したが、これは大きな一歩である。今後特殊な指示薬を用い、内耳におけるミトコンドリアの役割について多くの情報を得ることが期待できる。

E. 結論

MIDD 患者の難聴進行予防を目的とし、水素水とタウリンを投与して検討したが、まだ有意な結果は得られていない。OCT を用い、モルモットの摘出蝸牛を脱灰して観察したところ、蝸牛軸以外の組織（血管条、ラセン靭帯、コルチ器など）の詳細な観察が可能であり、側壁の萎縮、コルチ器の変性、内リンパ水腫が確認できた。高速撮影が可能な Nikon A1R 共焦点顕微鏡を基盤とした生体内リアルタイム共焦点顕微鏡を構築し、内耳有毛細胞の in vivo イメージングが可能となった。

F. 健康危険情報

特になし

G. 研究発表

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H. 知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（難治性疾患等克服研究事業）
分担研究報告書

意識障害を繰り返す母子例におけるミトコンドリア $tRNA^{Val}$ 遺伝子上の heteroplasmic m.1624C>T 変異に関する研究

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研究要旨　ミトコンドリア $tRNA^{Val}$ 遺伝子上の m.1624C＞T 変異についてはこれまでに世界で一家系例の報告しかなかった。その家系例では、全例が変異を homoplasmic に有し、臨床表現型は多様で多くは生下後死に至り、生存しても重篤な Leigh 脳症類似の臨床症状を引き起こしていた。今回我々は成人発症で精神運動興奮を伴う一過性の意識障害を繰り返し、m.1624C＞T 変異を heteroplasmic に持つ母子例を経験した。本家系例において同変異の heteroplasmy の割合の定量を行い、既報の homoplasmic にもつ家系例と臨床症状を比較検討し、相違点について考察した。

A．研究目的

ミトコンドリア遺伝子（mtDNA）上の m.1624C＞T 変異の報告はこれまで世界で一家系例の報告のみしかなかった。その家系症例は全例変異を homoplasmic に有しており、臨床表現型は多様で、母親は軽症であったがその子らの多くは胎生致死もしくは生下後数時間で死亡し、生存患児も Leigh 脳症類似の重篤な臨床症状を引き起こした(Robert McFarland et al. Nat Genet. 30, 145-6, 2002)。これまでに heteroplasmic に m.1624C＞T 変異を持つ症例の報告はない。今回我々は成人発症で精神運動興奮を伴う一過性の意識障害を繰り返し、m.1624C＞T 変異を heteroplasmic に持つ母子例を経験した。本家系例において同変異の heteroplasmy の割合の定量を行い、既報の homoplasmic にもつ家系例と臨床症状を比較検討し、相違点について考察した。

B．症例と研究方法

発端者となった症例は、36 歳男性。25 歳時に、せん妄による精神運動興奮を伴う一過性の意識障害を呈し入院加療となった。脱抑制、知的機能低下、人格変化などの精神神経症状を認めた。脳脊髄液では乳酸、ピルビン酸の上昇を繰り返し認めた。この症例に対して、WAIS-R、WAIS-Ⅲ、Trail-Making test、Modified Stroop test、Wiscontin card sorting test、Word fluency test などの神経心理学的検査を行い、経過を追った。また、母親は 37 歳時より精神運動興奮、幻視、幻聴やてんかん発作を伴う一過性の意識障害を繰り返し、頭部 CT 上に一過性に出現する低吸収域を繰り返し認めた。

文章によるインフォームドコンセントを患者、患者母親から得た後に採血を行い、白血球、患者筋肉から常法を用いて mtDNA を抽出した。患者白血球、患者筋肉、患者母親白血球の mtDNA について直接シークエンス法で変異検索を行った。

また、ARMS（Amplification Refractory Mutation System）法を用いた real-time PCR 法により heteroplasmic m.1624C>T の

定量を行った。定量の際には、p-GEM-T vector を利用した wild-type1624C と mutation1624T の plasmid により検量線を作成した。定量の際の蛍光試薬には SYBR Green を使用した。

（倫理面への配慮）

本症例の報告に当たっては、個人が特定されないよう十分に配慮した。また、本研究は鹿児島大学医学部生命倫理・遺伝子解析研究倫理委員会、遺伝子組み換え実験安全委員会の承認を得た。

C．研究結果

患者白血球、患者筋肉、両者の白血球から抽出した mtDNA において、全 mtDNA の配列解析を行い、m.1624C>T heteroplasmy 変異を見出した。また、ARMS 法を用いた real-timePCR により得られた Ct 値から、検量線を利用して変異比率の定量を行った。m.1624C＞T 変異比率は患者筋肉　88.8%(29 歳時)>患者筋肉 59.7%(36 歳時)>患者白血球 47.8% (29 歳時)>患者白血球 34.0% (36 歳)>母親白血球 17.2%>>健常者白血球 0% という結果を得た。m.1624T の変異比率は 7 年間で患者白血球、患者筋肉で共に減少していた。

