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EGF receptor transactivation by urokinase receptor stimulus through a mechanism involving Src and matrix metalloproteinases

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Abstract

Urokinase-type plasminogen activator receptor (uPAR) and epidermal growth factor receptor (EGFR) are ubiquitous receptors involved in the control of a variety of cellular processes frequently found altered in cancer cells. The EGFR has been recently described to play a transduction role of uPAR stimuli, mediating uPA-induced proliferation in highly malignant cells that overexpress uPAR. In the present work, we found for the first time that uPAR stimulation with the amino-terminal fragment (ATF) of urokinase devoid of proteolytic activity transactivates the EGFR in mammary MCF-7 cells through a mechanism involving Src and a metalloproteinase, as indicated by its sensitivity to selected inhibitors. In these cells, which express low levels of uPAR and malignancy, both ATF and EGF stimuli induced an interaction of the EGFR with uPAR and ERK activation. However, EGFR activation by uPAR stimuli mediated cellular invasion rather than proliferation, while EGFR activation by EGF led to a proliferative response. These results revealed a complex modulation of EGFR function toward different cellular responses according to the status of uPAR activity. On the other hand, we also found that MMP-mediated activation of EGFR can occur in an autocrine manner in cells which secrete uPA. All this reveals novel regulatory systems operating through autocrine loops involving uPAR stimuli, Src, MMP and EGFR activation which could mediate fine control of physiological processes as well as contribute to the expression of proliferative and invasive phenotypes of cancerous cells.
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Keywords: EGFR; Urokinase receptor; Src

Introduction

Cross-talk between different signaling pathways is a hallmark of the networking organization of cell surface receptors and transducing molecules through which cells interpret and integrate environmental stimuli both in normal and pathological conditions [1–3]. The epidermal growth factor receptor (EGFR) and the urokinase-type plasminogen activator receptor (uPAR) generate signals that control processes of cell proliferation, differentiation and migration, and their functions are frequently found altered in cancer cells, contributing to the invasive phenotype and malignancy [4–8]. Because EGFR and uPAR signaling pathways can elicit similar cellular responses and share elements converging toward activation of mitogen-activated kinases of the ERK family [2,9,10], it seems likely that cross-talk between these ubiquitous receptors might exist and modulate distinct functions depending on the cellular context. In spite of this, such cross-talk has remained largely unexplored.

An interesting aspect is that the EGFR might serve as downstream element in the signaling mechanism triggered by uPAR stimuli [11]. One of the mechanisms by which uPAR accomplishes a role in cell migration and invasion is by binding the serine proteinase urokinase-type plasminogen activator (uPA) which then becomes a membrane-associated ectoenzyme with proteolytic activity restricted to the immediate pericellular environment [2,10]. Macrophages and malignant cells degrade matrix proteins during tissue invasion using such uPAR-associated uPA. However, uPA binding to uPAR also generates intracellular signals that in certain cells, such as MCF-7 cells, provokes a migratory response whereas in other cells, such as the highly malignant T-Hep3

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cells, elicits proliferation, in both cases involving activation of the Ras–ERK pathway [12–15]. The signaling-mediated effects of uPAR stimulation do not require uPA proteolytic activity since they are also produced by a non-proteolytic amino-terminal fragment (ATF) of uPA [2]. The signaling mechanism is still enigmatic because uPAR is a glycosylphosphatidylinositol (GPI)-anchored protein and therefore, lacking transmembrane and intracytosolic domains, it should establish ligand-induced interactions with transducing proteins able to convey signals across the membrane [2,8].

It seems that uPAR can use a variety of signaling mechanisms, yet poorly understood. Integrins have been the most studied partners of uPAR transduction [16–19]. uPAR interacts directly with integrins [2,10,20,21], which link uPAR stimuli to the pathway mediated by FAK, Shc, Src and ERK [12–14]. In certain cells, gp130 seems to mediate uPAR responses leading to activation of the JAK–STAT pathway [22]. A different mechanism has been recently disclosed by the finding that a soluble fragment of uPAR, naturally produced during its interaction with uPA, constitutes a ligand for the G protein-coupled chemotactic receptor FPRL1, which then acts as a uPAR transducer for cell migration [23]. Recently, evidences have involved the EGFR in the signaling pathway of uPAR stimuli. One report described that the EGFR forms uPA-inducible complexes with $\alpha5\beta1$ integrin and is required as a uPAR downstream signaling mediator leading to ERK activation and cell proliferation [11]. More recently, another report appeared describing that uPA signaling is sensitive to a specific inhibitor of the EGFR tyrosine kinase [24]. These two studies suggested that involvement of the EGFR in uPAR signaling might be restricted to cells overexpressing uPAR. The functional importance of this cross-talk in different kinds of cells, the mechanism by which the EGFR becomes activated by uPAR stimuli and whether this could occur and lead to ERK activation in cells expressing low levels of uPAR remain unknown.

