLLIS M. RINEHART  
Deputy Chief of Staff for  
Information Management

Encl

ADB270849  
ADB263653  
ADB282255  
ADB262232  
ADB277418  
ADB285664  
ADB281628  
ADB284965  
ADB265796  
ADB282152  
ADB285670  
ADB257200  
ADB266081  
ADB285703  
ADB285699  
ADB285481  
ADB285668  
ADB283633