D.考察

今回我々は、繰り返す意識障害を伴う精神神経症状を呈し、脳脊髄液中の乳酸、ピルビン酸の上昇を認める家系例に対して、heteroplasmic な m.1624C>T 変異を同定した。既報の m.1624C>T 変異を homoplasmic に有する症例では、COX 欠損がみられていたが、本患者筋肉において、88.8%(29 歳)、59.7%(36 歳)といった高率の m.1624C>T 変異が見られたにもかかわらず、筋病理所見では、大小不同の筋線維以外の異常所見やミオパチーは認めなかった。m.3243A>G 変異では、COX-deficient fiber や ragged-red fiber がみられる変異率の閾値があると報告されている (Jeppesen et al., Arch. Neurol., 2006)。m.1624C>T 変異に関しても同様に筋病理所見や筋症状が出現する変異率の閾値があり、本症例は閾値以下の変異率であることが示唆された。

低比率の heteroplasmy の検出には RFLP や Taqman probe による real-time PCR よりも ARMS 法を使用した real-time PCR が有用であると報告されている (Bai and Wong, Clin. Chem., 2004)。直接シークエンス法では、低比率の変異は周囲のノイズと見間違う可能性がある。

胎生致死もしくは Leigh 脳症類似の重篤な臨床症状を引き起こしている homoplasmic な家系例に対して、我々の症例は発症年齢や臨床症状において、比較的軽症例と言える。Homoplasmic 家系では母親のみが片頭痛、易疲労感、筋力低下などの軽症となっており、Rorbach らは、VARS2 (valyl-tRNA synthetase 2) の発現が本変異の障害を rescue する可能性を考察していた (Rorbach et al., Nucleic Acids Res., 2008)。今回の結果から既報の homoplasmic に変異をもつ家系に比較すると、heteroplasmic に変異を有する本家系例の臨床症状は軽症であり、heteroplasmy の比率が表現型決定因子の一つであると考えられた。

発端者の 7 年間の経過で白血球と筋ともに m.1624C>T の変異率が低下していた。白血球においては経時的に m.3243A>G 変異率が低下することが知られている。Olsson, t' Hart らの研究では rapid turn over によるものではないかと考察されていた (Olsson et al., J. Hum. Genet., 2001; t' Hart et al., Hum. Mutat., 1996)。しかし、筋細胞では turn over の説明はできず、筋細胞における heteroplasmic 変異率の経時的減少の報告はほとんどない。Zhang らは筋において経時的な m.13167A>G 変異率低下を報告しており、変異細胞の複製の遅さによるのではないかと考察していた (Zhang et

al., Hum.Mutat.,1998)。本例の筋においても同様の機序で m.1624C>T 変異率低下が起こっている可能性が示唆された。

E.結論

今回我々は、繰り返す意識障害を伴う精神神経症状を呈し、脳脊髄液中の乳酸、ピルビン酸の上昇を認める日本人母子症例において、heteroplasmic な m.1624C>T 変異を同定し、筋や白血球由来の mDNA における ARMS 法により heteroplasmy 比率を計測した。既報の homoplasmic に同変異をもつ家系に比較すると、heteroplasmic に変異を有する本家系例の臨床症状は軽症であり、heteroplasmy の比率が表現型決定因子の一つであると考えられた。

F．健康危険情報

なし

G．研究発表

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H．知的財産権の出願・登録状況

(予定も含む。)

１．特許取得

なし

２．実用新案登録

現在のところなし

３．その他

特記すべき事項なし

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書　籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
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雑　誌

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Taurine Ameliorates Impaired the Mitochondrial Function and Prevents Stroke-like Episodes in Patients with MELAS

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Abstract

Objective Post-transcriptional taurine modification at the first anticodon ("wobble") nucleotide is deficient in A3243G-mutant mitochondrial (mt) $tRNA^{Leu(UUR)}$ of patients with myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). Wobble nucleotide modifications in tRNAs have recently been identified to be important in the accurate and efficient deciphering of codons. We herein examined whether taurine can alleviate mitochondrial dysfunction in patient-derived pathogenic cells and prevent clinical symptoms in MELAS patients.

Methods and Results The addition of taurine to the culture media ameliorated the reduced oxygen consumption, decreased the mitochondrial membrane potential, and increased the oxidative stress in MELAS patient-derived cells. Moreover, high dose oral administration of taurine (0.25 g/kg/day) completely prevented stroke-like episodes in two MELAS patients for more than nine years.

Conclusion Taurine supplementation may be a novel potential treatment option for preventing the stroke-like episodes associated with MELAS.

Key words: MELAS, post-transcriptional modification, taurine, stroke-like episodes

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Introduction

An A3243G or T3271C transition in the mitochondrial (mt) $tRNA^{Leu(UUR)}$ gene has been identified in approximately 80% and 10% respectively, of patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) (1). Nearly 90% of patients with myoclonus epilepsy associated with ragged-red fibers (MERRF) possess an A8344G transition in the mt $tRNA^{Lys}$ gene (1). These mutations are located in the cloverleaf structure of each mt tRNA. However, it remains unknown how such point mutations in mt tRNAs induce mitochondrial dysfunction leading to the wide variety of MELAS or MERRF symptoms.

