HB-EGF release mediates glucose-induced activation of the epidermal growth factor receptor in mesangial cells

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Uttarwar L, Peng F, Wu D, Kumar S, Gao B, Ingram AJ, Krepinsky JC. HB-EGF release mediates glucose-induced activation of the epidermal growth factor receptor in mesangial cells. Am J Physiol Renal Physiol 300: F921–F931, 2011. First published February 2, 2011; doi:10.1152/ajprenal.00436.2010.—Glomerular matrix accumulation is a hallmark of diabetic nephropathy. We showed that transactivation of the epidermal growth factor receptor (EGFR) is an important mediator of matrix upregulation in mesangial cells (MC) in response to high glucose (HG). Here, we study the mechanism of EGFR transactivation. In primary MC, EGFR transactivation by 1 h of HG (30 mM) was unaffected by inhibitors of protein kinase C, reactive oxygen species, or the angiotensin II AT1 receptor. However, general metalloprotease inhibition, as well as specific inhibitors of heparin-binding EGF-like growth factor (HB-EGF), prevented both EGFR and downstream Akt activation. HB-EGF was released into the medium by 30 min of HG, and this depended on metalloprotease activity. One of the metalloproteases shown to cleave proHB-EGF is ADAM17 (TACE). HG, but not an osmotic control, activated ADAM17, and its inhibition prevented EGFR and Akt activation and HB-EGF release into the medium. siRNA to either ADAM17 or HB-EGF prevented HG-induced EGFR transactivation. We previously showed that EGFR/Akt signaling increases transforming growth factor (TGF)-β1 transcription through the transcription factor activator protein (AP)-1. HG-induced AP-1 activation, as assessed by EMSA, was abrogated by inhibitors of metalloproteases, HB-EGF and ADAM17. HB-EGF and ADAM17 siRNA also prevented AP-1 activation. Finally, these inhibitors and siRNA prevented TGF-β1 upregulation by HG. Thus, HG-induced EGFR transactivation in MC is mediated by the release of HB-EGF, which requires activity of the metalloprotease ADAM17. The mechanism of ADAM17 activation awaits identification. Targeting upstream mediators of EGFR transactivation including HB-EGF or ADAM17 provides novel therapeutic targets for the treatment of diabetic nephropathy.

diabetic nephropathy; high glucose; TGF-β1; ADAM17

DIABETIC NEPHROPATHY is an important cause of end-stage renal disease, the pathogenesis of which has not been fully elucidated. Clinically, the major treatment and preventive options for diabetic nephropathy are control of blood pressure and serum glucose levels, as well as blockade of the renin-angiotensin system. However, progressive disease may occur despite these measures (27). A better understanding of the pathogenesis is thus required.

Our previous studies showed that the epidermal growth factor receptor (EGFR) is relatively rapidly (within 10–30 min) transactivated by high glucose in mesangial cells (MC) (52). EGFR transactivation mediated downstream signaling through Akt to activate the transcription factor activator protein (AP)-1 and upregulate transforming growth factor-β1 (TGF-β1) and collagen I synthesis by high glucose (52, 53). In vascular smooth muscle cells, prolonged (48 h) high glucose altered the glycosylation status of the EGFR and sensitized it to transactivation by G protein-coupled ligands including angiotensin II (22). How the EGFR is transactivated by high glucose, however, particularly in a much shorter time frame, is as yet unknown.

Stimuli with direct relevance to diabetes that have been shown to transactivate or to be required for transactivation of the EGFR include the generation of reactive oxygen species (ROS), angiotensin II, and PKC (4, 32, 41). Furthermore, it has increasingly been recognized that stimuli thought to transactivate the EGFR (such as angiotensin II or TGF-β) may in fact lead to processing and release of soluble ligands, which subsequently activate the receptor (36, 47). EGFR family ligands include EGF, the heparin-binding EGF-like growth factor (HB-EGF), TGF-α, and others. These are synthesized as membrane-anchored precursors that can be processed by specific metalloproteases to release soluble bioactive factors from the cell surface, known as “ectodomain shedding” (33). HB-EGF is of particular interest in the diabetic setting, since increased transcript levels were found in diabetic [streptozotocin (STZ)-induced] kidneys (26). Furthermore, smooth muscle cells obtained from STZ diabetic rats showed a heightened mitogenic response to HB-EGF compared with cells from nondiabetic or insulin-treated rats. This increased responsiveness was associated with greater EGFR activation (9). A role for HB-EGF in matrix elaboration has also been seen, with HB-EGF stimulating collagen I production in MC (44).

