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Advances in Brief

Regulation of Extracellular Matrix Metalloproteinase Inducer and Matrix Metalloproteinase Expression by Amphiregulin in Transformed Human Breast Epithelial Cells

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Abstract

Amphiregulin (AR) and epidermal growth factor effects on expression and activity of the extracellular matrix metalloproteinase inducer (EMMPRIN) were examined in NS2T2A1 breast tumor cells. Both growth factors induced mRNA and protein expression of EMMPRIN, and matrix metalloproteinase (MMP) -2 and -9 enzymatic activity. The induction of EMMPRIN by AR was mediated by epidermal growth factor receptor (EGFR) tyrosine kinase activation and inhibited by ZD1839. AR and EGFR antisense (AS) cDNAs inhibited EMMPRIN expression and MMP activity. Coculture of NS2T2A1V expressing AR- or EGFR-AS with fibroblasts and endothelial cells showed a decreased MMP activity. In parallel, nude mice tumors derived from AR and EGFR-AS cells revealed reduced level of EMMPRIN and MMP activity. AR and epidermal growth factor, therefore, regulate EMMPRIN and its MMP-mediated expression, identifying EGFR signaling as critical to this regulation.

Introduction

The metastatic process requires the degradation of the extracellular matrix both at the primary tumor site and at the secondary colonization site. This degradation process is dependent on the activity of specific endopeptidases, MMPs,[4] of which >20 species have been identified. These MMPs are expressed by tumor and stromal microenvironment cells including fibroblasts, and endothelial and inflammatory cells. The MMP levels and activity are negatively regulated by direct inhibition through specific tissue inhibitors of MMPs, and positively via proteolytic activation and induction through EMMPRIN/CD147, identified recently as a cell surface inducer of various MMPs, including collagenase 1 (MMP-1), gelatinase A (MMP-2), gelatinase B (MMP-9), and stromelysin 1 (MMP-3). EMMPRIN is a highly glycosylated member of the immunoglobulin super-family molecules expressed on the cell surface of most tumor cells (1, 2), and its expression was reported to correlate with tumor progression and invasion (3, 4). Overexpression of EMMPRIN in human MDA-MB-436 breast cancer cells resulted previously in marked enhancement of tumor growth and metastasis occurrence in nude mice. The latter was associated with high levels of MMP-2 and MMP-9 expression in the developed tumors (5). Accordingly, EMMPRIN was found to be the most frequently expressed protein in primary tumors and in micrometastatic cells isolated from bone marrow of cancer patients (6). In addition, antibodies to EMMPRIN and CD147-Fc fusion protein were shown to block the production and secretion of MMP-2, and inhibit tumor cells invasion *in vitro* (7). EMMPRIN was reported recently to mediate the induction of MMP-2 expression through activation of 5-lipooxygenase and phospholipase A1 activation (8). EGFR was reported to be involved in tumorigenesis and metastasis. The activation of EGFR is mediated through its interaction with its specific ligands, EGF and AR, that share 38% amino acid homology. AR was reported to act as an autocrine growth factor for various human mammary epithelial cells (9, 10) and tissues, and mediate EGF-independent growth in a breast cancer cell line overexpressing EGFR (11). Using the highly tumorigenic cell line NS2T2A1, an SV40 T immortalized human mammary epithelial cell line, we showed previously that stable expression of antisense cDNA of AR reduced tumorigenesis (12) as well as tumor vascular density, affirming the role of AR in tumorigenesis. In the present study, we showed that activation of EGFR by AR induced EMMPRIN expression, suggesting that in addition to their growth factor activity, AR and its receptor EGFR can promote tumor progression by increasing tumor cell invasion and metastasis, through interaction with fibroblasts and endothelial cells, which leads to increased MMP production and matrix degradation.

Materials and Methods

Cell Culture and Transfection. Transformed NS2T2A1 cell line was derived from normal human breast epithelial cells after immortalization by SV40 T antigen and nude mouse tumor selection (13, 14). The cells were maintained in DMEM/F12 (1:1) medium without calcium supplemented with 4 mM glutamine, 10 μg/ml insulin, 5 μg/ml hydrocortisone, 2 ng/ml EGF, and 5% horse serum Ca^{2+} depleted by Chelex 100 (13). At 50–60% of cell confluence, the medium was replaced with growth factor-free DMEM/F12 containing 2% of carbon-dextran-depleted FCS and 4 mM glutamine (M2) for 24 h. The effect of EGF and AR was studied in M2 in all of the studies except for zymography analysis, where cells were first incubated in M2 medium for 24 h and then transferred to serum-free medium (M0) 24 h before treatment. In some experiments, the cells were treated with 10 μM of the specific tyrosine kinase inhibitor of EGFR ZD1839 (Iressa, kind gift from Astra-Zeneca Inc.) for 2 h before the addition of growth factors. Transfection of the NS2T2A1 cells with the 1.1 kb AR cDNA fragment and the 1.8 kb EGFR cDNA resulting in AR antisense RNA expressing AR-AS1 and AR-AS3 cell clones, and EGFR-antisense expressing EGFR-AS cell clone (12, 14), respectively, were described previously. Control cell line NS2T2A1V was obtained by transfection of the parental NS2T2A1 cells with pCEP4M vector. Transfected cells were maintained in culture with 30 mg/liter hygromycin B (Roche, Mannheim, Germany). Human dermal fibroblast cultures, established by explanting tissue specimens obtained from neonatal foreskins, were used in passages 2–3. Cells were cultured in DMEM supplemented with 10% FCS and 2 mM glutamine. The human microvascular endothelial cell line (HMEC-1) was kindly provided by Dr. Thomas J. Lawley (Emory University, Atlanta, GA). These cells