Post-transcriptional modifications in tRNAs play critical roles in modifying the genetic code. In 1966, Francis Crick proposed that the first anticodon ("wobble") nucleotide recognizes the third codon nucleotide through non-canonical Watson-Crick geometry; so-called "wobble" pairing (2). Growing evidence has shown that various post-transcriptional modifications at the wobble nucleotides in tRNAs are required to recognize their cognate codons accurately and efficiently (3). In normal human mt $tRNA^{Leu(UUR)}$ or mt $tRNA^{Lys}$, uridine at the wobble position is modified with taurine, a sulfur-containing β-amino acid (4-6). In contrast, the taurine modification is deficient in mutant mt $tRNA^{Leu(UUR)}$ or mutant mt $tRNA^{Lys}$ derived from clinical specimens of MELAS or MERRF patients (4-8). The taurine modification defect in the mutant mt tRNAs causes a deficiency in deciphering codons (1, 9). These findings have given rise to the intriguing possibility that MELAS and

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MERRF are tRNA-modification disorders associated with the impairment of correct mitochondrial gene translation.

We hypothesized that high-dose taurine supplementation could restore the taurine modification of the mutant tRNAs in MELAS or MERRF patients. In the current study, we explored the potential therapeutic effect of taurine by examining the mitochondrial functions in patient-derived pathogenic cells and by observing the clinical symptoms in MELAS patients receiving taurine supplements.

Materials and Methods

The local ethics committee approved this study (No. 787) and all patients gave their informed consent for participation.

Construction of cybrid cells harboring mutant mtDNA

Immortalized cells possessing patient-derived mitochondrial (mt) DNA were constructed by the intercellular transfer of a patient's mtDNA to ρ^0 HeLa cells (EB8), which are mtDNA-less immortalized cells (10). EB8 cells were isolated by the long-term treatment of HeLa cells with ethidium bromide. Primary dermal fibroblasts were isolated from skin biopsy samples from an A3243G-MELAS, a T3271C-MELAS, and an A8344G-MERRF patient. The fibroblasts were enucleated by centrifugation in the presence of cytochalasin B. Then, the enucleated fibroblasts were fused with EB8 cells by treatment with polyethylene glycol. Control cytoplasmic hybrid (cybrid) strains (Ft2-11, A2) were constructed by fusing mtDNA-less HeLa cells with enucleated normal human fibroblasts.

The resulting cybrids were maintained in Dulbecco's modified Eagle's medium/Ham's F12 medium supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 50 μg uridine, 100 units/mL penicillin, and 100 μg/mL streptomycin (Invitrogen/Life Technologies Japan, Tokyo, Japan). Cybrids with more than 95% mutant mtDNA were used for the experiments. To decrease the endogenous taurine, the cells were also cultured in media with limited amounts of the taurine precursor, L-cysteine (1 mg/mL), and the taurine intermediate, L-methionine (high glucose, L-glutamine-minus, sodium pyruvate-minus Dulbecco's modified Eagle's medium; Gibco) supplemented with L-glutamine, sodium pyruvate, and uridine. The growth rate of mutant cybrids was unchanged after culture in limiting media for seven days.

Cell lines and in vitro analyses

Primary dermal fibroblasts obtained from skin biopsy samples from an A3243G-MELAS, a T3271C-MELAS, and an A8344G-MERRF patient were enucleated and subsequently fused with mt DNA-less HeLa cells (10). The resulting cybrid cells were treated with or without taurine and then were used in subsequent *in vitro* analyses of the mitochondrial oxygen consumption (11), membrane potential (12), and reduction and oxidation (redox) status (10). Taurine powder was purchased from Taisho Pharmaceutical Co., Ltd. (Tokyo, Japan).

Mitochondrial oxygen consumption

Cybrid cells cultured with or without taurine were trypsinized and resuspended in serum-free medium. The cell suspension was continuously stirred at 37℃ with an oxygen electrode (11). The cell concentration was determined using a hemocytometer. The oxygen consumption rates were measured using an Oxygen Meter Model 781 and a Mitocell MT200 closed respiratory chamber (Strathkelvin Instruments, North Lanarkshire, UK). The oxygen respiration rate was directly measured for the 40 mM taurine experiments. After treatment with the limiting media described above, the oxygen consumption was examined in the presence of 1 μM carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), a mitochondrial protonophore used to measure electron transport activity. The consumption value was subtracted from the 1 mM potassium cyanide-independent oxygen consumption value.

