AD NUMBER

ADB220022

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies only; Proprietary Info.; Sep 96. Other requests shall be referred to Commander, Army Medical Research and Materiel Command, Attn: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012.

AUTHORITY

Army Medical Research and Materiel Command ltr dtd 9 Jun 98

AD_____

GRANT NUMBER DAMD17-94-J-4448

TITLE: The Role of Matrilysin, a Matrix Metalloproteinase, in Mammary Tumorigenesis

PRINCIPAL INVESTIGATOR: Lynn M. Matrisian, Ph.D.
Laura A. Rudolph

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center
Nashville, TN 37232-2104

REPORT DATE: September 1996

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1996	Annual (1 Sep 95 - 31 Aug 96)

4. TITLE AND SUBTITLE The Role of Matrilysin, a Matrix Metalloproteinase, in Mammary Tumorigenesis

5. FUNDING NUMBERS
DAMD17-96-J-4448

6. AUTHOR(S)
Lynn M. Matrisian, Ph.D.
Laura A. Rudolph

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Vanderbilt University Medical Center
Nashville, TN 37232-2104

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick
Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only; Proprietary Information, Sep 96. Other requests for this document shall be referred to Comander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

The goal of this project is to test the hypothesis that the expression of matrilysin in mammary epithelial cells plays a causal role in the progression and metastasis of mammary carcinomas using a transgenic mouse model system. We have successfully generated transgenic mice expressing the metalloproteinase matrilysin, under the control of the mammary specific MMTV promoter/enhancer. In this report we demonstrate that matrilysin accelerates the development of mammary tumors in the MMTV-*neu* transgenic animals. Current studies involve investigating the possibility that matrilysin is proteolytically processing members of the EGF/erbB signal transduction pathway, thereby constitutively activating this pathway and accelerating mammary tumor formation.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer	9
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

LR Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

LR In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

LR In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

LR In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

LR In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Laura Rudolph 9-27-96

PI - Signature Date

TABLE OF CONTENTS

INTRODUCTION

Cell-matrix interactions are an important aspect to many biological processes. During processes such as mammary growth and neoplasia the extracellular matrix (ECM) is continuously degraded and remodeled. Proteins that degrade the extracellular matrix, such as matrix metalloproteinases (MMPs), clearly play a role in the interactions that occur within the extracellular environment. We have previously hypothesized that matrilysin, an epithelial specific MMP, is partly responsible for remodeling of the ECM during mammary development and tumorigenesis. To test our hypothesis, transgenic mice expressing matrilysin under the control of the mouse mammary tumor virus (MMTV) promoter/enhancer are being evaluated to investigate if overproduction of matrilysin alters mammary development and/or mammary tumorigenesis. In addition, we are also investigating the possibility of matrilysin altering growth factor and growth factor receptor interactions in mammary tumorigenesis by proteolytic processing.

Our previous annual report described the successfully generated transgenic animals that express native, active, and inactive human matrilysin in the mammary epithelium (*specific aim #1*). For review, three separate human matrilysin constructs have been used to develop three different transgenic lines: 1) a native, or wild-type transgene, 2) a constitutively activated transgene, and 3) an inactive matrilysin transgene. The constitutively active construct contains a mutation that results in spontaneous activation of the enzyme, therefore circumventing any dependence on activation by exogenous factors. A comparison of the results from the native and active matrilysin constructs will give an indication of the availability of activators of matrilysin in the mammary environment. The third construct encodes a matrilysin protein that lacks proteolytic activity due to the presence of an inactivating mutation. The use of this mutant will determine if any observed effect of matrilysin in this model is due to its proteolytic activity.