Several different proteases belonging to a large family of zinc-binding endopeptidases have the ability to cleave the precursor form of HB-EGF (proHB-EGF), including both matrix metalloproteases (MMPs) and a disintegrin and metalloproteases (ADAMs) (4, 17, 19). Of these, ADAM17 expression has been confirmed in MC and was shown to mediate 5-HT-dependent transactivation of the EGFR by stimulating HB-EGF shedding (12). Of interest, in models of angiotensin II infusion and uninephrectomy, ADAM17 inhibition attenuated glomerular and interstitial lesions, although these effects were ascribed to decreased TGF-α, another ADAM17 cleavage product (25). With the exception of a report showing increased ADAM17 expression in glomeruli and tubules of patients with diabetes (29), the role of ADAMs in diabetic nephropathy has not been addressed.

In this study, we aimed to identify the mechanism by which glucose transactivates the EGFR in MC. Although angiotensin II, ROS, and PKC can mediate EGFR transactivation in other settings, they are not involved in this milieu. We subsequently assessed the possibility of a metalloprotease-mediated cleavage event in driving EGFR transactivation, and our studies now identify a prominent role for ADAM17-induced cleavage of
HB-EGF in glucose-induced early transactivation of the EGFR. We further show the relevance of this early signaling event in mediating downstream upregulation of the profibrotic cytokine TGF-β. Our studies provide a basis for further exploration of the therapeutic potential of targeting ADAM17 in diabetic kidney disease.

MATERIALS AND METHODS

Cell culture. Sprague-Dawley primary rat MC (passages 6–15) were cultured in DMEM supplemented with 20% fetal calf serum (Invitrogen), streptomycin (100 μg/ml), and penicillin (100 U/ml) at 37°C in 95% air-5% CO₂. Medium contained 5.6 mmol/l glucose. Either 24.4 mmol/l glucose or mannitol (final concentration 30mmol/l) was added for high glucose or osmotic control, respectively. MC were made quiescent by serum deprivation for 24 h before treatment. Pharmacologic inhibitors were added before glucose as follows: PMA (200 nM, 24 h; Sigma), bisindolylmaleimide I (2 μM, 30 min; EMD), N-acetyl-cysteine (4 μM, 30 min; Sigma), ebenezol (50 μM, 60 min; EMD), losartan (10 μM, 30 min; Merck & Co.), GM6001 (20 μM, 60 min; EMD), heparin (100 U/ml, 30 min; PCC), CRM197 (500 ng/ml, 60 min; Sigma), TAPI2 (100 μM, 60 min; EMD), GM6001 (2 μM, 60 min; Sigma), G254023X (5 μM, 30 min; R&D Systems), and goat IgG (5 μM, 30 min; Jackson Immuno-Research).

Protein extraction and analysis. Cells were lysed and protein was extracted as we published (24). Cell lysates were centrifuged at 16,000 × g for 10 min to pellet cell debris. Supernatant (50 μl) was then added to 50 μl of 2× sample buffer, boiled, and analyzed by immunoblotting.

Alkaline phosphatase assay. HB-EGF cleavage was assessed using pRC/CMV-HBEGF-AP, kindly provided by Dr. M. Freeman (Harvard University) (20). MC stably expressing this vector were generated by antibiotic selection with neomycin after plasmid transfection using Effectene (Qiagen). Serum-deprived MC were washed with PBS and replacement 0% serum medium was collected after 1 h to assess for baseline shedding. This was replaced with new medium containing high glucose with or without drug, and medium was collected after 1 h of glucose treatment. Alkaline phosphatase activity in the medium was measured using a kit according to the manufacturer’s specifications (AnaSpec). Shedding induced by treatment was compared with baseline shedding for each well.