Mitochondrial membrane potential

To evaluate the mitochondrial membrane potential, cybrid cells were incubated for 30 minutes at 37℃ with 20 nM MitoTracker Red (Molecular Probes, Invitrogen, Carlsbad, CA, USA), a red-fluorescent dye that accumulates at the mitochondrial membrane (12) in response to the membrane potential. The MitoTracker Red signal increases in a membrane potential-dependent manner. The images were visualized with a confocal laser-scanning microscope (Fluoview FV300; Olympus, Tokyo, Japan) at an excitation wavelength of 594 nm. For the flow cytometric analysis, cells stained with MitoTracker Red were washed in phosphate-buffered saline, trypsinized, and analyzed using a Cell Lab Quanta™ instrument (Beckman Coulter, Inc., Brea, CA, USA). The fluorescent signal of more than 10,000 cells was examined for each experiment.

Mitochondrial redox status

The redox-sensitive green fluorescent protein, roGFP1, generates a unique fluorescence image after the formation (oxidation) of the disulfide bonds adjacent to the barreled β-sheets in the GFP protein (11). To allow real-time visualization of mitochondrial redox status, cybrid cells were stably transfected with the roGFP1 expression vector containing a mitochondrial-targeting sequence. Fluorescence images were recorded using a multi-dimensional imaging workstation (AS MDW; Leica Microsystems, Wetzlar, Germany) consisting of a tunable light source (Polychrome IV monochromator; Till Photonics, Gräfelfing, Germany), an inverted epifluorescence microscope (DM IRE2; Leica Microsystems) contained in a climate chamber maintained at 37℃, and a cooled charge-coupled device camera (CoolSnap HQ; Roper Scientific, Princeton, NJ, USA). The dual excitation ratio of roGFP1 from a single cell was recorded. The ratio of the reduced form of roGFP1 (roGFP1-SH) to the oxidized form

