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PRINCIPAL INVESTIGATOR: Lynn M. Matrisian, Ph.D.

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center
Nashville, Tennessee 37232-2301

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The overall goal of this project is to test the hypothesis that the action of growth factors on mammary neoplasia and tumor progression is mediated, at least in part, by their action on the genes for matrix-degrading metalloproteinases (MMPs). This hypothesis will be tested using transgenic mouse model systems of breast cancer. Initial studies involved the generation of transgenic mice expressing the MMP matrilysin under the control of the MMTV promoter/enhancer. One line of transgenic mice demonstrated the induction of an alveolar phenotype in virgin female mice similar to that observed in MMTV-TGFα transgenic mice. These results provide support for the initial hypothesis that TGFα may be affecting mammary gland development by enhancing MMP gene expression, although further confirmation of the results is required. These studies will assist in determining the most effective use of synthetic inhibitors of MMPs as potential therapeutic agents for the treatment of breast cancer.

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INTRODUCTION

The matrix-degrading metalloproteinases (MMPs) have been implicated in tumor progression, invasion, and metastasis by virtue of their ability to degrade extracellular matrices. The genes for these enzymes are regulated both positively and negatively by growth factors. We hypothesize that the action of growth factors on mammary neoplasia and tumor progression is mediated, at least in part, by regulating MMP levels. Specifically, we propose that the action of TGFα on accelerating breast cancer tumor progression, and the ability of TGFβ to repress breast tumor formation, is due to the positive (TGFα) and negative (TGFβ) effects of these growth factors on MMP transcription.

To test this hypothesis, transgenic mouse model systems of breast cancer will be used to evaluate the effect of a single gene on the complex processes of tumor development and progression. Transgenic mice expressing TGFα , TGFβ, or both, under the control of the MMTV promoter/enhancer, will be evaluated for the expression of mammary MMPs matrilysin, stromelysin-1, and stromelysin-3 in normal and tumor tissue. Transgenic mice expressing the tumor-specific MMP matrilysin or the MMP inhibitor TIMP, and matrilysin null mice, will be generated to determine if matrilysin expression is necessary and/or sufficient for mammary tumor progression. Finally, transgenic mice overexpressing the oncogene c-*erb*B2/*neu* will be used as a model of human breast cancer to examine the role of MMPs in c-*erb*B2/*neu* induced carcinogenesis. The results of these studies should provide insights into molecular mechanisms regulating mammary tumor progression and could lead to new therapeutic approaches.

The specific aims of this project are: 1) Examine the expression of matrilysin, stromelysin-1, and stromelysin-3 in transgenic mice overexpressing TGFα and/or TGFβ in the mammary gland under the control of the MMTV promoter. 2) Characterize MMTV-matrilysin transgenic mice for effects of the transgene on mammary gland development and DMBA-induced tumorigenicity. 3) Examine the effects of inhibiting matrilysin levels in mammary tumorgenesis using MMTV-TIMP mice and matrilysin null mice. 4) Examine the effect of MMP alterations and TGFβ on tumorigenicity in MMTV-*neu* transgenic mice.

PROGRESS

The focus of the past year was on generating and characterizing MMTV-matrilysin transgenic mice (specific aim 2). Three separate constructs have been used to express 1) the native, or wild-type matrilysin protein, 2) matrilysin in a constitutively activated form, and 3) catalytically inactive matrilysin. All constructs were made by inserting the matrilysin cDNA into the MMTV-LTR expression vector (Figure 1A). Mutations resulting in a constitutively activated enzyme or catalytically inactive enzyme are indicated in Figure 1B, and the resulting protein had the anticipated response to chemical activation as indicated in Figure 1C. Plasmids containing each transgene were purified and microinjected into FVB fertilized murine eggs. Transgenic mice were identified by Southern blotting, and founder animals mated to establish transgenic lines. At least two lines per construct have been or will be established to control for insertional variation (Figure 1D). The resulting lines are referred to as MMTV-ActMAT (expressing activated protein), MMTV-InMAT (expressing inactive protein), and MMTV-NatMAT (expressing native matrilysin).

Mammary glands were dissected from female transgenic and nontransgenic littermates

at various times during development. Transgene expression was detected in at least one line of mice for each construct (Figure 2). Additional mice are being generated to insure that two lines/construct expressing the transgene are available for these studies. Whole mount preparations of the mammary glands of MMTV-NaMat-3 mice revealed induction of alveolar structures in virgin transgenic mice when compared to nontransgenic littermates (Figure 3). This phenotype resembles that of MMTV-TGFα mice (Matsui et al., 1990), and is a preliminary observation that supports our initial hypothesis that the action of TGFα on mammary tissue is mediated through its action on metalloproteinase expression.

