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Cell migration in response to the amino-terminal fragment of urokinase requires epidermal growth factor receptor activation through an ADAM-mediated mechanism

Andrew M. Bakken, MD, Clinton D. Protack, MD, Elisa Roztocil, BS, Suzanne M. Nicholl, PhD, and Mark G. Davies, MD, PhD, MBA, *Houston, Tex*

Background: Cell migration is an integral component of intimal hyperplasia development and proteases are pivotal in the process. Understanding the role of urokinase signaling within the cells of vasculature remains poorly defined. The study examines the role of amino-terminal fragment (ATF) of urokinase on a pivotal cross-talk receptor, epidermal growth factor receptor (EGFR). EGFR is transactivated by both G-protein-coupled receptors and receptor tyrosine kinases and is key to many of their responses. We hypothesize that A Disintegrin and Metalloproteinase Domains (ADAM) allows the transactivation of EGFR by ATF.
Objective: To determine the role of ADAM in EGFR transactivation by ATF in human vascular smooth muscle cells (VSMC) during cell migration.
Methods: Human coronary VSMC were cultured in vitro. Assays of EGFR phosphorylation were examined in response to ATF (10 nM) in the presence and absence of the matrix metalloprotease (MMP) inhibitor GM6001, the ADAM inhibitors TAPI-0 and TAPI-1, heparin binding epidermal growth factor (HB-EGF) inhibitor, CRM197, HB-EGF inhibitory antibodies, epidermal growth factor (EGF) inhibitory antibodies, and the EGFR inhibitor AG1478. The small interference ribonucleic acid (siRNA) against EGFR and ADAM-9, ADAM-10, ADAM-12, and adenoviral delivered Gbg inhibitor, βARK_{CT} were also used.
Results: ATF produced concentration-dependent VSMC migration (by wound assay and Boyden chamber), which was inhibited by increasing concentrations of AG1478. ATF was shown to induce time-dependent EGFR phosphorylation, which peaked at fourfold greater than control. Pre-incubation with the G$\beta\gamma$ inhibitor βARK_{CT} inhibited EGFR activation by ATF. This migratory and EGFR response was inhibited by AG1478 in a concentration-dependent manner. Incubation with siRNA against EGFR blocked the ATF-mediated migratory and EGFR responses. EGFR phosphorylation by ATF was blocked by inhibition of MMP activity and the ligand HB-EGF. The presence of the ADAM inhibitors, TAPI-0 and TAPI-1 significantly decreased EGFR activation. EGFR phosphorylation by EGF was not interrupted by inhibition of MMP, ADAMs, or HB-EGF. Direct blockade of the EGFR prevented activation by both ATF and EGF. Incubation with siRNA to ADAM-9 and -10 significantly reduced HB-EGF release from VSMC and EGFR activation in response to ATF. The siRNA against ADAM-12 had no effect.
Conclusion: ATF can induce transactivation of EGFR by an ADAM-mediated, HB-EGF-dependent process. Targeting a pivotal cross-talk receptor such as EGFR is an attractive molecular target to inhibit cell migration. (J Vasc Surg 2009;49: 1296-303.)

Clinical Relevance: Cell migration is an integral component of intimal hyperplasia development and proteases are pivotal in the process. Understanding the role of urokinase signaling within the cells of vasculature remains poorly defined. The study examines the role of ATF of urokinase on a pivotal cross-talk receptor, EGFR. EGFR is transactivated by both G-protein-coupled receptors and receptor tyrosine kinases and is key to many of their responses. ATF can induce transactivation of EGFR by an ADAM-mediated, HB-EGF-dependent process. Targeting a pivotal cross-talk receptor such as EGFR, which can be transactivated by both G-protein-coupled receptors and receptor tyrosine kinases is an attractive molecular target.

Vessels remodel during atherogenesis in response to altered flow and following injury. This remodeling has been shown to involve an integrative program of cell proliferation, migration, and extracellular matrix modulation.[1] The migration of vascular smooth muscle cells (VSMC) involves the complex regulation of proteases, integrins, and extracellular molecules leading to the sequence of attachment, detachment, and contraction events, which allow a cell to move through the extracellular matrix. Urokinase plasminogen activator (uPA) is a serine protease that is the primary plasminogen activator in tissue remodeling processes and increased serum uPA is associated with development of restenosis after coronary angioplasty.[2] In addition to its

extracellular proteolytic activity, uPA is also capable of mediating cell signaling. Plasminogen activation is a complex system at the level of the cellular microenvironment.[3] The uPA is primarily responsible for plasmin generation in tissue remodeling processes, and is localized to the membrane by its receptor, uPAR.[4] The uPA induces cell migration through urokinase plasminogen activator receptor (uPAR) and it appears to be receptor-initiated and G-protein mediated; migration is dependent on ERK1/2 activity. The uPAR is involved in a multi-protein complex containing integrins, low-density lipoprotein (LDL)-related protein (LRP), FPRL1 (a G-protein coupled receptor [GPCR]) and epidermal growth factor receptor (EGFR).[5] Interaction with the components of the complex may allow differential cell signaling. EGFR is transactivated by both GPCRs and receptor-linked tyrosine kinases and is the key to many of their responses. The uPA induces time-dependent phosphorylation of the EGFR.[6] Inhibition of EGFR reduces ERK1/2 activation and cell migration.[6]