In this report we focus on the studies to address whether mammary tumorigenesis can be modified by overexpression of native matrilysin (*specific aim #2*). To address this question, we have mated mice expressing the wild-type matrilysin protein with those expressing the oncogene *neu* under the control of the MMTV promoter (1). Mice expressing the active and inactive matrilysin are currently being mated to the MMTV-*neu* animals. *neu*/c-erbB2 has been observed to be amplified and overexpressed in a significant number of human breast cancers (2). Several studies have shown that the degree of amplification is inversely correlated to a poor clinical outcome (2,3). Overexpression of the *neu* product in the murine mammary epithelium results in the appearance of focal mammary tumors in multiparous females by approximately 205 days that metastasized to the lungs in 70% of tumor bearing animals (1).

PROGRESS

Examining the effects of matrilysin overexpression on mammary tumor formation, growth, and progression (specific aim #2).

Transgenic mice expressing the wild-type matrilysin protein under the control of the MMTV promoter have been mated to the MMTV-*neu* transgenic animals. The resulting mammary tumors have been analyzed for the time and frequency of onset, growth rate, and presence of metastasis. As indicated in Figure 1, the matrilysin/*neu* females (closed diamonds) develop mammary tumors at an accelerated rate and higher frequency than the *neu* control females (open diamonds). However, we have observed no obvious difference in the growth rate or the development of metastasis in the matrilysin/*neu* mice when compared to the *neu* controls (Table I).

These results are remarkably similar to results recently obtained by Drs. R.J. Coffey, Jr., Vanderbilt University, and W. Muller, McMaster University, in which they crossed the MMTV-TGFα (4) transgenics with the MMTV-*neu* mice (1) and also observed significant acceleration in the onset of tumor development (personal communication). These results have led us to the possibility that there is a connection between matrilysin and the EGF/erbB receptor signal transduction pathways that may be related to accelerated mammary tumor growth. One potential mechanism to explain the similarities in the accelerated response of MMTV-*neu* tumors to both TGFα and matrilysin may be that matrilysin is responsible for the proteolytic processing of the EGF/erbB tyrosine kinase receptor family and/or their growth factor ligands.

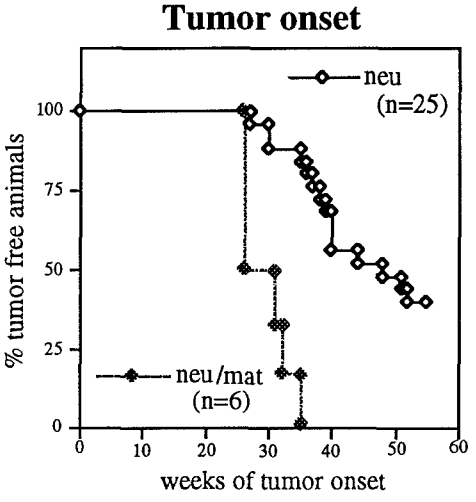

Figure 1: Mammmary tumor onset curve.
MAT/neu transgenics develop mammary tumors with an increased frequency and shorter latency period when compared to the neu animals. At 35 weeks of age, 100% of the MAT/neu females have developed mammary tumors, while only 17% of the neu females had tumors. Even at 50 weeks of age, only 54% of the neu females had developed mammary tumors.

	Mat/Neu	Neu
Tumor Growth Rate	14.0 ± 6.5 days (n = 12 tumors)	15.5 ± 6.7 days (n = 11 tumors)
Lung Metastasis	80% (4/5)	73% (8/11)
Ave. # Tumors per mouse	2.8 ± 1.7 tumors (n = 6 animals)	1.5 ± 0.5 tumors (n = 11 animals)
Ave. # of pregnancies	0.7 ± 0.8 preg. (n = 6 animals)	0.7 ± 0.7 preg. (n = 11 animals)

Table I: Comparison of MAT/neu and neu transgenic animals. The ***tumor growth rate*** was determined by physically measuring the tumors on a weekly basis. Tumor volume was then calculated and graphed against days of tumor growth. Using a log scale, the total number of days for the tumor to double in size was calculated. The percent ***metastasis*** was determined by both macro and microscopic analysis.