The EGFR is a member of the ERB tyrosine-kinase receptor family that binds a number of related peptide growth factors, including EGF, TGF-α and heparin-bound epidermal growth factor like (HB-EGF) ligand [25]. Ligand binding induces receptor dimerization, activation of the receptor protein tyrosine-kinase and transphosphorylation of several tyrosine residues at the carboxy terminal region of the receptor, which recruit and activate transducing elements of the Ras–ERK pathway [9]. A substantial amount of evidence led to consider the EGFR as both a transducer of its own ligand stimuli and a downstream element in signaling pathways triggered by a variety of other stimuli, thus playing a crucial role as cross-talk mediator of highly heterologous signaling systems [1,26]. Non-EGF-like stimuli able to transactivate the EGFR include G protein-coupled receptors (GPCRs), depolarization, UV light, and stressors [1]. At least for GPCRs, a mechanism of EGFR transmodulation involving metalloproteinase activity that causes HB-EGF release from the cell surface has been disclosed [27]. Whether such a mechanism might be used by other transmodulators, including uPAR, is unknown. It is also unknown whether transactivation of EGFR provides mechanisms to modulate different cellular responses or only reproduces those elicited by EGFR ligands.

In the present work, we found for the first time that ATF, the amino-terminal fragment of uPA lacking proteolytic activity, in cells expressing low levels of uPAR, activates the EGFR through a mechanism involving Src and a MMP activity. Furthermore, our results involve EGFR activation in uPAR signaling toward a migratory rather than a proliferative response contrasting with the proliferative effects elicited by EGF in the same cells. Thus, depending on whether the EGFR is either transactivated by uPAR or activated by its own ligand, different cellular responses can be evoked revealing complex modulation of the response of EGFR control system to cellular requirements.

Material and methods

Cells and antibodies

Mammary MCF-7 and MDA-MB-231 cells were purchased from the ATCC (Manassas, VA) and were grown in a phenol red-free DMEM/F12 plus 10% FCS. Polyclonal antibodies against peptides of residues 984–996 (EGFR984) and 1176–1186 (anti-C EGFR) have been previously characterized [28,29]. Monoclonal antibodies to phosphorylated and non-phosphorylated ERK were from Santa Cruz Biotechnology (Santa Cruz, CA), and the 4G10 monoclonal antibody used to assess EGFR tyrosine phosphorylation has been previously described [29]. The mouse monoclonal antibody against uPAR was from BD Pharmingen. GM6001 (Ilomastat) was purchased from Chemicon (Temecula, CA). The amino-terminal fragment of urokinase (ATF), EGF, AG 1478 (Tyrphostin) and PP2 were from Calbiochem (San Diego, CA).

Immunodetection of EGFR, uPAR and ERK

Before each treatment, cells were serum starved in FCS-free DMEM/F12 for 24 h. The EGFR was immunoprecipitated by the anti-C antibody as described [29] from cells extracts (10^7 cells per dish) prepared in 50 mM Hepes, pH 7.4, 150 mM NaCl, 2 mM MgCl2, 2 mM EGTA, 1% Triton X-100, 10% glycerol, 2 mM PMSF, 2 μg/ml pepstatin, 2 μg/ml leupeptin and 1 mM sodium orthovanadate at 4°C. Immune complexes were resolved by SDS-PAGE and analyzed by immunoblot with antibodies 4G10 for EGFR tyrosine phosphorylation and antibody EGFR984 for EGFR total mass [29] using the ECL chemiluminiscence detection kit (Amersham, Arlington Heights, FL). Tyrphostin (AG1478) was added (1 μM) 1 h before ATF or EGF stimuli. The metalloprotease inhibitor GM6001 (25 μM) and the PP2 Src inhibitor (15 μM) were added 2 and 1 h, respectively, before ligand stimuli. In the experiments with

MDA-MB-231 cells, a mild acid treatment to release endogenous ligands was made by incubating with Earle's balanced salt solution (EBSS), 10 mM HEPES, pH 7.4 for 2 min; 50 mM glycine–HCl, 100 mM NaCl pH 3.0 for 30 min; and 0.5 M HEPES, 0.1 M NaCl, pH 7,5 for 2 min, as described [30]. ERK 1,2 activation and total mass were assessed by immunoblots of cell lysates (30 μg) resolved by 8% SDS-PAGE using anti-ERK and anti-phosphotyrosine ERK, as described [31].