However, uPA is composed of three domains: amino-terminal fragment (ATF), kringle (K), and carboxyterminal (CTF) fragments, each of which is distinct and biologically active. The ATF and K fragments mediate VSMC migration.[7,8] With respect to ATF, the response is due to binding and cleavage of uPAR into a cell-bound D1 and a soluble D2/D3 fragment. The D2/D3 then binds to the low affinity receptor of fMPL, FPRL1, to induce a Gαi-mediated response.[9] We have shown that ATF can strongly induce plasmin-independent VSMC migration in vitro,[10] which is both PI3K-ERK1/2 and PI3K-akt dependent.[11] This migration can be blocked by inhibition of EGFR. At present, the triple membrane passing signaling (TMPS) mechanism of GPCR-induced EGFR activation is a widely accepted model of EGFR transactivation.[12-14] In the TMPS model, there is a sequence of three transmembrane signaling events: GPCR activation followed by transmembrane matrix metalloprotease (MMP) activation, and subsequent activation of the EGFR by release of heparin binding epidermal growth factor (HB-EGF), a tethered ligand of the EGFR. A potential transmembrane protease system that would convey a signal from intracellular domain to the extracellular domain is the A Disintegrin and Metalloproteinase Domains (ADAM) proteases, which span the membrane and can be activated intracellularly to mediate an extracellular MMP action (Fig 1). We hypothesize that ADAM allows the transactivation of EGFR by ATF. This study seeks to determine the role of ADAM in EGFR transactivation by ATF in human VSMC during cell migration.

METHODS

Experimental design. Human VSMC (passages 3-6, Clontech) were cultured in vitro. Lineage was confirmed by a-actin Western blotting. Assays of EGFR phosphorylation were examined in response to ATF in the presence and absence of the MMP inhibitor GM6001, the ADAM inhibitors TAPI-0 and TAPI-1, HB-EGF inhibitor, CRM197, HB-EGF inhibitory antibodies, EGF inhibitory antibodies
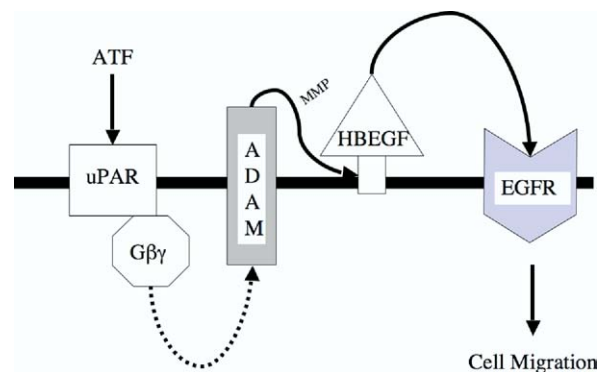
Fig 1. Proposed triple membrane passing system for amino-terminal fragment urokinase (ATF). ATF binds urokinase plasminogen activator receptor (uPAR) and this activates Gβγ and other intracellular molecules to activate A Disintegrin And Metalloproteinase Domains (ADAM) protease. Activation of ADAM associated matrix metalloproteinase (MMP) activity leads to release of the tethered ligand, heparin binding epidermal growth factor (HB-EGF), which in turn activates the epidermal growth factor receptor (EGFR). Activation of EGFR is necessary for ATF mediated migration.

and the EGFR inhibitor AG1478. Chemical inhibitors and antibodies were incubated for 1 hr before treatment. siRNA against EGFR and ADAM-9, ADAM-10, and ADAM-12 were also used.