ADAM17 activity assay. After treatment, protein was extracted from MC lysed in activity assay buffer (50 mM Tris-HCl, pH 7.4, 25 mM NaCl, 4% glycerol, 10 mM ZnCl₂). ADAM17 activity was measured in duplicate for each sample using 20 μg of protein and the TACE Substrate IV (Calbiochem). Cleavage of this substrate was measured in a fluorometer at 420 nm.

RNA interference. Rat HB-EGF and ADAM17 Silencer Select siRNA and control nontargeting siRNA were purchased from Applied Biosystems. MC were transfected with 100 nM using GeneEraser siRNA reagent (Stratagene) at 60% confluence. After 48 h, cells were serum deprived for 24 h, and cell response to high glucose was assessed. Protein expression was used to assess efficacy of downregulation by RNAi.

EMSA. After treatment, nuclear extracts were prepared as published (23). Cells were lysed in hypotonic buffer A, homogenized, and sedimented at 16,000 g for 20 min at 4°C. Pelleted nuclei were resuspended in buffer A with added 0.42 M NaCl and 20% glycerol, rotated for 30 min at 4°C, centrifuged as above, and supernatant containing nuclear proteins was collected. Nuclear proteins (3 μg) were incubated for 5 min at room temperature with a biotin-labeled AP-1 consensus oligonucleotide (Sigma) as per manufacturer’s instructions (Pierce). Reaction mixtures were electrophoresed in a 6% polyacrylamide gel, transferred and DNA cross-linked to a nylon membrane (Amersham), then probed with horseradish peroxidase-conjugated streptavidin antibodies (1:300; Pierce).

Northern analysis. Total RNA (20 μg), extracted using Trizol (Invitrogen), was separated on a formaldehyde-agarose gel and transferred to a nylon membrane (Hybond, Amersham Biosciences). Hybridization was performed with random primed digoxigenin-11-DUTP-labeled cDNA probe prepared from TGF-β cDNA amplified by PCR. Hybridized probes were detected using alkaline phosphatase-labeled anti-digoxigenin antibodies and CDP-star as substrate. Kits and reagents were from Roche Applied Science.

Luciferase assay. MC plated to 85% confluence were transfected with 0.5 μg of a TGF-β1 promoter (−1132 to +11)–luciferase construct (kindly provided by Dr. N. Kato) (46) and 0.05 μg PCMV-β-galactosidase (β-gal; Clontech) using LipofectAMINE (Qiagen). MC were serum-deprived overnight 24 h after transfection and then exposed to glucose for 24 h. Lysis was achieved with Reporter Lysis Buffer (Promega) using one freeze-thaw cycle, and luciferase and β-gal activities were measured on clarified lysate using specific kits (Promega) with a Berthold luminometer and a plate reader (420 nm), respectively. β-Gal activity was used to adjust for transfection efficiency.

Statistical analyses. Statistical analyses were performed with SPSS 14 for Windows using one-way ANOVA, with Tukey’s HSD for post hoc analysis for experiments with more than two groups. A t-test was used for analysis of two groups. A P value <0.05 (2-tailed) was considered significant. Data are presented as means ± SE. The number of repetitions for experiments, set up at different times with different passages of MC, is represented in figure legends by “n = “.

Fig. 1. Epidermal growth factor receptor (EGFR) transactivation by high glucose (HG) does not require protein kinase C (PKC), reactive oxygen species, or angiotensin II. Serum-deprived mesangial cells (MC) were pretreated with various inhibitors followed by HG for 60 min. EGFR transactivation was assessed by Western blotting for phosphorylation of Y1068. Inhibitors were PKC inhibitors PMA and bisindolylmaleimide (Bis, A), reactive oxygen species scavengers N-acetylcysteine (NAC) and ebenezol (Ebs; B), and angiotensin II receptor I antagonist losartan (Los; C).
RESULTS

HB-EGF release mediates glucose-induced EGFR transactivation. Various upstream mediators of EGFR transactivation have been identified, with the role of specific pathways dependent on cell type and stimulus. We thus used inhibitors of the most common potential mediators of EGFR transactivation to identify those involved in glucose-induced signaling. PKC transactivates the EGFR through release of HB-EGF in MC in response to both angiotensin II and TGF-β (32, 47, 48). We

Fig. 2. EGFR transactivation and downstream Akt activation by HG require heparin-binding EGF-like growth factor (HB-EGF). MC were treated with various inhibitors as outlined in MATERIALS AND METHODS. EGFR transactivation and Akt activation were assessed by Western blotting for phosphorylation of Y1068 and S473, respectively.

