HeLa, which proliferated without being affected by *mimitin* knockdown, contained high levels of c-Myc. c-Myc protein levels were relatively low and similar in TE-11, T98G, and Kyn2 cells. In this group, *mimitin* knockdown suppressed cell proliferation in TE-11 and T98G cells, but not in Kyn2 cells. The c-Myc protein level was very low in HEL cells, whose cell proliferation was weakly suppressed by *mimitin* knockdown.

Among the cells, proliferation of ESCC TE-11 cells was most severely suppressed by the reduction of Mimitin expression. To confirm this result, the expression of Mimitin in TE-11 cells was specifically suppressed by another siRNA molecule targeting a different position from the first one. Two kinds of siRNA specific to *mimitin* reduced the expression of Mimitin protein (Fig. 3D). As shown in Fig. 3E, si-mimitin-2 suppressed cell proliferation more severely than si-mimitin-1 did, showing that the rate of suppression of cell proliferation was proportional to the reduction of Mimitin expression (Fig. 3D).

When expression of c-*myc* was specifically suppressed in TE-11 cells (Fig. 3D), the expression of Mimitin reduced (Fig. 3D) and cell proliferation was severely suppressed (Fig. 3E). These results suggest that c-*myc* is involved in cell proliferation and the expression of *mimitin* in ESCC cells.

Expression of Mimitin in ESCC tissues - To investigate whether *mimitin* is related to tumorigenesis of ESCC, tumors from surgical ESCC specimens were resected, and Mimitin was detected immunohistochemically. HE staining was used to demarcate tumor areas. The section shown in Fig. 4A contains a moderately differentiated ESCC. Fig. 4B shows marked staining for Mimitin in tumor areas. Staining for Mimitin was found in the cytoplasmic region of cells, consistent with our observation that Mimitin is a mitochondrial protein (Fig. 1C and D). Specific staining was not observed when the first antibody was omitted (Fig. 4C). Staining indices of Mimitin in non-neoplastic epithelial areas from 23 patients were determined, and the averaged index for non-neoplastic epithelial areas was 0.81. Next, staining indices of Mimitin for ESCCs were determined in 35 specimens (Supplemental information, Table S1). Twenty-eight out of 35 tumor specimens had staining indices higher than 0.81. Therefore, 80% of the ESCC tissues highly expressed Mimitin, and high expression of Mimitin appears to be a characteristic feature of ESCC.

The level of Mimitin expression is related to cell proliferation - Because the expression levels of Mimitin differed between specimens, with staining indices from 0 to 3.92 (Supplemental information, Table S1), we investigated whether the level of Mimitin expression is associated with biological events in ESCC. When the staining indices of Mimitin were compared with the histopathological grade, stage of cancer, and age of patients (Supplemental information, Table S1), we did not observe any correlation (Table 1).

Next, the tissues were stained by anti-c-Myc antibody (Fig. 4D). The area stained by anti-c-Myc antibody overlapped well with that stained by anti-Mimitin antibody. When the staining indices of c-Myc were compared to those of Mimitin in 35 tumor tissues, a significant association was detected (Table 1). These results are consistent with our observation that *mimitin* is a Myc-target gene (Fig. 2).

Ki-67 is expressed in proliferating cells (45, 46), and anti-Ki-67 antibody is widely used to detect proliferating cells, including ESCC (47, 48). Fig. 4D shows that anti-Ki-67 antibody strongly stained the ESCC area, which was also stained by anti-Mimitin antibody (Fig. 4B). The staining indices of Mimitin correlated well with those of Ki-67 (Table 1). These results suggest that Mimitin is related to cell proliferation in ESCC.

DISCUSSION

Mimitin is a novel myc-target gene -We identified a novel gene, *mimitin,* which is up-regulated by c-Myc. The high conservation of amino acid sequences between human and mouse Mimitin suggests that *mimitin* has an important function in mammals. We showed here several lines of evidence that c-Myc directly induces the expression of *mimitin.* In brief, changes of *mimitin* expression followed those of c-*myc* (Fig. 2A and B). A significant association between Mimitin expression and c-Myc expression was observed in ESCC tissues (Fig. 4 and Table 1). Suppression of c-Myc expression by siRNA specific for c-*myc* reduced expression of Mimitin protein (Fig. 3D). When c-Myc was activated in the c-MycER

chimeric protein by OHT, stimulation of the expression of Mimitin protein (Fig. 1B) and *mimitin* mRNA (Fig. 2C) was observed. The stimulation of *mimitin* mRNA expression was maintained even in the presence of the protein synthesis inhibitor (Fig. 2C), suggesting that *mimitin* expression is directly controlled by c-Myc.

c-Myc protein binds to the endogenous *mimitin* genomic DNA that contains the CACGTG element in proliferating HL60 cells but not in HL60 cells treated with TPA (Fig. 2E and F). Experiments using reporter plasmids containing a human *mimitin* genomic DNA fragment suggest that c-Myc transactivates *mimitin* expression through the CACGTG element (Fig. 2D). Together, these results demonstrate that *mimitin* expression is controlled by c-Myc through the CACGTG element.

Mimitin protein is localized in mitochondria - Double staining with antibodies to Mimitin and a mitochondrial protein indicate that Mimitin is localized in mitochondria (Fig. 1C). Analysis by the SMART program showed that Mimitin has a domain called Complex1_17_2kD. This domain was found in NADH-ubiquinone oxidoreductase subunit B17.2 (Complex I-B17.2 or DAP13, GenBank accession No. AAF91224) (49, 50), which was originally identified as a subunit of complex I involved in oxidative phosphorylation in bovine heart mitochondria. Only one putative orthologous protein for Mimitin and Complex I-B17.2 was found in *C. elegans* (Y94H6A.8, GenBank accession No. NP_500247) or *D. melanogaster* (CG3214, GenBank accession No. AAF51238), suggesting that Mimitin and Complex I-B17.2 originated from the same gene. These results suggest that Mimitin may be involved in ATP generation in mitochondria. However, the similarity between Mimitin and Complex I-B17.2 is weak (28% identical), and it is not possible to directly compare the function of Mimitin with that of Complex I-B17.2 because the function of Complex I-B17.2 has not yet been specified. Further studies are necessary to clarify the function of Mimitin.