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[4] The abbreviations used are: MMP, matrix metalloproteinase; EMMPRIN, extracellular matrix metalloproteinase inducer; EGFR, epidermal growth factor receptor; EGF, epidermal growth factor; AR, amphiregulin; RT-PCR, reverse transcription-PCR; HMEC, human microdermal endothelial cell; VEGF, vascular endothelial growth factor; mAb, monoclonal antibody; QRT-PCR, quantitative real time PCR.

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were cultured in MCDB-131 medium (Sigma), supplemented with 10% FCS (Life Technologies, Inc.) and 2 mM glutamine.

Animal Studies. NS2T2A1 cells, NS2T2A1V, and AR and EGFR antisense expressing cells (10^7) were suspended in 0.2 ml PBS and injected s.c. into right flanks of 5-week-old female Swiss *nu/nu* mice (Iffa Credo, L'Arbresle, France). After 7 weeks, animals were sacrificed. Tumors were weighed and stored in liquid nitrogen before pathological studies, immunohistochemistry, RNA preparation, protein extraction for zymography, and immunoblotting.

Samples Preparation and Immunoblot Analysis. Cells were lysed in TBS-NP40 solution comprising 50 mM Tris buffer (pH 7.5), 150 mM NaCl, 1% NP40, 2 mM EDTA, and 1:100 protease inhibitor mixture Set V, EDTA free (Calbiochem, La Jolla, Ca). After incubation on ice for 1 h and scrapping, the lysates were centrifuged for 3 min at $15,000 \times g$ at 4°C, and the supernatants were quantified using the Bradford Bio-Rad Protein Assay. Ten-μm cryostat sections from excised tumors were immediately placed in the TBS-NP40 lysing solution containing the protease inhibitor mixture and incubated 1 h at 4°C with rotation. After clarification by centrifugation at $15,000 \times g$ for 5 min, the proteins extracted were quantified for protein content. Twenty μg of protein in Laemmli sample buffer were loaded on a 10% SDS-PAGE gel for electrophoresis, then transferred to nitrocellulose filters. EMMPRIN was detected using 0.2 μg/ml HIM6 (anti-CD147 mAb; Becton Dickinson) overnight at 4°C followed by 1-h incubation with mouse horseradish peroxidase-conjugated antibody (Jackson Laboratory) and visualized with ECL+ reagent (Amersham). Band intensities were quantified using a Scion Image for Windows (Scion Corporation) and normalized relative to actin.

Gelatin Zymography. The presence of the gelatinases MMP-2 and MMP-9 in the serum-free conditioned medium (10 μl) and tumor extracts (2 μg protein; see above for immunoblotting) was analyzed by zymography in 10% polyacrylamide gels containing 1 mg/ml gelatin (Sigma) as described previously (15). The gels were scanned using the Scion computer-based image analysis system.

QRT-PCR. To evaluate the expression levels of EMMPRIN, MMP-2, and MMP-9 normalized to the housekeeping β2 microglobulin gene, real-time quantitative PCR was performed using LightCycler (Roche Diagnostics) according to described techniques. Selected sets of primers and labeled probes (Eurogentec) are shown in Table 1. All of the experiments were performed in duplicate. Standards for EMMPRIN, MMP-2, MMP-9, and β2 microglobulin were prepared from total normal RNA, amplified by RT-PCR, and cloned using TOPO II TA cloning kit (Invitrogen) following the manufacturer's recommendations.

Immunohistochemistry. Cryostat sections (5 μm) were prepared from frozen tumors and fixed in cold acetone for 5 min, then treated for 20 min with 0.6% H_2O_2. The sections were incubated for 45 min in 3% BSA, then for 1 h with anti-EMMPRIN HIM6 mAb at 1:200 dilution, then for 1 h with horseradish peroxidase-conjugated antimouse IgG antibody. Staining was revealed using 3-amino-9-ethyl-carbazole. For the immunocytochemistry, cells were seeded on Lab-Tek chamber-slides, cultured for 2 days, and the medium changed for serum-free conditions for 24 h before 10 nM AR addition for 12 h. Slides were then processed as above.

