MiR-494-3p regulates mitochondrial biogenesis and thermogenesis through PGC1-α signaling in beige adipocytes.

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別紙様式３（課程博士・論文博士共用）

論 文 内 容 要 旨

※整理番号	833	（ふりがな） 氏　　名	シュカレ　メンギズツ　レメチャ Shukare Mengistu Lemecha
学位論文題目	MiR-494-3p regulates mitochondrial biogenesis and thermogenesis through PGC1-α signaling in beige adipocytes (Mir-494-3p は PGC1-α を介して脂肪細胞のベージュ化におけるミトコンドリアバイオジェネシスと体温調節を制御する)		

Background:
The primary role of white adipose tissue is storage or release of energy depending on the energy state. Brown adipose tissues are known to serve as heat production especially during cold environment. Recently beige adipocytes derived from white adipose tissue is receiving a lot of attention because of its promise as a new therapeutic target in the treatment of obesity and other metabolic disorders. Mitochondria is a key organelle through uncoupling protein-1 (Ucp-1) for heat generation in brown and beige adipocytes. Mitochondrial biogenesis in adipose tissue is activated by various situation such as cold exposure and catecholamine. PPAR-gamma coactivator 1-alpha (PGC-1α) is known as the master regulator of mitochondrial biogenesis. Previously, we have reported that microRNA-494 (miR-494) regulates mitochondrial biogenesis in the skeletal muscle. The regulatory mechanisms underlying cold-induced beige adipogenesis by microRNAs remains unknown. Because adipocytes and skeletal muscle cells share the same origin, we hypothesized that miR-494-3p may play a role in adaptive thermogenesis in beige cells through mitochondrial biogenesis. Therefore, here we examined this hypothesis using several beige adipogenesis models.

Purpose:
We aim to investigate the role of miR-494-3p on mitochondrial biogenesis during beige adipogenesis.

Methods:
We used three different models to justify our hypothesis. First, for the in vivo beige adipogenesis study, male C57BL/6J mice (8-week-old, n =5 each group) were subjected to cold exposure at 12°C for acute group (6 h), chronic group (6 h per day for 2 weeks) and control group maintained at 24°C. Second, for the in vitro model, we differentiated 3T3-L1 cells to matured white adipocytes and matured beige adipocytes. Third, we set up primary beige adipocytes by isolating primary stromal vascular cells from inguinal fat pad of male C57BL/6J mice (8-week-old). We collected data using different experimental approaches such as animal handling (proper anesthetizing of mice with sevoflurane), Tissue was harvested for histology (immunohistochemistry staining, immunofluorescence staining, hematoxylin and eosin staining), cell biology (cell culture, cell transfection and electroporation, immunocytochemistry, Oil O Red staining and cell live mitotracker staining), microscopy (bright field, fluorescence and confocal), rate of oxygen consumption,

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等を用いて印字すること。
２．※印の欄には記入しないこと。

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molecular biology (nucleic acid isolation, real-time qPCR analysis, mtDNA content quantification by PCR, miRNA quantification by TaqMan miRNA assays, luciferase reporter assay and vector cloning), biochemistry (western blotting), data analysis software (image j, SPSS and Excel).

Results:

Adipose tissues of C57BL/6J mice subjected to intermittent mild cold exposure induced PGC1-α and mitochondrial transcription factor A (TFAM), pyruvate dehydrogenase (PDH), adenine nucleotide translocator (ANT1/2), and Ucp1 proteins expression in inguinal white adipose tissue (iWAT). Ucp1 mRNA expression was significantly increased both in the acute ($p < 0.001$) and chronic cold ($p < 0.05$) exposure groups. miR-494-3p levels were significantly downregulated in iWAT upon cold exposure ($p < 0.05$). Oil Red O staining of 3T3-L1 beige adipocytes revealed a lower degree of lipid droplet accumulation compared with 3T3-L1 white adipocytes Ucp1 mRNA expression was approximately 17-fold higher in 3T3-L1 beige adipocytes compared with white adipocytes. In the in vitro model of both 3T3-L1 and primary beige adipocytes, β3 adrenergic activation with 10 μM isoproterenol potently down regulated miR-494-3p expression. miR-494-3p overexpression substantially reduced PGC1-α protein expression and its downstream targets TFAM, PDH and MTCO1 in 3T3-L1 white and beige adipocytes ($p < 0.05$). miR-494-3p inhibition in 3T3-L1 white adipocytes resulted in increased PDH ($p < 0.05$). PGC1-α, TFAM and Ucp1 mRNA levels were robustly downregulated by miR-494-3p overexpression in 3T3-L1 beige adipocytes. PGC1-α and Ucp1 proteins were downregulated by miR-494-3p in primary beige cells ($p < 0.05$), along with strongly decreased rate of oxygen consumption. Luciferase assays confirmed PGC1-α as a direct gene target of miR-494-3p. Our findings demonstrate that decreased miR-494-3p expression during browning regulates mitochondrial biogenesis and thermogenesis through PGC1-α.

Discussion:

This study has revealed three important findings. First, the expression of miR-494-3p was reduced in iWAT by cold exposure *in vivo* and was also reduced in response to beige induction, with a corresponding increase of mitochondrial proteins. Second, the expression of miR-494-3p was decreased by β_3-AR stimulation in beige adipocytes. Third, overexpression of miR-494-3p reduced PGC1-α mRNA and protein levels in adipocytes and attenuated mitochondrial biogenesis and oxygen consumption. These findings demonstrate that miR-494-3p directly inhibits the expression of PGC1-α and subsequently increase in mitochondrial biogenesis during beige adipogenesis. The decreased miR-494-3p expression during beige adipocyte differentiation removes its inhibitory effect, leading to stimulation of Ucp1 expression and mitochondrial biogenesis.

Conclusion:

Our findings demonstrated that decreased miR-494-3p levels during beige differentiation stimulated Ucp1 and mitochondrial biogenesis through reducing the inhibitory activity of miR-494-3p on PGC1-α gene expression.

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学位論文審査の結果の要旨

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論文審査委員			

（学位論文審査の結果の要旨）※明朝体１１ポイント、６００字以内で作成のこと

哺乳類の脂肪細胞には、これまでよく知られている褐色および白色脂肪細胞に加え、近年ベージュ脂肪細胞の存在が報告されたが、その形成過程には不明な点が多く残されている。本論文では、交感神経刺激などによって誘発されるMiR-494-3pのダウンレギュレーションが、白色脂肪細胞からベージュ脂肪細胞への変換に重要な意義を有するという仮説をたてて検証を行った結果、以下の点を明らかにした。

１）寒冷刺激に暴露したマウス脂肪組織において、MiR-494-3pの発現が低下するとともに、ミトコンドリア新生が亢進し、Ucp1やPGC1-αなどの発現上昇が認められた。
２）この現象はβ３アドレナリン受容体依存性であった。
３）培養細胞を用いた実験で、3T3-L1 細胞からベージュ脂肪細胞への分化誘導において、上記結果が確認された。
４）PGC1-αは、MiR-494-3p の直接の標的遺伝子であることが分かった。

本論文は、MiR-494-3p のダウンレギュレーションが、PGC1-αの発現上昇を介して、ミトコンドリア新生とベージュ脂肪細胞の分化誘導を制御していることについて新たな知見を与えたものであり、また、最終試験として論文内容に関連した試問を実施したところ合格と判定されたので、博士（医学）の学位論文に値するものと認められた。

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