We have initiated mating of these mice with the MMTV-*neu* mice (specific aim 4). We are breeding the mice and will select 25 female progeny expressing the *neu* transgene alone, the matrilysin transgene alone, and the matrilysin and *neu* transgenes, for a total of 75 animals/matrilysin line. We expect tumors to develop in half of the multiparous females harboring the *neu* transgene by approximately 200 days of life (Muller et al., 1988). We have determined that the tumors that develop in the MMTV-*neu* mice do express endogenous matrilysin, so these experiments will determine if tumor growth, invasion, and/or metastasis is accelerated by the introduction of exogenous matrilysin.

We have also initiated the mating of the MMTV-TGFα mice with the matrilysin null mice (specific aim 3). Progeny that are TGFα+/-, matrilysin +/+ and TGFα +/- and matrilysin -/- will be selected and examined for tumors in virgin and multiparous animals. MMTV-TIMP mice have been generated by Dr. Rama Khokha, Univ. of Western Ontario. We will attempt to obtain these mice for the proposed experiments rather than generate identical animals as proposed in specific aim 3.

CONCLUSIONS

The initial results from the native matrilysin transgenic animals (MMTV-NaMat-3) suggest that consequence of overexpression of matrilysin in virgin mammary glands is similar to that observed in the TGFα transgenic mice, providing initial support for our hypothesis that the effects of TGFα may be mediated, at least in part, by its induction of metalloproteinase mRNA. Further characterization of these animals is required as originally proposed.

This phenotype has not been observed in two lines of animals expressing the activated construct. It is possible that this is due to low levels of protein production off this mutated construct, or alterations in secretion as a result of the mutation. These possibilities will be explored. Additional lines of animals expressing the native transgene will be generated to be sure that this is a reproducible phenotype.

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Muller, W.J., Sinn, E., Pattengale, P.K., Wallace, R., and Leder, P. (1988). Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-*neu* oncogene. Cell *54*, 105-115.