MATERIALS

ATF was purchased from American Diagnostica, Inc (Greenwich, Conn). The EGF heparin was purchased from Sigma Chemical Co (St Louis, Mo). AG1478 and CRM197 were purchased from Calbiochem (La Jolla, Calif). GM6001 was purchased from Chemicon International, Inc (Temecula, Calif). CRM197, TAPI-0, and TAPI-1 were purchased from Biomol (Plymouth Meeting, Pa). The Anti-HB-EGF antibody was purchased from R&D Systems, Inc (Minneapolis, Minn). The anti-EGFR antibody (151-IgG) developed by Dr Ann Hubbard was obtained from the Developmental Studies Hybridoma Bank, developed under the auspices of the National Institute of Child Health and Human Development and maintained by The University of Iowa (Department of Biological Sciences, Iowa City, Iowa). The siRNA sequences for ADAMs were as follows: ADAM-9, AAUCACUGUG-GAGACAUUUGCdTdT and AAACUUCC AGUGUGU-AGAUGCdTdT; ADAM-10, AAUGAAGAGGGACACU-UCCCUdTdT and AAGUUGCCUCCUCCUAAACCAd-TdT; and ADAM-1, AACCUCGCUGCAAAGAAUGU-GdTdT and AAGACCUUGAUACGACUGCUGdTdT. Peroxidase-conjugated antirabbit IgG antibody (raised in goat) and peroxidase-conjugated antimouse IgG antibody (raised in goat) were purchased from Jackson Immuno Research Laboratories, Inc (West Grove, Pa). Phospho-EGFR (Y1068), total EGFR antibodies were obtained from Cell Signaling Technology, Inc (Beverley, Mass). Dulbecco modified Eagle minimal essential medium

(DMEM) and Dulbecco phosphate-buffered saline were purchased from Mediatech (Herndon, Va).

Boyden chamber. Chemotaxis was measured using a 48-well Boyden chamber (Neuroprobe Inc, Gaithersburg, Md) and polycarbonate filters (Neuroprobe Inc, 10 μm pore size, 25 × 80 mm, PVP free) with VSMC as previously described.[6,10] ATF (10 nM) was added to the lower wells. Trials included 8 or 12 wells per reagent per trial, and were repeated no fewer than three times.

Western blotting. Cells were allowed to grow to 80% confluence and starved for 48 hours. Cells were then stimulated with ATF (10 nM) or EGF (10 nM) alone and in the presence of pharmacological and peptide inhibitors; cells were harvested at time points from 0 to 30 minutes. Western blotting was performed as previously described.[15]

siRNA transfection. Pre-designed HPCL purified siRNA for gene knockdown for EGFR or control siRNA was purchased from Santa Cruz Biotechnology (Santa Cruz, Calif) and ADAM-9, ADAM-10, and ADAM-12 proteins from Ambion Inc (Austin, Tex). VSMC of 50% confluence in 60 mm plates are starved overnight in 4 mL Opti-MEM reduced serum medium (Gibco, Langley, Okla). The siRNA is transfected using Lipofectamine 2000 (Invitrogen, Inc, Carlsbad, Calif) following product protocol. Briefly, 22 μl of Lipofectamine 2000 is first incubated in total volume of 250 μl of Opti-MEM for 5 minutes at room temperature. It is then added to 250 μl of Opti-MEM containing 440 pmoles of siRNA. The solution is mixed gently and incubated for 20 minutes at room temperature, after which it is added to the starved plates. The medium can be changed after 4-6 hours of incubation. The cells may now be used between 24-72 hours after transfection for EGFR assays. Scrambled siRNA served as a control. Using the methodologies described, we conducted concentration-dependent experiments with siRNA against EGFR, ADAM-9, ADAM-10, and ADAM-12 and demonstrated a concentration-dependent decrease in protein expression that was specific for the protein targeted without altering the expression of the other proteins.

Adenoviral infection. Adenoviral vectors were constructed by Welgen, Inc (Worcester, Mass) using purified plasmid encoding βARK$_{CT}$, obtained from Guthrie cDNA Resource center. VSMC were plated at 70% confluence in 100 mm dishes and allowed to grow overnight. Recombinant adenovirus was then added at the appropriate concentrations (βARK$_{CT}$; 100 MOI) in a reduced volume of media (1.5-2 mL). After 48 hour incubation, the media was changed and cells grown for an additional 24 hours. The cells were then used for EGFR assays. Empty vector served as control.

