

Short Communication

Tumorigenic Potential of Extracellular Matrix Metalloproteinase Inducer

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Extracellular matrix metalloproteinase inducer (EMMPRIN), a glycoprotein present on the cancer cell plasma membrane, enhances fibroblast synthesis of matrix metalloproteinases (MMPs). The demonstration that peritumoral fibroblasts synthesize most of the MMPs in human tumors rather than the cancer cells themselves has ignited interest in the role of EMMPRIN in tumor dissemination. In this report we have demonstrated a role for EMMPRIN in cancer progression. Human MDA-MB-436 breast cancer cells, which are tumorigenic but slow growing *in vivo*, were transfected with EMMPRIN cDNA and injected orthotopically into mammary tissue of female NCr nu/nu mice. Green fluorescent protein was used to visualize metastases. In three experiments, breast cancer cell clones transfected with EMMPRIN cDNA were considerably more tumorigenic and invasive than plasmid-transfected cancer cells. Increased gelatinase A and gelatinase B expression (demonstrated by *in situ* hybridization and gelatin substrate zymography) was demonstrated in EMMPRIN-enhanced tumors. In contrast to *de novo* breast cancers in humans, human tumors transplanted into mice elicited minimal stromal or inflammatory cell reactions. Based on these experimental studies and our previous demonstration that EMMPRIN is prominently displayed in human cancer tissue, we propose that EMMPRIN plays an important role in cancer progression by increasing synthesis of MMPs. (Am J Pathol 2001, 158:1921–1928)

Extracellular matrix metalloproteinase inducer (EMMPRIN) was originally designated tumor collagenase stimulating factor (TCSF) by Biswas et al¹ after isolation and purification of the 58-kd glycoprotein from the plasma membrane of cancer cells and demonstration of its function in stimulating fibroblast synthesis of collagenase-1 (MMP-1). The subsequent finding that EMMPRIN also induced fibroblast synthesis of gelatinase A (MMP-2) and stromelysin-1 (MMP-3) indicated a more general effect on the production of MMPs.² Recent studies have documented the capacity of recombinant EMMPRIN or EMMPRIN purified from cancer cells to stimulate fibroblast production/secretion of stromelysin-1, collagenase-1, and gelatinase A *in vitro*.^{2,3} After secretion from fibroblasts, collagenase-1 is able to bind to EMMPRIN on the tumor cell surface.⁴ The demonstration by *in situ* hybridization (mRNA localization) that peritumoral fibroblasts synthesize most of the MMPs (collagenases, gelatinases, stromelysins, and membrane type-MMPs) in human tumors rather than the cancer cells themselves has ignited interest in the role of EMMPRIN in tumor dissemination.^{5,6} The association of intense EMMPRIN expression in neoplastic cells within invasive human tumors^{7,8} further supports a role for EMMPRIN in cancer dissemination. These data are consistent with a central function for EMMPRIN in stimulating stromal cell production of MMPs which, after pericellular activation, directly degrade the extracellular matrix.¹

Peptide sequencing and cDNA isolation of EMMPRIN from tumor cells^{1,9} led to the recognition that EMMPRIN is identical to human basigin¹⁰ and M6 antigen,¹¹ proteins of previously unknown function that were identified by other investigators in embryonic and inflammatory tissues. A knockout mouse has been produced in which the murine homologue of basigin/EMMPRIN is lacking.¹² The null mutant is, in most cases, unable to undergo oocyte implantation, presumably due to the requirement for MMPs in this process. It is apparent that although many embryonic and adult tissues express EMMPRIN, the level of EMMPRIN expression and glycosylation in tumors is

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much greater than in corresponding normal tissues.^{7,13-15}

In the current study we have examined the function of EMMPRIN in a cancer model in immunodeficient mice. Human MDA-MB-436 breast cancer cells that are tumorigenic, estrogen independent, and moderately invasive *in vitro*, but slow growing *in vivo*,¹⁶ were transfected with EMMPRIN cDNA and injected orthotopically into the mammary fat pad of nude mice. We took advantage of the observation that the 29-kd green fluorescent protein (GFP) of the jellyfish *Aequoria victoria* retains its fluorescent properties when recombinantly expressed in eukaryotic cells¹⁷ along with EMMPRIN cDNA and can be used as a powerful marker for gene expression and cancer dissemination *in vivo*. Cancer cells transfected with both EMMPRIN cDNA and GFP cDNA were compared with cancer cells transfected with GFP cDNA alone for tumorigenic behavior. The results demonstrated that tumor growth in nude mice was considerably enhanced by EMMPRIN/GFP-transfected breast cancer cells as compared to cells transfected with GFP alone.