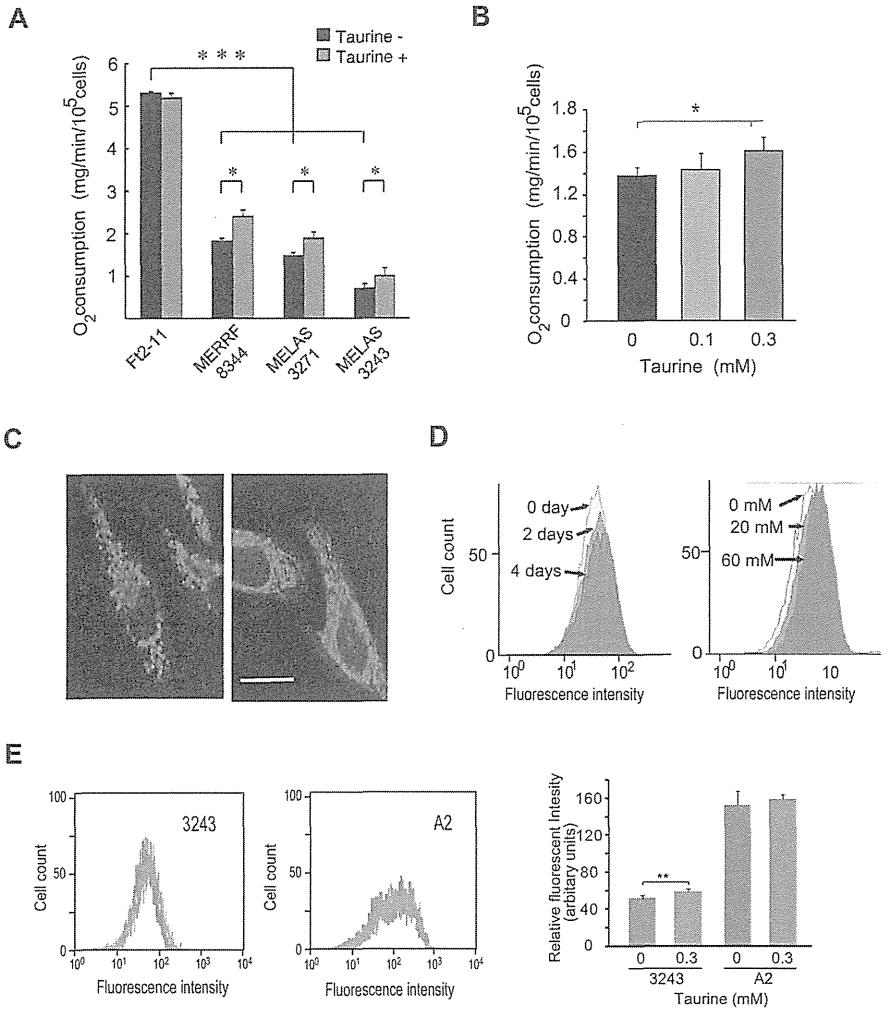

Figure 1. **Taurine ameliorates the impaired mitochondrial function in patient-derived cybrid cells. (A) Patient-derived cybrid cells showed marked decreases in oxygen consumption (black bars). After four days in culture with taurine (40 mM), there was a significant increase in the oxygen consumption rates in patient-derived cybrids with mutant mtDNA, but not in wild-type control Ft2-11 cells (red bars) (*p < 0.05). (B) Cybrids were cultured in media with limited amounts of the taurine intermediate, L-methionine (1 mg/mL), and the taurine precursor, L-cysteine (5 mg/mL), for two days, followed by an additional four day culture with or without taurine (0, 0.1, or 0.3 mM). Taurine (0.3 mM) improved the oxygen consumption in the A3243G-MELAS cybrids cultured in the limiting media (*p < 0.05). (C) Cybrids were cultured in the presence (right) or absence (left) of 40 mM taurine for 4 days. Staining with the membrane potential-sensitive red-fluorescent dye MitoTracker Red (100 nM for 30 min) revealed an increased mitochondrial membrane potential with morphological improvement in the A3243G-MELAS cybrid cells. Scale bar: 100 μm. (D) The mitochondrial membrane potential was determined by a flow cytometric analysis after staining with 100 nM of MitoTracker Red for 30 min. The profiles in the left-hand panel show a time-dependent increase in membrane potential after incubation with 40 mM taurine. The right-hand profiles indicate that there was a dose-dependent shift in the membrane potential after four days of culture with the indicated amounts of taurine. (E) Cybrid cells were cultured in the limiting media described in (B). The reduced mitochondrial membrane potential in the A3243G-MELAS cybrid cells (3243) was significantly improved as judged by a flow cytometric analysis after a four-day incubation with 0.3 mM taurine (*p < 0.05). In contrast, the membrane potential in the control cybrid cells (A2) was unchanged after taurine treatment.**

of roGFP1 (roGFP1-SS-) was obtained. The fluorescence ratio at 410:490 nm was used as the index of oxidation (11).

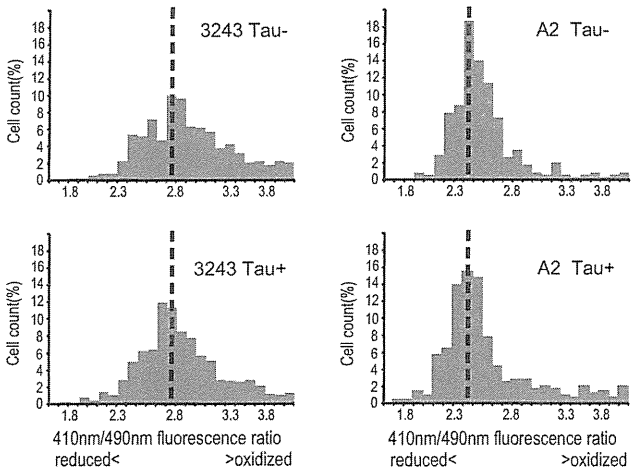

Figure 2. **Taurine reduces the oxidative stress in patient-derived cybrid cells. The A3243G-MELAS cybrid cells (3243, left) and the control cybrid cells (A2, right) were stably transfected with a mitochondria-targeting redox-sensitive green fluorescent protein (roGFP). The histograms show the distribution of cells according to their 410:490 nm fluorescence ratio, an indicator of the oxidation status. Compared with the A2 cybrid cells, the ratio was increased in the 3243 cybrid cells, suggesting an increase in oxidative stress (green, upper) The addition of taurine (3 mM; red, lower) caused a shift towards a reduced status in the 3243 cybrid cells, but not in the A2 cybrid cells (red, lower). The data represent the mean values from eight independent experiments. *$p < 0.05$ between culture conditions with and without taurine.**

Oral administration of taurine to patients with A3243G-MELAS

Taurine powder was orally administered three times a day, after a meal, to two patients with A3243G-MELAS at a dose of 0.25 g/kg/day. This corresponds to the maximal dose previously employed for Japanese patients with biliary obstructions (13).

Statistical analyses

Paired observations were statistically analyzed using a one-way analysis of variance followed by Bonferroni's test. p values <0.05 were considered to be statistically significant.

Results

Taurine restores the reduced mitochondrial oxygen consumption in patient-derived cells

The cybrid cells harboring the disease-causing mutant mtDNAs showed lower oxygen consumption rates than the control cells (Fig. 1A). The addition of 40 mM taurine to the culture media increased the oxygen consumption rate in patient-derived cybrid cells, but not in control cells. Moreover, 0.3 mM taurine was also effective when the cybrid cells were cultured in limiting media lacking cysteine and methionine, which are a precursor and an intermediate, respectively, of taurine biogenesis (Fig. 1B).

Taurine improves the reduced mitochondrial membrane potential in A3243G-MELAS cells

MitoTracker Red-labeled mitochondria in the A3243G-MELAS cybrid cells displayed a weak signal with a granular appearance, suggesting that they had a decreased mitochondrial membrane potential compared to normal cells (Fig. 1C, left) (12). When the cybrid cells were cultivated in the presence of 40 mM taurine for four days, the mitochondria underwent changes in their morphology to a normal filamentous appearance, which was accompanied by an increase in the membrane potential (Fig. 1C, right) (12). The reduced mitochondrial membrane potential in the A3243G-MELAS cells was reversed by taurine in a time- and concentration-dependent manner (Fig. 1D). Moreover, 0.3 mM taurine increased the membrane potential in the A3243G-MELAS cybrids that were cultured in limiting media (Fig. 1E). In contrast, taurine did not alter the membrane potential in the control A2 cybrid cells.

