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Translocase of inner mitochondrial membrane 44 alters the mitochondrial fusion and fission dynamics and protects from type 2 diabetes

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ABSTRACT

Objective. In obesity and type 2 diabetes, the impairment of mitochondrial function in white adipose tissue (WAT) is linked to a reduction in whole body insulin sensitivity. *Timm44* is upregulated in the kidneys of streptozotocin-induced diabetic mice. In the inner mitochondrial membrane, Timm44 anchors mitochondrial heat-shock protein 70 (mtHsp70) to the translocase of inner mitochondrial membrane 23 (TIM23) complex and facilitates the import of mitochondria-targeted preproteins into the mitochondrial matrix dependent on the inner membrane potential and ATP hydrolysis on ATPase domain of mtHsp70.

Methods. We generated the aP2-promoter driven Timm44 transgenic (Tg) mouse model and investigated whether Timm44 Tg mice fed high-fat/high-sucrose (HFHS) chow are protected from type 2 diabetes and obesity.

Results. The body weight of aP2-promoter driven Timm44 Tg mice was lower than that of wild type mice, and insulin sensitivity was greater in Timm44 Tg mice than in wild type mice. Although WAT weight was not altered in Timm44 Tg mice fed HFHS chow, adipocyte

Abbreviations: Actb, β-actin; BAT, brown adipose tissue; Cidea, cell death-inducing DFFA-like effector a; Cox7a1, cytochrome c oxidase subunit VIIa polypeptide 1; Dnm1l, dynamin 1-like; ETC, electron transfer chain; FA, fatty acid; Fabp4, fatty acid binding protein 4; Fis1, fission 1; HFHS chow, high-fat/high-sucrose chow; Mfn1, mitofusin 1; Mfn2, mitofusin 2; mtHsp70, mitochondrial heat-shock protein 70; Opa1, optic atrophy 1; OXPHOS, oxidative phosphorylation; PAM, presequence translocase-associated motor; Ppargc1a, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; PPARγ, peroxisome proliferator-activated receptor gamma; PRDM16, PR domain containing protein 16; ROS, reactive oxygen species; STD chow, standard chow; TFAM, mitochondrial transcription factor A; Timm44, translocase of inner mitochondrial membrane 44; TIM23, translocase of inner mitochondrial membrane 23; Ucp1, uncoupling protein 1; WAT, white adipose tissue.

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size was reduced, and mitochondrial fusion associated with decreased expression of fission genes, such as *Dnm1l* and *Fis1*, was observed. In addition, when fed standard (STD) chow, the expressions of the fusion genes *Opa1*, *Mfn1* and *Mfn2*, and *Mfn1* were significantly increased in Timm44 Tg mice compared to wild type mice, and fused mitochondria were also observed in Timm44 Tg mice fed STD chow.

Conclusions. The *Timm44* gene may be a new target for the treatment of type 2 diabetes.
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1. Introduction

In obesity and type 2 diabetes, the impairment of mitochondrial function in white adipose tissue (WAT) has received increasing attention, and it is now considered a major factor in the pathobiology of the disease. The regulatory roles of mitochondria in adipose tissues and adipocytes are closely linked to the regulation of systemic energy homeostasis and whole body insulin sensitivity [1]. Nutritional intake drives mitochondria to generate sufficient ATP for energy consuming lipogenic pathways and acetyl-CoA, a substrate for fatty acid (FA) synthesis. Mitochondria act as a crossroad of β-oxidation, the glyceroneogenic pathway and FA esterification and store lipids in the form of triglycerides (TGs). Primarily, mitochondrial dysfunction is defined as the incapability to produce sufficient ATP levels through oxidative phosphorylation (OXPHOS). In WAT of diabetic mice with a sustained excessive nutrient supply, mitochondrial numbers, mitochondrial DNA content, and enzymatic activities of the electron transfer chain (ETC) were reduced, and these reductions all contributed to a decline in OXPHOS activities in the WAT of these mice [2]. In addition to the classical concept of mitochondrial dysfunction, the mitochondrial ETC is a major source of reactive oxygen species (ROS), which are mainly produced by complexes I and III [3]. Excess calorie and lipid intakes are linked to mitochondrial substrate overload, which subsequently results in increased ROS production. ROS oxidizes various lipids, and the reactive lipid aldehydes produced by ROS oxidation further modify a variety of intracellular proteins, carbohydrates, and lipids. This finally results in insulin resistance [4].