Statistical Analysis. Data are expressed as mean ± SD. ANOVA and Student's *t* test were used to compare differences between groups in various experiments.

Table 1 *Oligonucleotide primer and probe sequences used*

Gene and oligonucleotide	Location	Sequence
EMMPRIN		
Upper primer	766U	5′-GCAGCGGGCAGCACC-3′
Lower primer	833L	5′-CCACCTGCCTCAGGAAGAGTT-3′
Probe	788U	5′-CAAAGGCAAGAACGTCCGCCAGAG-3′
MMP-2		
Upper primer	380U	5′-CCGTCGCCCATCATCAA-3′
Lower primer	450L	5′-AGGTATTGCACTGCCAACTCTTT-3′
Probe	406U	5′-CGATGTCGCCCCCAAAACGGA-3′
MMP-9		
Upper primer	610U	5′-CATTCAGGGAGACGCCCA-3′
Lower primer	673L	5′-AACCACGACGCCCTTGC-3′
Probe	629U	5′-TTCGACGATGACGAGTTGTGGTCCCT-3′
β2 microglobulin		
Upper primer	20U	5′-CGCTCCGTGGCCTTAGC-3′
Lower primer	86L	5′-GAGTACGCTGGATAGCCTCCA-3′
Probe	39U	5′-TGCTCGCGCTACTCTCTCTTTCTG-3′

Results

AR and EGF Induce EMMPRIN, MMP-2, and MMP-9 Expression in NS2T2A1 Breast Cancer Cells. The NS2T2A1 human breast transformed cell line has been selected after immortalization of normal mammary epithelial cells and two subsequent passages in the nude mouse for selection of highly tumorigenic cells. These cells express AR, EGFR, EMMPRIN, MMP-2, and MMP-9 at low levels. Incubation of NS2T2A1 cells with various concentrations of AR and EGF induced the transcription of both EMMPRIN and the two MMPs (Fig. 1). The mRNA levels of EMMPRIN as measured by QRT-PCR, rose 2–3-fold, 2 h after growth factor addition (Fig. 1, *A* and *B*). A similar but delayed 2-fold rise of MMP-2 and a dramatic 9-fold increase of MMP-9 transcripts occurred after 6 h. These results were confirmed at the protein levels by immunoblotting. As illustrated (Fig. 1*C*), the increase of EMMPRIN protein expression is observed as early as 1 h after addition and reached a maximum at 4 h. The expression of EMMPRIN was AR dose-dependent (0.1–10 nM). In parallel, the activities of MMP-2 and MMP-9, measured in the conditioned medium by gelatin zymography 24 h after the addition of AR, were increased in a dose-dependent manner. (Fig. 1*D*).

The Tyrosine Kinase Inhibitor ZD1839 Reduces EMMPRIN Protein Expression. To assess the importance of EGFR signaling pathway in the mediation of EMMPRIN expression, we used the specific inhibitor of the EGFR tyrosine kinase ZD1839. As illustrated in Fig. 1*D,* the addition of ZD1839 to cells 2 h before growth factor addition mediated 50% reduction of EMMPRIN levels when measured after 2- and 5-h incubation of tumor cells with EGF.

EMMPRIN and MMPs Are Reduced in Cells Expressing AR and EGFR Antisense cDNA. To confirm the role of EGFR and its ligands in the regulation of EMMPRIN, and MMP-2 and MMP-9 expression and activity, we used different cell clones stably transfected with the control empty vector (V cells), antisense cDNA for AR (clones AR-AS1 and AR-AS3), and antisense cDNA for EGFR (EGFR-AS). These clones have been described previously and shown to have a reduced expression of AR (50%) and EGFR (20%), respectively, as compared with control (12, 14). In all of these clonal cell lines, the levels of EMMPRIN transcripts as measured by QRT-PCR were markedly reduced to 20–30% of the control cells. This reduction was confirmed at the protein level on immunoblot with an 85–95% decrease of EMMPRIN expression in AR-AS1 and 3 cells, and 70% in EGFR-AS cells (Fig. 2*A*). The reduction of the MMP inducer was associated with a parallel reduction of both transcription and gelatinase activity for MMP-2 and -9 (Fig. 2*B*). In all of the antisense clones, the reduction of transcripts levels of MMP-2 and -9 reached 80%. Therefore, a very low to undetectable enzyme activity was observed in the antisense expressing cell lines (Fig. 2*B*). Exogenous AR partially restored the levels of EMMPRIN and MMPs in the AR antisense cell clones as observed using immunocytochemistry (Fig. 2*D*).

Transformed Epithelial Cells Regulate MMP Activity of Normal Fibroblast Cells in Coculture Experiments. Because EMMPRIN expressed by the epithelial cells is supposed to act *in vivo* through a paracrine loop to stimulate MMP expression, we cocultured control and antisense-expressing cells with primary cultures of human dermal fibroblasts or HMEC-1 (ratio of cells 1:1). Under the experimental conditions tested (3×10^5 cells/10 cm^2), the levels of MMP-2 and MMP-9 secreted in the conditioned medium were at the limit of detection in epithelial cells, and only MMP-2 was found to be expressed by fibroblasts and HMEC-1. The coculture of NS2T2A1V cells with fibroblasts and HMEC-1 cells induced MMP-9 activity (Fig. 3). This induction was markedly reduced when coculture was performed with AR antisense or EGFR antisense cells.