Recently it was reported that several mitochondrial proteins are candidates for Myc-target genes (51, 52), suggesting that control of mitochondrial functions may be one of the important classes of Myc-functions. Mitochondrial serine hydroxymethyl transferase (mSHMT), a major source of the one-carbon unit for cell metabolism, partially restored cell proliferation of the c-*myc*-null fibroblasts, and was reported to be a Myc target (53). Myc induces mitochondria-mediated apoptosis, and a mitochondrial peroxidase redox protein PRDX3 (Peroxiredoxin III) was shown to be a Myc target and involved in apoptosis (54). mSHMT and PRDX3 were found in *S. cerevisiae, C. elegans, D. melanogaster,* and mammals. Mimitin was required for some types of cells to proliferate. Mimitin and/or proteins with significant similarity to Mimitin have been found in *C. elegans*, *D. melanogaster*, and mammals, but not in *S. cerevisiae.* These results suggest that Myc elevates expression of a variety of mitochondrial proteins, which would contribute to the multi-functional character of Myc. Further thorough studies are necessary to provide a complete picture of Myc control of mitochondrial functions.

Mimitin and cell proliferation - Specific inhibition of *mimitin* expression by an RNA interference (RNAi) method severely suppressed cell proliferation in ESCC cells, which require c-*myc* for proliferation (Fig. 3). A clear correlation was found in ESCC tissues between the Mimitin staining index and cell proliferation and between the indices of Mimitin staining and c-Myc staining (Table 1). These results suggest that *mimitin* is involved in Myc-dependent cell proliferation in ESCC.

Mimitin siRNA reduced c-Myc levels in ESCC TE-11 cells (Fig. 3C). However, *mimitin* siRNA did not reduce c-Myc expression level in SW620 cells (data not shown), in which *mimitin* siRNA did not suppress cell proliferation (Fig. 3B). c-*myc* expression is associated with cell proliferation and is down-regulated in quiescent cells. Therefore, the reduced expression of c-Myc by *mimitin* siRNAs in TE-11 cells could be the indirect effect via the suppression of cell proliferation induced by *mimitin* siRNA.

The suppression of Mimitin expression did not reduce cell proliferation in some kinds of cultured cells (Fig. 3B). One possible reason is the existence of a functionally redundant protein. *Cyclin D2* is a well-established Myc-target gene. Mice lacking the *cyclin D2* gene grow into adults with few abnormalities (55), showing that cyclin

D2 is not always necessary for cell proliferation. The existence of the functionally redundant proteins cyclin D1 and cyclin D3 apparently can compensate for the absence of cyclin D2. Therefore, *cyclin D2* is a Myc target gene that is necessary only in specific types of cells, and *mimitin* appears to belong to this class of Myc-target genes. A protein homologous to Mimitin, Complex I-B17.2 (49) described above, may have a redundant function with Mimitin, and may be expressed in some cells that do not require Mimitin to proliferate.

Another possibility is the existence of a functionally redundant mechanism. The cell lines SW620 and HeLa, which were resistant to *mimitin* knockdown, expressed c-Myc highly compared to the other cell lines. Therefore, it is possible that overexpression of Myc triggers alternative Myc proliferating pathway(s), conferring resistance to *mimitin* knockdown. If Mimitin is involved in ATP generation, glycolysis is a candidate pathway. Because a number of glycolytic genes are up-regulated by c-Myc (40, 56) and elevation of the glycolysis level would produce an elevated amount ATP, the effect of *mimitin* knockdown may be blunted in Myc-overexpressing cells.

Mice lacking a myc-target gene *ODC* die in early embryogenesis (57). Loss of *ODC* in yeast results in a cessation of growth (58), and yeast cells lacking *RCC1*, a Myc-target gene (12), do not grow (59). These Myc-target genes appear to be generally important for all types of cells to proliferate. Thus, a treatment to inhibit the function of one of these general type Myc target genes in a body would suppress cell proliferation of not only cancer cells but also normal cells, and would have serious side effects. On the other hand, the other class of Myc-target genes, such as *cyclin D2* and *mimitin*, are necessary for only specific types of cells to proliferate. Thus, inhibition of the function of one of these specific Myc-target genes may hamper only some restricted types of cells and would have fewer side effects and more potential than a general type for cancer therapy. Further identification and characterization of these specific Myc-target genes may lead to the discovery of new methods for cancer therapy and would help elucidate the mechanisms by which Myc controls cell proliferation in the highly complex animal system.

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FOOTNOTES

The nucleotide sequences studied here were deposited in the GenBank database: cDNA sequence for human Mimitin, AB183433; DNA sequence for the *mimitin* gene promoter, AB183435; cDNA sequence for mouse Mimitin, AB183434. During our experiments, similar sequences for human *mimitin* were deposited in the GenBank database (LOC361894).

The abbreviation used are: ESCC, esophageal squamous cell carcinoma; PBS, phosphoate buffered saline; ODC, ornithine decarboxylase; OHT, 4-hydroxytamoxifen; CHX, cycloheximide; RNAi, the RNA interference; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; cad, the trifunctional enzyme carbamoyl-phosphate synthase/aspartate carbamoyltransferase/dihydroorotase.