Invasion and proliferation assays

The ability of MCF-7 cells to migrate through Matrigel-coated filters was measured by using Transwell chambers (Costar, Cambridge, MA) with 8.0-μm-pore polycarbonate filters coated with 30 μg of Matrigel in the upper side of the filter (Collaborative Research, Bedford, MA). Cells resuspended in serum-free medium were seeded on the upper compartment of the chamber and incubated for 48 h in the presence or in the absence of 10 nM ATF or 25 ng/ml of EGF. Both ligands were added in the upper and lower compartments of Transwell chambers. Tyrphostin treatment was initiated 1 h before ATF or EGF. The bioactivity of ATF, EGF and Tyrphostin was maintained along the assay by readdition every 12 h. After 48 h of incubation, the number of cells present in each chamber was determined with MTT (Sigma, St. Louis, MO), as described [32]. To test the effect of ATF and EGF on cell proliferation, we cultured MCF-7 cells (5×10^4) in serum-free medium in the presence or absence of 10 nM ATF or 25 ng/ml of EGF for 48 h, and then the number of viable cells was evaluated by MTT assay.

Results

ATF causes activation of the EGFR leading to tyrosine phosphorylation of the receptor, formation of a complex between EGFR and uPAR, and ERK activation in MCF-7 cells

To analyze transactivation of the EGFR by ATF in MCF-7 cells, we first compared the kinetics of EGFR tyrosine phosphorylation induced by either ATF or EGF. Both 10 nM ATF and 25 ng/ml of EGF activates the EGFR with similar time courses, eliciting maximal responses between 2 and 15 min of incubation (Figs. 1A and 1B). In the subsequent experiments we used 10 min of stimulation. Strikingly, after stimulation with either EGF or ATF, we detected uPAR in the EGFR immunoprecipitates (Fig. 2A, lanes 3 and 5), suggesting that upon stimulation, both receptors established, either directly or indirectly, some kind of interaction. Because MCF-7 cells express uPAR at a relatively low level [13], compared with highly malignant cells [11], this results indicates that EGFR transactivation does not require overexpression of uPAR, as suggested by previous reports [11,24].

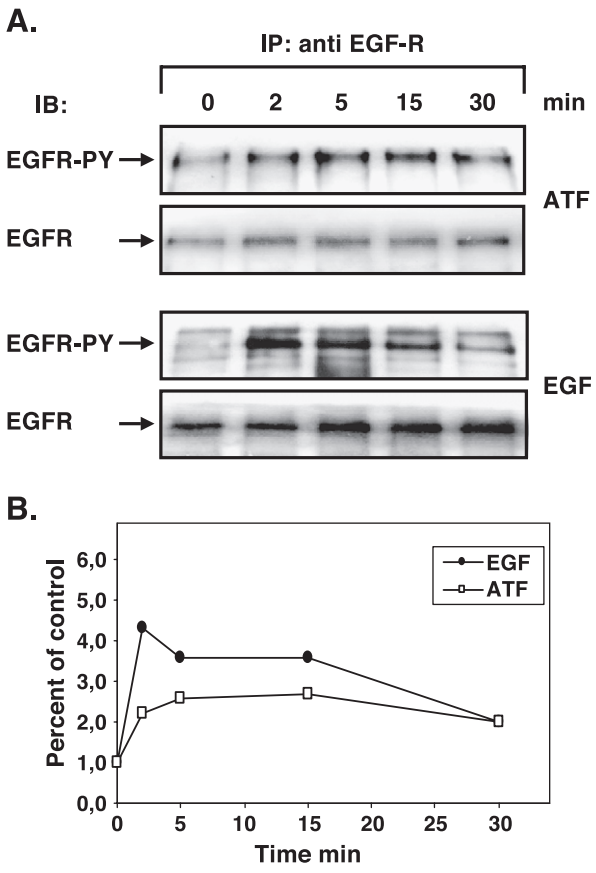

Fig. 1. ATF and EGF induce EGFR activation with similar kinetics. Serum-starved MCF-7 cells were incubated at 0, 2, 5, 15 and 30 min in the presence of 10 ng/ml ATF or 25 ng/ml EGF. In A, the EGFR was immunoprecipitated (IP) and the immune complexes analyzed by immunoblots for EGFR tyrosine phosphorylation (PY) and EGFR total mass; B shows a densitometric analysis of digitalized bands depicted in A and plotted as a function of time.