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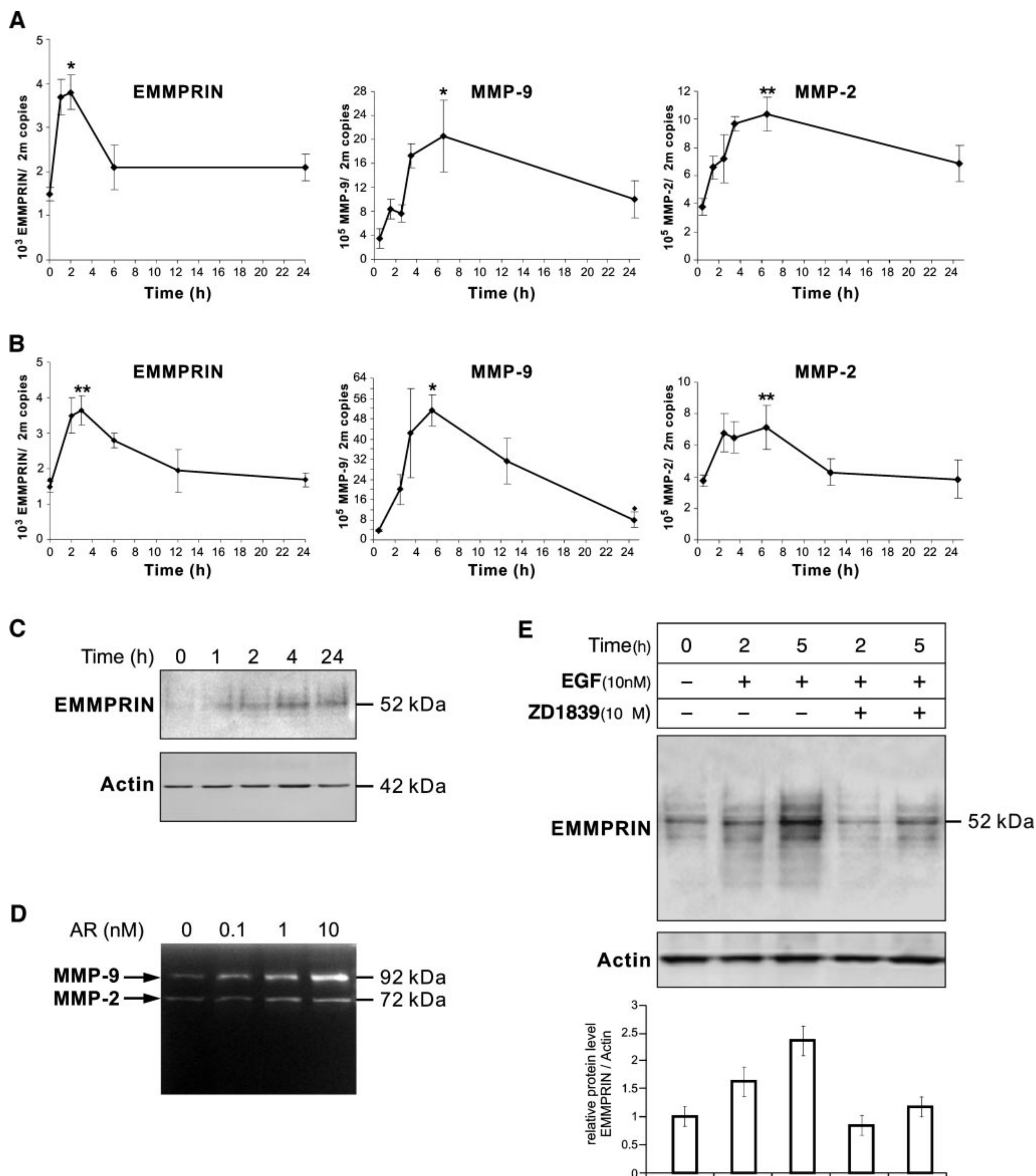