Taurine improves the impaired redox status in patient-derived cells

We transfected the MELAS-cybrid cells with a gene encoding a redox-sensitive green fluorescent protein, roGFP, to monitor their redox status as judged by the ratio of fluorescence signals at 410 and 490 nm (11). The ratio in the A3243G-MELAS cybrid cells increased in comparison to that in the control cells, thus suggesting that they had an increased degree of oxidative stress (Fig. 2, upper). The addition of taurine to the culture media reduced the ratio in the A3243G-MELAS cybrid cells, but not in the control cells (Fig. 2, lower).

Taurine prevents stroke-like episodes in A3243G-MELAS patients

Case 1: A 29-year-old woman had an abrupt onset of generalized seizures and was admitted to our hospital in February 2001 (Fig. 3A). The lactate and pyruvate levels in her serum were elevated to 48.3 mg/dL (normal range, 3.0-17.0 mg/dL) and 1.7 mg/dL (normal range, 0.3-0.9 mg/dL), respectively. Brain magnetic resonance imaging (MRI) revealed a stroke-like lesion in the left occipital region (Fig. 3B). A biopsy from the left biceps brachii muscle showed a MELAS-like pattern, with cytochrome c oxidase-negative ragged-red fibers and succinate dehydrogenase-reactive blood vessels. A molecular genetic analysis of the mitochondrial DNA confirmed an A3243G transition. Treatment with coenzyme Q10 (180 mg daily) and phenytoin (250 mg daily) was commenced in February 2001. The anticonvulsant was switched from phenytoin to valproate (600 mg daily) in April 2001 because of repeated generalized seizures. A follow-up MRI in August 2001 revealed an additional right occipitotemporal lesion (Fig. 3C). The patient continued to experience epileptic seizures and had a stroke-

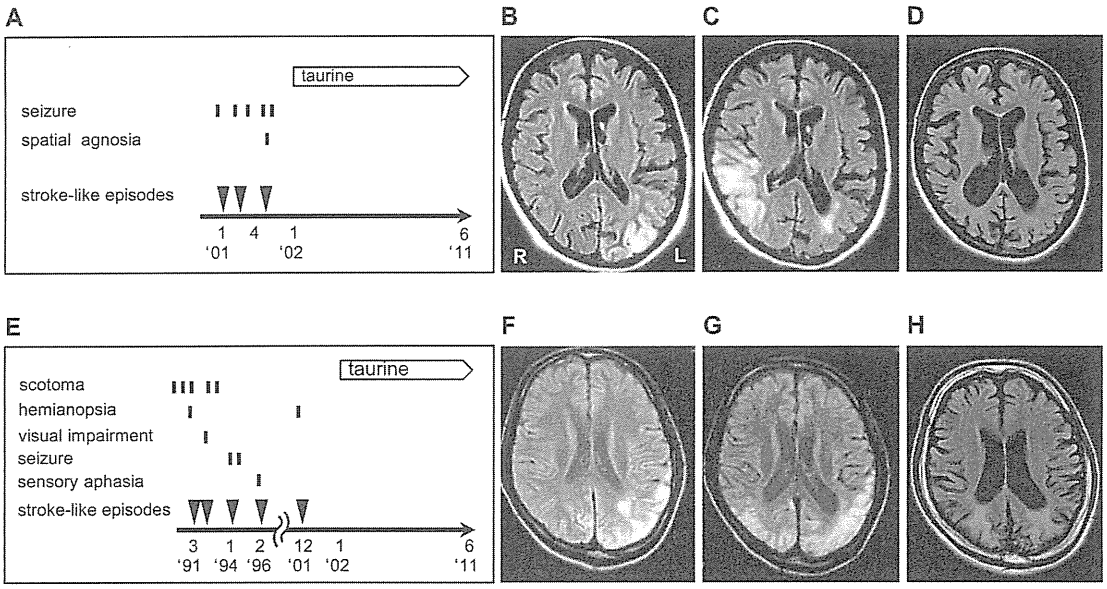

Figure 3. **Oral administration of taurine reduces the stroke-like episodes in MELAS patients. The clinical courses of two MELAS patients (Cases 1 and 2) harboring the A3243G mutation in the mt $tRNA^{Leu(UUR)}$ (A, E) are shown. Taurine administration completely prevented stroke-like episodes in both patients for more than nine years. Fluid Attenuated Inversion Recovery (FLAIR) images of brain MRI revealed that multiple stroke-like lesions had developed in the occipitotemporal region before oral taurine administration (B, C, F, G). The most recent MRI showed no additional stroke-like lesions after taurine treatment in both patients (D, H).**

like episode presenting hemispatial agnosia over the next seven months. Oral taurine supplementation was started in January 2002. From the beginning of the taurine treatment, her epileptic and stroke-like episodes ceased completely. In July 2007, her blood concentration of taurine was 481.3 μM, more than 5-fold higher than the normal range (39.5-93.2 μM). In December 2010, the elevated levels of serum lactate and pyruvate had declined to near normal levels, at 24.3 mg/dL and 0.9 mg/dL, respectively. The most recent brain MRI exhibited no new lesions, but mild cerebral atrophy was present (Fig. 3D). The patient has been doing well for the last nine years with the taurine treatment still ongoing.