Materials and Methods

Reagents

Restriction enzymes were purchased from Stratagene (La Jolla, CA). EMMPRIN was purified from LX-1 lung cancer cells using affinity column chromatography.¹⁸ Monoclonal antibodies to EMMPRIN (clone 1G6.2) were produced in collaboration with Dr. D. Dembro at Chemicon International, Inc. (Temecula, CA). The F4/80 rat anti-mouse macrophage antibody was purchased from Serotec (Raleigh, NC). Thrombin was a kind gift from Dr. J. Jesty. Phorbol 12-myristate-13 acetate (PMA) was purchased from Sigma Chemical Co. (St. Louis, MO).

Cell Lines and Culture Conditions

Human MDA-MB-436 breast cancer cells were maintained in Richter's improved minimal essential medium supplemented with 10% donor calf serum.¹⁶ Immunostaining of MDA-MB-436 cells was performed using a primary mouse monoclonal antibody to EMMPRIN (1G6.2) and a secondary goat anti-mouse IgG (H&L) horseradish peroxidase-labeled antibody (Kirkegaard & Perry Laboratories, Gaithersburg, MD).

Construction of Plasmids and Transfection into Cells

A 1.6-kb cDNA¹ representing the entire EMMPRIN sequence encoding 269 amino acid residues was placed at an *EcoRI* site under the control of the CMV promoter in pcDNA3 (Invitrogen, Carlsbad, CA). To facilitate identification of transfected cells *in vitro* and metastases *in vivo*, GFP (GFPmut1 variant) cDNA (Clontec Laboratory, Inc., Palo Alto, CA) was inserted into the EMMPRIN-containing plasmid. The GFP cDNA, along with a separate upstream

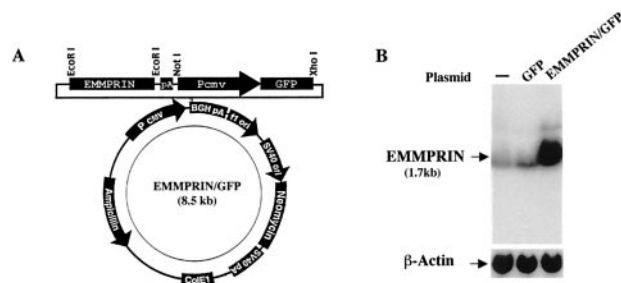


Figure 1. A: Schematic illustration of the EMMPRIN/GFP plasmid. A 1.6-kb cDNA representing the entire EMMPRIN sequence was placed at an *EcoRI* site under the control of the CMV promoter in pcDNA 3. GFP cDNA was inserted along with an upstream CMV promoter into the EMMPRIN expression vector between *NotI* and *XhoI* sites. A polyadenylation (PA) signal was placed downstream. **B:** Northern blot analysis of EMMPRIN. Approximately 20 μ g of total cellular RNA from plasmid alone-transfected, GFP-transfected, and EMMPRIN/GFP-transfected MDA-MB-436 breast cancer cells (from experiment 3) was size fractionated in a 1% denaturing agarose gel, transferred to a nylon membrane, and incubated with 1.7 kb of ³²P-radiolabeled EMMPRIN cDNA as a probe. Blots were analyzed by autoradiography. A single 1.7-kb mRNA transcript corresponding to the known EMMPRIN band was detected at $\sim 20\times$ greater intensity in EMMPRIN/GFP-transfected cells as compared to plasmid alone or GFP-transfected cells.

cytomegalovirus promoter from pEGFP-C1 plasmid (Clontec), was inserted into the EMMPRIN expression vector between *NotI* and *XhoI* sites as shown in Figure 1A. An additional polyadenylation signal from pSG5 (Stratagene) was placed downstream of the *EMMPRIN* gene to provide balanced expression of both recombinant genes under control of CMV promoters. The resulting plasmid was named EMMPRIN/GFP. As a control plasmid, GFP cDNA alone was subcloned into pcDNA3 without EMMPRIN cDNA. In experiment 2, EMMPRIN cDNA was subcloned into pcDNA3 without GFP; the control plasmid was pcDNA3 alone.