Fig. 1. *A–D,* EGF and AR up-regulate EMMPRIN, MMP-9, and MMP-2 in the transformed breast epithelial cells NS2T2A1 cells. *A* and *B,* quantitative real-time RT-PCR analysis was performed on RNA extracted from cells at different times, 1–24 h after addition of 10 nM EGF (*A*) or 10 nM AR (*B*), to determine transcript copy numbers of EMMPRIN, MMP-2, and MMP-9 relative to the reference gene $\beta 2$ microglobulin (b2m). The data are mean of four different experiments; *bars,* ±SD. *, $P \leq 0.03$; **, $P \leq 0.006$; *P*s were calculated at peak levels. *C,* immunoblot analysis of EMMPRIN protein expression after addition of 10 nM EGF at different indicated times. Cells were lysed in 1% NP40 in the presence of protease inhibitors, and 10 μg protein samples were subjected to SDS-PAGE and blotted with the HIM6 mAb. *D,* gelatin zymography analysis of the serum-free conditioned medium 24 h after addition of different indicated concentrations of AR. *E,* inhibition of EGF induced EMMPRIN protein expression by the tyrosine kinase inhibitor ZD1839. Cells were pretreated with 10 μM ZD1839 for 2 h before the addition of 10 nM EGF. Cell extracts (10 μg) prepared at the times indicated were subjected to immunoblot analysis. *Bottom* shows densitometry quantification of EMMPRIN protein levels normalized relative to actin of 3 different experiments. Values represent means of density variation relative to control; *bars,* ±SD.

EMMPRIN and MMP Levels in Tumors Obtained from the AR-AS and EGFR-AS Expressing Cells. We have shown previously that the AR-antisense and EGFR antisense-expressing cells, when injected into nude mice, give rise to smaller tumors than those observed with control-transfected cells. Not only the size and weight of tumors were lower (5–20% of control tumors at day 50 for EGFR antisense and AR antisense cells), but also their angiogenesis, as measured in the microvessel area of tumors (12). Because angiogenesis and MMP activity

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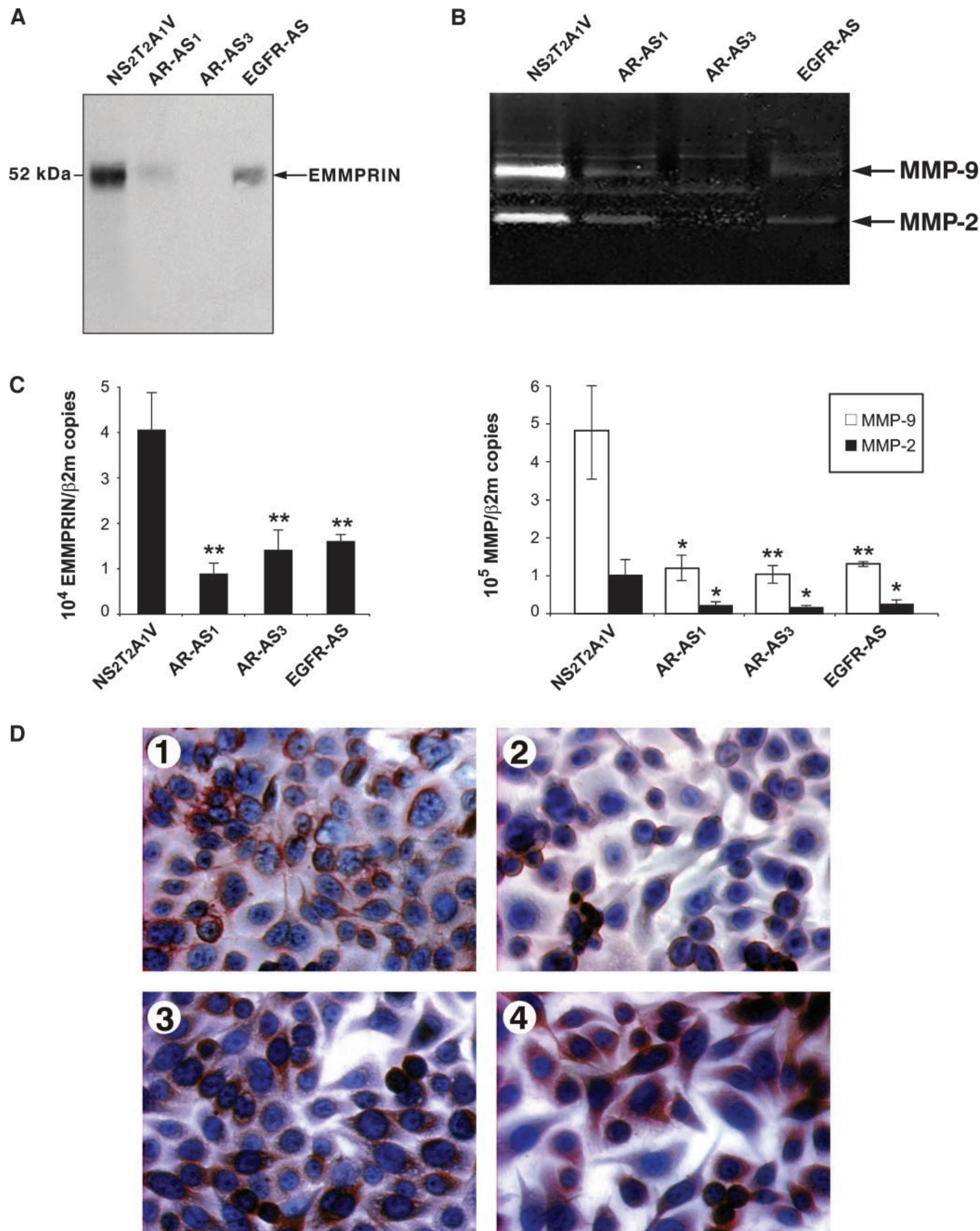