Case 2: A 21-year-old man was admitted to another hospital in March 1991 because of repeated scintillating scotoma and right homonymous hemianopsia (Fig. 3E). He was diagnosed with A3243G-MELAS based on typical muscle biopsy findings and a mtDNA analysis. He was treated with coenzyme Q10 (120 mg/dL) and phenytoin (150 mg daily); however, he soon developed vision loss on the right side. He was admitted to our hospital in July 1991. The serum levels of lactate and pyruvate were elevated to 38.7 mg/dL and 1.2 mg/dL, respectively. The anticonvulsant was switched from phenytoin to valproate (600 mg daily) in January 1994 because of repeated generalized seizures. Over the next eight years he suffered from several stroke-like episodes, including sensory aphasia and visual impairment. Brain MRI scans in October 1991 and January 1994 revealed an accumulation of stroke lesions in the bilateral occipital regions (Fig. 3F, G). In December 2001 he had a stroke-like episode presenting with left hemianopsia. Taurine supplementation was started in January 2002, and since then, no stroke-like episodes have occurred. In September 2007, his blood taurine concentration was 996.0 μM, approximately 10-fold higher than the normal range. In February 2010, the serum values of lactate and pyruvate had declined to 29.1 mg/dL and 0.38 mg/dL, respectively. The most recent brain MRI exhibited no additional stroke-like lesions (Fig. 3H).

Discussion

Post-transcriptional modifications at the wobble nucleotide are crucial for the maturation mechanisms of tRNAs and they are required for the correct decoding of codons. In A3243G-MELAS patients, the taurine modification is defective at the wobble nucleotide in the mutant mt $tRNA^{Leu(UUR)}$ (5). In the present study, we showed that taurine ameliorates the mitochondrial dysfunction in patient-derived pathogenic cells carrying mutant $tRNA^{Leu(UUR)}$, but did not reinforce the normal mitochondrial function in control cells. Oral taurine administration also achieved long-term prevention of stroke-like episodes in two patients with MELAS.

We previously showed that when taurine (τ) is added to the culture media of HeLa cells, it is transported to the mitochondria and used as a substrate to synthesize taurine-modified uridine, 5-taunomethyluridine (τm^5U), in mt $tRNA^{Leu(UUR)}$ (Fig. 4A) (1, 4-7). Considering that τm^5U formation proceeds through an enzymatic reaction, the present results suggest that an increased concentration of taurine ac-