Measurement of tethered ligand HB-EGF. Cultured VSMC were deprived of serum for 24 hours and then cultured in phenol red-free Dulbecco's modified Eagle's medium H21. Following exposure to ATF (10 nM) alone and in the presence of pharmacological inhibitors, siRNA, and adenoviral agents, medium was collected and concentrated using a Centricon centrifugal filter device (YM-3, Millipore, Billerica, Mass). Samples were centrifuged at
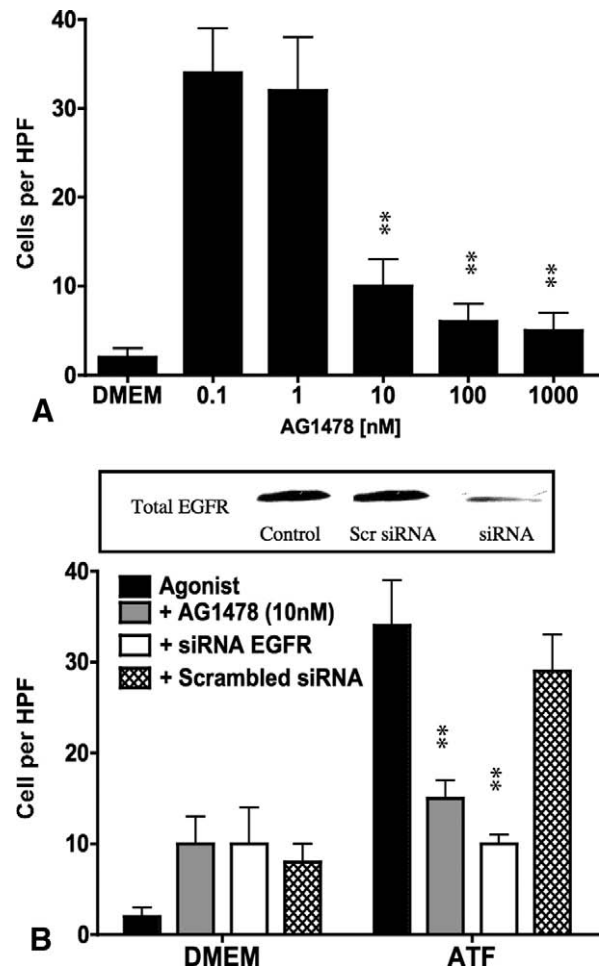
Fig 2. Amino-terminal (ATF) (10 nM) induces significant smooth muscle cell migration in the Boyden chamber migration assays. **A,** Migration in response to ATF was decreased in a concentration-dependent manner by the epidermal growth factor receptor (EGFR) inhibitor (AG1478, 1 hour pre-stimulation and 12 hours stimulation); **(B)** Pre-incubation (4-6 hours) with small interference ribonucleic acid (siRNA) to EGFR inhibited the migratory response. Insert shows the efficacy of the siRNA in knocking down the total EGFR protein present. Values are the mean ± SEM (n = 6, **P < .01 compared to control).

6500 × g for at least 3 hours until volume was reduced from 4 to 0.5 mL. Concentrates were incubated with 2.5 μg of anti-HB-EGF antibody and protein A-agarose overnight at 4°C. Immunoprecipitates were washed three times, boiled, and electrophoresed on 15% SDS-polyacrylamide gels. Using conventional Western immunoblotting techniques, HB-EGF levels were assessed using goat polyclonal anti-HB-EGF IgG and donkey anti-goat IgG.

Data and statistical analysis. All data are presented as the mean ± standard error of the mean (SEM) and statistical differences between groups were tested with a Kruskal-Wallis nonparametric test with post hoc Dunn's multiple comparison correction, where appropriate. A *P* value less than .05 was regarded as significant.
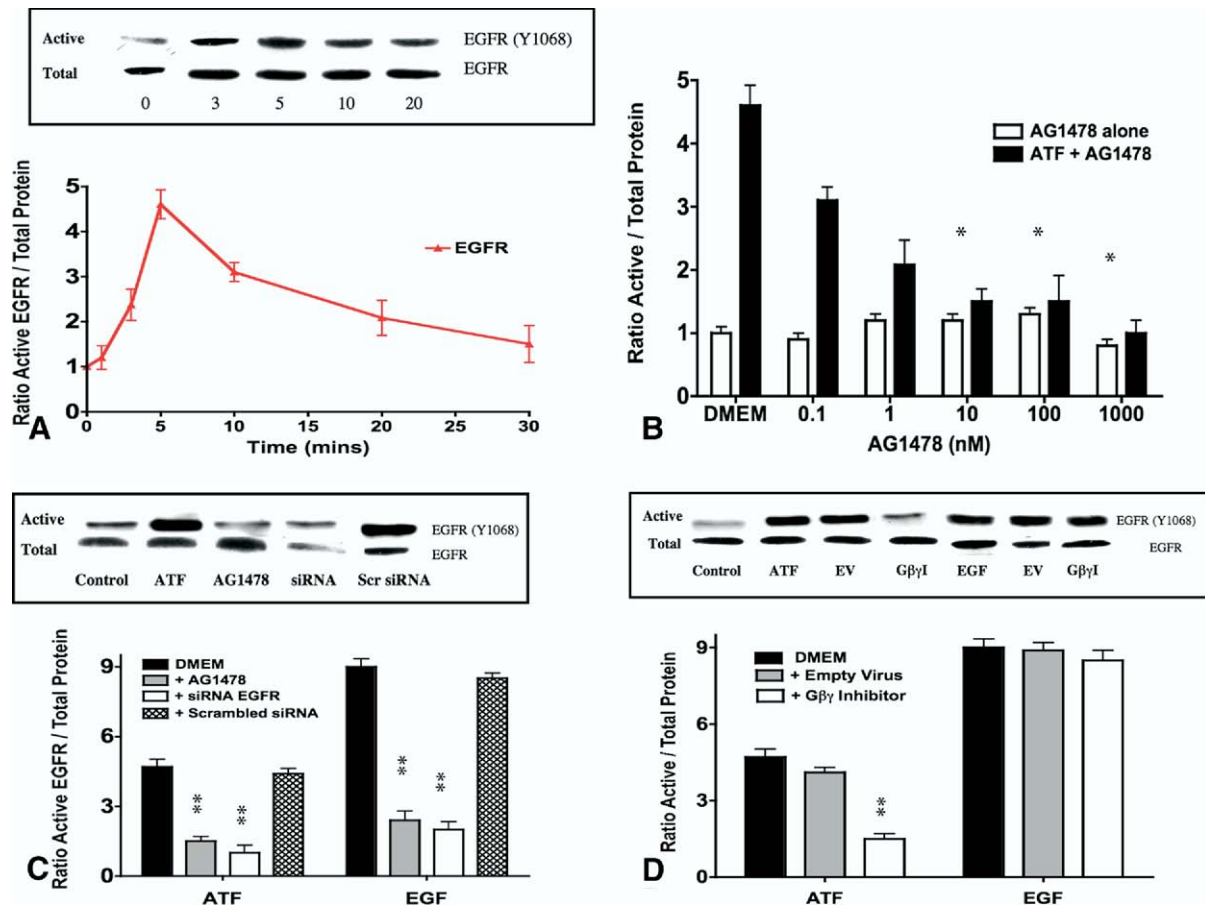
Fig 3. A, Amino-terminal (ATF)-induced a fourfold increase in epidermal growth factor receptor (EGFR) phosphorylation and **(B)** this response was inhibited in a concentration-dependent manner by increasing concentrations of AG1478 (0.1-1000 nM, 1 hour pre-stimulation). **C,** Pre-incubation with small interference ribonucleic acid (siRNA) to EGFR inhibited EGFR activation in response to ATF and also inhibited EGFR phosphorylation in response to EGF. Response to AG1478 is shown for comparison **(D)**. Transfection with adenovirus containing the Gβγ inhibitor βARK$_{CT}$ (GβγI) inhibited EGFR activation in response to ATF, but had no effect in response to epidermal growth factor (EGF). Values are the mean ± SEM of the ratio of the phosphorylation of EGFR relative to the total unphosphosphorylated EGFR (n = 6, *P < .05, **P < .01 compared to Dulbecco's modified Eagle's medium [DMEM] control). A representative Western blot is shown above the graphs.