FIGURE LEGENDS

Fig. 1. Amino acid sequence, expression, and subcellular localization of Mimitin protein. A, Amino acid sequence of human Mimitin protein, which was predicted from the cDNA isolated in this study, is shown. Only those residues of the mouse protein that are divergent are shown below. The ATP/GTP-binding site motif A (P-loop) and a region called Complex1_17_2kD domain are surrounded by solid and dotted lines, respectively. B, Western blotting analysis of Mimitin. T98G and T98Gmycer-2 cells were cultured in the presence or absence of 0.2 μM OHT for 40 h (left panel). T98G cells were transfected with a Mimitin expression plasmid (mimitin), c-myc-tagged Mimitin expression vector (mimitin-mychis), or control vector (contr) (right panel). Lysates from these cells were subjected to Western blotting using a specific antibody against Mimitin protein (upper panel). The blotting membrane detecting Mimitin was re-probed with anti-β-actin antibody (lower panel). The same amounts of protein analyzed by Western blotting were electrophoresed and stained for total protein to confirm that a similar amount of the protein was analyzed in this study (data not shown). C, The subcellular localization of human Mimitin protein. The localization of Mimitin in HeLa cells was visualized by indirect immunofluorescence staining with antibodies against Mimitin (Mimitin) and Tom20 (Tom20). An overlapped image is also shown (merge). Scale bars, 20 μm. D, T98G cells were fractionated into cytosolic and mitochondrial fractions, and analyzed by Western blotting using antibodies against Mimitin, MAPK/ERK, and Tom22.

Fig. 2. Control of *mimitin* expression by c-Myc. A-C, RNA was isolated at the indicated time points, and then analyzed by Northern blotting to detect *mimitin, c-myc* (except C), and GAPDH mRNA. 28S and 18S ribosomal RNAs are also shown. Serum-starved T98G cells were stimulated by the addition of serum at a final concentration of 10% (A). Human promyelocytic leukemia HL60 cells were exposed to 10 nM TPA (B). After T98Gmycer-2 cells (mycer) and parental T98G cells (contr) were cultured in the medium supplemented with 0.25% serum for 40 h, cells were treated with 200 nM 4-hydroxytamoxifen (OHT) to activate c-MycER. When indicated as +, 20 μg/ml cycloheximide (CHX) was added 20 min before the addition of OHT (C). D, Transient expression assay for the *mimitin* promoter. The reporter plasmid (pmimitin(W)luci, wild) was constructed from a DNA fragment of *mimitin* (containing upstream of exon 1, exon 1 and part of intron 1) that contained a CACGTG element and firefly *luciferase*. The CACGTG element was mutated to CACCCG in pmimitin(mE)luci (mutant). After normalization, firefly luciferase activities were expressed as the ratio to activities without MycER activation. Values are the means of four separate experiments. The bars indicate the standard errors. E and F, Chromatin immunoprecipitation experiments. HL60 cells in the proliferating phase (-TPA) and those treated with 10 nM TPA for 24 h (+TPA) were fixed with 1% formaldehyde, nuclear extracts were collected, and chromatin fragments were immunoprecipitated using antibodies against c-Myc (anti-c-Myc antibody-1 and anti-c-Myc antibody-2), control antibody, or no antibody (- antibody). Mock immunoprecipitation, in which no nuclear extracts were added, was also performed (- nuclear extract). Following DNA purification, samples were subjected to PCR with primers designed to amplify a DNA fragment of the *mimitin* gene (mimitin primers) or primers to specifically amplify a DNA fragment containing a chromosome 22 E-box (control primers) (14, 15) (E), and with the mimitin primers or primers to specifically amplify a DNA fragment containing the *cad* gene (cad primers) (F).

Fig. 3. Mimitin is involved in cell proliferation of cultured ESCC cells. A, Reduction of Mimitin protein expression by siRNA specific to *mimitin*. Human cells, colon adenocarcinoma SW620, cervical carcinoma HeLa, esophageal squamous cell carcinoma TE-11, glioblastoma T98G, human embryonic lung HEL, and hepatocellular carcinoma Kyn2 were transfected with siRNA specific to *mimitin* (si-mimitin-2) or control siRNA (contr) without serum. Forty hours after adding serum, cells were collected, and cell extracts from the same number of cells in each set were analyzed by Western blotting using anti-Mimitin and anti-β-actin antibodies. B, Cells in (A) were transfected with si-mimitin-2 (●) or a control siRNA (○) without serum. Then cells were cultured in the presence of serum (arrow) from day 0, and cell numbers were counted on various days and expressed on the *y*-axis. Experiments were performed at least 3 times, and

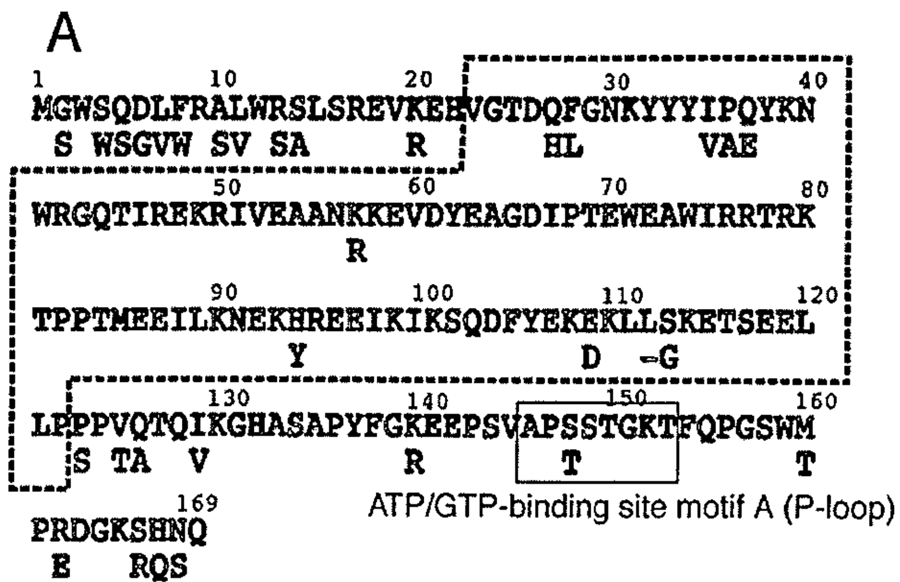
error bars are shown. C, c-Myc protein expression. Cells used here were collected, lysed, and the protein concentration was determined. Similar amounts of protein were analyzed by Western blotting using anti-c-Myc antibody. The blotting membrane was re-probed with anti-β-actin antibody. D, Reduction of Mimitin protein expression by siRNA specific to c-*myc* or *mimitin* in ESCC cell line TE-11. Cells were transfected with a control siRNA (contr), an siRNA duplex specific for c-*myc* (myc), and siRNA duplexes specific for *mimitin*, si-mimitin-1, or si-mimitin-2 (mimitin-1 or mimitin-2, respectively) without serum. Forty hours after adding serum, cell extracts were processed for Western blot analysis using anti-c-Myc and anti-Mimitin antibodies. The blotting membrane detecting Mimitin was re-probed with anti-β-actin antibody. Aliquots of the same amounts were electrophoresed, transferred, and stained for protein to confirm that similar amounts of proteins were used in this experiment (data not shown). E, Cell proliferation of siRNA transfected TE-11 cells. TE-11 cells were transfected with siRNA duplexes specific for *mimitin* (si-mimitin-1 (◆) and si-mimitin-2 (▲)), c-*myc* (●) or a control siRNA (○) without serum. Then cells were cultured in the presence of serum (arrow) from day 0. On various days, cell numbers were counted and expressed on the *y*-axis. Experiments were performed 3 times, and error bars are shown.