A: †P < 0.05, n = 4; *P < 0.01, n = 6. B: †P < 0.05, n = 4; *P < 0.01, n = 4. C: †n = 3 for EGFR and n = 4 for Akt. D: *P < 0.01 vs. control and α-HB-EGF, n = 6 for EGFR and n = 4 for Akt.
used the nonspecific PKC inhibitor PMA (24-h pretreatment) as well as the conventional PKC inhibitor bisindolylmaleimide I to assess the role of PKC in glucose-induced EGFR transactivation, as determined by autophosphorylation at Y1068. Figure 1A shows that neither inhibitor blocked glucose-induced EGFR transactivation. ROS mediate EGFR transactivation in various settings (7, 41). However, the ROS scavengers ebselen and N-acetylcysteine did not affect EGFR transactivation by glucose (Fig. 1B). Given the important role of angiotensin II in diabetic nephropathy and its ability to transactivate the EGFR through its type 1 receptor (AT1R) (2, 54), we also tested the effects of the AT1R inhibitor losartan. As seen in Fig. 1C, losartan did not prevent EGFR transactivation by glucose. The release of EGFR ligands by metalloproteases is also a well-described mechanism for EGFR transactivation (33). Using the broad spectrum metalloprotease inhibitor GM6001, we next assessed whether metalloprotease activity was required for EGFR transactivation by glucose. Figure 2A shows that EGFR transactivation was inhibited by GM6001. We previously showed that Akt is activated downstream of the EGFR by high glucose and mediates matrix upregulation (52, 53). As seen in Fig. 2A, Akt activation, as assessed by phosphorylation on S473, was also inhibited by GM6001.

Although several ligands exist for the EGFR, HB-EGF was shown by others to be upregulated in diabetic nephropathy and to mediate angiotensin II signaling in MC (26, 32). We thus tested whether it was involved in the regulation of EGFR activation by glucose. We first assessed the effects of heparin, which inhibits EGFR transactivation by competing with cell surface-associated heparan sulfate proteoglycans, coreceptors for HB-EGF and amphiregulin binding to EGFR (8). Figure 2B shows that heparin prevented glucose-induced EGFR transactivation and Akt activation. We then confirmed a role for HB-EGF using more specific inhibitors. CRM197 is a mutated nontoxic form of diphtheria toxin that binds the extracellular domain of HB-EGF and blocks its biological activity (37). CRM197 effectively blocked both EGFR transactivation and Akt activation (Fig. 2C). Furthermore, a neutralizing antibody to soluble HB-EGF also blocked glucose-induced EGFR transactivation and Akt activation, while a nonspecific antibody was without effect (Fig. 2D). Together, these data suggest that EGFR transactivation by glucose, along with...
activation of a downstream signaling pathway, is dependent on HB-EGF release.

To further confirm that HB-EGF is necessary for glucose-induced EGFR transactivation, MC were stably transfected with an AP-tagged proHB-EGF construct, which has previously been used to measure shedding of HB-EGF (40). HB-EGF shedding was assessed by detection of AP activity in the medium, with the baseline shedding rate serving as the comparison control. Figure 3A shows that high glucose (1 h) led to significantly increased HB-EGF shedding. Short-term PMA pretreatment (30 min), which does not inhibit PKC, but is known to induce HB-EGF shedding (8), was used as a positive control. We then confirmed that the general metalloprotease inhibitor GM6001 blocked high glucose-induced HB-EGF release into the medium (Fig. 3B). Next, we downregulated the expression of HB-EGF using siRNA. Successful downregulation is shown in Fig. 3D, in which mature HB-EGF characteristically appears as multiple bands between 20 and 30 kDa due to glycosylation (16, 36). As shown in Fig. 3C, untransfected MC and those transfected with control siRNA showed similar degrees of EGFR transactivation by 1 h of high glucose. This response was prevented in MC transfected with HB-EGF siRNA. Thus, HB-EGF mediates high glucose-induced EGFR transactivation and downstream Akt activation in MC.

