Ratfish liver oil prevents diabetic nephropathy via regulation of TET enzymes and DNA demethylation

Miao-Yi Wu[1], Ali Mallakin[2], Hans-Uwe Dahms[3], Tusty-Jiuan Hsieh[1]

1. Graduate institute of Medicine, College of Medicine, Kaohsiung Medical University, Taiwan
2. West Coast Biomedius, West Vancouver, British Columbia, Canada
3. Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Taiwan

Abstract

Diabetic nephropathy (DN) is a leading cause of end-stage renal disease (ESRD) in Taiwan. However, available therapies have not been fully effective in the treatment of DN. Administration of omega-3 fatty acid to DN patients had favorable effects on insulin levels, serum triglycerides and VLDL-cholesterol; however, its effect did not influence biomarkers of inflammation and oxidative stress. Epigenetic mechanisms have been reported to involve in the renal cell injury and the progression of DN. Abnormal DNA methylation has been observed in the renal proximal tubules of diabetic mice but the mechanism is not fully understood. Ten-eleven translocation (TET) enzyme family plays key roles in DNA demethylation pathway. TET enzymes can oxidize the 5-methylcytosine (5-mC) of DNA to generate 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine and 5-carboxylcytosine and dynamically regulate global or locus specific 5-mC and 5-hmC levels by facilitating active DNA demethylation. Our previous study has shown TET-1 protein was upregulated in the renal proximal tubules of db/db diabetic mice. We also found methylation level of histone 3 lysine 4 (H3K4me) was increased in high-glucose stimulated HK-2 cells and in renal proximal tubules of db/db mice. Our previous study indicates TET-1 may be a novel pathological molecule in modifying epigenome in DN. Therefore, we hypothesized that omega-3 fatty acid rich oils may prevent the progression of DN via regulation of epigenome.

In this study, we tested three kind of oils in db/db diabetic (DM) mice, including soybean oil (SO), ratfish oil (RLO) and flaxseed oil (FO). Ratfish oil and flaxseed oil have been reported rich in omega-3 fatty acid. Our result demonstrates that RLO could inhibit the progression of diabetes in the db/db mice. By real-time PCR and western blotting, we find TET-1, TET-2 and TET-3 mRNAs and proteins are increased in the kidney of db/db mice. Interestingly, TET1 and TET2 proteins are decreased by RLO, FO and SO. DNA methylation is decreased in 16-week DM mice but H3K4 protein methylation is increased, indicating a more active genome in DM mice. Methylation levels of DNA and histone 3 lysine 4 (H3K4me) are also changed by RLO, FO and SO, indicating their influence on epigenome. The protein level of cleaved caspase-3, the cell apoptosis marker, is decreased in the RLO and FO treated mice. Active form of Transforming Growth Factor beta (TGFβ), the renal fibrosis marker, is also inhibited by RLO and FO treatment. Our results demonstrate that omega-3 fatty acid rich oils, RLO and FO, could reduce kidney apoptosis and fibrosis to prevent DN, but SO has the similar effect. However, whether the mechanism is via regulating active DNA demethylation by TET enzymes still needs further investigation.

Result

		DM-8W	DM-16W	DM+SO	DM+RLO	DM+FO
Weight (g)	0w	34.3±3.2	34.4±3.3	34.2±2.6	34.2±1.0	28.7±11.5
	8w	34.5±5.1	35.4±5.6	32.7±2.7	31.1±2.0	28.8±11.6
	16w	-	31.7±7.1	26.4±2.8	27.5±3.0	26.3±10.7
Blood Glucose (mg/dl)	0w	447.7±62.1	393.1113.1	492.7±88.7	516.3±48.2	487.5±79.8
	8w	549.8±36.4	533.5±50.0	553.3±55.1	547.2±65.4	561.0±34.1
	16w	-	524.1±62.2	522.0±6.0	390.5±86.5	590.5±9.5

Table 1. Effects of RLO on body weight and blood glucose in diabetic db/db mice (DM). Data are mean ± SD from 5~9 mice. RLO reduced the blood glucose in the DM mice.

	DM-8W	DM-16W	DM+SO	DM+RLO	DM+FO
BUN	36.2±6.9	35.9±7.3	45.0±11.2	37.9±11.3	37.6±13.1
TRIGL	217.8±52.2	220.1±54.6	202.3±23.9	204.3±26.5	206.7±17.5
ALT	217.7±235.0	94.8±78.8	44.9±18.1	63.2±22.4	68.8±15.3
CHO2I	108.9±14.8	123.6±11.1	107.4±21.7	110.0±23.7	102.0±9.6
AST	793.5±882.7	301.3±312.1	209.4±48.1	255.7±102.2	221.0±61.6
CREA2	0.2±0.1	0.3±0.2	0.3±0.1	0.3±0.1	0.3±0.1
Kidney weight / Tibia length	18.5±2.2	18.8±2.4	18.0±1.5	18.3±2.4	15.8±5.7

Table 2. Serum biochemistry profiles of the DM and treatment mice. Data are mean ± SD from 5~9 mice.