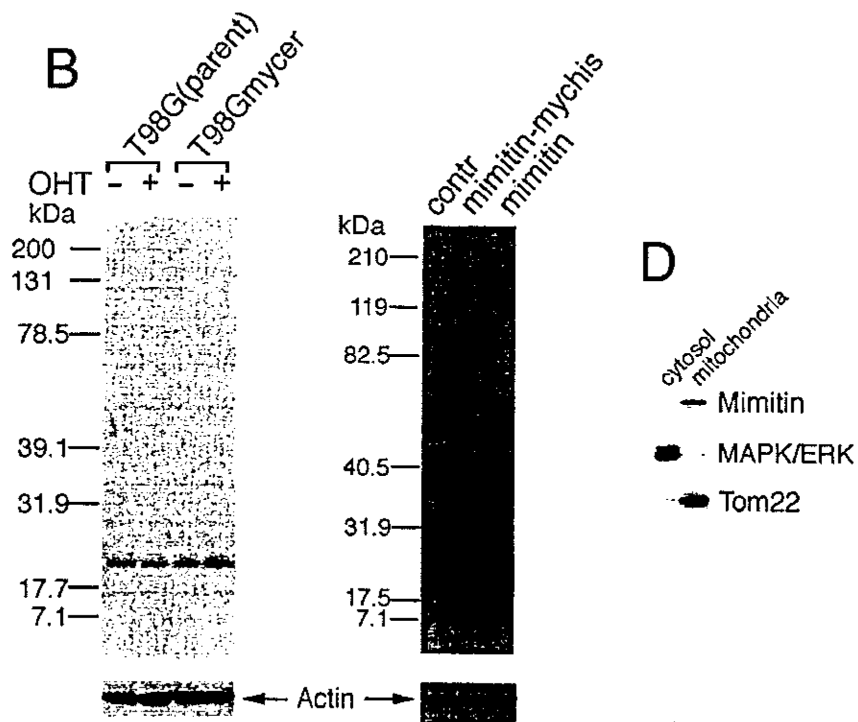
Fig. 4. Expression of Mimitin, c-Myc and Ki-67 in surgically resected human ESCC tissues. A, HE staining of a section that contained moderately differentiated ESCC. B, A serial section of (A) stained by anti-Mimitin antibody showing the expression of Mimitin in the tumor area. C, A control serial section, in which the primary antibody was omitted. D, A serial section of (A) stained by anti-c-Myc antibody. E, A serial section of (A) stained by anti-Ki-67 antibody. Positive staining is brown and counterstained nuclei are blue (B-E). Scale bars, 50 μm.

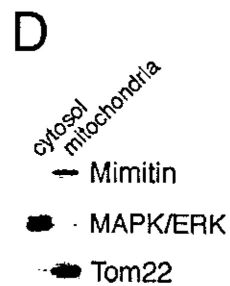
Table 1. Relationship between expression of Mimitin and biological events in ESCC. The Mimitin staining index was statistically compared with histopathological grade, stage of cancer, age of patient, the staining index for c-Myc, and the cell proliferation index (the staining index for Ki-67).

Factor	r	*P*
Histopathological grade	0.070	0.6901
Stage of cancer	0.131	0.4545
Age of patients	-0.069	0.6973
Staining index of c-Myc	0.435	0.0084
Cell proliferation (staining index of Ki-67)	0.728	<0.0001

