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The object of this proposal is to understand the tumor suppressor function of maspin in prostate, and to explore maspin's role in normal prostate development. Maspin transgenic mouse model will be employed to study the effects of maspin overexpression on mouse prostate tumorigenesis and metastasis. Prostatic tumorigenesis and normal prostate development will be studied using a variety of established techniques, including organ culture, histopathology, and molecular biology.

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FOREWORD

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Zesheng Liu, Ph.D. 6/30/03

PI - Signature Date

6/27/03

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Introduction

Tumor growth and metastasis is a complex pathophysiological process comprising of various interactions between tumor cells and the host. A significant approach in understanding tumor invasion and metastasis is by identifying the genes involved in tumor growth and suppression, and investigating their molecular mechanisms and eventually in their role as therapeutic agent, if any.

Cancer progression is facilitated by local proteolysis of the basement membrane, by proteases secreted by the tumor cells or bound to its surface, and several of these proteases are regulated by protease inhibitors. Maspin is a tumor suppressor gene related to the serine protease inhibitor (serpin) family. It is expressed in normal prostate epithelial cells, but is down regulated as normal prostate cells progress from a pre-neoplastic to an invasive state. This down regulation is mediated *in vitro* at the transcriptional level through cis-acting elements and trans-acting factors in the maspin promoter[i]. Further, recombinant maspin is shown to inhibit prostate tumor-induced growth and neovascularization in a xenograft nude mouse model and inhibit tumor invasion and motility of prostatic cell lines *in vitro*.

This proposal aims to investigate the tumor suppressor function of maspin *in vivo,* and thereby as a novel molecular target in the treatment of prostate cancers. The specific aims for this two-year proposal are:

Specific Aim 1. To investigate the effect of maspin in a syngeneic xenograft mouse model of prostate cancer.
Specific Aim 2. To establish a transgenic mouse overexpressing maspin in the prostate to study the role of gain of function of maspin in prostate cancer.

Objective 1: To generate a bitransgenic mice expressing maspin in the prostate in the context of an inducible system

Objective 2: To cross the bigenic maspin mice with TRAMP mice to investigate the tumor suppressor function of maspin *in vivo*.

Body

Materials and methods

Animals

Syngeneic C57BL/6 mice (for implantation of TRAMP tumor cells) were purchased from Harlan, Inc. All animals were maintained within the PI's animal facility at Baylor.
TRAMP cell lines were obtained from Dr. Norm Greenberg at Baylor College of Medicine.

Antibodies

Polyclonal anti-maspin antibody was made by Zymed, Inc. as a custom service. All secondary antibodies were purchased from Zymed, Inc.

Northern and Western analysis

RNAs and proteins were isolated from cells, prostate tissues. Total RNAs were isolated using Gibco/BRL Trizol regent. For northern blot, roughly 20 ug RNA will be loaded each lane. For Western blot analysis, protein extracts were prepared by lysing the cells in non-ionic detergent-containing buffers. Total 100 ug protein extract will be loaded for electrophoresis.

Immunohistochemical analysis

Prostate tissues were removed from male mice and dissected. Tissues were fixed in 10% neutral formalin buffer and embedded in paraffin and sectioned at 5 µm. For maspin immunostaining, tissues were boiled in citrate buffer (Zymed, Inc.) for ten minutes for antigen retrival. The antibody was produced in rabbit against a fifteen amino acid peptide located in the reactive site loop of maspin, a region designated as AbS4A. The antibody was purified using an AbS4A sulfo-linked affinity column (Sulfolink kit, Pierce, IL). The sections were stained with the affinity purified maspin antibody at a dilution of 1:400, followed by a secondary goat anti-rabbit antibody staining, and the color was developed by Zymed's AEC (3-Amino-9-Ethylcarbazole) chromogen kit. For specific peptide blocking, a concentration of 10 nM of AbS4A peptide was preincubated with antibody for thirty minutes at room temperature. For proliferating cell nuclear antigen (PCNA) staining, a PCNA staining kit was purchased from Zymed (Zymed, Inc., CA) and slides were stained following the instruction of the kit.