The human MDA-MB-436 breast cancer cell line was stably transfected using the calcium phosphate precipitation method.¹⁹ Selected G418-resistant clones were screened by fluorescent appearance using a Nikon microscope equipped with a xenon lamp power supply and a GFP filter set. Fluorescent positive clones were further analyzed by Northern blot analysis probed with an EMMPRIN cDNA fragment.

RNA Isolation and Northern Blot Hybridization

Total RNA was extracted from MDA-MB-436 cells stably transfected with desired plasmids by guanidine solubilization, phenol/chloroform extraction, and serial precipitation.^{1,20} Approximately 20 μ g of total RNA was resolved by denaturing gel electrophoresis followed by Northern transfer to nylon membranes (Schleicher and Schuell, Keene, NH). Blots were hybridized to ³²P-radiolabeled EMMPRIN cDNA (1.7 kb) at 42°C as described²⁰ and analyzed after overnight exposure with an intensity screen at -80°C . The amount of the samples applied to the lanes was normalized by β -actin RNA.

Labeling of RNA Probes

Antisense and sense digoxigenin-labeled RNA probes for EMMPRIN, gelatinase A, and gelatinase B were syn-

thesized by reverse-transcribing 1 μg of cDNA from a polymerase chain reaction that had used gene-specific primers that contain the T7 or T3 phage promoter sequence followed by 20–25 bases of the mRNA sequence.²¹ The probes for human EMMPRIN (bases 319–701), human gelatinase A (bases 42–436), and mouse gelatinase B (bases 56–361) were designed based on published nucleotide sequences (GenBank accession numbers AH007299, J03210, and Z27231, respectively). Homology between the human and mouse nucleotide sequences for gelatinase A and gelatinase B are 91 and 78%, respectively, as determined by BLAST 2 sequence alignment (www.ncbi.nlm.nih.gov/gorf/bl2.html). *In vitro* transcription of the amplified DNA template was performed using the digoxigenin RNA labeling kit (Roche Molecular Biochemicals, Indianapolis, IN). Labeled probes were purified and sequences were verified.

In Situ Hybridization

Serial sections of paraffin-embedded mouse tumors were prepared for *in situ* hybridization according to the method of Komminoth.²² Slides were processed for immunodetection using anti-digoxigenin alkaline phosphatase conjugate antibody (Roche Molecular Biochemicals) and then incubated with substrate solution (Wash and Block Set; Roche Molecular Biochemicals).

Cell Proliferation in Vitro

Cell proliferation assays were performed by plating MDA-MB-436 cells at 4×10^4 cells per well (Costar, Corning, NY) and then switched to serum-free medium. After 48 hours, serum-enriched medium was added again and cells were cultivated for 4 additional days. Cell counts were performed daily.

Tumor Formation in Mice and Preparation of Tissue Extracts

Four-week-old female athymic NCr *nu/nu* mice were obtained from Taconic Farms (Germantown, NY). Cancer cells (1×10^6) were injected into the mammary fat pad of nude mice. Tumor growth was monitored weekly. Tumor volume was calculated using the formula: (length)(width²)/2. At termination of experiments, mice were sacrificed, and autopsied; and tissue sections of the primary tumor, lungs, liver, lymph nodes, gastrointestinal tract, and other suspicious areas were prepared for histological/microscopic examination (hematoxylin and eosin staining of paraffin-embedded sections). Tissue sections were also stored in liquid nitrogen for subsequent *in situ* hybridization (see above) and extraction of MMPs. The extraction procedure for tumor tissue involved detergent and heat-extraction steps.²³

Zymography and Immunohistochemistry

Primary cell cultures were transferred to serum-free medium and cultivated for 18 hours with or without the

addition of thrombin or PMA. Serum-free spent medium was then collected and tested by gelatin zymography. Gelatin substrate zymography was performed in 10% polyacrylamide gels that had been cast in the presence of 0.1% gelatin (NOVEX, San Diego, CA).^{24,25} Protein determinations were made using the bicinchoninic acid reagent (Pierce, Rockford, IL).

Immunohistochemistry for mouse macrophages, monocytes, and dendritic cells was performed using the rat anti-mouse F4/80 antibody (Serotec) as described by Tsuruga et al.²⁶ A biotinylated rabbit anti-rat IgG was used as the secondary antibody. Immunoreactivity was visualized by the avidin-biotin peroxidase complex method (Vectastain ABC kit; Vector Laboratories, Burlingame, CA).

