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14. ABSTRACT Tissue Factor (TF) is the cell surface receptor that activates coagulation by binding the serine protease coagulation factor VIIa (VIIa). The activation of the coagulation cascade leads to thrombin generation, fibrin formation and platelet activation which together may aide tumor growth and metastasis. While the role of TF in metastasis through thrombin pathways is well established, evidence is increasing that TF may drive tumor development dependent on cell signaling pathways that involve either the cytoplasmic domain or proteolytic activation of protease activated receptors by TF associated proteases. A newly developed breast cancer model with a tetracycline regulated TF expression-cassette shows that TF enhances breast cancer tumor growth. This model will be useful to study mechanisms by which TF enhances breast cancer progression. Transgenic models are ongoing to test whether the TF cytoplasmic domain overall supports of suppresses breast cancer progression.					
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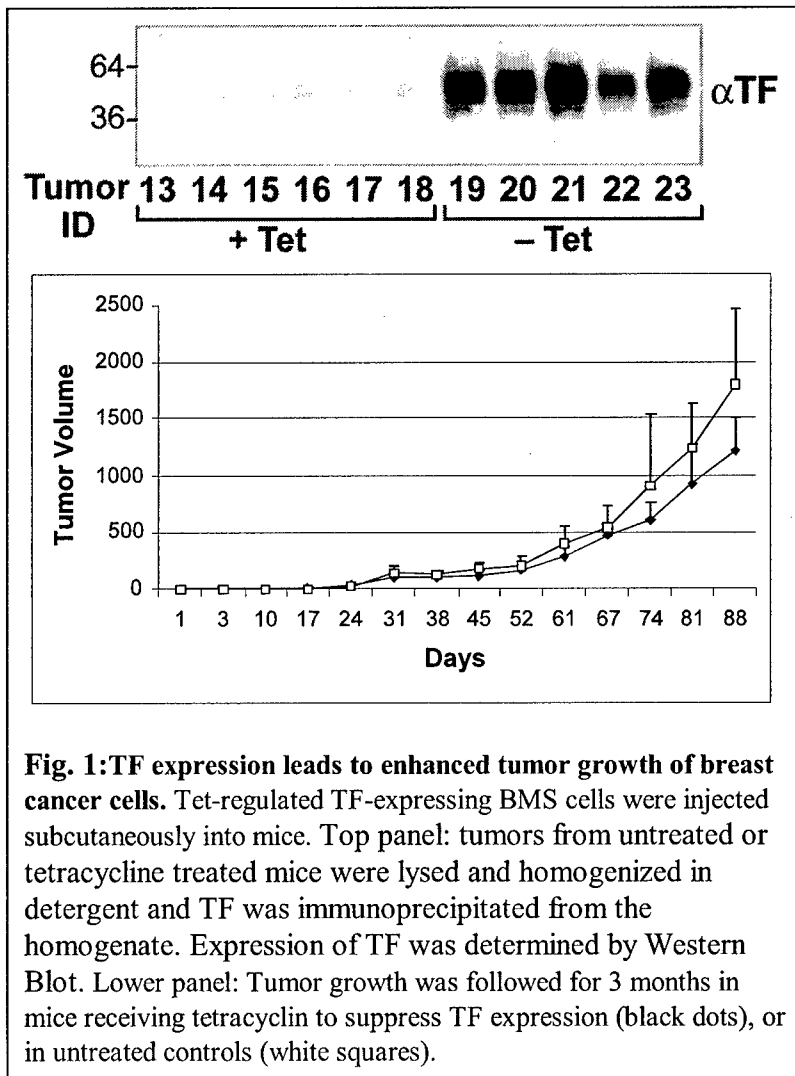
Introduction

Tissue Factor (TF) is the cell surface receptor that activates coagulation by binding the serine protease coagulation factor VIIa (VIIa). The activation of the coagulation cascade leads to thrombin generation, fibrin formation and platelet activation which together may aid tumor growth and metastasis. While the role of TF in metastasis through thrombin pathways is well established, evidence is increasing that TF may drive tumor development dependent on cell signaling pathways that dependent either on the cytoplasmic domain or proteolytic activation of protease activated receptors by TF associated proteases. This grant specifically addresses the question whether the TF cytoplasmic domain acts as a brake of breast cancer progression by transfection studies as well as spontaneous tumor development in transgenic animals.

