ADAM17 mediates Nox4 expression and NADPH oxidase activity in the kidney cortex of OVE26 mice

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ADAM17 mediates Nox4 expression and NADPH oxidase activity in the kidney cortex of OVE26 mice. Am J Physiol Renal Physiol 305: F323–F332, 2013. First published May 15, 2013; doi:10.1152/ajprenal.00522.2012.—Matrix protein accumulation is a prominent feature of diabetic nephropathy that contributes to renal fibrosis and decline in renal function. The pathogenic mechanisms of matrix accumulation are incompletely characterized. We investigated if the matrix metalloprotease a disintegrin and metalloprotease1 7 (ADAM17), known to cleave growth factors and cytokines, is activated in the kidney cortex of OVE26 type 1 diabetic mice and the potential mechanisms by which ADAM17 mediates extracellular matrix accumulation. Protein expression and activity of ADAM17 were increased in OVE26 kidney cortex. Using a pharmacological inhibitor to ADAM17, TMI-005, we determined that ADAM17 activation results in increased type IV collagen, Nox4, and NADPH oxidase activity in the kidney cortex of diabetic mice. In cultured mouse proximal tubular epithelial cells (MCT), high glucose increases ADAM17 activity, Nox4 and fibronectin expression, cellu-

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colorimetric dye-binding assay was purchased from Accurate Chemical & Scientific Corporation (Westbury, NY).

Animals and tissue culture. Age- and weight-matched male type 1 diabetic OVE26 mice (14) and nondiabetic FVB control mice were purchased from Jackson Laboratories. Five-month-old mice were randomly assigned to three groups that received vehicle or drug by oral gavage: group 1: FVB mice given vehicle buffer (2% Tween 80 and 0.5% methylcellulose) twice daily; group 2: OVE26 mice that received vehicle buffer twice daily; and group 3: OVE26 mice that received the ADAM17 MMP inhibitor TMI-005 at a dose of 10 mg/kg twice daily. After 3 wk of drug administration, 24-h urine was collected in metabolic cages and urine albumin was measured by a mouse albumin ELISA kit (Bethyl Laboratories; Montgomery, TX). Following urine collection, animals were killed by exsanguination while they were under anesthesia. Both kidneys were removed and weighed, and portions of kidney cortex were flash-frozen in liquid nitrogen for microscopy and biochemical analyses or formalin fixed for morphometric imaging.

All animal studies followed a protocol that was approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

Immortalized mouse renal cells (glomerular endothelial cells, glomerular epithelial cells, mesangial cells, and proximal tubular epithelial cells) were cultured in DMEM supplemented with fetal bovine serum and antibiotic/antifungal solution or RPMI medium supplemented with fetal bovine serum, antibiotic/antifungal solution (glomerular epithelial cells). When cells reached 90% confluence, tissue culture plates were serum starved overnight before pretreatment with or without 1 μM TMI-005 for 1 h followed by exposure to 25 mM D-glucose for 4 or 24 h. Immortalized mouse proximal tubular epithelial cells (MCTs) were a gift from Eric Neilson (23). Transient transfection using RiboJuice siRNA transfection reagent and 20 nM siRNA was performed when tissue culture plates reached 30% confluence. Cells were incubated with siRNA and transfection reagent for 24 h before overnight serum starvation and subsequent D-glucose exposure (25 mM).

Western blot analysis. Kidney cortex homogenates were prepared in 500 μl of radioimmune precipitation assay buffer (20 mM Tris·HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1 mM Na2VO4, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, 20 μg/ml leupeptin, and 1% NP-40) using a Dounce homogenizer. Samples containing equivalent amounts of protein were separated by 7.5% SDS-polyacrylamide gel electrophoresis and transferred to 0.45-μm nitrocellulose membranes before immunoblotting with antibodies. Blots were incubated with anti-collagen IV (1:1,000; Santa Cruz Biotechnology), anti-Nox4 (1:500; Santa Cruz Biotechnology), anti-fibronectin (1:1,000; Sigma Aldrich), and polyclonal anti-ADAM17 (1:250; Abcam). Secondary antibodies conjugated with horseradish peroxidase were employed to detect signals (1:5,000; Bio-Rad). Results were visualized with enhanced chemiluminescence detection reagents (Amersham GE Life Sciences and Pfenix X-ray film). Quantitation of Western blot data was performed using ImageJ software analysis (Wayne Rasband, National Institutes of Health).