**ADAM17 is required for HB-EGF release and EGFR transactivation by glucose.** HB-EGF shedding can be affected by several MMPs/ADAMs, with the specific sheddase being highly dependent on cell type and agonist (7, 45). We thus sought to identify the metalloprotease responsible for HB-EGF release. Given that ADAM17 is the major convertase for HB-EGF (40), that high glucose activates ADAM17 in vascular smooth muscle cells (38), and that MC express ADAM17 (12), we first studied the effects of the ADAM17 inhibitor TAPI2. Figure 4A shows that TAPI2 almost completely prevented high glucose-induced HB-EGF release into the medium as assessed by AP activity in MC overexpressing AP-tagged proHB-EGF. We next assessed ADAM17 activity using a fluorogenic substrate specific for this protease. As seen in Fig. 4, B and C, high glucose (1 h), but not the osmotic control mannitol, activated ADAM17. This was prevented by TAPI2. As expected, given these results, TAPI2 prevented high glucose-induced transactivation of the EGFR (Fig. 4D). Finally, to confirm the necessity of ADAM17 in glucose-induced EGFR transactivation, we assessed the effects of ADAM17 siRNA. Figure 4E shows that ADAM17 downregulation did prevent EGFR transactivation by high glucose. Successful knockdown of ADAM17 is shown in Fig. 4F. Since ADAM10 has also been described to lead to HB-EGF release (19), we assessed its involvement using its specific inhibitor GI254023X (18). As seen in Fig. 4G, ADAM10 inhibition did not prevent glucose-induced EGFR transactivation. In aggregate, these data demonstrate that high glucose leads to the activation of ADAM17, which cleaves HB-EGF, leading to EGFR transactivation.

**TGF-β1 upregulation by glucose is dependent on ADAM17-induced HB-EGF release.** We previously demonstrated that EGFR transactivation and downstream Akt signaling are required for the upregulation of TGFβ1 by high glucose, and this is mediated by the transcription factor AP-1 (53). We thus next studied the involvement of ADAM17 and HB-EGF in these downstream events. Figure 5A shows that the glucose-induced activation of AP-1 was blocked by metalloprotease inhibition with GM6001. Neutralization of HB-EGF in the medium also decreased AP-1 activation by high glucose, while a nonspecific antibody was without effect (Fig. 5B). Furthermore, the ADAM17 inhibitor TAPI2 also prevented glucose-induced AP-1 activation (Fig. 5C), as did siRNA downregulation of either ADAM17 or HB-EGF (Fig. 5D).

Next, we confirmed that TGF-β1 upregulation by glucose was also dependent on ADAM17 activation and HB-EGF signaling. Using a luciferase construct, which contains the promoter region of TGFβ1, we showed in Fig. 6A that ADAM17 inhibition with TAPI2 prevented glucose-induced promoter activation. Figure 6, B and C, further shows that siRNA downregulation of HB-EGF and ADAM17, respectively, also prevented TGF-β1 promoter activation by glucose. These effects were confirmed at the transcript level using Northern blotting. Figure 6D shows that the metalloprotease inhibitor GM6001 and the HB-EGF inhibitor heparin both blocked TGF-β1 transcript upregulation by high glucose, and Fig. 6E shows inhibition of TGF-β1 transcript upregulation by the ADAM17 inhibitor TAPI2. Taken together, these data indicate that the glucose-induced activation of AP-1 and upregulation of TGF-β1 are dependent on ADAM17-mediated HB-EGF release. Given the prominent role of AP-1 in TGF-β promoter upregulation by glucose (50), these studies suggest that ADAM17/HB-EGF mediate TGF-β promoter activation through effects on this transcription factor. However, other factors have been described to regulate TGF-β promoter activation by glucose, including upstream stimulatory factors, which bind to glucose response elements in the TGF-β promoter (49, 56). Our data do not exclude their contribution to promoter activation.