Analysis of variance and Student's *t*-test were used to compare differences between groups in various experiments; $P < 0.05$ was considered significant. Survival experiences between groups were compared by the Wilcoxon χ^2 test.

Results

Cell Transfection and Proliferation

Northern blot analysis using EMMPRIN cDNA as a probe detected ~20-fold enhanced EMMPRIN expression by EMMPRIN/GFP-transfected cells as compared to GFP or non-transfected cells (Figure 1B). Similar results were achieved with each of the three transfected clones used in experiments 1 to 3 (see below). Immunostaining of MDA-MB-436 cells using specific mouse monoclonal antibodies to EMMPRIN documented intense staining of EMMPRIN/GFP-transfected cells and infrequent weak staining of GFP-transfected or vector transfected cells (data not shown).

There were no significant differences in cell doubling times between GFP and EMMPRIN/GFP cDNA-transfected cells (~18 hours) in medium with or without serum. These data are inconsistent with EMMPRIN acting as an autocrine growth factor for tumor cells *in vitro*.

Tumor Growth in Nude Mice

Three independent experiments, each using a different clone of EMMPRIN-transfected MDA-MB-436 cells, were performed. In experiments 1 and 2, the GFP-alone or vector-transfected clones did not form palpable tumors by the time of the experiment's termination at 12 weeks; however, ~0.01-cm³ noninvasive tumors were identified at autopsy in 18 of 18 mice. In contrast, the EMMPRIN/GFP- or EMMPRIN (alone)-transfected clones formed palpable breast tumors at the site of mammary injection by week 6 in 18 of 18 mice which grew progressively to >1.7 cm³ in diameter by week 12, at which time the animals were sacrificed. Histological examination of tissue sections revealed local cancer invasion, but no metastases.

In experiment 3, groups of 10 mice were injected with transfected MDA-MB-436 cells into mammary tissue. The

tumors emanating from the EMMPRIN/GFP cDNA-transfected MDA-MB-436 cells grew relatively rapidly, and all mice expired or had to be sacrificed within 12 weeks (Figure 2A). Extensive metastases to the liver, mediastinum, pleura, spleen, lymph nodes, and mesentery were present in 3 of 10 mice. In contrast, injection of the GFP cDNA-transfected tumor cells into mice resulted in tumors that grew considerably more slowly than EMMPRIN/GFP expressing tumors. Tumor diameter was $<0.3 \text{ cm}^3$ and no metastases were noted at week 15 in 9 of 10 GFP-transfected mice. One mouse in the GFP-transfected group developed a 1.4 cm^3 primary tumor by week 12. EMMPRIN/GFP and GFP expressing tumors (primary tumors and metastases) were readily visible by their expression of green fluorescence when examined grossly with fluorescent light (Figure 2B). The enhancement effect of GFP on tumor visualization has been previously described.²⁷

Gelatinolytic Activity Extracted from Tumor Tissues and Cells

Gelatin zymograms of conditioned medium from 18-hour cultivated MDA-MB-436 tumor cells (Figure 2C, left panel) revealed that cells transfected with EMMPRIN/GFP cDNA secreted more than threefold more progelatinase A (72 kd) than did GFP cDNA-transfected cells. Treatment of both sets of transfected cells with thrombin (20 nmol/L) enhanced both secretion and activation of progelatinase A (more prominently displayed in the EMMPRIN/GFP-transfected cells). Treatment of cells with PMA (100 nmol/L) resulted in the appearance of weak gelatinolytic bands at 92 kd consistent with human progelatinase B.

Extracts of tumors derived from EMMPRIN/GFP cancer cell injections in mice displayed intense gelatinolytic bands localized at 105, 92, 85, 72, and 64–62 kd. Figure 2C (right panel) is representative of both groups of mice in experiment 3; the primary tumors from mice with metastases did not display higher levels of gelatinases than the nonmetastatic group (data not shown). The 105-kd band is consistent with mouse latent gelatinase B; human latent gelatinase B and activated mouse gelatinase B migrates at $\sim 92 \text{ kd}$.²⁸ The 72-kd and 62-kd gelatinolytic bands could represent human or mouse latent and activated gelatinase A, respectively. Tumor extracts from GFP alone-injected mice revealed weaker gelatinolytic bands (with minimal activated gelatinolytic bands) than EMMPRIN/GFP-injected mice.