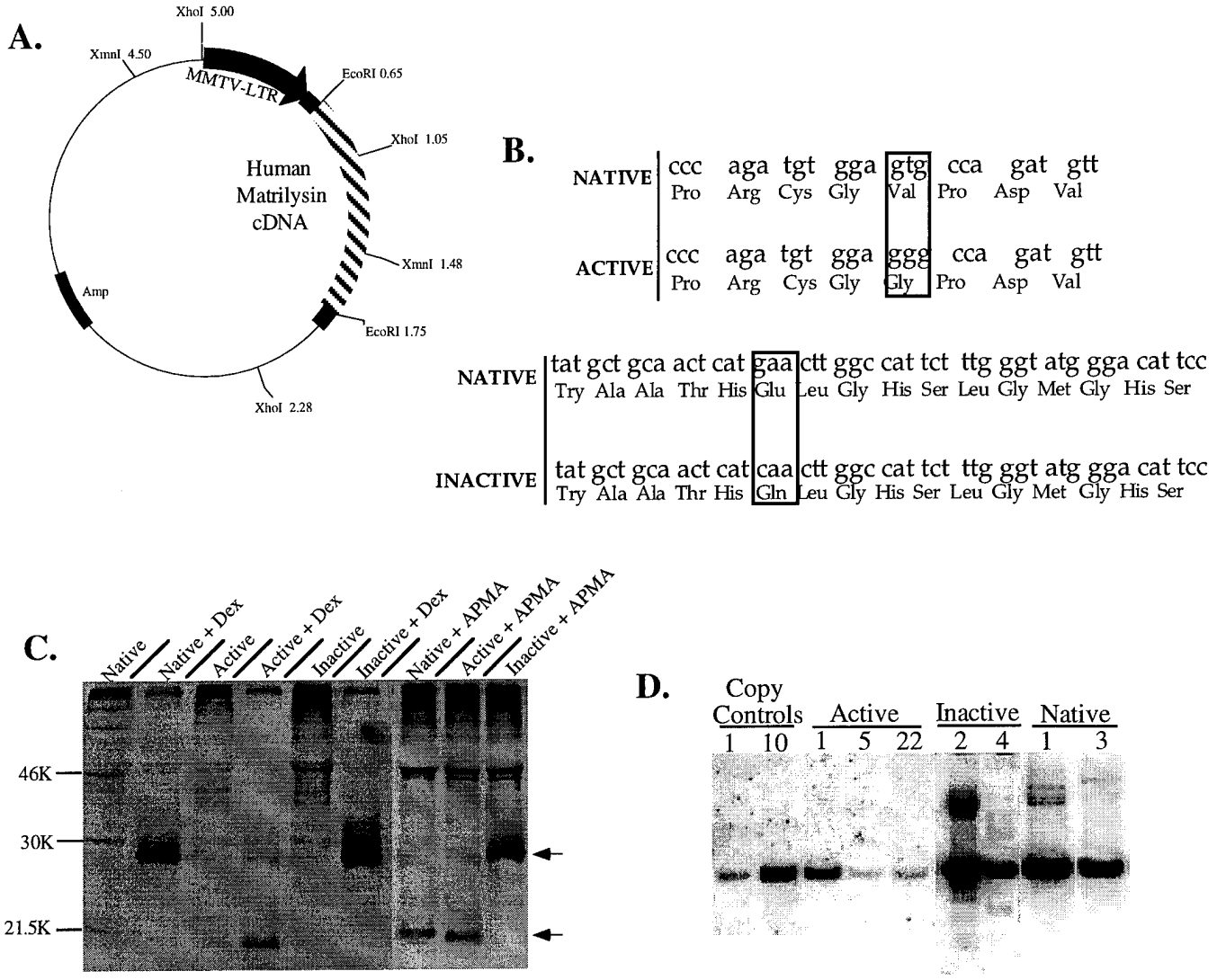

Figure 1: Generation of MMTV-matrilysin transgenic animals. A) Diagram of the plasmid construct utilized for creation of transgenics. Shaded region corresponds to the 1.5kb of the MMTV-LTR. The filled regions correspond to the rabbit b-globin gene. Three forms (inactive, active, and native) of the human matrilysin cDNA was inserted into the third exon of the b-globin gene. The open region corresponds to the expression vector pKCR sequence, with the gene for ampicillin resistance indicated. B) Native, active, and inactive matrilysin cDNA sequence and corresponding amino acids. Boxed areas indicate the position of the nucleotide mutation and amino acid substitution. C) Immunoprecipitation of the matrilysin protein. The breast cancer cell line Hs578t was transiently transfected with each MMTV-matrilysin construct, and the matrilysin protein immunoprecipitated from the conditioned media as indicated by the arrows. The active construct exhibits an increased tendency to undergo spontaneous conversion to the lower molecular weight mature form. Addition of 1μM of dexamethasone to the culture media had an inductive affect on all the MMTV-matrilysin constructs. Addition of APMA, a known MMP activator, to the condition media converted only the native and active forms to the lower molecular weight mature forms indicating that the inactive mutation prevents the enzyme from becoming cleaved and therefore fully activated. D) MMTV-matrilysin transgenic lines. Southern hybridization of 10μg of genomic DNA from founder animals, probed with a 1.1kb full length 32P-labelled human matrilysin cDNA probe.

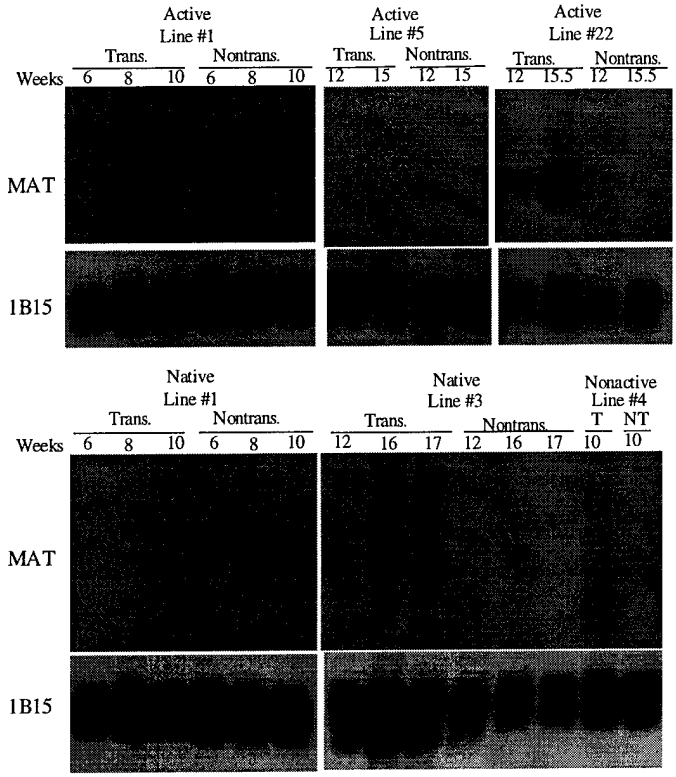

Figure 2: Expression of the human matrilysin transgene in developing mammary tissue. Northern analysis of poly A+ selected RNA (4μg) from nontransgenic and transgenic female mammary tissue at various weeks during mammary development. To identify the human matrilysin transgene (MAT), blots were probed with a 1.1kb full length 32P-labelled human matrilysin cDNA probe. Cyclophilin (1B15) cDNA probe was used to control for equal loading of the RNA.