The phosphorylation status of ERK1,2 showed that both EGF and ATF stimuli led to activation of the MAPK pathway. The specific inhibitor of the EGFR tyrosine-kinase, Tyrphostin (AG 1478), completely abolished ATF-induced EGFR tyrosine phosphorylation as well as induced co-immunoprecipitation of EGFR and uPAR (Fig. 2A, lane 4), and ERK activation (Fig. 2B, lane 4). Therefore, the major activity responsible for all these events was the activation of the EGFR.

ATF-induced MCF-7 cell invasion is mainly mediated by EGFR activation

It is well known that uPA stimulates MCF-7 cell invasion in Transwell chambers [13]. Using this assay, we found here that Tyrphostin inhibited by more than 70% the ATF-induced 3-fold increase in the invasive capacity of MCF-7 cells (Fig. 3A). Thus, activation of the EGFR kinase is a crucial event involved in uPAR signaling leading to stimulated invasion. Interestingly, in spite of a similar level of

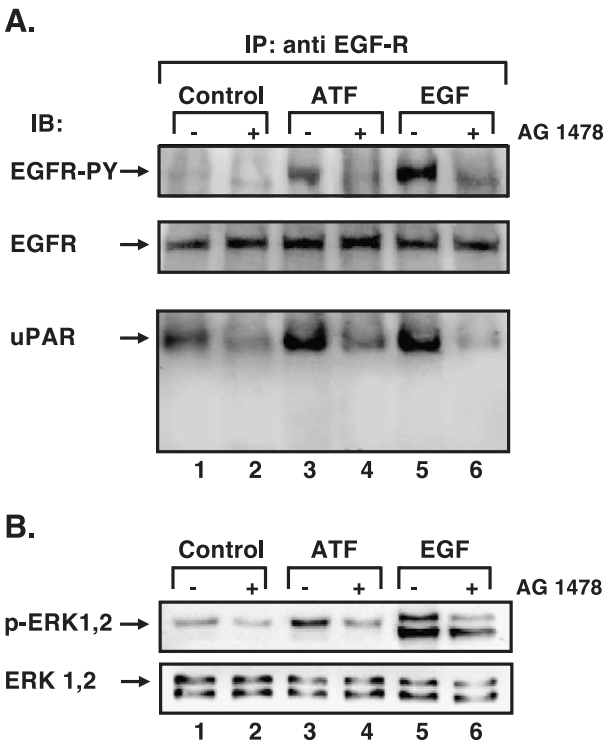

Fig. 2. ATF induces EGFR activation leading to co-immunoprecipitation with uPAR and ERK 1,2 activation. Serum-starved MCF-7 cells were incubated during 5 min in the absence (lanes 1 and 2), or presence of 10 ng/ml ATF (lanes 3 and 4) or 25 ng/ml EGF (lanes 5 and 6) either without or with 1 μM AG1478, as indicated. In A, the EGFR was immunoprecipitated (IP) and the immune complexes analyzed by immunoblot (IB) for EGFR tyrosine phosphorylation (PY), EGFR total mass and uPAR, as indicated; B shows ERK activation, measured by its phosphorylation (p-ERK1,2), and ERK 1,2 total mass, both assessed by immunoblot in aliquots of the same cell extracts.

EGFR tyrosine phosphorylation induced by either ATF or EGF (compare lanes 3 and 5 of Fig. 2A), EGF evoked instead a proliferative response (Fig. 3B). ATF had no effect on MCF-7 proliferation, as previously reported [13]. In addition, these results showed that EGFR activation can exhibit different functions in the same cell depending on the activation status of the uPAR control system.

ATF-induced activation of the EGFR requires Src and a metalloproteinase activity

Activation of the EGFR via GPCRs stimuli has been described to be sensitive to inhibitors of metalloproteinases [27]. Furthermore, transmodulation of the EGFR induced by stimuli acting via MMP has been described to involve Src activation [33]. Therefore, we tested whether EGFR activation induced by ATF could be affected by the MMP inhibitor GM6001 and/or the Src inhibitor PP2.