Results and Discussion

Task 1. Examination of maspin as a tumor suppressor in prostate.

We have overexpressed maspin in TRAMP (TRansgenic Animal Model of Prostate cancer) prostate tumor cells by retrovirus infection. Retroviral stable transfectants were selected. We used a C2N mouse prostate tumor cell line that was initially isolated from TRAMP tumors in Dr. Norman Greenburg's laboratory. Parental C2N cells and maspin stable cells were injected subcutaneously into C57BL/6 mice. However, none of the maspin transfectants developed into any palpable tumors in mice. Almost all of C2N cells inoculated developed into palpable tumors. However, none of the maspin stable clones have developed any tumors after extended time of observation, demonstrating those maspin functions to inhibit prostate tumor in vivo. When both C2N and C2N-maspin clones were cultured in vitro, both cells grew rapidly in the presence of 5% fetal bovine serum medium. In 3D spheroid culture, both cells grew into similar size of spheres. Thus, the reason for the tumor inhibition in maspin clones in vivo may be due to the inhibition of angiogenesis. Therefore, we collected the condition medium from C2N-maspin cells and carried out western blot analysis with maspin antibody. Our data showed that C2N-maspin medium contained detectable maspin protein. Using the collected conditional medium we showed that endothelial cell migration was significantly inhibited.

Task 2. To generate a bitransgenic mice expressing maspin in the prostate in the context of an inducible system

We have made a DNA construct to target maspin overexpression in prostate. This construct uses a actin promoter to direct beta-geo gene expression. This beta-geo is floxed by LoxP sites

(locus of X-over of P1, which is the 34-bp on the P1 genome recognized by bacteriophage P1Cre protein (cyclization recombination)). Mouse maspin is ligated to the construct following the LoxP sites. In the absence of cre recombinase, only beta-geo is expressed. However, when cre is targeted by a prostate specific promoter, such as the androgen responsive region (ARR2) promoter (developed by Bob Matusik), maspin will be turned on in prostate tissue. Major progresses have been made in the generation of such tissue specific transgenic mice. Firstly, we have made the DNA construct and injected the construct for the generation of transgenic mice. The transgenic mice were born and preliminary data showed that beta-geo was expressed in the tissue of prostate. Secondly, through a collaboration with Dr. Feng Wang at Texas A& M University, we have obtained a strain of ARR2-cre mice. This strain has been crossed with ROSA26 reporter line to demonstrate the prostate specific expression of cre recombinase. ROSA26 reporter line is a transgenic mouse strain that upon cre cleavage expresses beta-galactosidase (beta-gal). We will breed our floxed beta-geo-maspin transgenic mice with Dr. Wang's ARR2-cre to generate bitransgenic mice in the near future. The goal of this specific aim is to study the effect of maspin overexpression on prostate development and eventually on tumorigenesis.

Key research accomplishments

Clones of prostate tumor cells without (C2N) and with (C2NM) maspin expression grew equally well in liquid and semisolid culture media, but only the C2N cells developed into palpable tumors in C57BL/6 mice. Condition medium from C2N-maspin cells were collected for western blot analysis with maspin antibody. Our data showed that C2N-maspin medium contained detectable level of maspin protein. Using the collected conditional medium we showed that endothelial cell migration was significantly inhibited. These data indicate that maspin prevents tumor angiogenesis in mice.

Reportable outcome

Products

Animal models developed

- Bitransgenic mice were developed to express maspin and the beta-geo marker.
- Bitransgenic mice were developed to express the ROSA26 reporter and thereby demonstrate expression of cre recombinase by ARR2-cre mice.

We are currently focusing to study the effect of maspin overexpression on prostate development and the transgenic mice will be crossed with prostate oncogenic mice to examine the role of maspin tumorigenesis.

Conclusion

Two tasks proposed in the grant were initiated in the first year of proposal. Key reagents have been obtained and transgenic mice have been developed. Continuation of the tasks in the next few years will help us understand the role of maspin in prostate development and tumor metastasis, and hopefully leading to the development of new therapies for the treatment of prostate cancer.

Reference

None

Appendices

None