Fig. 2. Down-regulation of EMMPRIN, MMP-2, and MMP-9 production in AR and EGFR antisense-transfected NS2T2A1. *A,* immunoblot analysis of 10 μg cell extracts of vector transfected (*NS2T2A1V*) and antisense (*AS*) -transfected cells (*AR-AS1, AR-AS3,* and *EGFR-AS*). *B,* gelatin zymography of serum-free conditioned medium collected from vector and AS-transfected cells after 24-h incubation. *C,* quantitative real-time RT-PCR of EMMPRIN, MMP-2, and MMP-9 copies relative to the β2 microglobulin reference transcripts. The data represent means of three different experiments; *bars,* ±SD. * and ** denote significant differences with $P \leq 0.04$ and $P \leq 0.008$, respectively, between the expression values with and without antisense cDNA. *D,* immunocytochemical detection of EMMPRIN in NS2T2A1V (*1* and *3*) and EGFR-AS (*2* and *4*) cells by the HIM6 mAb. Cells were seeded in Lab-Tek chamber slides and treated without (*1* and *2*) and with (*3* and *4*) 10 nM AR for 12 h.

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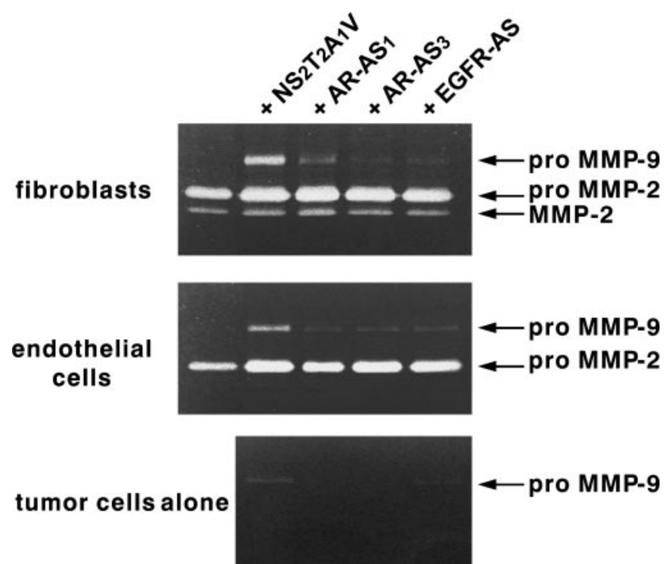
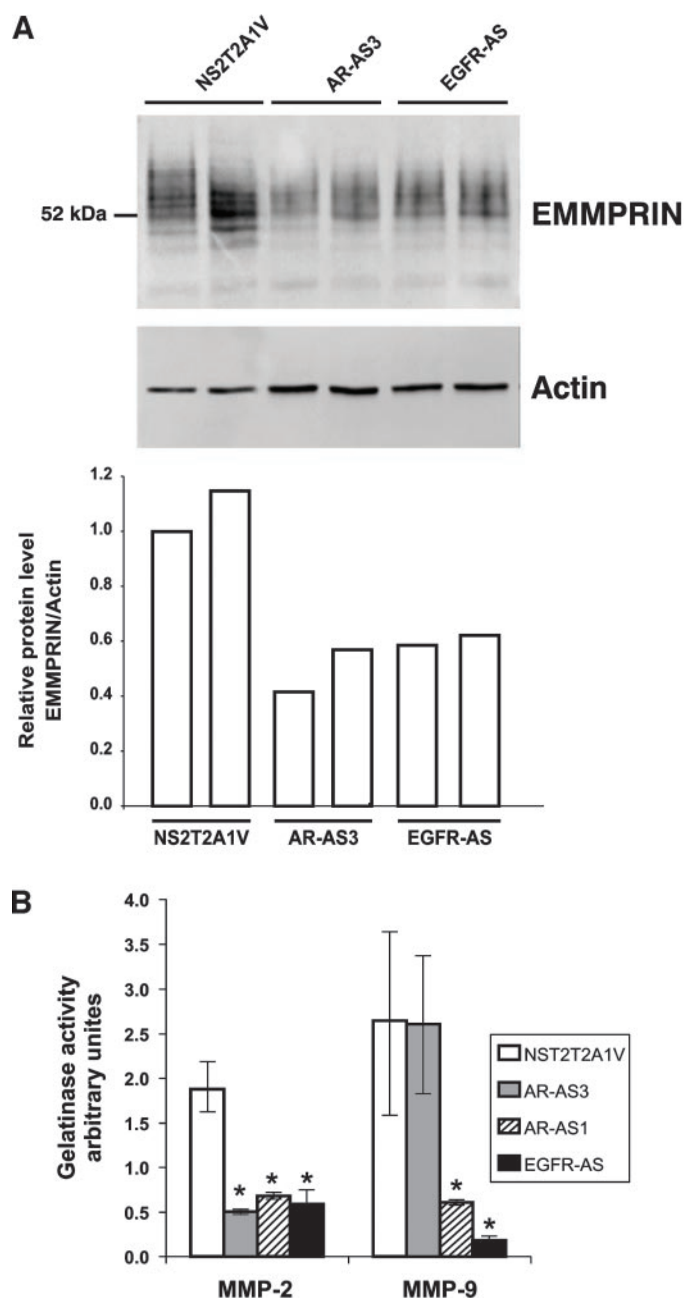