Treatment of MCF-7 cells with GM6001 completely blocked both EGFR auto-phosphorylation and ERK activation induced by ATF but not by EGF (Fig. 4), suggesting that ATF provoked a MMP-dependent release of a membrane-bound precursor ligand of the EGFR, similar to that reported for EGFR transactivation induced by GPCRs stimuli [27]. On the other hand, PP2 also inhibited the ATF-induced, but not the EGF-induced, tyrosine phosphorylation of the EGFR and activation of ERK (Fig. 5). It is interesting to note that PP2 decreased only by 40% the ATF-induced EGFR activation whereas it almost completely inhibited the activation of ERK, suggesting, among other possibilities, that Src could act in a paralleled route to EGFR.

Cellular responses can differ greatly when the same stimulus is presented either in autocrine or in exogenous (paracrine) manners [6]. MDA-MB-231 cells, which constitutively express a high level of activated ERK, attributed to overexpression of endogenous uPA [30], allowed to test whether uPA signaling via MMP-mediated EGFR activation also occurs in an autocrine manner. We first subjected these

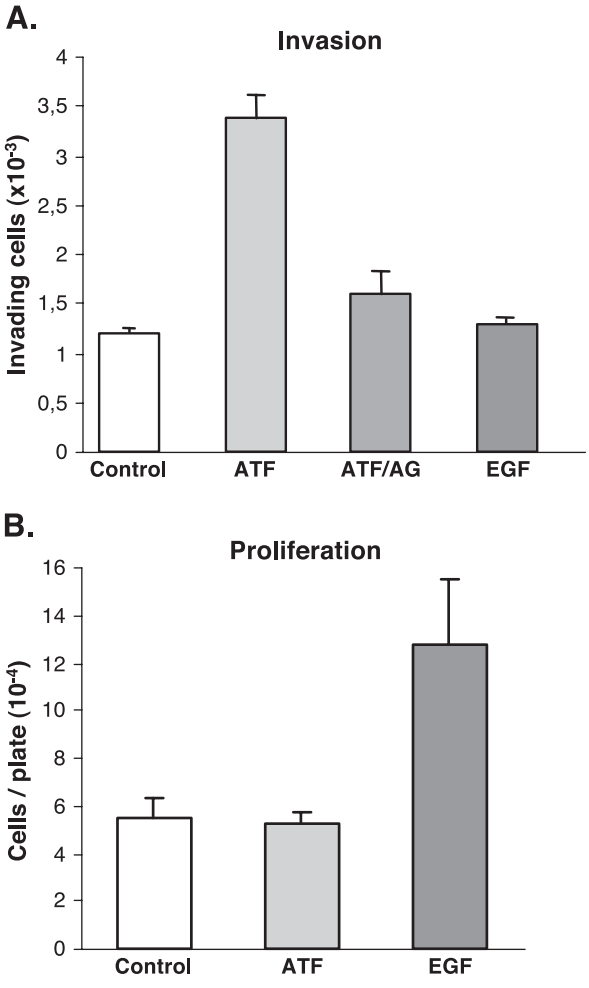

Fig. 3. ATF increases invasiveness of MCF-7 cells through a mechanism involving EGFR activation. (A) Invasion assays made in serum-starved MCF-7 cells plated in Matrigel-coated Transwell chambers and incubated with 10 ng/ml ATF, 10 ng ATF plus 1 μM Tyrphostin or 50 ng/ml EGF for 48 h. Values are expressed as number of invading cells by insert ± SD of three determinations; (B) cell proliferation in serum-free medium determined by MTT assay in the absence (control) or presence of 10 nM ATF or 50 ng/ml EGF for 48 h, expressed as number of cells per dish.