Histochemistry and in Situ Hybridization

Hematoxylin and eosin staining of resected breast masses revealed extensive replacement of normal mammary tissue with carcinoma in tumors originating from mice injected with EMMPRIN/GFP- or GFP cDNA-transfected MDA-MB-436 cells; other than size of the tumor masses, the EMMPRIN-transfected and vector-transfected tumors were indistinguishable by routine staining. Minimal fibrosis and inflammatory cell infiltration were

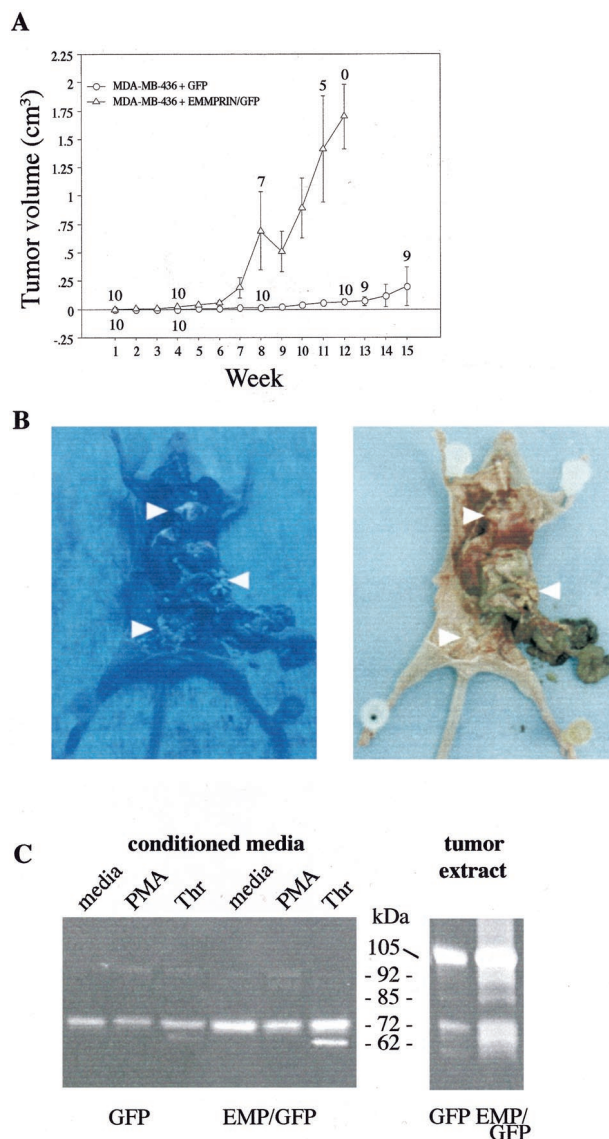


Figure 2. A: MDA-MB-436 breast cancer cells transfected with EMMPRIN/GFP cDNA resulted in enhanced rate of tumor growth after tumor cell implantation into the mammary fat pad of nude mice as compared to GFP-transfected cells. The tumorigenicity of transfected cells was assessed by weekly measurement of tumor size. The data represent the mean \pm SE observed in 10 animals in each group injected with 1×10^6 transfected cancer cells. The numbers associated with each symbol refer to the number of mice alive at each time point (ie, at week 8, three mice in the EMMPRIN/GFP group had large tumors and were sacrificed, hence the number seven is listed). **B:** GFP-transfected tumors are readily visible under fluorescent light. EMMPRIN/GFP-transfected MDA-MB-436 breast cancer cells were injected into the mammary tissue of a female NCr *nu/nu* mouse. Eight weeks later, the mouse was sacrificed and extensive green colored metastatic tumors in the peritoneum, liver, spleen, and mediastinum were visible under fluorescent light (**left** photo). The photo on the right demonstrates the same tumors visualized by bright light (**arrowheads** identify tumors). **C:** Comparison of gelatinases secreted by MDA-MB-436 cells cultivated in serum-free medium and extracts of nude mouse tumors. Spent serum-free conditioned medium from primary cells cultivated for 18 hours with vehicle (medium), PMA, and thrombin (**left**) and tumor cell extracts (**right**) were assessed by gelatin substrate zymography. Protein concentration ($15 \mu\text{g}/\text{well}$) of tissue samples was equalized within each group. Conditioned medium and tumor extracts from the EMMPRIN/GFP group displayed more gelatinolytic activity than did the GFP-alone group of mice. The displayed extract from the GFP-alone tumor is from the largest tumor (1.4 cm^3) in this group of mice. The intensity of tumor gelatinolytic activity demonstrated in each group of mice did not correlate with tumor size (data not shown). Molecular weights were calculated using protein standards. The conditioned medium of HT-1080 cells was used to confirm the molecular weight of human gelatinase A and gelatinase B (data not shown).

noted in tumor tissue and surrounding normal-appearing mammary tissue. The sparsity of inflammatory cells in the tumors was confirmed using an antibody (F4/80) that recognizes mouse macrophages, monocytes, and dendritic cells (data not shown).