Immunoperoxidase and immunofluorescent staining. Frozen kidney cortex samples were sectioned with a Leica cryostat at a thickness of 5μm and processed for immunoperoxidase staining. Digital images were obtained with an Olympus AX70 research microscope and a DP70 digital camera. Immunoperoxidase staining was performed using Vector Laboratories DAB substrate kit SK-4100, avidin/biotin blocking kit (SP-2001), and ABC Vectastain standard kit (PK-4000). Anti-collagen IV antibodies (Millipore) were used at 1:500. Image Pro Plus software (Media Cybernetics) was utilized to quantify staining within the glomeruli and interstitium.

ADAM17 enzymatic activity assay. Kidney cortex homogenates were prepared in 200 μl of assay buffer (20 mM Tris·HCl, pH 7.5, 150 mM NaCl, and 1% NP-40) using a Dounce homogenizer. Protein estimation was performed by the Bradford method, and 25 μg of homogenate were used in the assay. ADAM17 activity was measured using EMD Bioscience’s InnoZyme ADAM17 activity kit. The kit utilizes an internally quenched fluorogenic substrate, MCA-KPLGL-Dpa-AR-NH2, which when cleaved specifically by ADAM17, causes

Fig. 1. A disintegrin and metallopeptase 17 (ADAM17) expression is upregulated in response to high glucose (HG) exposure in renal cells. A: glomerular epithelial cells were exposed to 25 mM D-glucose for varying time points before lysis and collection of cells for Western blot analysis. Normal glucose (NG) was used as a control (5 mM); 50 μg protein were resolved on a 7.5% SDS-PAGE and immunoblots were probed for anti-ADAM17. B: glomerular endothelial cells were exposed to 25 mM D-glucose for varying time points before lysis and collection of cells for Western blot analysis. NG was used as a control (5 mM); 50 μg protein were resolved on a 7.5% SDS-PAGE and immunoblots were probed for anti-ADAM17. C: glomerular mesangial cells were exposed to 25 mM D-glucose for varying time points before lysis and collection of cells for Western blot analysis. NG was used as a control (5 mM); 50 μg protein were resolved on a 7.5% SDS-PAGE and immunoblots were probed for anti-ADAM17. D: proximal tubular epithelial cells were exposed to 25 mM D-glucose for varying time points before lysis and collection of cells for Western blot analysis. NG was used as a control (5 mM); 50 μg protein were resolved on a 7.5% SDS-PAGE and immunoblots were probed for anti-ADAM17.
fluorophore release. The resultant fluorescence is measured at an excitation wavelength of 320 nm and an emission wavelength of 405 nm. Activity was expressed as relative fluorescence units per milligrams of protein.

**NADPH oxidase assay.** NADPH-dependent superoxide production was determined using the lucigenin-enhanced chemiluminescence method as described previously (7, 13). Kidney cortex or MCT cell homogenates were prepared in 1 ml or 250 μl, respectively, of lysis buffer (20 mM KH₂PO₄, pH 7.0, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 0.5 μg/ml leupeptin) by using a Dounce homogenizer. Protein content was estimated using the Bradford method, and 25 μg of homogenate were added to 50 mM phosphate buffer, pH 7.0, containing 1 mM EGTA, 150 mM sucrose, 5 μM lucigenin, and 100 μM NADPH at a final volume of 1 ml. Photon emission was measured every 30 s for 5 min in a luminometer. Superoxide production was expressed in relative light units per milligrams of protein (RLU/mg).

Statistical analysis. Results are expressed as the means ± SE. Statistical significance was calculated by either student’s unpaired t-test (see Fig. 4D) or one-way ANOVA with post hoc Tukey analysis (see Figs. 2A; B, C, E; and G, H) and determined as P < 0.05.