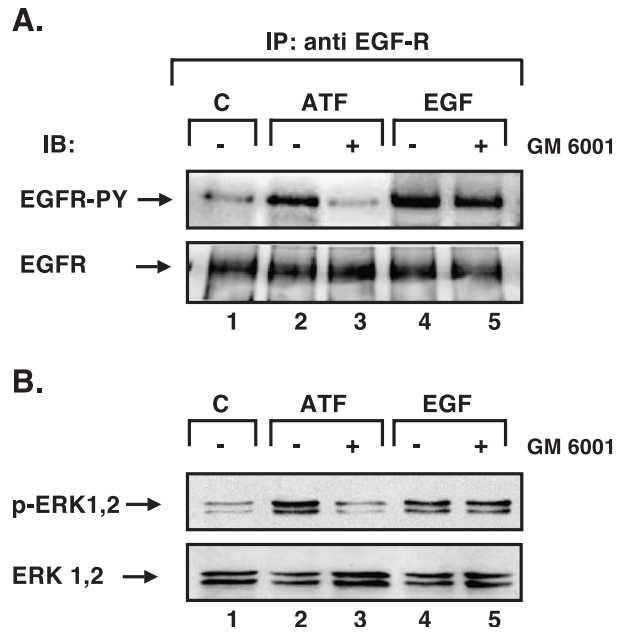

Fig. 4. ATF-induced activation of EGFR and ERK requires MMP activity. Serum-starved MCF-7 cells preincubated in the absence (lanes 1, 2, 4) or presence (lanes 3 and 5) of the MMP inhibitor GM6001 were then stimulated with 10 ng/ml ATF (lane 2 and 3) or 25 ng/ml EGF (lanes 4 and 5) for 5 min. (A) The EGFR was then immunoprecipitated and analyzed by immunoblot for tyrosine phosphorylation or total mass; B shows ERK activation and total mass assessed by immunoblots in aliquots of the same cell extracts.

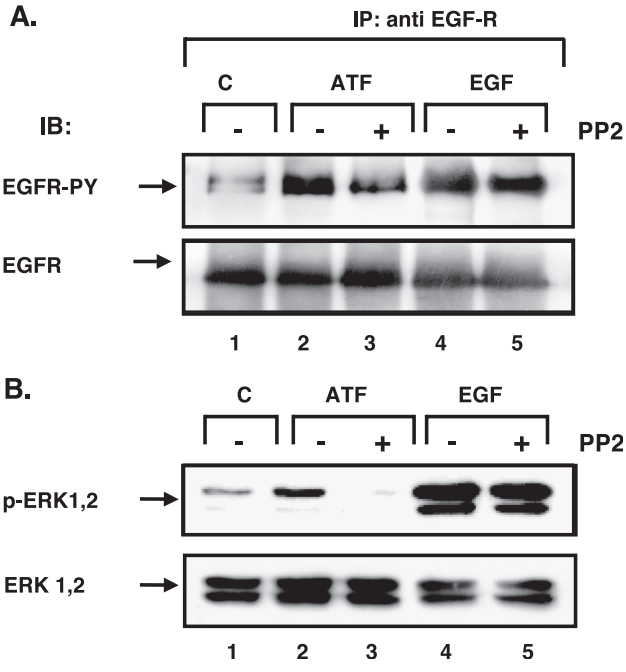

Fig. 5. ATF activation of EGFR and ERK is mediated by Src. Serum-starved MCF-7 cells pre-incubated during 1 h in the absence (lanes 1, 2, and 4) or presence (lanes 3 and 5) of the Src inhibitor PP2 (25 μM) were then stimulated with either 10 ng/ml ATF (lanes 2 and 3) or 25 ng/ml EGF (lanes 4 and 5) for 5 min and then the EGFR was immunoprecipitated and analyzed by immunoblot for phosphotyrosine and total mass (A), and phosphorylated and total mass of ERKs (B) analyzed as before.

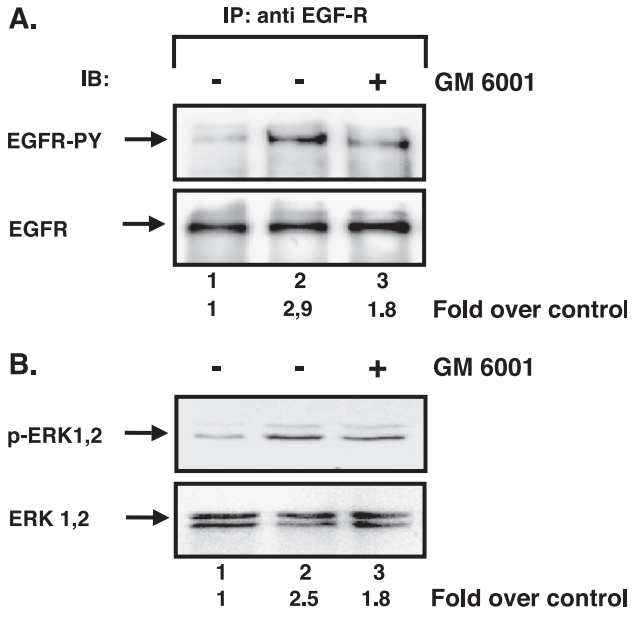

Fig. 6. MMP activity is required for uPA autocrine activation of EGFR and ERK in MDA-MB-231 cells. Serum-starved MDA-MB-231 cells which produces much endogenous uPA were first subjected to mild acid treatment to eliminate endogenous ligands (lane 1) and then incubated in serum-free medium for 16 h (lanes 2 and 3) in the absence (lane 2) or presence (lane 3) of 25 μM GM6001, a MMP inhibitor. (A) Activation of EGFR, reflected in its tyrosine phosphorylation, and total mass of receptors were assessed as before; (B) ERK activation (p-ERK 1,2) and total mass. Inhibition of MMP decreased both EGFR and ERK activation caused by the autocrine stimuli of endogenously produced ligands. Densitometry analysis was expressed as percentage of the control.