Figure 1







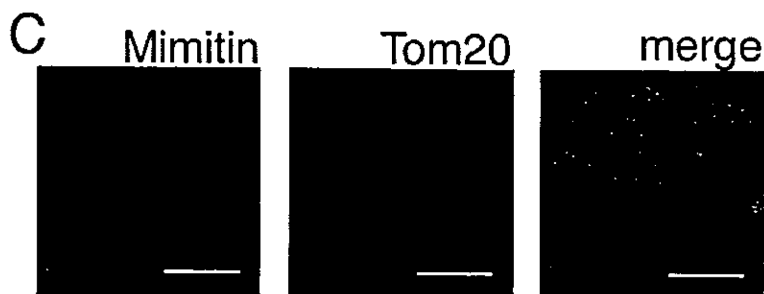


Figure 2

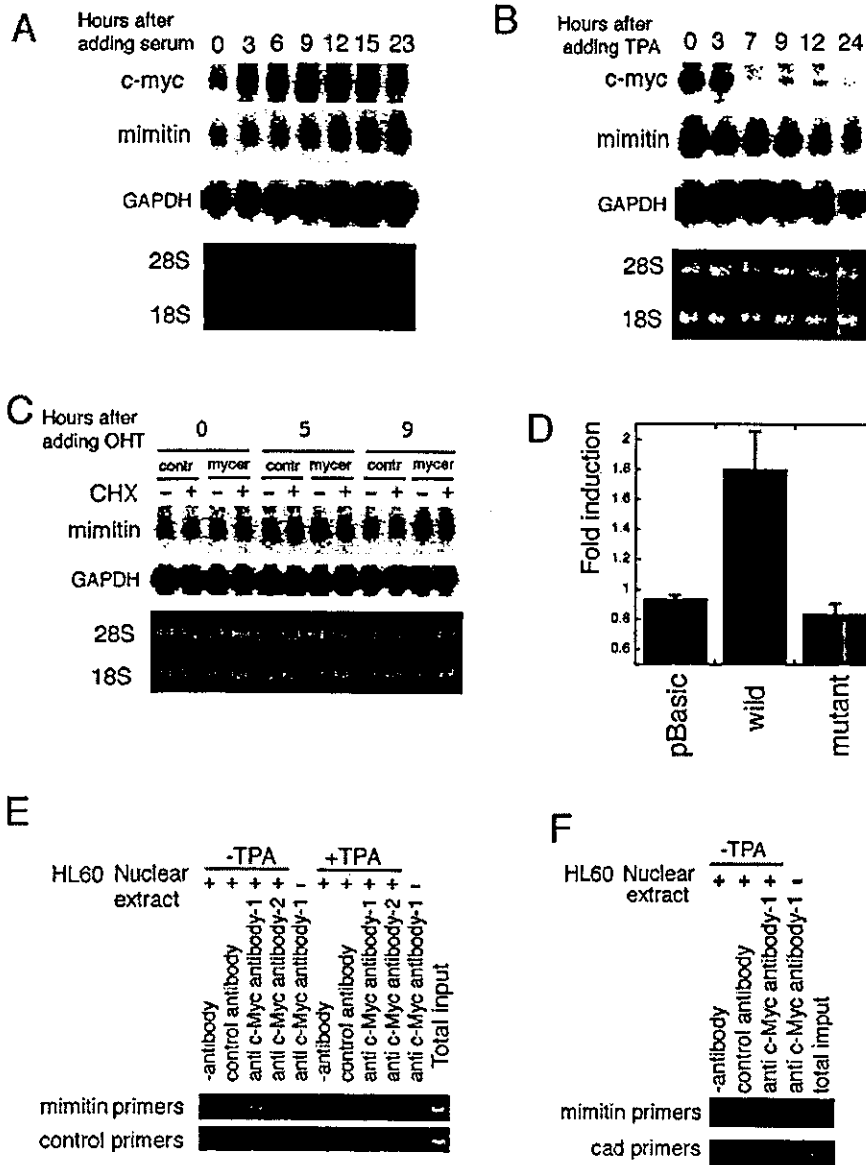


Figure 3

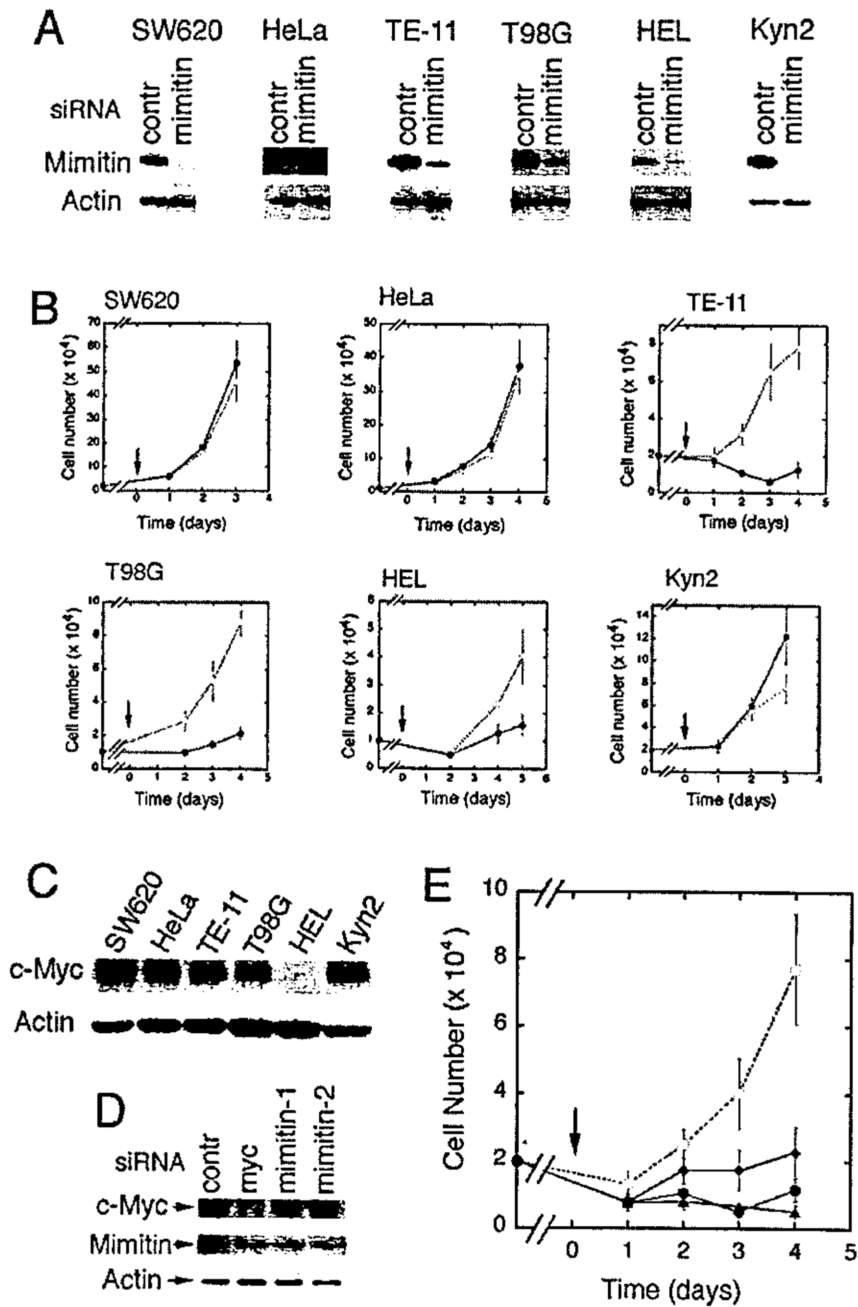
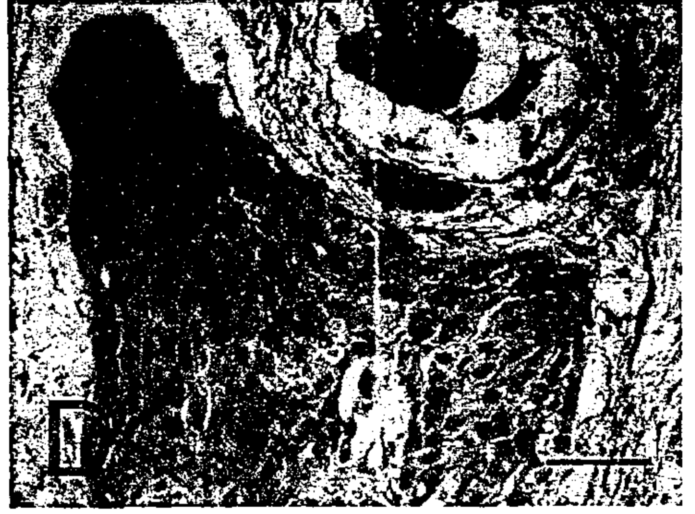
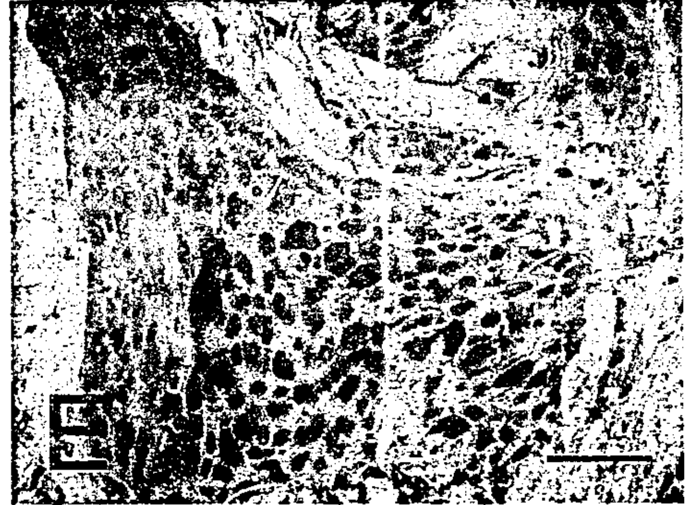


