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Enhanced alpha-kinase 1 accelerates multiple early nephropathies in streptozotocin-induced hyperglycemic mice

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ABSTRACT

Alpha-kinase 1 (ALPK1) is associated with chronic kidney disease (CKD), type 2 diabetes mellitus and gout. Elevated ALPK1 levels have been observed in the kidneys of patients with diabetes and the white blood cells of patients with gout. As renal injury is a common outcome of CKD, diabetes and gout, the aim of this study was to investigate the effect of ALPK1 in the development of renal injury in a hyperglycemic condition. Hyperglycemia was induced in wild-type and *ALPK1* transgenic mice by an intraperitoneal injection of streptozotocin (STZ). Functional and histological examinations were performed after 3 weeks. STZ-treated *ALPK1* transgenic mice exclusively showed arteriolar sclerosis and fibrous thickening of the Bowman's capsule in the kidney. This was accompanied by body weight loss, severe hyperglycemia, and low serum insulin levels. Renal renin and serum renin protein levels were higher in STZ-treated *ALPK1* transgenic mice, whereas cGKII protein level was decreased by ALPK1 in human embryonic kidney 293 (HEK293) cells. ALPK1 up-regulated TGF-beta1 levels and transcription of fibrosis-related genes, including *MMP-9*, *FIBRONECTIN*, and *TIMP1*. MSU crystals increased ALPK1 transcription in cultured kidney cells. Finally, ALPK1 enhanced production of MSU crystals-induced IL-1beta in mice. Stimulation of soluble sodium urate induced *IL-1beta* and *Alpk1* mRNA production in mice kidney. Taken together, these data show that an increase in ALPK1 results in accelerated fibrotic nephropathies, primarily through the enhancement of renin, TGF-beta1, and IL-1beta. Renal or blood ALPK1 levels are involved in the induction of fibrotic renal injury in an experimental model of hyperglycemia.

1. Introduction

Alpha-kinase 1(ALPK1), also known as lymphocyte α-kinase or alpha-protein kinase 1, is a member of the alpha-kinase family. This kinase family shares little sequence similarity to other conventional protein kinases [1]. Mice with a mutation in the *Alpk1* gene exhibit severe defects in motor coordination and those with overexpressed-ALPK1 exhibit lower testosterone levels [2,3]. ALPK1 is a component of apical transport vesicles involved in myosin 1A phosphorylation in apical protein transport [4]. To date, no additional cellular functions have been identified.

A series of studies have previously demonstrated that *ALPK1* is strongly associated with gout [5–8], but is not found in a study of Japanese [9]. Gout, a prevalent disease associated with hyperuricemia, is a painful arthritic condition resulting from monosodium urate (MSU) crystal-induced inflammatory response in the joints [10]. Deposition of urate crystals in the kidney has been observed in patients with chronic gouty arthritis, which causes the obstruction of renal tubules and inflammatory conditions in kidney [11–13]. Higher ALPK1 levels were detected in the white blood cells of patients with gout than in those of healthy individuals [8]. MSU crystals stimulate ALPK1 expression in human monocytic THP1 cells, which is involved in the production of pro-inflammatory cytokines, including IL-1 beta, IL-8, and TNF-

Abbreviations: ALPK1, Alpha-kinase 1, Alpha-protein kinase 1 or lymphocyte α-kinase; hALPK1, human ALPK1; mALPK1, endogenous mouse ALPK1; MSU, monosodium urate; STZ, streptozotocin.

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alpha [8]. ALPK1 also increases IL-1beta and TGF-beta1 levels in cultured murine TM3 Leydig, and human embryonic kidney 293 (HEK293) cells [2,8]. This suggests a regulatory role of ALPK1 in inflammation.

An increasing number of recent studies suggest that the *ALPK1* gene increases susceptibility to many chronic diseases, including chronic kidney disease (CKD), myocardial infarction, coronary artery disease, and type 2 diabetes mellitus (type 2 DM) [14–18]. CKD, type 2 DM and cardiovascular disease are also associated with gout [19]. Two studies from Taiwan and Japan indicated that *ALPK1* gene variants are involved in approximately 42%–51% of cases of gout and 32% of type 2 DM, respectively [7,20]. Up-regulation of ALPK1 has also been shown in the renal tubular epithelial cells of patients with diabetic glomerulosclerosis [14]. Renal injury results in nephropathy and is common in patients with chronic diseases. Diabetic nephropathy is a common leading cause of end-stage kidney failure worldwide [21]. Renal fibrosis is a major pathological feature of progressive kidney disease and is also central to the development of diabetic nephropathy [22,23]. It is well known that sustained inflammation plays a key role in the development and progression of renal fibrosis [24]. Pro-inflammatory cytokines including IL-1beta and TGF-beta1 are increased at the site of kidney injury in diabetes [25]. TGF-beta1 has long been considered a critical mediator of renal fibrosis [26].