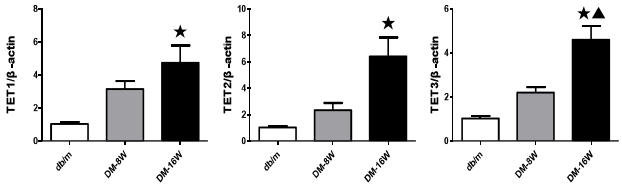


Figure 1. mRNA expression of TETs in db/m (control) mice and db/db mice (8 and 16 weeks). $p < 0.05$, ★:compared with db/m mice, ▲: compared with db/db mice-8w. Data are mean ± SD from 5~9 mice. TETs mRNA are increased in the kidney of DM mice.

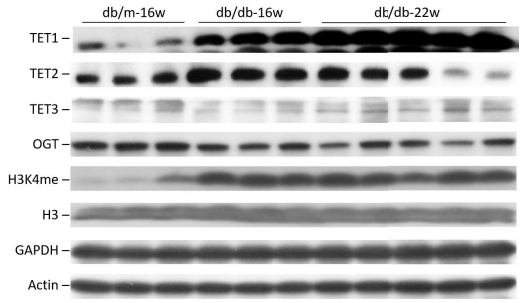


Figure 2. Protein expression of TET-1,TET-2, TET-3, OGT, H3K4me and histone 3 (H3) in the kidney of mice. TET1, TET2 and methylated H3K4 proteins are increased in the kidney of DM mice.

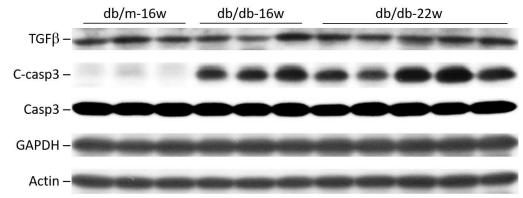


Figure 3. Protein expression of TGFβ, cleaved caspase-3 (C-casp3), and caspase-3 (Casp3) in the kidney of mice. Cleaved caspase-3 and TGF-β protein are increased in the kidney of DM mice, indicating apoptosis and fibrosis occurred in the kidney.

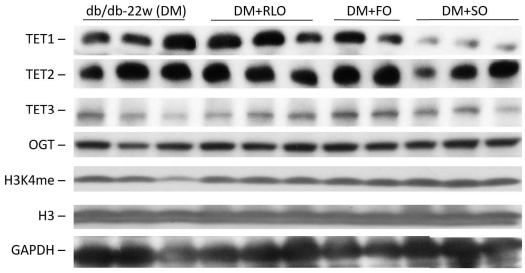


Figure 4. Protein expression of TET-1,TET-2, TET-3, OGT, H3K4me and H3 in the kidney of db/db mice and treatment mice. TET1 and TET2 proteins are decreased but H3K3me is increased in the kidney of treatment mice, indicating RLO, FO and SO maybe could influence epigenetic change of the genome in the diabetic kidney.

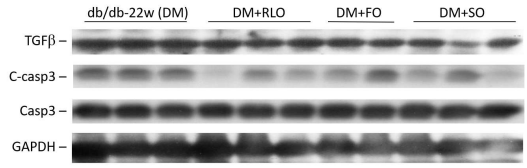


Figure 5. Protein expression of TGFβ, cleaved caspase-3 and caspase-3 in the kidney of db/db mice and treatment mice. Cleaved caspase-3 and TGF-β proteins are decreased in the kidney of treatment mice, indicating RLO, FO and SO could prevent apoptosis and fibrosis occurred in the diabetic kidney.

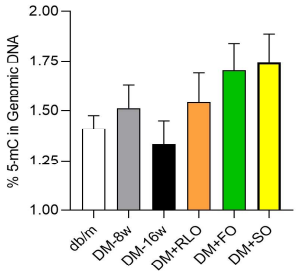
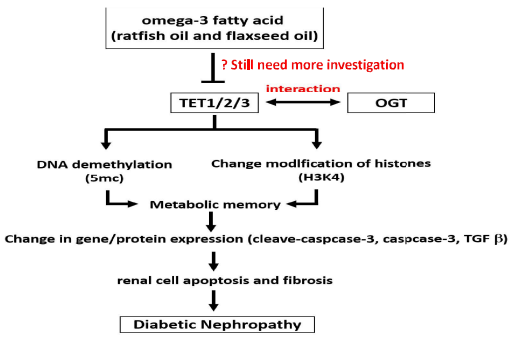


Figure 6. Quantification of 5-methy-cytosine (5-mC) in the kidney of db/db mice and treatment mice. Data are mean ± SD from 5~9 mice. DNA methylation is decreased in the kidney of 16-week DM mice. The decrease is reversed by RLO, FO and SO.

Summary



This research was funded by the grant from Ministry of Science and Technology, R.O.C. (MOST 105-2314-B-037-042-MY2)