Fig. 3. MMP-2 and MMP-9 enzymatic activities are decreased in AR and EGFR antisense transfected cells compared with control NS2T2A1V cells when cocultured with fibroblasts and endothelial cells. Primary human dermal fibroblasts or human microvascular endothelial cells, HMEC-1, at 3×10^5/35-mm dish were allowed to adhere for 18 h in 10% FCS-containing DMEM. The tumor cells (*NS2T2A1, NS2T2A1V, AR-AS1, AR-AS3,* and *EGFR-AS*) were added to the fibroblast monolayer and allowed to attach in the same medium for an additional 6 h. The cells were then incubated for 48 h in serum-free DMEM, and the conditioned media were harvested for zymography analysis. The *first lane* denotes fibroblasts alone (*top zymogram*) or HMEC-1 alone (*middle zymogram*). The *bottom zymogram* shows the gelatinolytic activity expressed by the same number of the tumor cells cultured alone (corresponding to approximately 30–40% confluence).



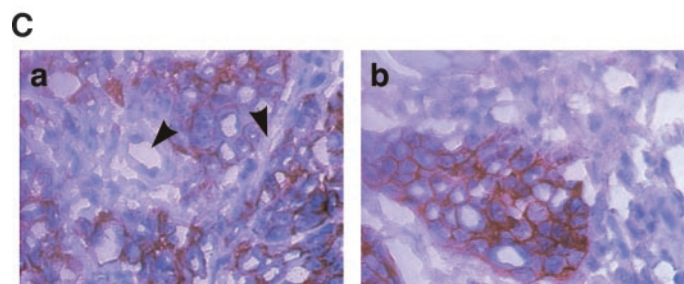


Fig. 4. EMMPRIN protein levels, and MMP-2 and MMP-9 enzymatic activities in nude mice tumor extracts resulting from NS2T2A1V, AR-AS, and EGFR-AS s.c. cell injection. *A,* immunoblot analysis of EMMPRIN in tumor extracts. Ten μm cryostat sections of excised tumors were extracted in the presence of protease inhibitor mixture during 1 h at 4°C. *Bottom* shows densitometry quantification of EMMPRIN normalized to actin expression. *B,* densitometry quantification of MMP-2 and MMP-9 lysis bands obtained by zymography analysis of 2 μg tumor extracts; *bars,* ±SD. The data represent the mean of four different tumors in each mouse group. * denotes a difference with $P \leq 0.01$ between the values for tumors with and without antisense cDNA. *C,* EMMPRIN immunostaining of cryostat sections of tumors derived from NS2T2A1V (*a*) and EGFR-AS cells (*b*).

were reported previously to be related, we evaluated the level of EMMPRIN and both gelatinases in cryostat sections prepared from tumors derived from NS2T2A1V, AR-AS, and EGFR-AS cells developed in nude mice. These sections were extracted in 2% NP40 containing a mixture of protease inhibitors including 2 mM EDTA to inhibit degradation or possible activation of the MMPs during extraction. The extracts were then analyzed for EMMPRIN by immunoblot and by gelatin zymography for MMPs. As shown in Fig. 4*A,* EMMPRIN expression was reduced 50% as compared with controls. Different bands reflecting the highly glycosylated profile of EMMPRIN in tumors can be observed (less evident in cell lines). In the same tumors (Fig. 4*B*) the gelatinase activities were also reduced except for MMP-9 in one clone. This reduction reached 90% and 60% for MMP-9 and -2, respectively, in the EGFR antisense clone. No reduction in the activation status of the gelatinases could be observed in the tumors.

Immunohistochemistry for EMMPRIN was performed in tissue sections of control tumors, and EGFR and AR antisense tumors. An EMMPRIN heterogeneous staining of the epithelial tumor cells was observed in antisense tumors as compared with a more homogenous staining in control tumors associated with abundant negatively stained blood vessels (Fig. 4*C*).