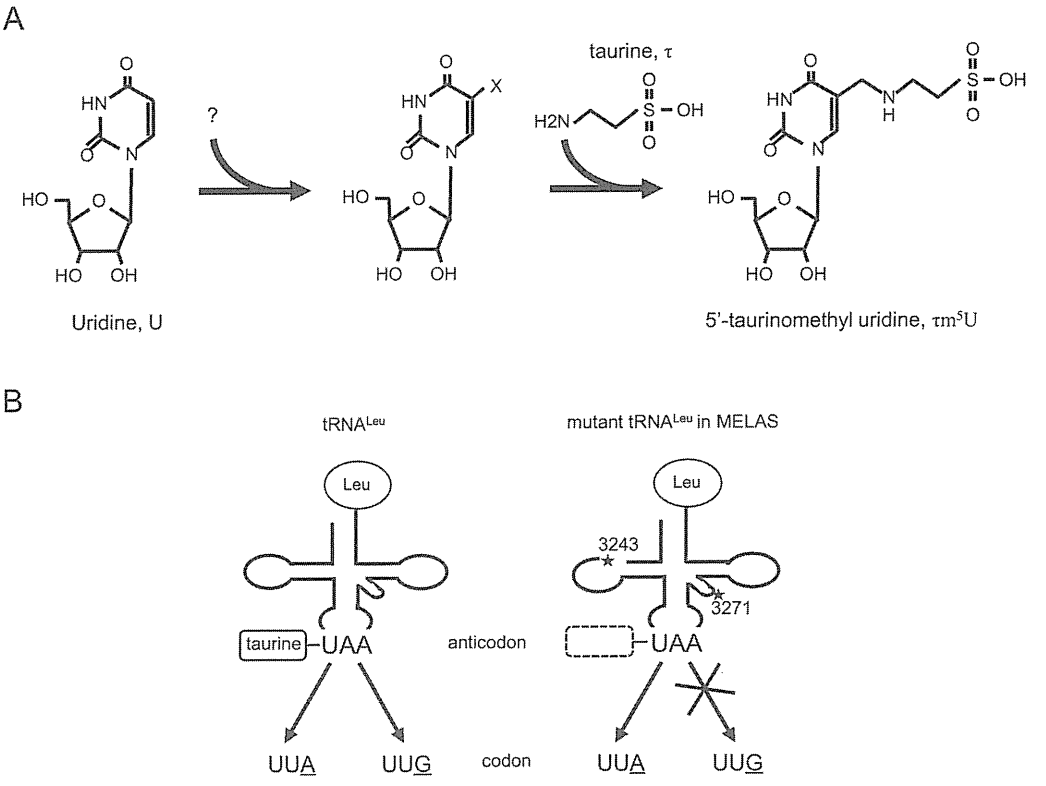

Figure 4. A proposed pathomechanism of MELAS, an RNA-modification disorder. (A) A mechanism of post-transcriptional taurine modification at the first wobble anticodon [uridine (U)] in normal mt $tRNA^{Leu(UUR)}$. Taurine (τ) is incorporated into the C5 position of the uracil ring to generate the final modification product, 5-taurinomethyluridine (τm^5U) (4). (B) Taurine modification functions to stabilize the wobble anticodon-codon pairing. Normal mt $tRNA^{Leu(UUR)}$, with a taurine-modification at the wobble uridine (U), efficiently pairs with codons UUA and UUG (right). In contrast, the MELAS-causing mutant mt $tRNA^{Leu(UUR)}$ lacks the wobble taurine modification, resulting in a specific reduction of UUG codon-specific translation but not UUA codon-specific translation. Defective taurine modification in the mutant mt $tRNA^{Leu(UUR)}$ results in a deficiency in mitochondrial protein synthesis caused by an inability to decipher codons (left) (7).

celerates the enzymatic formation of τm^5U, thereby reversing impaired codon recognition by the mutant mt $tRNA^{Leu(UUR)}$ (Fig. 4B). The pathogenic mutations in MELAS and MERRF might hinder the specific recognition by an RNA-modifying enzyme (4-7). Further studies will be required to clarify the precise molecular mechanisms underlying the wobble taurine modification in mt $tRNA^{Leu(UUR)}$, and how much supplemented taurine incorporates into the wobble uridine in mutant mt $tRNA^{Leu(UUR)}$ in clinical samples from patients.

Low plasma concentrations of taurine induce cardiomyopathy in cats. This particular species has no biosynthetic pathway for endogenous taurine (14). In agreement with our results, high-dose oral administration of taurine to cats increased the plasma and cardiac concentrations, and ameliorated the cardiac dysfunction. Because the cardiac muscles are composed of slow myofibers that are rich in mitochondria (14), taurine supplementation could alleviate the cardiomyopathy via increased τm^5U formation in mt tRNAs.

The present results provide new insight into our understanding of MELAS, and possibly MERRF, as putative RNA-modification disorders that lack the wobble taurine modification. Our results also suggest that the oral administration of taurine may be an effective therapy for these disorders.

The authors state that they have no Conflict of Interest (COI).

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Case report

Liver-specific mitochondrial respiratory chain complex I deficiency in fatal influenza encephalopathy

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Abstract

We report on a 4-year-old boy who died from influenza encephalopathy. The clinical course and microscopic findings of the autopsied liver were compatible with Reye's syndrome. We examined the mitochondrial respiratory chain function by blue native polyacrylamide gel electrophoresis (BN-PAGE), western blotting, and respiratory chain enzyme activity assays. The activity of liver respiratory chain complex (CO) I was markedly decreased (7.2% of the respective control activity); whereas, the other respiratory chain complex activities were substantially normal (CO II, 57.9%; CO III, 122.3%; CO IV, 161.0%). The activities of CO I–IV in fibroblasts were normal (CO I, 82.0%; CO II, 83.1%; CO III, 72.9%; CO IV, 97.3%). The patient was diagnosed with liver-specific complex I deficiency. This inborn disorder may have contributed to the fatal outcome. We propose that relying only on fibroblast respiratory chain complex activities may lead to the misdiagnosis of liver-specific complex I deficiency.

Keywords: Influenza encephalopathy; Reye's syndrome; Mitochondria; Complex I deficiency; Liver-specific

1. Introduction

Influenza encephalopathy is a critical complication of influenza infection. Although the pathological mechanism is poorly understood, mitochondrial malfunction is suggested to play a role in the pathogenesis [1]. We describe a boy with liver-specific mitochondrial respiratory chain complex I deficiency who developed fatal encephalopathy associated with influenza A infection. The possible contribution of the mitochondrial respiratory chain disorder to the clinical course is discussed.

2. Case report

A 4-year-old Japanese boy developed pyrexia. He was treated with acetaminophen once and visited the family doctor. Influenza A infection was diagnosed by nasal antigen test in a clinic and he was treated with oseltamivir. He was admitted to a nearby hospital due to a generalized seizure in the evening; then, he was transferred to our institute because of highly elevated serum transaminase. He was the first child born to healthy parents with no consanguinity. No other child had died in early

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