cells to an acid wash step to reduce background autocrine stimulation and then studied the effect of GM6001 upon both EGFR autophosphorylation and ERK activity after a 16-h recovery period. Activation of EGFR and ERK returned to their constitutively higher levels after 16 h of incubation in the absence of serum, but was significantly reduced in the presence of GM6001 (Fig. 6), suggesting that uPA induction of a MMP accounts, at least partially, for an autocrine transactivation of EGFR which consequently determines the increased ERK activity. The known endogenous production of TGFα by these cells [34] is presumably responsible for the remaining GM6001-insensitive activities of EGFR and ERK.

Discussion

We found here for the first time that uPAR stimulus leading to ERK activation and increased invasive capacity of MCF-7 cells is predominantly mediated by transactivation of the EGFR through a mechanism involving Src and MMP activity. These results revealed a novel functional link among uPAR, Src, and EGFR that should be considered in the mechanisms by which malignant cells express an invasive phenotype. We also presented evidence suggesting that endogenously produced uPA can transactivate the EGFR

leading to ERK activation. These observations suggest the existence of previously unexpected uPA autocrine loops with perpetuating positive feedback mediated by EGFR. Furthermore, EGF induced proliferation instead of invasion in the MCF-7 cells, also indicating that EGFR can play different roles depending on whether it becomes activated by its own ligand or as a downstream element of uPAR stimulation. To our knowledge, this is the first example of differential EGFR-mediated processes (e.g. invasion versus proliferation) elicited either by its heterologous transactivation or activation by its own ligand.

Transactivation of the EGFR induced by uPAR stimulus has been recently reported but exclusively in cells overexpressing uPAR [11,24]. In the highly malignant T-Hep3 cells, Liu et al. [11] described that uPA induced an activation of EGFR which resulted in a proliferative response. Cells expressing low levels of uPAR did not show an increased tyrosine phosphorylation of the EGFR. On the other hand, in transfected Cos-7 cells expressing exaggerated levels of uPAR, Jo et al. [24] reported that Tyrphostin inhibited uPA-induced cell migration. Our present results demonstrated that uPAR transactivation of the EGFR is not restricted to cells overexpressing uPAR, and therefore could depend on the cellular context. We found here that the ATF fragment of uPA, lacking proteolytic activity, transactivated the EGFR in MCF-7, which are known to express about 3400 uPA receptors [13,30]. Thus, the role of the EGFR as a crucial element of the uPAR signaling pathway seems to be more extensive than previously thought. Because it does not require overexpression of uPAR it could play a role in physiological processes. Moreover, together with previous reports [11,24], our present observations indicate that uPAR signaling through the EGFR could occur in cells independently of their malignancy state, and therefore, could also play a role in different stages of cancer progression, contributing to enhance either the invasive or proliferative cellular phenotype.

Although one of the previous reports [11] suggested that an association of the EGFR with $\alpha5/\beta1$ integrin could be important in the EGFR activation by uPAR stimulation, at least in cells expressing high levels of uPAR, the actual mechanism linking uPAR stimuli to EGFR activation is still unknown. It has been reported that integrin-mediated adhesion induces EGFR activation as part of the mechanism involved in extracellular matrix-dependent cell survival and proliferation [35,36]. Integrins also interact with uPAR [16,21]. Therefore, a possible functional link between uPAR and the EGFR might indeed be mediated by an inducible association of the EGFR with integrins. We observed that neither ATF-dependant activation of EGFR nor ERK phosphorylation occurred in non-adherent MCF-7 cells (data not shown), indirectly suggesting the participation of integrins in these effects. Furthermore, we showed that ATF-induced a co-immunoprecipitation of the EGFR with uPAR, indicating that these receptors can directly or indirectly interact in cells expressing low levels of both. Integrins, uPAR and the EGFR could eventually form tripartite complexes with variable stoichiometry depending on their relative expression levels. The significance of such interactions for uPAR signaling would need to be elucidated in more detail, specially considering the roles of Src and MMP that we found here.