**RESULTS**

ADAM17 expression is upregulated in response to high glucose exposure in renal cells. To first determine if glucose regulates ADAM17, mouse renal cells were exposed to a high concentration of D-glucose (25 mM) to mimic the diabetic milieu. Glomerular epithelial cells or podocytes showed a robust increase in ADAM17 protein expression after 6-h exposure to high glucose, and its expression decreased at 12-h exposure but increased once more at 24 h and maintained

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Table 1. Glucose level, body weight, kidney weight, kidney weight/body weight ratio, and urine albumin (albuminuria) levels in vehicle-dosed FVB and OVE26 mice and TMI-005-dosed OVE26 mice

<table>
<thead>
<tr>
<th></th>
<th>FVB Vehicle</th>
<th>OVE26 Vehicle</th>
<th>OVE26 + TMI-005, 10 mg/kg</th>
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<tbody>
<tr>
<td>Blood Glucose, mg/dl</td>
<td>151 ± 8.14</td>
<td>565 ± 6.00*</td>
<td>571.5 ± 23.63</td>
</tr>
<tr>
<td>Body Weight, g</td>
<td>28.66 ± 1.39</td>
<td>17.29 ± 1.07</td>
<td>16.55 ± 0.60</td>
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<tr>
<td>Kidney Weight, mg</td>
<td>227.86 ± 7.05</td>
<td>241.25 ± 12.74</td>
<td>241.67 ± 15.75</td>
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<tr>
<td>Kidney Weight/Body Weight, mg/g</td>
<td>7.95 ± 3.17</td>
<td>13.95 ± 1.02*</td>
<td>14.60 ± 1.43</td>
</tr>
<tr>
<td>Albuminuria, μg/24 h</td>
<td>0.08 ± 0.01</td>
<td>10.73 ± 4.05†</td>
<td>5.34 ± 2.56†</td>
</tr>
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Values are the means ± SE from 4 animals from each group. *P < 0.05 vs. FVB group; †P < 0.01 vs. FVB group; ‡P < 0.05 vs. OVE26 vehicle-treated group.

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**Fig. 2.** ADAM17 protein expression and enzymatic activity are increased in OVE26 kidney cortex. Five-month-old diabetic OVE26 mice were administered TMI-005, a pharmacological inhibitor to ADAM 17 (Pfizer-Wyeth), or vehicle control (2% Tween 80 and 0.5% methylcellulose) by oral gavage for an interval of 4 wk. FVB nondiabetic mice were only given vehicle control during the same duration. A: dounce homogenized whole kidney cortex was used to determine ADAM 17 activity levels by measuring cleavage of the internally quenched fluorogenic substrate MCA-KPLGL-Dpa-AR-NH₂. Shown are means of 4 animals/group ± SE. *P < 0.05 vs. the FVB control group; †P < 0.05 vs. the OVE26 diabetic group. B: kidney cortex lysates were subjected to Western blot analysis and immunoblotted with anti-ADAM 17 antibody and anti-actin for loading control. Western blot is representative of 4 animals from each group. C: 5-μm thick frozen kidney cortex sections were stained with anti-ADAM 17 antibody using an immunoperoxidase method. Digital images are representative of 4 animals from each group.
expression at 48-h exposure to high glucose (Fig. 1A). Western blot analysis of glomerular endothelial cells incubated with a high concentration of glucose revealed a slight increase in ADAM17 protein expression at 6- and 12-h exposure to high glucose and a strong increase 24 and 48 h (Fig. 1B). Mouse mesangial cells also showed increased ADAM17 expression at 3-, 6-, and 24-h exposure to 25 mM glucose with the highest expression levels at 6 and 24 h (Fig. 1C). Proximal tubular epithelial cells exhibited an increase in ADAM17 protein expression in response to high glucose incubation at 4, 6, and 24 h (Fig. 1D). Collectively, these experiments show that ADAM17 is expressed ubiquitously in the kidney cortex and that it is induced in all four cell types with high glucose.

**ADAM17 protein expression and enzymatic activity are increased in OVE26 kidney cortex.** Initial reports indicate ADAM17 and cleavage of its substrates may be involved in kidney disease and can also contribute to the progression of diabetes (1, 3, 9, 10, 15, 20, 21, 26, 29, 33, 35, 40, 42, 45, 47). We hypothesized that ADAM17 plays a key role in ECM accumulation in DN. To examine this hypothesis, 5-mo-old OVE26 transgenic mice and FVB nondiabetic control mice were segregated into the following groups with four mice per group: FVB control mice, OVE26 diabetic mice, and OVE26 diabetic mice receiving 10 mg/kg TMI-005 by oral gavage twice daily. TMI-005 is a potent and selective thiomorpholine sulfonamide hydroxymate inhibitor of ADAM17, generously provided by Pfizer (formerly FVB OVE26 OVE26 + TMI-005 Collagen IV α2

