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研究課題名（和文）Identification and clinical implication of biomarkers for mitochondrial diseases

研究課題名（英文）Identification and clinical implication of biomarkers for mitochondrial diseases

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研究成果の概要（和文）：本研究では、薬剤によるミトコンドリアの機能の変化を調べるため、mitofilin過剰発現細胞を作成した。また、腎臓の近位尿細管にミトコンドリアを多く含まれ、ミトコンドリア病では尿細管障害などの腎疾患が合併する。よって、腎疾患の研究にも着目した。腎不全マウスを用いてSGLT阻害薬が腸内環境の変化をもたらし、血中の尿毒素濃度の減少作用を明らかにした。一方、糖尿病性腎症では腎臓の近位尿細管に脂肪滴の蓄積が起こることが報告され、近位尿細管由来細胞を用いて、細胞内の脂質蓄積とミトコンドリアの断片化を観察し、ATP産生の低下とともに過剰な活性酸素種が生じることにより、細胞死に至ると考えられる。

研究成果の学術的意義や社会的意義
本研究では、腸内細菌叢の変化により腎障害の改善作用や、腎臓の近位尿細管細胞の脂肪滴の減少により、活性酸素種の消去およびミトコンドリア障害の改善作用を示した、将来的にはミトコンドリアのエネルギー代謝機構を解明することで、新たな治療戦略になり、あるいは新たなバイオマーカーを見つけることができると考え、ミトコンドリア病や腎疾患の患者の減少、医療費削減に貢献することができると考える。

研究成果の概要（英文）：For seeking a novel therapeutic approach for the treatment of mitochondria disorder, several studies have been performed. First, the function of mitofilin, which play an indispensable role in the maintenance of mitochondrial function. Second, for the reason of high mitochondrial content and oxygen consumption in the kidney, the continuous mitochondrial dysfunction plays an essential role in progression of renal diseases. Here, we also investigated a new candidate drug for chronic kidney disease and focused on the correlation between lipotoxicity and mitochondrial dysfunction in the kidney.
The current study found that a SGLT1 inhibitor decreased the accumulated uremic toxins in the renal failure mice, through altering the gut microbiota composition without changing renal function. Moreover, using a proximal tubule epithelial cell line, lipid droplets accumulated in fatty acids treated cells accompanied by decreased ATP production and mitochondrial dysfunction.

研究分野：腎臓内科学

キーワード：Mitochondria　chronic kidney disease

１．研究開始当初の背景

Mitochondrial defects are a central factor in human health and disease, it is one kind of the intractable disease that the diagnosis is still complicated by a distinct lack of sensitive and specific biomarkers. As the mitochondria are responsible for producing energy, mitochondrial dysfunction has been related to a wide range of diseases, including kidney disease and diabetes. The aim of the research is to find out the role of mitochondria-specific protein, mitofilin, in the cells treated with a new therapeutic candidate drug for mitochondrial diseases, MA-5 [1-3]. As kidney disease also have been associated with mitochondrial dysfunction, we here examined the effect of a SGLT1 inhibitor as a candidate drug for reducing the uremic toxins in a chronic kidney disease (CKD) mice model according to our recent study, which indicated that a SGLT2 inhibitor exerts intestinal effects that reduce the accumulation of uremic toxins [4]. Moreover, other study showed that the lipid droplets accumulated in the renal proximal tubule cell of patient with diabetic nephropathy, we also investigated the correlation between the accumulated lipids and mitochondrial function using the renal proximal tubule cells. Improving mitochondrial function may provide a novel therapeutic approach to minimize renal injury and ameliorate progression of CKD.

２．研究の目的

(1) To seek a novel therapeutic approach for the treatment of mitochondria disorder.

(2) To examine the effect of a SGLT1 inhibitor (mizagliflozin, MIZA) on the renal function and uremic toxins in a CKD mice model.

(3) To assess the correlation between lipotoxicity and mitochondrial dysfunction in the kidney.

３．研究の方法

(1) To seek a novel therapeutic approach for the treatment of mitochondria disorder.

Mitofilin, which play an indispensable role in the maintenance of the MICOS complex stability and the mitochondrial cristae morphology. MA-5 is a novel drug plays a role on mitochondrial function that our group recently invented. For investigating the relationship between mitofilin and MA-5 in mitochondria, we established the mitofilin overexpression cells.

(2) To examine the effect of a SGLT1 inhibitor (mizagliflozin, MIZA) on the renal function and uremic toxins in a CKD mice model.

The adenine-induced renal failure mice were administrated with MIZA for 10 days, the uremic toxins of plasma were analyzed by LC-MS and the fecal short-chain fatty acids were analyzed by GC-FID. To further investigate the effect of MIZA on the intestinal microbiome, we performed 16S rRNA gene sequencing of feces of the mice with or without MIZA by Next Generation Sequencer.