**DISCUSSION**

In previous studies, we demonstrated the importance of EGFR transactivation in the fibrogenic responses of MC to high glucose (52, 53). Our current work extends these findings by revealing the mechanism whereby EGFR transactivation occurs. Here, we identified the release of the EGFR ligand HB-EGF as an important mediator of EGFR transactivation by high glucose. We further defined a critical role for the metalloprotease ADAM17 in the cleavage and release of HB-EGF. Targeting HB-EGF or ADAM17 thus represents potential new therapeutic options for the treatment and/or prevention of diabetic nephropathy.

The activation of EGFR by mechanisms other than initial direct ligand binding, a process known as transactivation, has been recognized as an important event in signaling (13). Several stimuli known to be involved in the pathogenesis of diabetic nephropathy, including ROS, angiotensin II, TGF-β, and PKC, were shown to transactivate the EGFR (4, 32, 41, 47). We thus initially tested the involvement of those mediators that were potentially activated in the short time frame in which we observe EGFR transactivation by glucose (within 1 h). However, our data demonstrate that oxidative stress, angiotensin II, and PKC are not involved in glucose-induced EGFR transactivation in MC. We cannot, however, exclude the possibility that these mediators play a role in the maintenance of EGFR transactivation with prolonged exposure to high glucose.
More recently, it has been recognized that stimuli thought to transactivate the receptor, such as angiotensin II, may in fact lead to processing and release of soluble EGFR ligands (36, 47). These ligands are synthesized as membrane-anchored precursors that can be processed by specific metalloproteases to release soluble bioactive factors from the cell surface. These events are largely still referred to as transactivation since transmembrane signaling events, such as ligand activation of a
G protein-coupled receptor, followed by generation of an intracellular signal that activates a protease, leads to the processing and release of the ligand. Our data show for the first time that high glucose also activates metalloprotease-dependent release of the EGFR ligand HB-EGF to affect EGFR transactivation.

In addition to its role in EGFR signaling to mediate high glucose-induced matrix upregulation in MC (52), HB-EGF release may also amplify or perpetuate renal injury through other mechanisms. For example, HB-EGF increased hexokinase activity in MC (39). The consequent increase in net glucose uptake may lead to increased matrix production, as seen in MC overexpressing the glucose transporter glut-1 (15). It is also of interest that hyperglycemia may sensitize cells to respond to HB-EGF (9, 10), possibly amplifying responses to low levels of secreted growth factor. Although the mechanisms underlying this heightened sensitivity were not explored, increased glycosylation of the EGFR was observed with longer-term glucose incubation (48 h) in vascular smooth muscle cells, increasing its transactivation by angiotensin II. Although HB-EGF was not involved (22), similar posttranslational modification of the EGFR may alter its susceptibility to HB-EGF signaling by glucose.

HB-EGF shedding may be affected by several metalloproteases. Although ADAM17 has been identified as its major sheddase (40), the particular sheddase involved in proHB-EGF cleavage is highly dependent on cell type and agonist (7, 33, 36). For example, oxidative and osmotic stress transactivate the EGFR mostly through ADAM10 and 17-mediated cleavage of proHB-EGF in several different tumor cells (7), but in rat gastric cells these stimuli did not activate ADAM17 (30). In MC, ADAM9 and ADAM17 were responsible for HB-EGF release in response to phorbol ester-induced PKC activation and serotonin, respectively (12, 14). Our data show that ADAM17 is the enzyme responsible for glucose-induced HB-EGF shedding in MC. Conversely, ADAM17 itself has other