Figure 4





Supplemental information

Table S1. Characterization of ESCC patients and tissues. Tumors from surgical ESCC specimens were immunohistochemically analyzed for Mimitin, Ki-67, and c-Myc. G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.

Patient	Stage	Pathological	Mimitin staining			Ki-67 staining			c-Myc staining		
No. (Sex, Age)		Grade	Intensity	%	Index	Intensity	%	Index	Intensity	%	Index
1 (M, 62)	I	G2	2	86	1.72	3	89	2.67	1	58	0.58
2 (M, 72)	III	G1	1	56	0.56	3	83	2.49	2	82	1.64
3 (M, 77)	I	G3	2	71	1.42	1	12	0.12	2	79	1.58
4 (M, 56)	III	G1	3	93	2.79	3	84	2.52	2	90	1.8
5 (M, 59)	III	G1	2	82	1.64	3	64	1.92	2	85	1.7
6 (M, 60)	IIa	G1	2	91	1.82	3	63	1.89	2	87	1.74
7 (M, 67)	IV	G2	2	91	1.82	3	49	1.47	0.5	19	0.1
8 (M, 67)	III	G2	2	85	1.7	3	39	1.17	0	0	0
9 (M, 69)	III	G2	3	85	2.55	4	71	2.84	3	81	2.43
10 (M, 51)	I	G2	3	34	1.02	0	0	0	0.5	56	0.28
11 (M, 49)	III	G2	3	67	2.01	3	84	2.52	3	94	2.82
12 (M, 47)	I	G3	3	95	2.85	3	65	1.95	3	92	2.76
13 (M, 57)	IV	G1	3	92	2.76	4	60	2.4	0.5	55	0.28
14 (M, 69)	III	G2	3	95	2.85	3	71	2.13	3	90	2.7
15 (F, 71)	IIa	G2	2	95	1.9	1	26	0.26	3	90	2.7
16 (M, 64)	III	G1	2	63	1.26	3	42	1.26	2	97	1.94
17 (M, 59)	III	G2	1	30	0.3	0	0	0	3	98	2.94
18 (M, 46)	III	G2	2	60	1.2	3	51	1.53	3	75	2.25
19 (M, 70)	I	G2	2	82	1.64	4	40	1.6	3	98	2.94
20 (M, 68)	IV	G2	2	44	0.88	0	0	0	2	64	1.28
21 (F, 76)	I	G2	2	95	1.9	4	55	2.2	3	90	2.7
22 (M, 68)	IIa	G1	1	43	0.43	0.5	6	0.03	2	47	0.94
23 (M, 41)	IIa	G3	2	43	0.86	3	30	0.9	1	46	0.46
24 (M, 66)	III	G2	2	32	0.64	2	18	0.36	1	53	0.53
25 (M, 65)	III	G2	3	92	2.76	2	18	0.36	3	57	1.71
26 (M, 66)	IIa	G1	3	88	2.64	4	72	2.88	3	90	2.7
27 (F, 71)	III	G3	3	94	2.82	4	95	3.8	2	79	1.58
28 (M, 50)	IV	G1	2	63	1.26	2	49	0.98	2	91	1.82
29 (M, 61)	IV	G1	4	98	3.92	4	93	3.72	3	93	2.79
30 (M, 67)	IV	G3	2	98	1.96	2	48	0.96	0	0	0
31 (M, 66)	IIb	G3	1	72	0.72	0	0	0	0	0	0
32 (M, 57)	IV	G1	2	78	1.56	4	47	1.88	3	81	2.43
33 (M, 61)	III	G1	0	0	0	0.5	6	0.03	0	0	0
34 (M, 80)	IIa	G1	0	0	0	0	0	0	0	0	0
35 (M, 63)	III	G2	2	95	1.9	3	47	1.41	3	96	2.88