****Note: This page contains confidential unpublished results.**

Current studies involve the examination of members of the EGF/erbB signalling pathway as potential substrates for matrilysin's catalytic activity. Immunoprecipitations and Western blot analysis have been used to determine the levels, processed and phosphorylation state of erbB-1 (EGF receptor), erbB-2 (*neu*), erbB-3 and erbB-4 in extracts from mammary glands and mammary gland tumors from the matrilysin/*neu* and *neu* control animals (Figure 2). As shown in figure 2, there seems to be an increase in erbB-4 protein, and the presence of smaller, potentially proteolytically processed forms of the receptor in the matrilysin/*neu* glands. Additionally, there is an increase in tyrosine phosphorylated proteins when compared to the *neu* control glands.

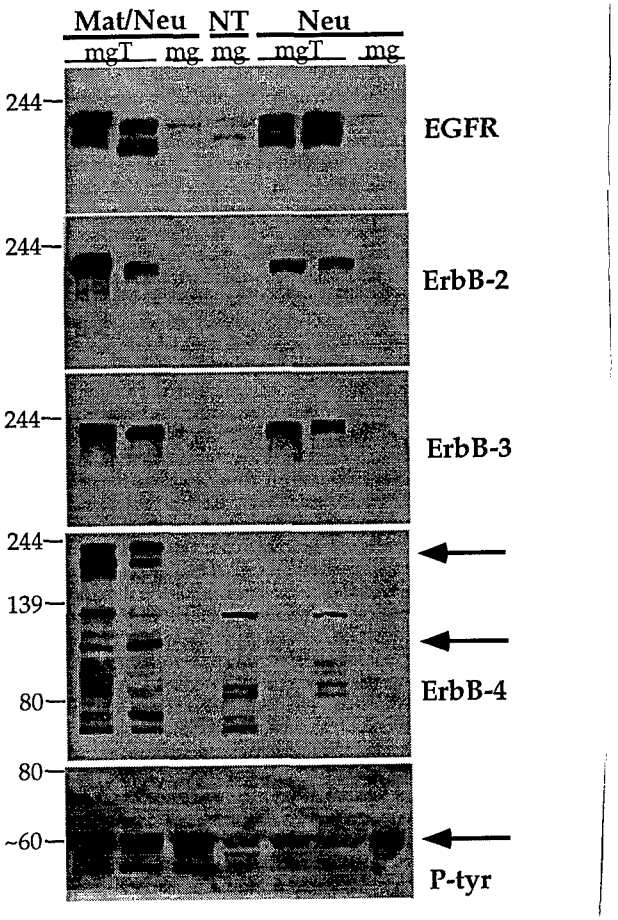

Figure 2: Analysis of erbB receptor expression in transgenic tumors.
Mammary glands (mg) or mammary gland tumors (mgT) were removed from nontransgenic control (NT), MMTV-*neu* (*Neu*) or MMTV-matrilysin/*neu* (Mat/*Neu*) females. 100µg of protein was separated on an 8% acrylamide gel, transferred to nitrocellulose, and probed using antibodies specific for each erbB family member (Santa Cruz) or for mouse phosphotryosine (Upstate Biotechnology).

****Note: This page contains confidential unpublished results.**

To support our preliminary findings, recent reports have demonstrated that metalloproteinase activity is involved in the processing of tumor necrosis factor-a (5), and heparin binding-epidermal growth factor (HB-EGF) (6), releasing this growth factor to become a soluble, paracrine factor (7). Most recently, processing of the erbB-4 receptor as a result of protein kinase C activation has been reported (8), which further supports the possibility that matrilysin may be involved in this proteolytic event.

We plan to pursue our findings by using a cell culture system derived from the matrilysin/*neu* and the *neu* mammary tumors. In addition, we will continue our experiments using mammary tumor extracts on the EGF/erbB receptors and also expand our efforts to the growth factor receptor ligands, namely EGF, TGFα, and heregulin to determine if their expression or processing is altered by overexpression of matrilysin.