![Fig. 3. Inhibition of ADAM17 with TMI-005 reduces collagen IV protein expression in OVE26 kidney cortex. Five-month-old diabetic OVE26 mice were administered TMI-005, a pharmacological inhibitor to ADAM 17 (Pfizer-Wyeth) or vehicle control (2% TWEEN 80 and 0.5% methylcellulose) by oral gavage for an interval of 4 wk. FVB nondiabetic mice were only given vehicle control during the same duration. A: frozen kidney cortex sections were stained with anti-type IV collagen /H9251 by immunoperoxidase method. Digital images are representative of 4 animals from each group. B: glomerular collagen IV α2-protein expression was calculated using Image-Pro Plus software. Shown are means ± SE of 20 imaged glomeruli per treatment group. ***P < 0.001, compared with FVB control group; ###P < 0.001, compared with OVE26 diabetic group. C: interstitial collagen IV α2-protein expression was calculated using Image-Pro Plus software. Shown are means ± SE of 20 imaged fields per treatment group. ***P < 0.001, compared with FVB control group; #P < 0.05, compared with OVE26 diabetic group. D: kidney cortex lysates were subjected to Western blot analysis and immunoblotted with anti-collagen IV α5-antibody and anti-actin for loading control. Western blots represent 4 animals from each group. E: pixel densitometry was performed using ImageJ software to quantitate differences in protein expression levels. Values are representative of the means of 4 animals from each group ± SE. *P < 0.05, compared with FVB control group.**
Wyeth-Ayerst Pharmaceuticals). TMI-005 has an IC₅₀ of 20 nM and exhibits 89% inhibition of lipopolysaccharide-induced TNFα production in THP-1 monocytic cells (30).

Table 1 displays the blood glucose levels, body and kidney weights, and urine albumin (albuminuria) levels in the three different groups of mice (Table 1). After 3 wk of TMI-005 administration, there was no significant difference in blood glucose levels and body weight between the OVE26 group and the control FVB group. Mice treated with TMI-005 had a significant reduction in ADAM17 activity (Fig. 2A). OVE26 kidney cortex exhibited a significant increase in ADAM17 activity compared with the control FVB group. Moreover, ADAM17 protein expression increased in OVE26 kidney cortex compared with FVB nondiabetic kidney cortex (Fig. 2B) and treatment with TMI-005, while inhibiting enzyme activity, increased ADAM17 protein expression (Fig. 2B). The increase in the urine albumin levels was observed in OVE26 mice treated with TMI-005. ADAM17 activity and protein expression were assayed in kidney cortex samples of OVE26 type 1 diabetic mice (Fig. 2A). OVE26 kidney cortex exhibited a significant reduction in ADAM17 activity (Fig. 2A). After 3 wk of TMI-005 administration, there was no significant difference in blood glucose levels and body weight between the OVE26 group and the control FVB group. Mice treated with TMI-005 had a significant reduction in ADAM17 activity (Fig. 2A). Moreover, ADAM17 protein expression increased in OVE26 kidney cortex compared with FVB nondiabetic kidney cortex (Fig. 2B) and treatment with TMI-005, while inhibiting enzyme activity, increased ADAM17 protein expression (Fig. 2B). The increase in the urine albumin levels was observed in OVE26 mice treated with TMI-005.

Fig. 4. ADAM17 activation mediates the effect of glucose on fibronectin protein expression and collagen content in mouse proximal tubular epithelial cells (MCTs). A: proximal tubular epithelial cells were serum-starved for 24 h and pretreated with 1 µM TMI-005 before exposure to 25 mM D-glucose for 4 h. B: proximal tubular epithelial cells were serum-starved for 24 h and pretreated with 1 µM TMI-005 before exposure to 25 mM D-glucose for 4 h; 20 µg of cellular lysates were resolved on a 7.5% SDS-PAGE and immunoblotted for fibronectin. C: pixel densitometry was performed using ImageJ software to quantitate differences in protein expression levels. Values are representative of the means of 4 experiments ± SE. *P < 0.05, compared with the control cells; #P < 0.05, compared with HG-treated cells. B: proximal tubular epithelial cells were serum-starved for 24 h and pretreated with 1 µM TMI-005 before exposure to 25 mM D-glucose for 4 h; 20 µg of cellular lysates were resolved on a 7.5% SDS-PAGE and immunoblotted for fibronectin. C: pixel densitometry was performed using ImageJ software to quantitate differences in protein expression levels. Values are representative of the means of 4 experiments ± SE. *P < 0.05, compared with the control cells; #P < 0.05, compared with HG-treated cells. B: proximal tubular epithelial cells were serum-starved for 24 h and pretreated with 1 µM TMI-005 before exposure to 25 mM D-glucose for 4 h; 20 µg of cellular lysates were resolved on a 7.5% SDS-PAGE and immunoblotted for fibronectin. C: pixel densitometry was performed using ImageJ software to quantitate differences in protein expression levels. Values are representative of the means of 4 experiments ± SE. *P < 0.05, compared with the control cells; #P < 0.05, compared with HG-treated cells.
in ADAM17 protein levels was confirmed by immunoperoxidase staining (Fig. 2C).