In situ hybridization of tumor tissue from six mice injected with EMMPRIN/GFP-transfected cells revealed widely distributed, specific staining for EMMPRIN mRNA in cancer cells (Figure 3, panel 2). Surrounding normal-appearing mammary ductal cells and scattered periductal cells also expressed EMMPRIN mRNA (panel 6). Gelatinase A mRNA was found in both cancer cells (panel 3) and the surrounding non-malignant tissue, including normal-appearing mammary ducts and adipose cells (panel 7). There was specific staining for gelatinase B mRNA in the tumor sections (panel 4), but not as widely distributed as gelatinase A. By counting the number of stained cells in serial sections of EMMPRIN cDNA-transfected tumors, the ratio of cells immunotyped as macrophages (F4/80 antibody) versus gelatinase B mRNA-expressing cells was ~1:70. Intense staining for gelatinase B was also noted in small aggregates of cells (negative staining for mouse macrophages using F4/80 antibody) scattered around normal-appearing ducts (panel 8). Similar *in situ* hybridization results were found on examination of metastatic tumors in the EMMPRIN/GFP-treated mice (data not shown). Similar results were achieved using either human or mouse gelatinase A and gelatinase B mRNA probes. Specific staining was abolished by pretreatment of tissues with RNase (data not shown). No staining was detected in any of the tumor tissues that were hybridized with EMMPRIN, gelatinase A, or gelatinase B sense probes (data not shown).

In the GFP-alone-transfected tumors (seven mice examined), virtually no EMMPRIN, gelatinase A, or gelatinase B mRNA was identified in the tumor cells or in the surrounding normal-appearing mammary tissue (Figure 3, panels 10 to 12 and 14 to 16).

Discussion

The current report describes a direct effect of EMMPRIN expression on tumorigenicity in an animal model. Transfection of EMMPRIN cDNA or EMMPRIN/GFP cDNA into human MDA-MB-436 breast cancer cells resulted in marked enhancement of tumor growth in nude mice after orthotopic injection of tumor cells as compared to injection of vector or GFP alone-transfected tumor cells. High levels of gelatinase A and gelatinase B mRNA expression were demonstrated by *in situ* hybridization in EMMPRIN-transfected tumors as compared to vector- or GFP-transfected tumors. Regardless of the size of the tumors, enhanced gelatinase B and gelatinase A levels were identified in zymograms from extracts of EMMPRIN/GFP-transfected tumors as compared to GFP tumors. The 105-kd gelatinolytic band represents mouse gelatinase B,²⁹ but the 92-kd band could be either activated mouse gelatinase B or latent human gelatinase B.

It is noteworthy that human MDA-MB-436 cells propagated *in vitro* readily secreted progelatinase A, but se-