MELASの新しい治療法—L-アルギニン

古賀靖敏[1)]

〔KEYWORDS〕 ミトコンドリア病，MELAS，L-アルギニン，脳卒中，電子伝達系酵素欠損，ミトコンドリアDNAの変異，NO，血管内皮機能，ADMA，治療法

1. はじめに

ミトコンドリア病(ミトコンドリア脳筋症)は，細胞のなかのエネルギー産生の中核であるミトコンドリアの異常で脳や筋肉の機能が低下する病気である．この一病型であるMELAS(mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes)は，小児期に脳卒中様の発作を繰り返し，ひいては，精神運動面の退行をきたし，早期に死に至る慢性進行性の難病である．本症における脳卒中発作の成因は，血管説および細胞機能不全説などいまだ不明な点が多い．われわれは，脳卒中発作の成因に血管説が大きく関与しているという仮説のもと，L-アルギニンを投与し，脳卒中に起因する種々の症状が劇的に改善することを発見報告した．MELAS患者では，血管内皮機能が有意に低下しており，本来もっているはずの動脈の拡張機能が傷害されていた．さらに，MELAS患者急性発作時には，血漿中のL-アルギニンや生体内での動脈拡張機能に中心的役割を果たす一酸化窒素(NO)の代謝産物(NOx)が有意に低下しており，かつADMA(asymmetrical dimetyl arginine)が相対的に増加していることがわかった．MELAS患者の脳卒中様発作急性期にL-アルギニンを静注することで，脳虚血からくる神経症状が注射後30分以内に劇的に改善した．また，脳卒中様発作寛解期の患者で，L-アルギニンを内服することで，患者の脳卒中様発作の重症度および頻度を有意に低下することが判明した．MELASに対するL-アルギニン療法は，発作急性期の静注による特効薬的効果のみでなく，発作間歇期の予防的内服薬剤としても期待される．

2. MELASとは？

小児期に発作性の頭痛，嘔吐，半身けいれんで発症し，脳卒中様の発作を特徴とするミトコンドリア病の一病型である．本疾患は，1986年，コロンビア大学の神経内科医Pavlakisにより初めて臨床的に報告された[1)]．2003年の全国調査で，日本においては約233名が罹患し，122名が小児科でフォローアップされている[2)]．80%の患者でミトコンドリアDNAのtRNA$^{Leu(UUR)}$遺伝子のA 3243 G変異が[3)]，また，10%の患者で同じ遺伝子のT 3271 C遺伝子の変異が報告され[4)]，その後多くの点変異が見いだされた．典型的な症状は，20歳前の一過性脳卒中様症状(頭痛，嘔吐，半身けいれん，視野異常，閃輝暗点，視力障害，麻痺など)を特徴とし，同時に筋力低下，感音性難聴，心刺激伝導障害，心筋症を合併するものもある．血液検査では，乳酸ピルビン酸の高値，代謝性アシドーシス，高アラニン血症がみられ，筋生検でミトコンドリアの異常集積像(ragged-red fibers)と中小動脈壁の異常染色性(SSV：strongly SDH hyperreactive blood vessels)が観察される．このSSVは，筋内の中小動脈のみでなく，中枢神経の動脈でも観察され，本症が血管障害を

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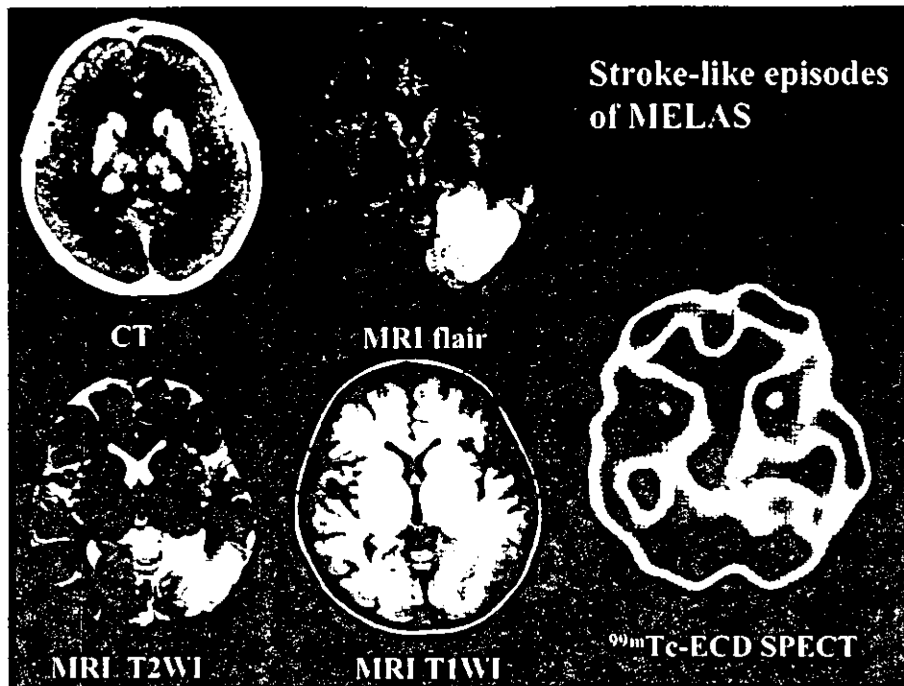

図1　MELAS の頭部画像

図2　MELAS 患者の筋内動脈の電子顕微鏡所見

有することを示している.