Inhibition of ADAM17 with TMI-005 reduces collage IV protein expression in OVE26 kidney cortex. To determine the role of ADAM17 activation in ECM accumulation, the basement membrane protein collagen IV was investigated. Immunoperoxidase staining demonstrated a significant increase in collagen IV within the glomeruli and tubule/interstitium of OVE26 kidney cortex (Fig. 3, A–C). OVE26 mice treated with TMI-005 exhibited a significant reduction in collagen IV α5-protein expression in both compartments (Fig. 3, A–C). Additionally, these findings were supported by Western blot analysis where collagen IV α5-expression was significantly increased in kidney cortex of OVE26 mice compared with control mice. Collagen IV α5 had a trend to decrease in TMI-005-treated mice (Fig. 3D). These data suggest that ADAM17 plays a role in ECM accumulation in DN.

ADAM17 activation mediates the effect of glucose on fibronectin protein expression and collagen content in mouse proximal tubular epithelial cells (MCTs). To determine the role of ADAM 17 in matrix accumulation in mouse proximal tubular epithelial cells (MCTs; Ref. 23) were exposed to a high concentration of glucose (25 mM) and ADAM17 activity was assayed. After 4 h of exposure to 25 mM glucose, ADAM17 activity was significantly increased and pretreatment with TMI-005 inhibited enzyme activity (Fig. 4A). Fibronectin protein expression was also examined using Western blot analysis. Fibronectin increased after 4-h glucose exposure, and inhibition of ADAM17 with TMI-005 prevented the glucose-induced increase in fibronectin (Fig. 4, B and C). To confirm the role of ADAM17 in mediating ECM accumulation in cells treated with glucose, a pool of siRNAs targeting ADAM17 was transfected into MCTs. ADAM17 mRNA and protein expression were reduced with siRNA-targeted knockdown as assessed by quantitative real-time PCR and Western blot, respectively (Fig. 4, D and E). Knockdown of ADAM17 blocked the increase in fibronectin protein expression in response to glucose (Fig. 4, F and G). RNA interference of ADAM17 also blocked the accumulation of all collagen isoforms as assessed by a Sircol colorimetric assay (Fig. 4H). These data strongly implicate ADAM17 in the accumulation of ECM in mouse proximal tubular epithelial cells exposed to glucose.

ADAM17 mediates Nox4 protein expression and NADPH oxidase activity in OVE26 kidney cortex. To identify a potential downstream target of ADAM17 that results in increased ECM expression in DN, we examined Nox4, a member of the NADPH oxidase family of enzymes. Numerous studies, including several from our group, have identified Nox4 in mediating renal injury including renal hypertrophy and ECM accumulation in mesangial cells and proximal tubular epithelial cells as well as glomerular epithelial cell apoptosis and glomerular endothelial cell dysfunction (2, 7, 8, 13, 31, 44). We hypothesized that ADAM17 increases ECM accumulation in the diabetic kidney, at least partially, through the actions of Nox4. To investigate this possibility, Western blot analysis of Nox4 was performed on cortical lysates of FVB, OVE26, and OVE26 mice treated with TMI-005. Nox4 protein expression was increased in OVE26 kidney cortex and significantly reduced when mice were administered the ADAM17 inhibitor (Fig. 5, A and B). It is important to note that previous work has shown Nox4 exists in a conformation that allows for spontaneous transfer of electrons from NADPH to FAD conferring constitutive activity; hence, the enzyme is regulated partially at the level of protein expression with an increase in Nox4 protein expression translating to an increase in enzymatic activity (46). NADPH oxidase activity was also measured using a lucigenin-enhanced chemiluminescence assay. There was a significant increase in fibronectin protein expression in response to glucose (Fig. 4, B). These data suggest that ADAM17 increases fibronectin protein expression and collagen content in mouse proximal tubular epithelial cells.