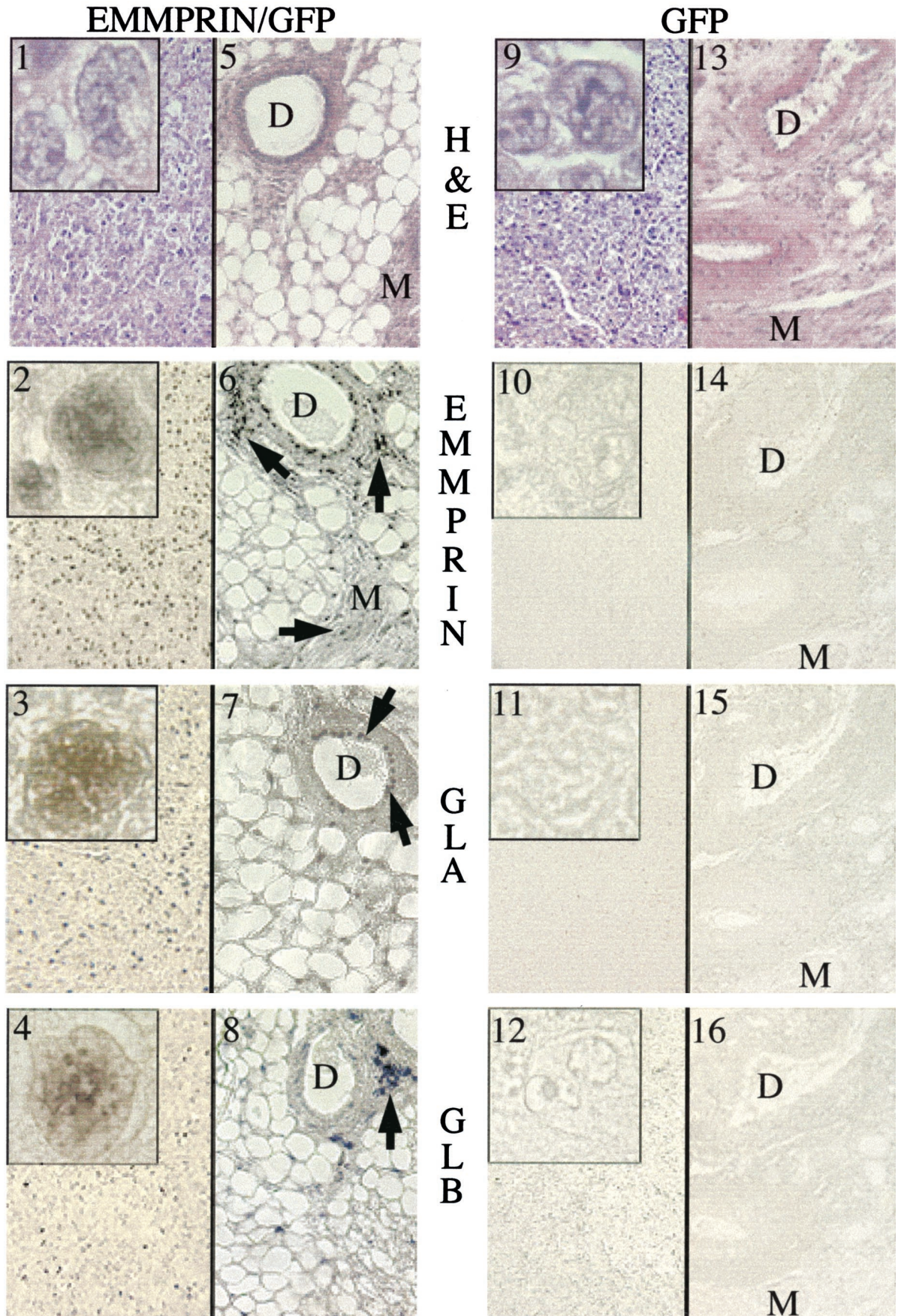
creted minimal gelatinase B, whereas extracts of tumors removed from nude mice injected with these tumor cells contained higher levels of gelatinase B than gelatinase A. Treatment of these breast cancer cells with thrombin and PMA *in vitro* resulted in increased secretion and activation of progelatinase A and progelatinase B, respectively; this is consistent with the stimulatory effects of these agents described with other types of cells.^{25,30} These observations are consistent with the concept that both mouse host cells and transplanted human cancer cells are responsible for the production of gelatinase A and gelatinase B in nude mouse tumors. An association between expression of EMMPRIN and gelatinase B in benign and malignant pigment cell skin lesions in humans has been reported,³¹ but a direct stimulatory effect of EMMPRIN on gelatinase B expression or activation has not been previously described. EMMPRIN expression has also been linked to the activation of progelatinase A through a MT-MMP mechanism.³²

Another important observation in this study was that EMMPRIN-transfected MDA-MB-436 cancer cells secreted higher levels of gelatinase A *in vitro* than vector-transfected cells; this presumably represents autocrine stimulation. These cancer cells also displayed a more invasive phenotype than control transfectants when examined in a modified Boyden chamber (S. Caudroy, M. Polette, B. Nawrocki-Raby, B. Toole, S. Zucker, and P. Birembaut, submitted manuscript).

