Blocking lysophosphatidic acid receptor 1 signaling inhibits diabetic nephropathy in db/db mice

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Lysophosphatidic acid (LPA) is known to regulate various biological responses by binding to LPA receptors. The serum level of LPA is elevated in diabetes, but the involvement of LPA in the development of diabetes and its complications remains unknown. Therefore, we studied LPA signaling in diabetic nephropathy and the molecular mechanisms involved. The expression of autotaxin, an LPA synthesis enzyme, and LPA receptor 1 was significantly increased in both mesangial cells (SV40 MES13) maintained in high-glucose media and the kidney cortex of diabetic *db/db* mice. Increased urinary albumin excretion, increased glomerular tuft area and volume, and mesangial matrix expansion were observed in *db/db* mice and reduced by treatment with ki16425, a LPA receptor 1/3 antagonist. Transforming growth factor (TGF)β expression and Smad-2/3 phosphorylation were upregulated in SV40 MES13 cells by LPA stimulation or in the kidney cortex of *db/db* mice, and this was blocked by ki16425 treatment. LPA receptor 1 siRNA treatment inhibited LPA-induced TGFβ expression, whereas cells overexpressing LPA receptor 1 showed enhanced LPA-induced TGFβ expression. LPA treatment of SV40 MES13 cells increased phosphorylated glycogen synthase kinase (GSK)3β at Ser9 and induced translocation of sterol regulatory element-binding protein (SREBP)1 into the nucleus. Blocking GSK3β phosphorylation inhibited SREBP1 activation and consequently blocked LPA-induced TGFβ expression in SV40 MES13 cells. Phosphorylated GSK3β and nuclear SREBP1 accumulation were increased in the kidney cortex of *db/db* mice and ki16425 treatment blocked these pathways. Thus, LPA receptor 1 signaling increased TGFβ expression via GSK3β phosphorylation and SREBP1 activation, contributing to the development of diabetic nephropathy.

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Diabetic nephropathy is the major microvascular complication of both type 1 and type 2 diabetes.[1] High expression of extracellular matrix (ECM) proteins, such as collagen, fibronectin, and laminin in the mesangial cells of glomeruli, leads to thickening of the basement membrane[2] and results in glomerulosclerosis, which is a characteristic pathological feature of type 2 diabetes–induced nephropathy.[3,4] Transforming growth factor–β (TGF-β) is a key mediator for high expression of ECM proteins[5–8]; therefore, increased TGF-β expression in mesangial cells promotes ECM accumulation and hypertrophy during progression of diabetic nephropathy. Treatment with renin-angiotensin system inhibitors or tight control of glucose levels can prevent diabetic nephropathy, but they are not completely effective in preventing disease progression. Renin-angiotensin inhibitors are not tolerated by all patients with diabetic nephropathy[1]; therefore, early prevention or delaying progression to diabetic nephropathy is necessary.

Lysophosphatidic acid (LPA) is a small, ubiquitous phospholipid involved in cellular processes such as proliferation, survival, migration, and suppression of apoptosis.[9] LPA is released from activated platelets at sites of injury, and increased LPA production during inflammation mediates proinflammatory effects on several cell types within the kidney.[10,11] Moreover, LPA has been demonstrated to be a profibrotic mediator in various organs, such as the liver, lung, kidney, peritoneum, skin, retina, and heart.[12–14] LPA mediates its cellular effects via binding to at least 6 G-protein–coupled receptors (LPAR1–LPAR6).[15,16] Pharmacological tools specifically targeting LPA receptor subtypes have been developed, including ki16425, which specifically blocks LPAR1 and LPAR3 subtypes,[17] and is used for various LPA-related disorders.[18,19]

It was reported that the plasma level of LPA was increased in high-fat, diet-induced obese mice[20] and in the glomeruli of diabetic mice.[21] Moreover, the expression of autotaxin (ATX), a hydrolysis enzyme that produces LPA from lysophosphatidyl choline, was significantly increased in the kidney cortex of *db/db* mice compared with control mice,[22] as well as in the serum of patients with diabetic nephropathy.[23] These observations

led us to hypothesize that LPA could be involved in the development of glomerulosclerosis and could contribute to the progression of diabetic nephropathy.

In the present study, we found that ki16425 reduced the progression of diabetic nephropathy in *db*/*db* mice. Moreover, glycogen synthase kinase 3β (GSK3β) (Ser9) phosphorylation and subsequent sterol regulatory element–binding protein (SREBP) 1 activation was involved in TGF-β production induced by LPA in the diabetic condition.

RESULTS

LPAR1 expression is upregulated in both the renal cortex of diabetic *db*/*db* mice and simian virus-transformed mouse mesangial cells maintained in high glucose

It was reported that LPA was elevated in the serum of diabetic animal models,[20] and that the mRNA levels of ATX were significantly increased in the adipose tissue of patients with diabetes.[24] We first examined the expression of ATX, the enzyme required for LPA production, in the kidney cortex of *db*/*db* mice. mRNA (Figure 1a) and protein (Figure 1b and c) levels of ATX were significantly increased in *db*/*db* mice compared with wild-type mice, which was consistent with a previously reported study.[22] mRNA expression of lipid phosphate phosphatases, which are enzymes associated with extracellular LPA degradation, were not different between the 2 groups (Supplementary Figure S1). Next, we examined the expression of LPAR subtypes, and found that LPAR1, LPAR2, and LPAR3 were expressed, but other receptor subtypes (e.g., LPAR4 and LPAR 5) were not detected in the kidney cortex of both *db*/*db* and wild-type mice (data not shown). The mRNA (Figure 1a) and protein (Figure 1b and c) levels of LPAR1 were significantly increased in *db*/*db* mice compared with