Fig. 6. Transforming growth factor (TGF)-β upregulation by HG is dependent on ADAM-17-induced HB-EGF release. A-C: TGF-β upregulation by HG (24 h), as assessed by luciferase, was prevented by ADAM-17 inhibition with TAPI2 (†P < 0.05, n = 10; A), siRNA to HB-EGF (†P < 0.05, n = 6; B), and siRNA to ADAM-17 (†P < 0.01, n = 5; C). D and E: TGF-β upregulation by HG (24 h), as assessed by Northern analysis, was prevented by metalloprotease inhibition with GM or HB-EGF inhibition with heparin (Hep; *P < 0.01, n = 6; D), and by ADAM-17 inhibition with TAPI2 (†P < 0.05, n = 4; E).
major substrates, including TGF-α and TNF-α, some of which act as EGFR ligands (33). However, we implicated HB-EGF in several ways, and our data thus support the primary involvement of HB-EGF in EGFR transactivation by high glucose.

The mechanism whereby ADAM17 is activated by glucose in MC remains to be defined. A recent study in vascular smooth muscle cells implicated aldose reductase-induced PKCδ activation in ADAM17 activation by glucose (38). PKC was required for HB-EGF shedding in response to TGF-β in MC, although the sheddase was not identified (47), while oxidative stress activated ADAM17 in monocytes, perhaps through dissociation of its inhibitory and catalytic domains (55). However, our data do not support a role for ROS or PKC in ADAM17 activation. The MAPKs have also been implicated in ADAM17 activation in response to osmotic or oxidative stress (7). ADAM17 may also be regulated through phosphorylation of its cytoplasmic domain by Erk (1, 5, 43). Whether MAPKs play a role in glucose-induced ADAM17 activation remains to be addressed.

A few studies recently addressed the role of ADAM17 in diabetes, with increased ADAM17 staining observed in endothelial cells, MC, and proximal tubules in renal biopsies from seven patients with diabetic nephropathy. Glomerular ADAM17 staining was associated with mesangial matrix expansion (29). It is of interest that impaired regulation of ADAM17 by its inhibitor TIMP3 was observed in skeletal muscle of obese type 2 diabetic patients and suggested to contribute to the development of insulin resistance (31). In support of this construct, TIMP3 deficiency in insulin receptor haploinsufficient mice was associated with increased ADAM17 activity and soluble TNF-α and greater development of diabetes (6). Contrary to the effects on Akt in these studies, however, we found that glucose-induced activation of Akt required ADAM17 activation. Thus, the effect of ADAM17/TIMP3 dysregulation on signaling pathways varies with cell and tissue type.

HB-EGF upregulation was also observed in type 1 diabetic kidneys at early (7 days) and later (3 mo) stages of diabetes (26), and a small increase in renal HB-EGF was seen in GK type 2 diabetic rats (35). In vitro, HB-EGF expression was increased in response to mechanical, oxidative, or osmotic stress (3, 21, 34), although the effects of high glucose have not as yet been studied. Interestingly, EGFR transactivation may itself lead to HB-EGF upregulation, suggesting that increased HB-EGF levels may perpetuate the profibrotic responses seen in our cells (30). Indeed, the HB-EGF gene has binding sites for several transcription factors that may be activated by glucose, including NF-κB, AP-1, and Spl (11, 42, 51). The AP-1 site in particular (as part of a composite AP-1/Ets site) was primarily responsible for its induction by several stimuli (21, 28, 34), and we demonstrated that EGFR/Akt signaling regulates AP-1 activation by glucose (53). Thus, HB-EGF upregulation may contribute to increased profibrotic signaling through EGFR transactivation.

In further defining the mechanism of glucose-induced EGFR transactivation in MC, these studies have potential therapeutic implications. Although EGFR inhibitors are available, their long-term use, as would be required for diabetic nephropathy, would be limited by their adverse effects. We have now narrowed the potential target to HB-EGF or ADAM17. Inhibition of ADAM17 in particular may have additional beneficial effects, albeit through mechanisms different from its effects on HB-EGF (namely reduction of circulating TNF-α levels and thus inflammation) (6). Finally, an alternate therapeutic option is the targeting of TIMP3, which would need to be increased to antagonize ADAM17 activity. Further studies are required to define whether HB-EGF or ADAM17 inhibition will prevent or reduce the development of diabetic nephropathy.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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