FUTURE STUDIES

Determining if matrilysin expression contributes to mammary tumor formation or progression (specific aim #3).

Initially we had intended to determine if metalloproteinase expression in general contributes to mammary tumor formation or progression by generating transgenic mice overexpressing the inhibitor of metalloproteinases, TIMP, and then testing the effect of TIMP expression on the progression of chemically-induced mammary tumors. Since that time, our laboratory has generated matrilysin null animals. Using this model system we can specifically test whether matrilysin contributes to mammary tumor formation or progression. We have begun mating the matrilysin null animals with MMTV-polyomavirus middle T oncogene (MT) transgenic animals that develop multiple mammary tumors by approximately 80 days (9). We have previously determined that the mammary tumors that develop in the MMTV-MT mice do express endogenous matrilysin, so these experiments were designed to address if tumor growth, and/or metastasis is decreased by the absence of matrilysin.

CONCLUSIONS

The initial results obtained from the matrilysin/*neu* transgenic animals demonstrate that matrilysin, like TGFα, accelerated the formation of mammary tumors. We are currently investigating the possibility that matrilysin may be responsible for the proteolytic processing of the EGF/erbB tyrosine kinase receptor family and/or their growth factor ligands, thereby accelerating mammary tumor formation. Future studies using matrilysin null animals will determine if matrilysin specifically contributes to the formation and progression of mammary tumorigenesis.

ABSTRACTS

Rudolph, L.A. and Matrisian, L.M. Overexpression of human matrilysin induces ErbB-4 expression and processing and results in a shorter latency period of mammary tumors in transgenic animals. *Clinical and Experimental Metastasis*, 14 (Supp. 1):64, 1996. Presented at the Sixth International Congress of the Metastasis Research Society, Aula of the University of Gent, Gent, Belgium. Sept., 1996.

Rudolph, L.A. and Matrisian, L.M. Overexpression of human matrilysin results in the aberrant development of the testis and mammary glands and accelerates mammary tumor formation. Presented at Protease and Protease Inhibitors Meeting, Panama City Beach, FL, March 1996.

Rudolph, L.A. and Matrisian, L.M. Alterations resulting from the overexpression of the matrix metalloproteinase matrilysin in the murine mammary gland. Presented at the Mammary Gland Biology Gordon Conference, New London, NH, June 1995.

REFERENCES

1. C. T. Guy, *et al, Proc. Natl. Acad. Sci. USA* **89**, 10578 (1992).

2. D. J. Slamon, *et al, Science* **235**, 177 (1987).

3. J. M. Varley, J. E. Swallow, W. J. Brammar, J. L. Wittaker, R. A. Walker, *Oncogene* **1**, 423 (1987).

4. Y. Matsui, S. A. Halter, J. T. Holt, B. L. M. Hogan, R. J. Coffey, *Cell* **61**, 1147 (1990).

5. A. J. H. Gearing, *et al, Nature* **370**, 555 (1994).

6. M. Lanzrein, O. Garred, S. Olsnes, K. Sandvig, *Biochemical Journal* **310**, 285 (1995).

7. K. Goishi, *et al, Mol. Biol. Cell* **6**, 967 (1995).

8. M. Vecchi, J. Baulida, G. Carpenter, *Journal of Biological Chemistry* **271**, 18989 (1996).

9. C. T. Guy, R. D. Cardiff, W. J. Muller, *Mol Cell Biol* **12**, 954 (1992).

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

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 9 Jun 98

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCP, 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 the technical report written for Grant DAMD17-94-J-4448. Request the limited distribution statement for Accession Document Number ADB220022 be changed to "Approved for public release; distribution unlimited." This report 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 email: judy_pawlus@ftdetrck-ccmail.army.mil.

FOR THE COMMANDER:

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management