(3) To assess the correlation between lipotoxicity and mitochondrial dysfunction in the kidney.

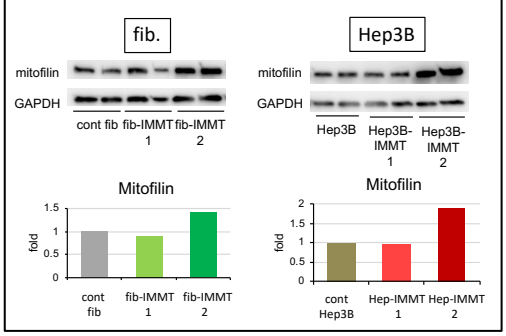
The renal proximal tubular HK-2 cells were treated with unsaturated fatty acids or saturated fatty acid, and lipid accumulation was then visualized by Oil Red O staining. Cardiolipin (CL) is a phospholipid localized in the mitochondrial membrane that CL plays a critical role in mitochondrial function. Here, we also measured CL composition using LC-MS. The ATP production also been measured using a commercial ATP Determination Kit (A22066, Thermo Fisher Scientific).

４．研究成果

(1) Established the mitofilin-overexpression cell models for the evaluation of mitochondrial functions.

We established the mitofilin-overexpression cells (Fig. 1), and will further evaluate its role on mitochondrial functions in MA-5 treated cells.

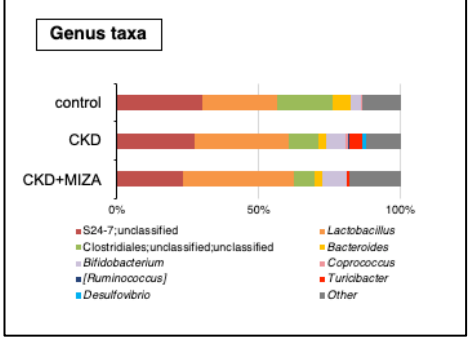
Fig. 1



(2) To examine the effect of a SGLT1 inhibitor (mizagliflozin, MIZA) on the renal function and uremic toxins in a CKD mice model.

Oral administration of MIZA did not significantly change the impaired renal function, although it significantly reduced the plasma level of indoxyl sulfate (an approximately 40% reduction compared with the control) without changing the glucose level. It also altered the composition of the fecal short fatty acid propionate suggesting a change in the bacterial carbohydrate fermentation. These data strongly suggested the alteration gut microbiota by MIZA. Although the principal coordinates analysis of the microbiome did not show a significant shift of the whole gut microbial composition between control and the MIZA renal failure group, at the genus level, some major taxa were significantly reduced in the renal failure mice were altered by MIZA (Fig. 2). These results suggest that SGLT1 inhibition reduced the accumulation of gut-derived uremic toxins through modification of the gut microbiota, providing a novel and potential therapeutic tool for CKD patients.

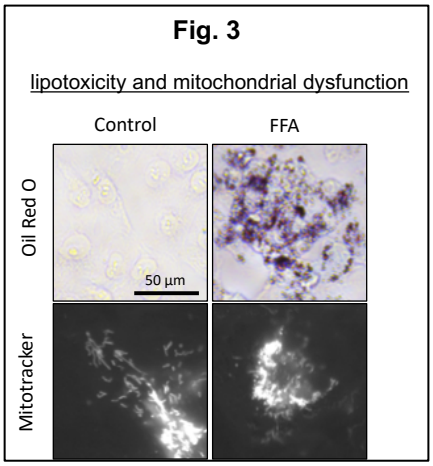
Fig. 2



(3) To assess the correlation between lipotoxicity and mitochondrial dysfunction in the kidney.

Moreover, studies also indicated that cholesteryl ester is accumulated in the urine of CKD patients. The accumulated lipids induce mitochondrial dysfunction in the kidney. Using a proximal tubule epithelial cell line, HK-2, lipid droplets accumulated in both saturated and unsaturated fatty acids in human proximal tubular HK-2 cells (Fig. 3), however, lipotoxicity accompanied by increased double bonds of unsaturated fatty acids but not saturated fatty acid (palmitic acid, 16:0) in HK-2 cells but not human liver HepG2 cells using cytotoxicity assay. Further, the production of ATP decreased in unsaturated fatty acid (oleic acid, 18:1) treated cells at 3 hours, suggesting that the function of mitochondria might decreased. Cardiolipin (CL) is a phospholipid localized in the mitochondrial membrane that CL plays a critical role in mitochondrial function. CL composition changed with the composition of saturated fatty acid (palmitic acid, 16:0) and unsaturated fatty acid (oleic acid, 18:1), the investigation of CL composition could be a potential tool to understanding the progression of CKD.

Fig. 3



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５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　0件）

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４．発表年 2018年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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