Our *in situ* hybridization data of tumors transplanted into nude mice differs from *de novo* human breast cancer. Gelatinase A mRNA was identified in EMMPRIN-transfected human cancer cells growing both in nude mice and in surrounding host stromal cells (Figure 3). Previous studies in patients with breast cancer demonstrated the expression of gelatinase A and gelatinase B almost exclusively in peri-tumoral, stromal, and inflammatory cells, respectively.^{6,8,33} However, a few reports have described gelatinase B expression in breast,³⁴ lung,³⁵ and liver carcinoma cells.^{35,36} In comparing experimental cancer models to the human counterpart, it needs to be emphasized that cancer cell lines propagated *in vitro* (eg, MDA-MB-436) that are selected for their invasive properties generally express high levels of gelatinases.³⁷ Furthermore, by comparison to *in situ* human breast cancers,³⁸ transplanted human tumors in nude mice demonstrate sparse inflammatory and fibrotic reactions; this represents an important distinction that is often overlooked. These differences between human and animal models of cancer need to be considered in predicting human responses to novel therapies developed in experimental animal models.

A technical aspect of this study that needs explanation relates to quantitative differences in expression of EMMPRIN, gelatinase A, and gelatinase B using different methodologies (Figures 1B, 2C, and 3). It should be noted that the nonradioactive digoxigenin-labeled RNA probes used in this study provide increased resolution and rapid detection of cellular messages, but are less sensitive than autoradiography³⁹ and substrate zymography.

In one of three sets of experiments, metastasis after orthotopic injection of tumor cells into nude mice oc-



curred more frequently with EMMPRIN-transfected cells than with vector-transfected cells, but the overall rate was low. As we reported previously,⁴⁰ EMMPRIN expression did not affect tumor cell proliferation *in vitro*. Based on the established role of EMMPRIN in enhancing MMP synthesis by stromal cells, it would appear that increased degradation of extracellular matrix permits more rapid tumor growth *in vivo*. The higher rate of tumor growth with EMMPRIN-transfected cancer cells and the associated matrix degradation may also occur by favored neoplastic cell survival in a tissue stroma environment initially not permissive for tumor growth. Enhanced extracellular matrix degradation may also release growth factor-like fragments of matrix components, resulting in an indirect effect on cell proliferation.⁴¹ A role for host-derived MMPs in tumor progression and angiogenesis has been supported by studies in gelatinase A-deficient (knockout) mice.⁴² In contrast to these findings with EMMPRIN, stromelysin-3 (an MMP with minimal proteolytic activity on extracellular matrix proteins) expression in cancer cells promoted tumor take, but not tumor growth in nude mice.⁴³ These studies with EMMPRIN reinforce the notion that cancer dissemination is a multistep process and that extracellular matrix degradation contributes to the process but is insufficient in itself to account for tumor metastasis.⁴⁴ Continued exploration of genes responsible for the metastatic process is warranted.⁴⁵

Acknowledgments

This article is dedicated to the memory of our friend and colleague, Chitra Biswas, whose career was dedicated to the discovery and exploration of EMMPRIN. Dr. Biswas died in August 1993, but her inspiration continues to guide us in our studies of EMMPRIN. We thank Dr. Serge Lyubsky for his contribution to the histopathological studies and Dr. Philippe Birembaut for helpful discussions.

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Figure 3. *In situ* hybridization of primary tumors from mice injected with EMMPRIN/GFP and GFP-transfected MDA-MB-436 breast cancer cells. Serial sections from tumor tissue (panels 1-4, and 9-12) and surrounding nonmalignant tissue (panels 5-8 and 13-16) were examined. Panels 1 and 9 represent H&E staining of cancer tissues from EMMPRIN/GFP and GFP tumors, respectively; panels 5 and 13 represent H&E staining of non malignant mammary tissues (tumor cells not identified) adjacent to the primary EMMPRIN/GFP and GFP tumors, respectively. Cells in the primary tumor mass from mice injected with EMMPRIN/GFP-transfected cells revealed widely distributed, specific staining with EMMPRIN, gelatinase A (GLA), and gelatinase B (GLB) antisense riboprobes (panels 2-4, respectively). Minimal cell staining for EMMPRIN, gelatinase A, and gelatinase B was seen in cancer cells from GFP-transfected MDA-MB-436 cells (panels 10-12). Nonmalignant tissues adjacent to the primary tumors from EMMPRIN/GFP mice demonstrated focal staining for EMMPRIN, gelatinase A, and gelatinase B in mammary ducts (D) and myocytes (M) (panels 6-8: arrows identify EMMPRIN-expressing cells). Nonmalignant tissue from GFP mice revealed no discernible staining for EMMPRIN, gelatinase A, or gelatinase B (panels 14-16). The insets in panels 1-4 display higher magnifications of mRNA-stained tumor cells; prominent nuclear staining is noted. Comparable cells (but minimally stained) in the GFP-transfected tumors are demonstrated in panels 10-12.

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