In fact, our evidences obtained with widely used selective inhibitors indicate that MMP and Src activity are upstream elements in the EGFR-mediated uPAR signaling. Previous evidence has led to the notion that EGFR transactivation by GPCRs occurs via activation of MMP and subsequent release of EGF-like ligands, such as HB-EGF, from growth factor precursors in the plasma membrane [27]. In T(84) intestinal epithelial cells, Src has been involved in the EGFR transactivation pathway triggered by carbachol [37]. Other studies have shown evidence that the mechanism of MMP activation requires the function of Src [33]. We showed here that the widely used MMP inhibitor GM6001 and Src inhibitor PP2 both strongly decreased the transactivation of EGFR induced by ATF in MCF-7 cells, leaving unaffected the EGF-dependent activation of EGFR and ERK. Therefore, Src activation resulting in increased MMP activity and cleavage of an EGF-like precursor seems to be not exclusive for the GPCR transmodulation of EGFR [38], but also the most likely mechanism linking uPAR and EGFR functions. Furthermore, in MDA-MB-231 cells, which endogenously produce and secrete high levels of uPA, we showed evidence that this MMP-mediated activation of EGFR can also occur in an autocrine manner [34].

In principle, Src might play a dual role in the uPAR signaling pathway. The recently demonstrated effect of prostaglandin E2 in colon cancer cells [33] showed that Src is able to activate MMP and consequently induce an EGFR-mediated ERK activation. On the other hand, there is a pathway linking Src with ERK which bypasses the EGFR [39], and which might include the participation of integrins and Src-mediated FAK phosphorylation [12,40,41]. Under distinct circumstances, these two pathways leading to ERK could reinforce each other or operate independently. In MCF-7 cells, we found that inhibition of EGFR kinase completely abrogated the ERK activation induced by ATF, indicating predominance of the EGFR-mediated pathway. However, Src inhibition by PP2 almost completely abolished ERK activation whereas it only decreased by 40% the EGFR transactivation. Two speculative possibilities can be envisioned to explain these results. First, there might be a threshold for the EGFR activity able to trigger the Ras–ERK pathway in response to uPAR stimuli. Second, Src could be involved in upstream processes leading to EGFR activation and also in a paralleled route to EGFR via the integrin–FAK pathway, which contributed to ERK activation independently of growth factors. Moreover, previous studies have shown that EGFR stimuli cause activation of Src [42] and that both EGFR and Src can operate synergistically [43].

Signals emerging from the EGFR could evoke either invasion or proliferation depending on the stimuli which activates its intrinsic kinase in MCF-7 cells. In fact, in these cells ATF induced invasion whereas EGF induced proliferation. Transduction pathways elicited by ATF and EGF stimuli should diverge at some point downstream from the activated EGFR or include additional elements from other pathways with the ability to modulate the signals emanating from the EGFR so that molecular programs responsible for invasion instead of proliferation could predominate upon uPAR stimuli. An attractive possibility is that these stimuli determine a different endocytic behavior of the EGFR. Migratory responses mediated by the EGFR can be elicited by signaling from the cell surface [6] whereas proliferation might require qualitatively and quantitatively distinct broadcasting from the cell surface and endosomal compartments [44–47].

Our results in the very low malignant and weakly invasive cell line MCF-7 assign a critical role for EGFR function in uPAR signaling pathways controlling cellular invasive capacity, whereas in other cells expressing high levels of uPAR, this role can be more directed to control cell proliferation [11]. We also showed that endogenously produced uPA can act in an autocrine manner to induce MMP-mediated transactivation of the EGFR leading to increased ERK activity, suggesting a previously unnoticed way to establish autocrine loops. Autocrine loops play crucial roles in regulating cell function within tissue contexts, operating in numerous physiological situations, which include wound healing, angiogenesis, chemotaxis, migration and tissue organization, and even in tumorigenesis [23,48–51]. A correlation between malignancy and uPA production has been demonstrated in breast carcinoma, indicating that an enhanced proteolytic potential is a critical element in the acquisition of malignancy [51,52]. All these findings suggest the possibility that cells under distinct circumstances can establish autocrine loops involving uPAR stimuli and EGFR transactivation, with functional implications for both normal processes as well as the expression of the invasive and proliferative phenotype of cancer cells.

Acknowledgments

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