It remains unclear whether ALPK1 activates or simply plays a part in the onset and pathogenesis of diabetes or CKD. Renal injury is frequent among patients with gout, diabetes or CKD. On the basis of blood and renal ALPK1 up-regulation in patients with gouty or diabetic conditions, the present study aimed to examine whether ALPK1 plays a pathogenic role in the development of renal injury in streptozotocin-induced diabetic mice. Because deposition of urate crystals in the kidney is common in patients with chronic gouty arthritis, we examined whether MSU crystal is able to enhance the ALPK1 effects related to renal injury. The regulation of ALPK1 in diabetes and other chronic diseases and its importance regarding kidney injury, are discussed.

2. Materials and methods

2.1. Animals and generation of diabetic animal models

The cytomegalovirus (CMV) promoter-driven h*ALPK1* transgenic mice in the C57BL/6 background were generated as previously

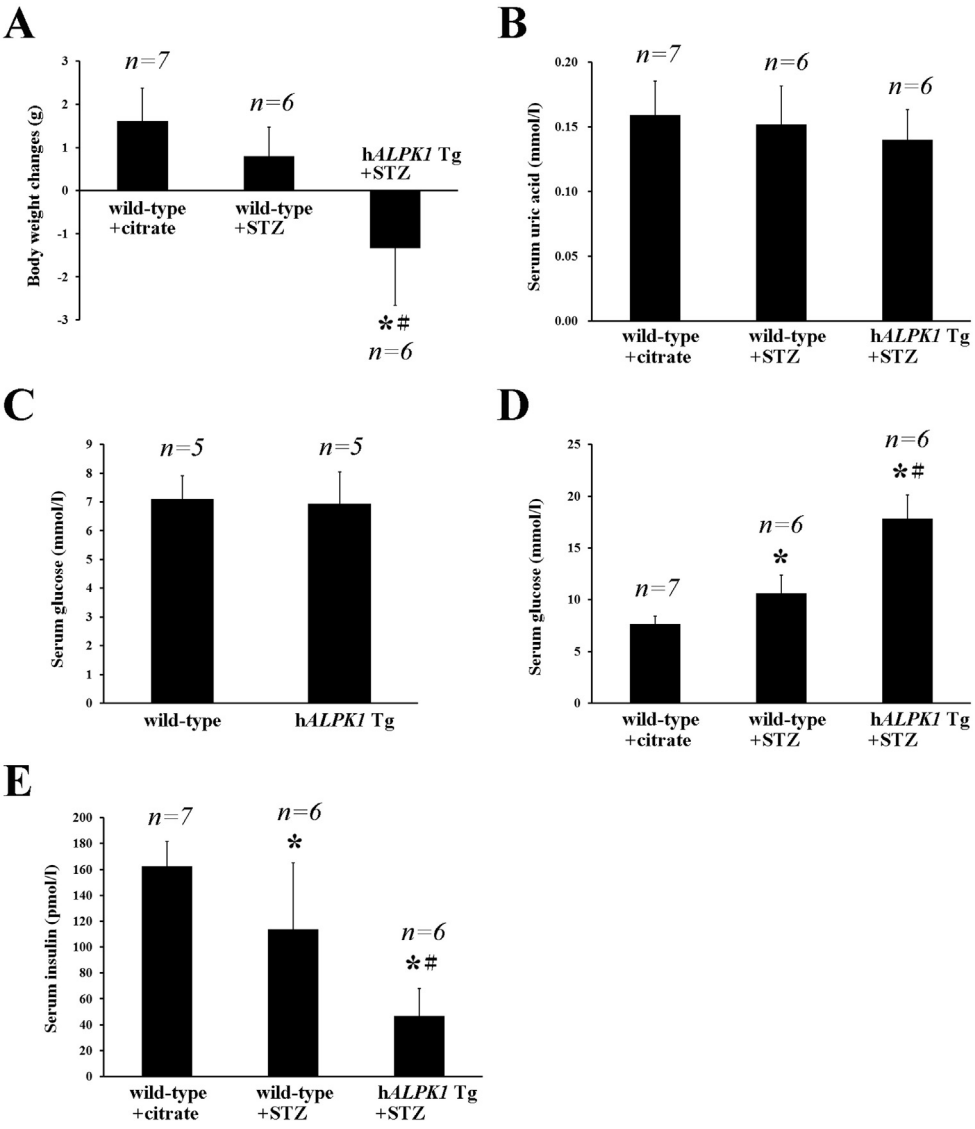

Fig. 1. *ALPK1* transgenic mice showing severe hyperglycemia after streptozotocin (STZ) injection. Age-matched wild-type and h*ALPK1*-Tg mice were injected with STZ or control citrate, as described in the materials and methods. (A) Body weight changes and (B) serum uric acid measured at 3 weeks after STZ injection. (C) Serum glucose concentration in non-treated wild-type and h*ALPK1*-Tg mice. (D) Serum glucose and (E) insulin measured in each group of STZ-treated mice. The n values represent the numbers of animals in each group. Data are mean ± SD. (*) $p < 0.05$ compared with citrate treated wild-type mice. (#) $p < 0.05$ compared with STZ treated wild-type mice.