Fig. 5. ADAM17 mediates Nox4 protein expression and NADPH oxidase activity in OVE26 kidney cortex. Five-month-old diabetic OVE26 mice were administered TMI-005, a pharmacological inhibitor to ADAM 17 (Pfizer-Wyeth) or vehicle control (2% Tween 80 and 0.5% methylcellulose) by oral gavage for an interval of 4 wk. FVB nondiabetic mice were only given vehicle control (2% Tween 80 and 0.5% methylcellulose) by oral gavage for an interval of 4 wk. FVB nondiabetic mice were only given vehicle control during the same duration. A: Kidney cortex lysates were subjected to Western blot analysis and immunoblotted with anti-Nox4 antibody and anti-Actin for loading control. Western blots represent 4 animals from each group. B: pixel densitometry was performed using ImageJ software to quantify protein expression levels. Values are representative of the means of 4 animals. C: dounce homogenized kidney cortex samples were used to determine NADPH oxidase levels using a lucigenin-enhanced chemiluminescent assay. Shown are means of 4 animals/group ± SE. *P < 0.05 vs. the FVB control group by one-way ANOVA with post hoc Tukey analysis.
decrease in NADPH-dependent superoxide production in kidney cortex homogenate from TMI-005-treated OVE26 mice compared with kidney cortex from the OVE26-vehicle-treated mice (Fig. 5C). These data suggest that ADAM17 is an upstream mediator of the Nox oxidases in the diabetic kidney.

Glucose-induced ADAM17 activation mediates Nox4 protein expression and NADPH oxidase activity in MCTs. After determining that glucose increased ADAM17 activity in mouse proximal tubular epithelial cells and that ADAM17 mediated amplified Nox4 protein expression and NADPH oxidase activity in diabetic mouse kidney cortex, we sought to determine if glucose is the factor involved in ADAM17-dependent NADPH oxidase activity and Nox4 protein expression. MCTs were pretreated with 1 μM TMI-005 before exposure to 25 mM D-glucose for 4 h. ADAM17 inhibition blocked glucose-induced Nox4 protein expression (Fig. 6, A and B). Concomitant with the increase in Nox4 protein expression, NADPH oxidase activity was significantly increased after 4-h exposure to high glucose (Fig. 6C). When MCTs were pretreated with TMI-005, there was an attenuation of the glucose-induced NADPH oxidase activity (Fig. 6C). Consistent with our pharmacological inhibitor results, siRNA-mediated knockdown of ADAM17 resulted in a reduction of glucose-induced Nox4 protein expression by Western blot analysis (Fig. 6, D and E). It is also noteworthy that ADAM17 knockdown also resulted in a reduction in basal Nox4 levels (Fig. 6, D and E). Finally, NADPH oxidase activity was measured in MCT cells transfected with nontargeting or ADAM17 siRNA with or without 4-h high glucose treatment (Fig. 6F). Basal NADPH-dependent superoxide production was diminished when ADAM17 was knocked down and siRNA-mediated knockdown of ADAM17 significantly decreased high glucose-induced NADPH oxidase activity (Fig. 6F). Taken together these data indicate that glucose is responsible for ADAM17-dependent increase in Nox4 expression and NADPH oxidase activity.

DISCUSSION

This study provides strong evidence that hyperglycemia in diabetes results in the activation of the metalloprotease...
ADAM17 to increase matrix protein expression in kidney cortex. Furthermore, ADAM17 appears to exert its effect on matrix accumulation through upregulation of Nox4 and increased generation of reactive oxygen species. Our conclusions are based on the following findings: ADAM17 is upregulated and activated in the kidney cortex of mice with type 1 diabetes; and inhibition of ADAM17 downregulated Nox4 expression, decreased NADPH oxidase activity, and reduced fibronectin expression. In cultured proximal tubular epithelial cells, high glucose concentration activated ADAM17, increased Nox4 protein expression and NADPH oxidase activity, and increased the expression of matrix proteins. The increase in fibronectin expression or cellular collagen content observed after glucose exposure was reversed when ADAM17 or Nox4 was blocked with pharmacological inhibitors and/or siRNA-mediated knockdown. This report is the first to show that the MMP ADAM17 is activated in the kidney in diabetes and that it acts upstream of Nox4 to enhance matrix accumulation (Fig. 7).