RESULTS

To examine the effect of EGFR on ATF mediated VSMC migration, we employed the Boyden chamber migration assay. ATF (10 nM) induced significant smooth muscle cell migration (Fig 2). A similar response was noted if hydroxyurea was added to prevent and control for proliferation. This response to ATF was inhibited by increasing concentrations of the EGFR inhibitor, AG1478, and by application of siRNA against EGFR (Fig 2), suggesting that EGFR plays a role in the pathways leading to ATF-mediated cell migration. ATF induced a fourfold increase in EGFR phosphorylation as determined by activation of Y1068 phosphorylation site; the phosphorylation response was inhibited in a concentration-dependent manner by increasing concentrations of AG1478 (0.1-1000 nM) (Fig 3, *A* and *B*). Pre-incubation with siRNA to EGFR inhibited

EGFR activation in response to ATF (Fig 3, *C*). ATF is known to mediate its response through Gαi G-proteins.[10] Gαi proteins are linked to Gβγ proteins, and Gβγ proteins are known to be associated with triple membrane passing system of tyrosine kinase-linked receptors such as EGFR. To examine the role of Gβγ, we blocked Gβγ with the inhibitor peptide βARK$_{CT}$ and showed that pre-incubation with βARK$_{CT}$ inhibited EGFR activation by ATF (Fig 3, *D*). No effect was seen with the empty vector or with EGF application. When we pre-incubated VSMC with βARK$_{CT}$ in the Boyden chamber, we saw an 85% decrease in migration in response to ATF (empty vector resulted in a 5% decrease).

To determine if ATF activation of EGFR is via a transactivation mechanism, we examined the role of HB-EGF on EGFR activation in response to ATF. EGFR phosphor-
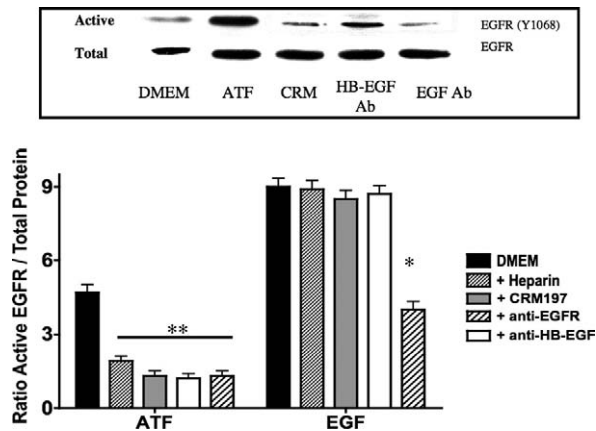
Fig 4. Epidermal growth factor receptor (EGFR) phosphorylation by amino-terminal (ATF) (10 nM) was inhibited by preincubation with heparin (100 U/mL), blockade of the ligand heparin binding epidermal growth factor (HB-EGF) with the HB-EGF inhibitor CRM197 and an Anti-HB-EGF antibody. IgG had no effect. EGF-mediated activation of EGFR was not blocked by these inhibitors. Direct blockade of the EGFR prevented activation by both ATF and EGF. Values are the mean ± SEM of the ratio of the phosphorylation of EGFR relative to the total unphosphophosphorylated EGFR (n = 6, **$P < .01$ compared to control). Representative Western blots are shown above the graphs.