In WAT, pharmacological interventions, such as peroxisome proliferator-activated receptor gamma (PPARγ) agonists, enhance brown adipose tissue (BAT) enriched genes, such as peroxisome proliferator-activated receptor γ, coactivator 1 alpha (PGC1α), mitochondrial transcription factor A (TFAM) and the PR domain of protein 16 (PRDM16), and induce the browning of WAT, which is associated with increased mitochondrial biogenesis, mitochondrial DNA content, and palmitate-stimulated oxidative capacity [2,5–7]. In addition to PPARγ agonists, chemical uncoupling agents may reduce ATP synthesis by increasing proton leakage and heat production and limiting ROS production. However, uncoupling agents can lead to uncontrolled thermogenesis and cytotoxicity in the central nervous system, and uncoupling agents are not available in clinical settings [1]. Interestingly, the adipose-specific deletion of TFAM in mice (F-TFKO) resulted in a decreased mtDNA copy number, perturbed mitochondrial function with decreased complex I activities, greater oxygen consumption, and uncoupling. F-TFKO mice are protected from age- and diet-induced obesity and insulin resistance [8]. This line of the evidence suggests that the mitochondrion is an important therapeutic target for the treatment of type 2 diabetes and obesity.

We identified translocase of inner mitochondrial membrane 44 (*Timm44*) through its upregulation in the kidneys of streptozotocin (STZ)-induced diabetic mice [9]. In the inner mitochondrial membrane, Timm44 anchors mitochondrial heat-shock protein 70 (mtHsp70) to the translocase of inner mitochondrial membrane 23 (TIM23) complex and is involved in the import of mitochondria-targeted preproteins into the mitochondrial matrix dependent on the inner membrane potential and ATP hydrolysis by the ATPase domain of mtHsp70. We also demonstrated that gene delivery of *Timm44* ameliorated diabetic nephropathy in both STZ-induced diabetic mice [10] and a carotid artery balloon injury STZ-induced diabetic WKY rat model [11] by reducing ROS production and ATP content. Here, we generated aP2-promoter driven Timm44 transgenic (Tg) mice and investigated whether Timm44 Tg mice fed high-fat/high-sucrose (HFHS) chow are protected from type 2 diabetes and obesity. When Timm44 Tg mice were fed HFHS chow, we observed fused mitochondria associated with decreased expression of the fission gene *Fis1*. As insulin sensitivity was higher in Timm44 Tg mice than in non-transgenic STZ-induced diabetic mice, the Timm44 gene may be a new target for the treatment of type 2 diabetes.

2. Materials and methods

2.1. *Generation of* ***Timm44*** *transgenic mice*

The aP2 promoter and β-globin intron were amplified using the PCR primers 5′-GGGGGGCTCGAG(*Xho*I)CCAGCAGGAATCAGGTAGCTGGAGA-3′ and 5′-GGGGGGGTCGAC(*Sal*I)GAATTC(*Eco*RI)TTTGCCAAAATGATGAGACAGCACA-3′ and were then ligated into the *Xho*I and *Sal*I sites of pBluescript KS(+) (Stratagene). Next, human GH poly A signal was amplified using the primers 5′-GGTCGAC(*Sal*I)ATCGAT(*Cla*I)GCATGC(*Sph*I)GAATTC(*Eco*RI)ACTCCTCAGGTGCAGGCTGC-3′ and 5′-GGGGGGGCGGCCGC(*Not*I)GGATCTCGATCTTCATAAGAGAAGA-3′ and was then ligated into the *Sal*I and *Not*I sites of pBluescript KS(+) (Stratagene). Finally, the full-length cDNA of *Timm44* was amplified using the primers 5′-GGGGGGGTCGAC(*Sal*I)CCAACATGGCGGCGGCACGT-3′ and 5′-GGGGGGGCATGCT(*Sph*I)GCCCTCAGAGGATCTGCTC-3′ and was then ligated into the *Sal*I and *Sph*I sites of pBluescript KS(+) (Stratagene). The insert was excised with *Xho*I and *Not*I, and the transgene was amplified. C57BL/6NJcl one-cell stage zygotes were microinjected with the transgene and then oviduct-transferred and permitted to develop to term, and the

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