Body

This application has two specific aims. Aim 1 is to analyze the role of the TF cytoplasmic domain by transfecting TF negative breast cancer cells.

Preliminary data with melanoma cells showed that transfection with full-length, but not cytoplasmic domain deleted TF suppressed tumor growth, indicating a regulatory role of the TF cytoplasmic domain in certain tumor cells. In response to the suggestions of the review committees, we identified TF negative breast cancer cells and transfected these cells with human TF under the control of tetracycline regulated promoters. Tumors cells were injected subcutaneously into immunodeficient Scid/Scid mice.



Mice were treated with or without tetracycline in their drinking water. Tetracycline administration lead to the expected loss of human TF expression in the tumors. Contrary to the melanoma model, TF expression enhanced tumor growth of breast cancer cells ($p < 0.05$ using a two-tailed t-test). We could not detect phosphorylation of the TF cytoplasmic domain in these tumors and could not establish whether phosphorylation of TF turned off suppressive functions of the TF cytoplasmic domain. Remaining tasks on Aim 1 were to test whether overexpression of WW-domains can release suppression of tumor growth by the TF cytoplasmic domain. The breast cancer tumor models indicates that the TF cytoplasmic domain may not suppress tumor growth in breast cancer and the originally proposed experiments to release suppression are considered not feasible. In conclusion, we established a breast cancer model that is suitable to study TF enhanced breast cancer growth.

Aim 2 is to generate tumor prone animals that lack the TF cytoplasmic domain. The first strategy was to cross hormone regulated C3-TAg mice with TF cytoplasmic domain deleted ($TF^{ACD\Delta CD}$) mice. As proposed, we generated 56 mutant and 40 littermate-derived wild-type control mice that are transgene carriers and are following the cohort. 15 and 5 mice, respectively, have died prematurely at an average age of ~ 7 months and the oldest mouse in the colony is currently 9 months of age. Survival is thus longer than described in the literature for the same transgene on the FVB/N background. Most mice that died showed signs of wasting with progressive weight loss, but we have not found palpable tumors in the mammary glands of these mice. We are addressing this inconsistency with published phenotypes of the same transgene on different genetic backgrounds by (1) obtaining routine pathology of 3 wasting mice for the development of tumors in other organs and possible metastatic disease; (2) confirming transgene expression in the mammary gland; and (3) analyzing whether male mice develop the expected adenocarcinomas of the prostate that is described for this transgene. These experiments will provide insight into the utility of this model for breast cancer research.

The grant proposed to generate an alternative breast cancer model using a cross with FVB/N-TgN(MMTVneu)202Mul mice. In considering the difficulties to control for strain effects resulting from the cross of the FVB strain with $TF^{ACD\Delta CD}$ in the C57BL/6 background, we decided to follow an alternative strategy. PyMT mice became recently available to us in a pure C57BL/6 genetic background. Breast cancer development in this model has been documented and the breast cancer pathology of polyoma middle T transgenic animals mirrors the stages of human breast cancer progression. The crosses are ongoing and a cohort of this cross instead of the originally proposed strain will be followed for the remaining funding period of this grant.

Key Research Accomplishments

- Demonstrated enhanced tumor growth upon TF expression in breast cancer cells
- Generated $TF^{ACD\Delta CD}$ /C3-TAg cohort and identified a strain specific shift in pathology with animals expressing the C3-TAg transgene.

Reportable Outcomes

none

Conclusions

We have identified a potential strain specific reduction in breast cancer development in C3-TAg mice. Alternative tumor models are used to establish the role of the TF cytoplasmic domain in breast cancer progression.