ylation by ATF was blocked by pre-incubation with heparin and by blockade of the ligand HB-EGF with the HB-EGF inhibitor CRM197 (Fig 4). This suggests that HB-EGF is involved and that it is being ligated by a protease, can bind the EGFR extracellularly and induce EGFR phosphorylation. Incubation with an antibody against HB-EGF blocked the ATF but not the EGF-mediated activation of EGFR (Fig 4). Direct blockade of the EGFR with an anti-EGFR antibody prevented activation by both ATF and EGF (Fig 4). We tested whether plasmin had a role in ATF signaling to EGFR. Blockade of metalloprotease activity with the general inhibitor GM6001, at a level that inhibited MMP-2/MMP-9 activity on gelatin zymography, did significantly inhibit EGFR phosphorylation (Fig 5). EGFR phosphorylation by EGF was not interrupted by the presence of inhibitors of plasmin or MMPs (Fig 5). Given the fact that we demonstrated that intracellular processes (Gβγ) and MMPs are involved, we tested whether ADAM are involved. ADAMs span the membrane and can be activated intracellularly to mediate an extracellular MMP action. VSMC were incubated with the ADAM inhibitors TAPI-0 (20 μM) and TAPI-1 (10 μM) and both blocked EGFR activation by ATF but had no effect on EGFR activation by EGF (Fig 5). The VSMC cells express ADAM-9, ADAM-10, and ADAM-12. We examined their contribution to EGFR activation by the use of siRNA to ADAM-9, ADAM-10, and ADAM-12. The siRNA reduced ADAM-9, ADAM-10, ADAM-12 protein levels by >50% (Fig 6). Incubation with siRNA ADAM-9 and ADAM-10 significantly reduced EGFR activation in response to ATF. Scrambled siRNA and siRNA against
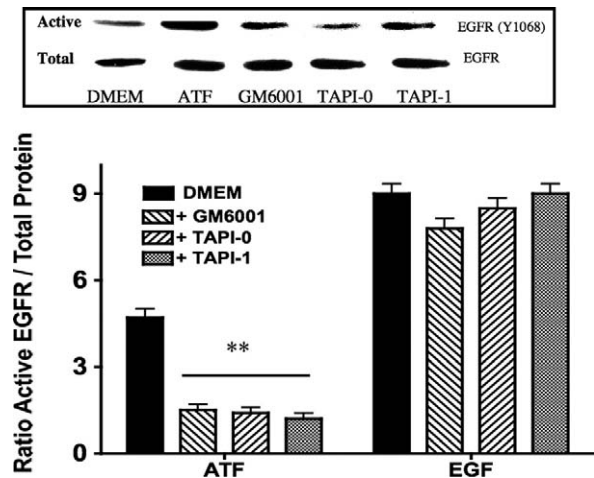

Fig 5. Amino-terminal (ATF) (10 nM) induced epidermal growth factor receptor (EGFR) phosphorylation which was inhibited by the matrix metalloprotease inhibitor (GM6001) and the A Disintegrin And Metalloproteinase Domains (ADAM) inhibitors (TAPI-0 and TAPI-1). Values are the mean ± SEM of the ratio of the phosphorylation of EGFR relative to the total unphosphosphorylated EGFR (n = 6, *$P < .05$ **$P < .01$ compared to control). Representative Western blots are shown above the graphs.

ADAM-12 had no effect (Fig 6). When the quantity of HB-EGF released by ATF was measured in the presence of the metalloprotease inhibitor, GM6001 and the ADAM inhibitors, TAPI-0 and TAPI-1, there was a significant reduction in the concentration of HB-EGF in the media (Fig 6). This response was reproduced in the presence of siRNA to ADAM-9 and ADAM-10; siRNA to ADAM-12, and a scrambled control had no effect (Fig 6). When we pre-incubated VSMC with siRNA to ADAM-9 and ADAM-10 in the Boyden chamber, we saw a 70% decrease in migration compared to the scrambled siRNA and control in response to ATF (Fig 6).