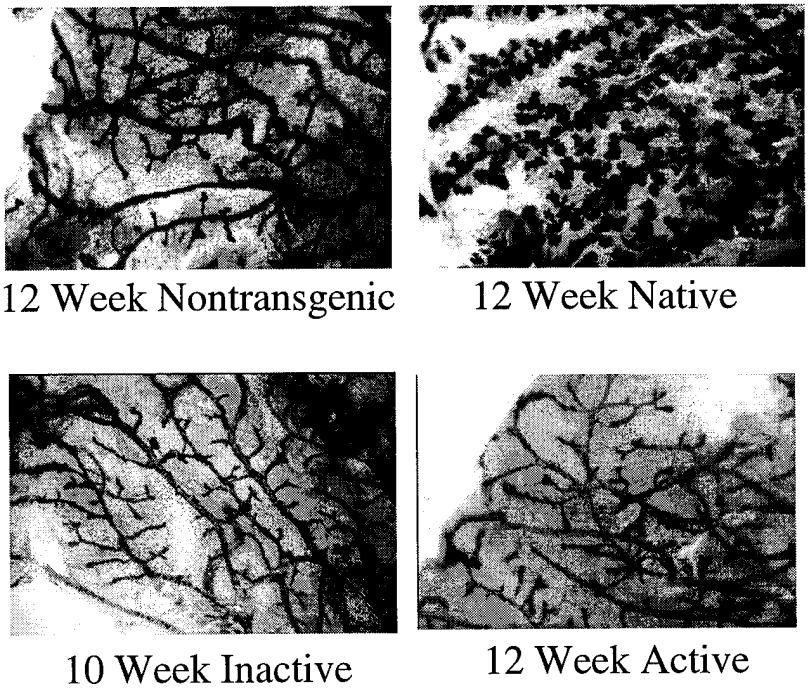

Figure 3: Morphological appearance of transgenic juvenile mammary glands. Whole mount preparations of mammary glands from 12 week native and active and 10 week inactive transgenics and littermate controls.

ABSTRACT

Presented at the Mammary Gland Gordon Conference, June, 1995

Alterations resulting from the overexpression of the matrix metalloproteinase matrilysin in the murine mammary gland.

Laura A. Rudolph and Lynn M. Matrisian
Vanderbilt University Medical Center
Department of Cell Biology
Nashville, TN

Cell-matrix interactions are an important aspect to many biological processes, including mammary gland growth and development. Proteins that degrade and remodel the extracellular matrix, such as matrix metalloproteinases (MMPs), clearly play a role in the interactions that occur within the mammary gland environment. MMPs are a family of secreted proteases thought to be responsible for normal extracellular matrix (ECM) remodeling. The expression and the activity of the MMPs are, in general, tightly controlled and restricted to specific processes such as mammary gland involution, endometrial cycling, and wound healing. Mis-regulation of MMPs is associated with several pathological conditions involving connective tissue breakdown, including arthritis, periodontal disease, and tumor invasion and metastasis.

All of the MMP family members, with the exception of matrilysin, have been shown to be expressed primarily in connective tissues. We have been particularly interested in matrilysin because of its normal expression in the epithelial cells of the murine small intestine and uterus, as well as its expression in adenocarcinomas of human prostate, colon, and breast. Several studies have indicated that matrilysin transcripts are found in early neoplastic tissues, whereas many other MMP family members appear at later stages of tumor onset. We therefore propose that the mis-expression of matrilysin in tumor cells may contribute to the progression, invasion, or metastasis of these cells.

Because of the epithelial localization of matrilysin expression in normal and malignant tissues, we were interested in studying the effects due to the overproduction of matrilysin on mammary growth and development as well as in pathological conditions leading to mammary malignancies. In order to study these processes, we have developed three different transgenic mice lines overexpressing distinct forms of the human matrilysin protein in mammary gland epithelial cells: 1) a normal, or wildtype protein, 2) a constitutively activated protein, and 3) a non-activatable matrilysin protein. The constitutively active construct contains a mutation that results in spontaneous activation of the enzyme, and is therefore not dependent on activation by exogenous factors. A comparison of the result of the wildtype and activated matrilysin constructs will give us an indication of the availability of activators of matrilysin in the mammary environment. The non-activatable transgene lacks proteolytic activity. The use of this mutant will determine if any observed effect of matrilysin in this model system is due to its proteolytic activity.

Preliminary results have shown a potential alteration in the branching patterns during mammary gland development in animals bearing the human matrilysin transgene. We are currently further investigating these branching modifications during mammary development, pregnancy and involution. In addition, we have obtained the MMTV-neu transgenic animals and plan to mate these animals with our MMTV-matrilysin animals. The MMTV-neu female mice have previously been shown to develop spontaneous malignant and metastatic mammary tumors at approximately 4 months of age. We will determine if the MMTV-neu/matrilysin double transgenic animals have altered tumor induction and/or progression when compared to the MMTV-neu animals to test the role of matrilysin in mammary tumor progression.