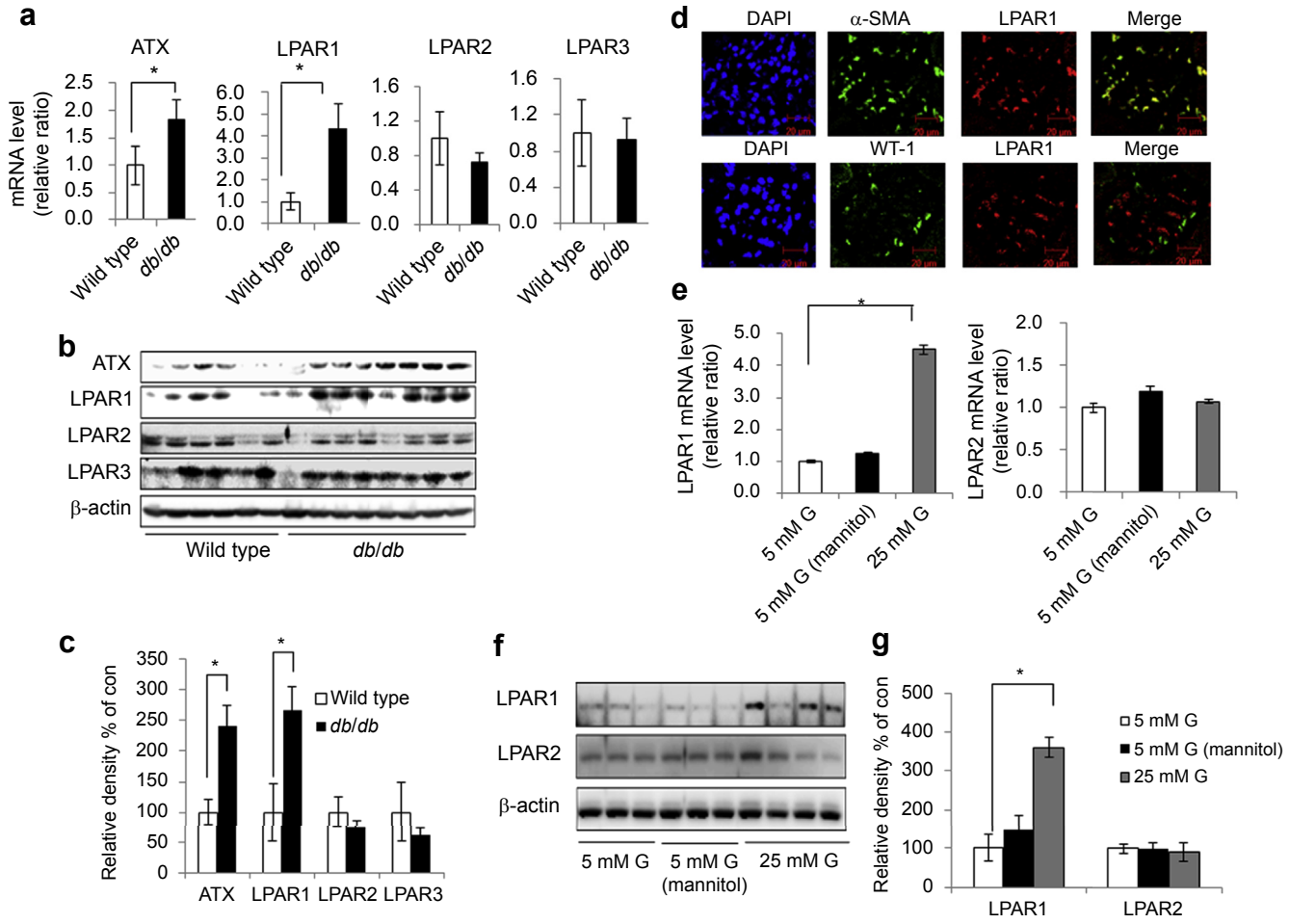

Figure 1 | mRNA and protein levels of lysophosphatidic acid receptor 1 (LPAR1) are upregulated in both the renal cortex of *db*/*db* mice and simian virus-transformed mouse mesangial (SV40 MES13) cells maintained in high glucose. mRNAs and proteins were isolated from the renal cortex of 16-week-old wild-type and *db*/*db* mice. (**a**) mRNA levels of autotaxin (ATX), LPAR1, LPAR2, and LPAR3 were measured by quantitative real-time polymerase chain reaction (qRT PCR) (n = 3–4). (**b**) Protein levels of ATX, LPAR1, LPAR2, and LPAR3 were measured by Western blot. (**c**) The results were quantified and β-actin was used as a loading control (n = 6–8). Representative pictures of (**d**) immunofluorescence staining for colocalization of α–smooth muscle actin (α-SMA) or Wilms' tumor 1 (WT-1) with LPAR1, which were examined in the kidney sections of 16-week-old *db*/*db* mice. Bars = 20 μm (n = 6). **(e,f)** mRNA and protein levels of LPAR1 and LPAR2 in SV40 MES13 cells maintained in low glucose (5 mM), low glucose (5 mM) + mannitol (19.44 mM), and high glucose (25 mM) were examined by qRT PCR and Western blot. (**g**) The results were quantified, and β-actin was used as a loading control (n = 3 independent experiments). *$P < 0.05$. Data represent the mean ± SEM. DAPI, 4′,6-diamidino-2-phenylindole.