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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Crucial steps in tumor progression and the process of metastasis, e.g. tumor growth, invasion through extracellular matrices and angiogenesis, involve proteolytic modification of the pericellular matrix surrounding tumor cells. A major class of proteases involved in these processes is the matrix metalloproteinases (MMPs), and inhibition of MMPs prevents progression and metastasis of several tumor types, including human breast carcinomas, in animal models. In vivo, tumor MMPs are often produced by stromal cells associated with tumors as well as the tumor cells. The tumor cell surface glycoprotein, EMMPRIN, stimulates MMP production by fibroblasts and endothelial cells, and may be an important regulator of MMP production during tumorigenesis in vivo. However no direct evidence for its role in tumor progression had been published prior to this study. The focus of this proposal has been to demonstrate directly whether or not EMMPRIN promotes breast cancer progression, whether a role for EMMPRIN in tumor progression may be to promote or induce angiogenesis, and whether approaches can be developed that may have future therapeutic potential. In this addendum we have shown that increased expression of EMMPRIN also induces MMP production and increased invasiveness in phenotypically normal human mammary epithelial cells. Thus this study has shown that EMMPRIN promotes tumor growth and invasion and that interference with the action of EMMPRIN may be an effective way to retard breast carcinoma progression in patients.

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INTRODUCTION

Crucial steps in tumor progression and the process of metastasis, e.g. tumor growth, invasion through extracellular matrices and angiogenesis, involve proteolytic modification of the pericellular matrix surrounding tumor cells. A major class of proteases involved in these processes is the matrix metalloproteinases (MMPs), and inhibition of MMPs prevents progression and metastasis of several tumor types, including human breast carcinomas, in animal models. In vivo, tumor MMPs are often produced by stromal cells associated with tumors as well as the tumor cells themselves. The tumor cell surface glycoprotein, EMMPRIN, stimulates MMP production by fibroblasts and endothelial cells, and may be an important regulator of MMP production during tumorigenesis in vivo. We recently published direct evidence for an important role for EMMPRIN in tumor progression (Zucker et al., 2001). The focus of this addendum has been to examine the effect of up-regulation of emmprin on invasiveness and MMP production in epithelial cells themselves rather than on stromal cell MMP production. This study adds to our knowledge of the role of EMMPRIN in cancer and may constitute a newly discovered aspect of breast carcinoma progression. Interference with EMMPRIN action may then be an effective way to retard breast carcinoma progression in patients.

Abbreviations used: EMMPRIN, extracellular matrix metalloproteinase inducer; MMP, matrix metalloproteinase; MMP-2, gelatinase A; MMP-9, gelatinase B; MT-MMP, membrane-type MMP.

BODY

The work performed during the extension period is an outcome of our previously revised **Statement of Work, Task #2:** To explore the use of recombinant adenoviral constructs for efficient delivery.

Other work has shown that EMMPRIN on the surface of tumor cells stimulates MMP production by fibroblasts (Guo et al., 1997) and endothelial cells (Caudroy et al., 2002). We have recently found that increased expression of EMMPRIN in tumor cells also stimulates MMP production in the tumor cells themselves (Zucker et al., 2001). We have now explored this phenomenon more extensively, using recombinant EMMPRIN adenovirus infection to stimulate EMMPRIN expression in normal epithelial cells, specifically MCF-10A human mammary epithelial cells.

First we showed that increased expression of EMMPRIN in MCF-10A cells caused an increase in production of MMP-2, compared to cells treated with the control recombinant β-galactosidase adenovirus (Fig. 1A). Since membrane-type MMP-1 (MT-MMP-1) is required for activation of MMP-2, we also examined its expression and found that it also increased compared to cells treated with the control recombinant β-galactosidase adenovirus (Fig. 1B). In addition we examined a variety of other MMPs involved in tumor cell invasion, namely MMP-1, MMP-3, MMP-7, MMP-9, MT2-MMP, MT3-MMP, MT4-MMP, and MT5-MMP, but found no increase in their expression.

Next, we examined whether increased expression of EMMPRIN affected invasiveness of the normal MCF-10A mammary epithelial cells, employing a Matrigel invasion chamber assay. We found that increased EMMPRIN expression caused increased invasion in this system, compared to cells treated with the control recombinant β-galactosidase adenovirus (Fig. 2). We also found that treatment with an MMP2/9 inhibitor reduced EMMPRIN-enhanced invasion to control levels (Fig. 2).