DISCUSSION

This study demonstrates that ATF requires EGFR for cell migration and that this response is mediated by Gbg G-proteins and by ADAM-9 and ADAM-10. ADAMs in turn release the tethered ligand HB-EGF by a metalloprotease mediated process to activate EGF. This is distinct from our published findings with the findings with the intact molecule.[6] Kalmes et al[16] have shown that thrombin, another coagulation factor, will also mediate EGFR-dependent VSMC migration and induce EGFR activation through an extracellular pathway, which involves MMP-mediated, HB-EGF release, and subsequent activation of the EGFR. The EGFR/ErbB family is composed of four receptor tyrosine kinases: ERB1/EGFR, ErbB2/neu, ErbB3, and ErbB4, each of which consists of a glycosylated ligand binding domain, a single transmembrane domain and a cytoplasmic tyrosine kinase domain.[17,18] Eleven different ligands have been identified for this receptor family; EGF, TGF-α, amphoregulin, HB-EGF, betacellulin, epi-
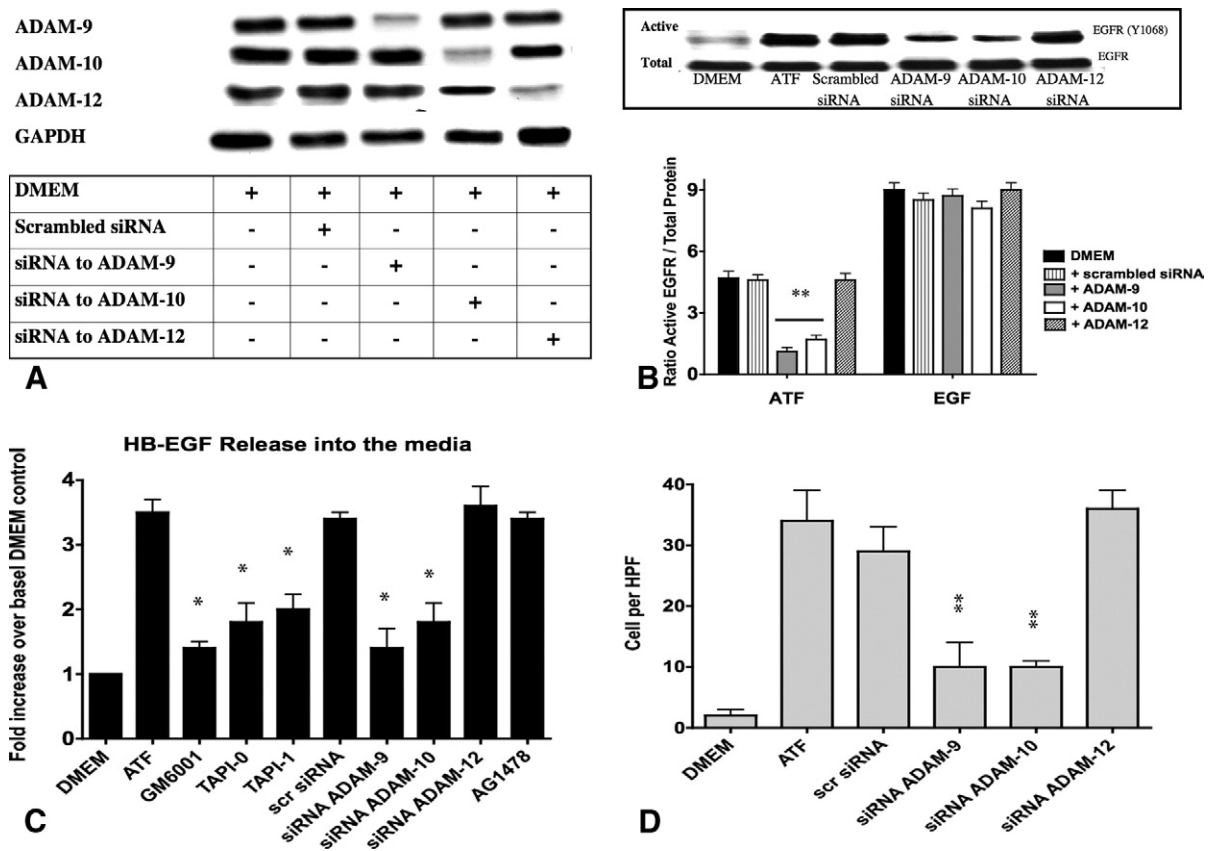
Fig 6. A, Small interference ribonucleic acid (siRNA) to A Disintegrin And Metalloproteinase Domains (ADAM)-9, ADAM-10 and ADAM-12 resulted in a ~50% decrease in their respective ADAM protein expression compared to Dulbecco's modified Eagle's medium (DMEM) control or scrambled siRNA. This was specific. **B,** Incubation with siRNA, ADAM-9, and ADAM-10 significantly reduced epidermal growth factor receptor (EGFR) activation in response to ATF. Scrambled amino-terminal (ATF) siRNA and siRNA against ADAM-12 had no effect. **C,** Incubation with GM6001, TAPI-0 and TAPI-1 and siRNA to ADAM-9 and ADAM-10 markedly decreased heparin binding epidermal growth factor (HB-EGF) release from smooth muscle cells into the media. **D,** Incubation with siRNA to ADAM-9 and ADAM-10 markedly decreased smooth muscle cells migration compared to scrambled siRNA in the Boyden chamber in response to ATF. Values are the mean ± SEM (n = 6, * $P < .05$, ** $P < .01$ compared to control).