Nox4 involvement in ECM accumulation has been reported by several groups (2, 6, 8, 22, 38, 44). Additionally, there is evidence that ROS activates ADAM17 (16, 37, 49). However, only one publication has shown upregulation of NADPH oxidases and subsequent ROS production, which results in shedding of known ADAM17 substrates (36). In this particular report, rat liver hepatocytes exposed to the growth factor TGFβ exhibited an increase in HB-EGF and TGFα transcript levels. Pretreatment with the flavoprotein inhibitor diphenyleneiodonium or the NADPH oxidase inhibitor apocynin reversed the increase in TGFα and HB-EGF mRNA (36). To the best of our knowledge, the present study provides the first evidence that ADAM17 also functions as an upstream regulator of Nox enzymes. ADAM17 activity is required for high glucose-induced Nox4 protein expression and subsequent ROS production. Note that ADAM17 appears to regulate Nox4 protein expression. This is important since Nox4 is constitutively active (2) and thereby ROS production by Nox4 is governed by changes in its expression levels. Utilizing pharmacologic and genetic approaches, we show that inhibition of ADAM17 in cultured tubular cells decreases Nox4 expression/activity and reduces matrix protein expression. Of interest is that while administration of ADAM17 inhibitor to the diabetic mice blocked enzyme activity, ADAM17 protein levels in the kidney cortex increased. It is possible that the inhibitor stabilizes enzyme protein levels by inhibiting its degradation by the proteasome; other mechanisms cannot be excluded at this time.

The mechanism by which ADAM17 regulates Nox oxidases in the diabetic kidney cortex or in response to glucose in cultured tubular cells remains to be determined. It is likely that ADAM17 activation by glucose results in cleavage of growth factors that in turn activate and upregulate Nox4. A potential direct effect of ADAM17 on the oxidase is unlikely but cannot be excluded.

Glucose increases ADAM17 activity and/or EGFR transactivation causing increased ECM or fibrotic factors within the kidney (48, 51–53). Exposure of primary cultures of rat mesangial cells to a high concentration of D-glucose activates ADAM17 and results in an increase in TGFβ promoter activity and an increase in TGFβ transcripts (48). Additional data in mesangial cells suggest that the increase in collagen I and fibronectin expression in response to glucose depends on the activation of ADAM17, EGFR transactivation, and activation of downstream signals such as the phosphoinositide 3-kinase-Akt pathway (48, 51–53). Other than these limited publications, glucose-induced ADAM17 expression or activity influencing the progression of DN has not been thoroughly investigated.

In addition to high glucose, several groups demonstrated a role for ADAM17 in mediating the profibrotic effects of angiotensin II (ANG II). In 2005, Lautrette et al. (29) published a seminal article linking ANG II-induced EGFR transactivation to ADAM17. Chronic infusion of ANG II in mice resulted in glomerulosclerosis and interstitial fibrosis, while mice deficient in EGFR (dominant negative isoform of EGFR) or TGFα−/− mice were protected from these lesions. Importantly, ANG II-induced renal lesions were reduced in wild-type mice administered a pharmacological inhibitor to ADAM17, WTACE2 (29). Interestingly, it appears that there may be cross-talk between glucose and AngII signaling in EGFR transactivation. In vascular smooth muscle cells cultured with high glucose, EGFR glycosylation increased. HG sensitized EGFR transactivation through AngII-mediated angiotensin type 1 receptor activation (27).

In conclusion, this study establishes a role for ADAM17 activation in ECM accumulation in DN in vivo and in mouse proximal tubular epithelial cells exposed to HG. Additionally, it provides a novel mechanism whereby the activation of ADAM17 in diabetes or in a high glucose medium induces oxidative stress through the upregulation of Nox4 protein expression and increased NADPH oxidase activity contributing to ECM accumulation.

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ADAM17 REGULATES Nox4

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ADAM17 REGULATES Nox4


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