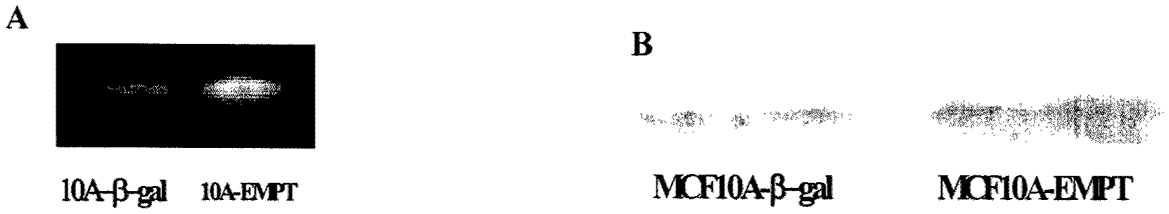

Figure 1. Effect of elevated EMMPRIN levels on MMP-2 and MT1-MMP production. EMMPRIN expression was increased in MCF10A human mammary epithelial cells via recombinant adenoviral infection.

A. Gelatin zymography of conditioned media from MCF10A cells infected with control recombinant β-galactosidase adenovirus (10A-β-gal) or with recombinant EMMPRIN adenovirus (10A-EMPT).

B. Western analysis of conditioned media from MCF10A cells infected with control recombinant β-galactosidase adenovirus (MCF10A-β-gal) or with recombinant EMMPRIN adenovirus (MCF10A-EMPT).

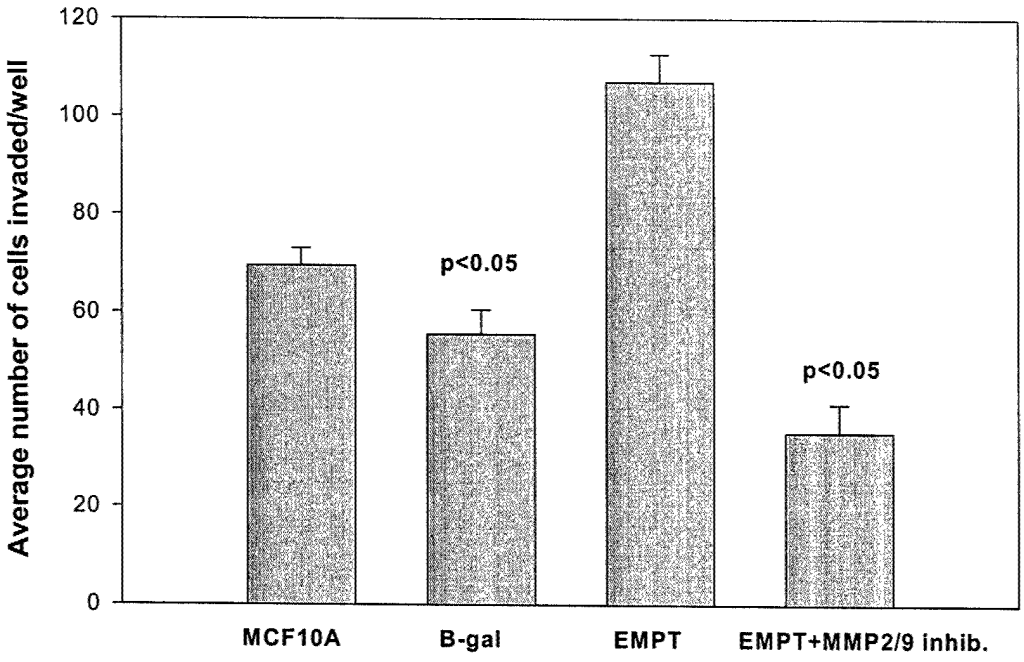

Figure 2. Effect of elevated EMMPRIN expression on cell invasion. EMMPRIN expression was increased in MCF10A human mammary epithelial cells via adenoviral infection. The cells were placed in Matrigel invasion chambers, allowed to invade for 24 hours, then invaded cells were counted. MCF 10A, untreated cells; B-gal, treated with control β-galactosidase adenovirus; EMPT, treated with recombinant EMMPRIN adenovirus; treated with recombinant EMMPRIN adenovirus + inhibitor of MMP-2 and MMP-9 activity.

KEY RESEARCH ACCOMPLISHMENTS

1) Demonstration that increased expression of EMMPRIN in normal epithelial cells induces increased production of specific MMPs, MMP-2 and MT1-MMP2.
2) Demonstration that increased expression of EMMPRIN in normal epithelial cells induces increased invasiveness.

REPORTABLE OUTCOMES

Thesis completed:

Ph.D. completed by Erica Marieb: "Effects of increased expression of a matrix metalloproteinase inducer, EMMPRIN, on malignant cell characteristics."

CONCLUSIONS

We conclude from the above work that EMMPRIN not only promotes mammary carcinoma progression via stimulation of MMP production in tumor stromal cells but also in the tumor cells themselves. In addition EMMPRIN induces invasive properties in normal epithelial cells. Thus inhibition of EMMPRIN action may be useful therapeutically.

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Zucker, S., Hymowitz, M., Rollo, E.E., Mann, R., Conner, C.E., Cao, J., Foda, H.D., Tompkins, D.C., and Toole, B.P.: Tumorigenic potential of extracellular matrix metalloproteinase inducer (EMMPRIN). Amer. J. Pathol., 158: 1921-1928, 2001.

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