Discussion

In this study we show that the expression of EMMPRIN is up-regulated by AR and EGF, and down-regulated by AR or EGFR RNA antisense expression *in vitro* and *in vivo* in a human breast epithelial transformed cell line, NS2T2A1. Using the same cells, we showed previously that AR, through its interaction with the EGFR, played a crucial role in the transformed phenotype. The role of the EGFR family members in cell invasion, migration, and tumor angiogenesis has been described by several authors (16). In addition to its direct role in these processes, EGFR activation was reported to induce various downstream effectors themselves involved in the processes of carcinogenesis and

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metastasis. These include VEGF, interleukin 8, urokinase-type plasminogen activator, plasminogen activator inhibitor 1, and MMPs. Activation of MMPs has been described extensively in cancers of different origin. Of the >20 MMPs, mainly MMP-2 and MMP-9 are likely to be regulated by EGFR activation through the activation of ets-1 and 2, mitogen-activated protein kinases, phosphatidylinositol 3′-kinase, and extracellular signal-regulated kinase 1 and 2 pathways (17–19). It was shown recently that MMPs are induced through the action of a specific inducer, EMMPRIN/CD147. We had earlier shown that the inhibition of AR expression by antisense transfection of transformed human breast epithelial cell line NS2T2A1 suppressed their growth and invasion in nude mice, and significantly decreased tumor vascularization, conferring a role for AR in tumor angiogenesis (12). Our data presented here suggest that the AR/EGFR system contributes to the molecular pathogenesis of breast cancer by increasing cancer cell invasion through up-regulation of EMMPRIN and MMPs. Indeed, exogenous AR increased EMMPRIN expression in the transformed epithelial mammary tumor cells NS2T2A1, both at the RNA and protein level. This up-regulation of EMMPRIN was rapid, reaching maximum for transcripts at 3 h, whereas protein peaked at 4 h. When AR expression was inhibited by stable transfection of the cells with AR antisense cDNA, a concomitant reduction of EMMPRIN, as well as MMP-9 and MMP-2, was observed. This was additionally confirmed using mAbs to EGFR (data not shown), cDNA antisense to EGFR, or ZD1839 tyrosine kinase inhibitors. Finally, the reduced EMMPRIN and gelatinase expressions were also observed in nude mouse tumors showing that the same mechanism of regulation persisted *in vivo*.

Lately there was a growing evidence for the key role of EMMPRIN in the processes of tumorigenesis and metastasis. EMMPRIN was reported as one of the most constantly up-regulated mRNA in metastatic cells (6). The data presented here demonstrate that the AR/EGFR system, by regulating EMMPRIN, also activates the machinery for MMP induction in the surrounding tissue and, hence, greatly increases the invasive potential of the tumor. Whether EMMPRIN also participates in the induction of the gelatinases in the tumor cell themselves, as suggested by Sun and Hemler (7), is a subject for additional investigation. However, the fact that the induction of EMMPRIN by exogenous AR was rapid, with RNA levels peaking at ~3 h compared with 6 h for the two gelatinases, is consistent with an induction of MMPs by EMMPRIN within the same cells. Coculture experiments have shown that NS2T2A1V stimulated production of MMP-2 and MMP-9 not only in fibroblasts but also in endothelial cells. This stimulation was greatly reduced in AR-AS and EGFR-AS cells. In our data published previously we have shown a significant decrease of tumor angiogenesis in the tumors derived from AR antisense RNA-expressing cells, suggesting a role for AR in angiogenesis (12). Inhibition of angiogenesis was also described recently in experimental human renal cell carcinoma in mice by blockade of EGFR signaling pathways using the EGFR tyrosine kinase inhibitor PK1166 (20). Although VEGF was suggested to be implicated in this process, we show for the first time that AR and EGF up-regulate tumor cell EMMPRIN and, hence, MMP production in the adjacent endothelial cells, mediating angiogenesis. ZD1839, a specific tyrosine kinase inhibitor of EGFR, reduced EMMPRIN expression in these cells. Numerous genes have been described as targets for EGFR-mediated transduction including VEGF, and more recently several mucin genes including 11p15 MUC2 and MUC5AC (21). These authors showed, as described for gastrin and apolipoprotein A-I, that the ubiquitous transcription factor Sp1 participated to the EGF-mediated up-regulation. Very recently, a 470-bp fragment upstream of the coding region of EMMPRIN has been shown to promote its transcription. A 30-bp element of this sequence (−142 to −112 bp), which contains a binding site for Sp1, was important for EMMPRIN transcription (22). Additional tests with such constructs will confirm the direct regulation of EMMPRIN transcription by EGFR.

In conclusion, we have shown that AR can modulate invasion of breast cancer cells by increasing expression of EMMPRIN and MMPs. Our study suggests that interruption of the AR-induced EMMPRIN up-regulation in breast cancer cells could be a target of anti-invasion therapy in breast cancer. At the same time, current works are in progress to explore the pathway involved in EGFR signaling.

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Regulation of Extracellular Matrix Metalloproteinase Inducer and Matrix Metalloproteinase Expression by Amphiregulin in Transformed Human Breast Epithelial Cells

Suzanne Menashi, Maria Serova, Lin Ma, et al.

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