regulin, epigen bind to activate EGFR/ErbB1; betacellu-lin, and in some cases HB-EGF, activate ErbB4 in addition to ErbB1. Neuregulins are ligands for ErbB3 and ErbB4. Although no ligand for ErbB2 has been identified, it appears to act as a cofactor for ErbB1, ErbB3, and ErbB4 forming heterodimers with the respective receptors. These heterodimers enhance and diversify downstream signal transduction pathways. ErbB1 has been studied in VSMC and is expressed in the injured vessel wall.[19,20] In the rat carotid injury model, blockade of EGF with an antibody will reduce intimal hyperplasia development.[21] In a murine injury model, deletion of uPA reduced intimal hyperplasia while deletion of PAI-1 enhanced intimal hyperplasia.[22-25] Thus, EGFR and uPA both appear to be necessary for vessel remodeling. All ErbB ligands are synthesized as transmembrane precursors and reside in a tethered fashion on the extracellular membrane. They can then in turn be proteolytically cleaved to release biologically active soluble growth factors, which act in an autocrine and/or paracrine manner. HB-EGF is expressed in atherosclerotic lesions[26,27] and in restenotic lesions following angioplasty.[28-30] The present study examined HB-EGF as HB-EGF has been shown to stimulate the growth and migration of VSMC under appropriate conditions.[29,31] Activation of HB-EGF requires that it be cleaved from the membrane and also requires cell surface heparan sulphate proteoglycans to act as a co-receptors; HB-EGF binding to EGFR can be antagonized in a dose-dependent manner by heparin.[32] We saw a similar inhibitory effect with heparin in this study. Our data using CRM197 and antibodies against HB-EGF and EGFR support the presence of the extracellular pathway using HB-EGF.

Receptor transactivation is the process whereby activation of a given receptor activates a heterologous receptor.[12] Both G-protein-coupled receptors and receptor-linked tyrosine kinases can induce rapid phosphorylation of the

EGFR, and suppression of this EGFR activation leads to reduced MAPK activation.[12-14] At present, the TMPS mechanism of GPCR-induced EGFR activation is a widely accepted model of receptor-linked tyrosine kinases transactivation.[12] In this model, there is a sequence of three transmembrane signaling events: GPCR activation followed by MMP activation, and subsequent activation of the EGFR by HB-EGF, or other latent ligands of the EGFR. Several different MMPs have been identified with HB-EGF release: ADAM-10, ADAM-12, ADAM-17 (TNF-α converting enzyme), and MMP3.[33] Activation of these proteases pathway has been shown to involve one or more of the following signaling molecules: src, intracellular calcium, and protein kinase C.[12] An alternative mechanism may be inactivation of protein tyrosine phosphatases due to the generation of oxygen free radicals by the NAD(P)H oxidase complex. In the current study, we have identified both intracellular and extracellular components of transactivation. Transmembrane molecules such as ADAM are attractive molecules for the transmembrane portion of the signaling pathway. ADAM are approximately 70 to 90 kDa (mature proteins; the unprocessed precursors are about 20 kDa larger due to their prodomain). They feature a common modular ectodomain structure, encompassing a variable stalk region; a cysteine-rich domain, a disintegrin domain binding to integrin-class cell adhesion molecules and a Zn-binding metalloprotease domain. ADAM-12, ADAM-9, ADAM-10, and ADAM-17 have been reported to be involved in the ectodomain shedding of pro-HB-EGF.[34,35] We have shown that the VSMC express ADAM-9, ADAM-10, and ADAM-12. Furthermore, we have identified that the predominant pathways that lead to HB-EGF release are ADAM-9 and ADAM-10 in these particular VSMC. Little is known of the role of ADAM in atherogenesis and vessel remodeling.

CONCLUSION

ATF requires EGFR activation during cell migration. ATF induces transactivation of EGFR by an ADAM-mediated, HB-EGF-dependent process. This is the first description of crosstalk via ADAM between ATF and EGFR in VSMC.

AUTHOR CONTRIBUTIONS

Conception and design: AB, MD
Analysis and interpretation: AB, MD
Data collection: AB, CP, ER, SN
Writing the article: AB, CP, SN, MD
Critical revision of the article: AB, CP, ER, SN, MD
Final approval of the article: AB, CP, ER, SN, MD
Statistical analysis: AB, MD
Obtained funding: MD
Overall responsibility: MD

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