3. 頭部画像所見

頭部 CT にて大脳基底核の両側対称性石灰化, 脳実質の低吸収域などがみられる. 急性期の頭部 MRI では, T 2 強調画像で大脳皮質の高信号域, 腫脹などがみられる(図 1). 発作は連続で起こるときもあれば, 数か月寛解期をみることもある. 発作時みられる症状は一過性の場合もあるが, 適切な治療がなされなければ症状は遷延し, 血流が障害された末梢脳組織の後遺障害として, 半身麻痺, 視野障害(同名半盲), 失明をきたす重篤な難治性進行性疾患である.

4. MELAS における脳卒中様発作の成因

MELAS における脳卒中様発作の成因は, 血管説および細胞機能不全説などいまだ不明な点が多い. しかし, われわれは, 脳卒中様発作の成因に血管説が大きく関与しているという仮説を立てるのに, 以下に挙げるようなエビデンスを重要視している.

MELAS 患者では,

（1）ミトコンドリアの機能異常の指標として筋肉での ragged-red fiber(RRF)があり,

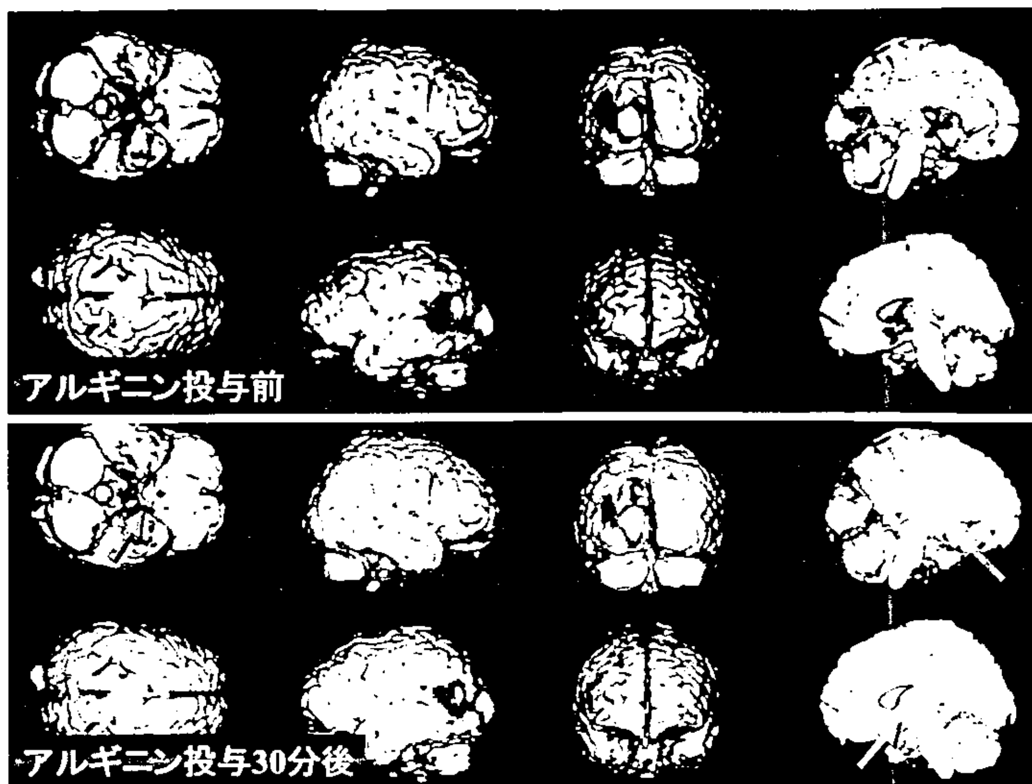

図3　L-アルギニン投与前後のSPM-SPECT画像

その部分の電子顕微鏡的検索ではミトコンドリアの形態異常がみられる．

(2) 筋内中小動脈にSSV(strongly SDH positive blood vessels)が観察され，電子顕微鏡的観察で異常ミトコンドリアが中膜平滑筋層および血管内皮細胞に存在し，これがSSVとして観察される．

(3) 血管の閉塞性病変が存在する(図2)[5]．

(4) 血管内皮の機能異常が存在する．MELAS患者の内皮機能を測定したところ，患者では，同性同年齢のコントロールに比較し，中小動脈の血管内皮依存性血管拡張機能が有意に低下していた．

(5) MELAS患者では，血漿中のL-アルギニンや生体内での動脈拡張機能に中心的役割を果たすNOの代謝産物(NOx)が有意に低下しており，かつADMAが相対的に増加している[6,7]．

(6) L-アルギニンは，動脈の拡張機能に中核的役割を行う．L-アルギニンは，中小動脈における血管の拡張機能において必須となる一酸化窒素産生のもととなるアミノ酸である．このアミノ酸は，MELAS患者の脳卒中様発作急性期では，有意に低下しており，したがって，NO産生の総量を表すNOxは有意に低下していた．ADMAは，虚血性心疾患のリスクファクターとして注目されている物質であり，悪玉L-アルギニンとして，NO合成酵素に抑制的効果を示す．この事実から，MELAS患者では，動脈が拡張しにくい状態にあるといえる[7]．

以上の生化学的基盤をもとに，MELASの脳卒中の成因に，血管障害(特に内皮機能不全)が大きく関与しており，血中のL-アルギニン低値，ADMAの相対的高値がさらなる脳卒中のリスクファクターになっていると考える．

5. MELASの脳卒中様発作急性期におけるL-アルギニン療法の効果

われわれは，脳卒中をきたすミトコンドリア病の急性期にL-アルギニンを投与し，速やかに脳卒中症状が改善したことを報告した[6]．主な内容として，投与量：L-アルギニン10%溶液で5 ml/kg/hr，投与方法：右上腕より点滴静注，有効性：閃輝暗点を除く脳卒中様症状に有効である．安全性：副作用としての頭痛が1例あった

が，肝障害，発疹などの重篤副作用は見られず，頭痛を訴えた症例も投与速度を落としたら頭痛の症状は消失した．血圧に対しては，投与前に比較し最高血圧の 10 mmHg 以下の血圧低下が投与開始後 30 分でみられたが，血圧低下による副作用の症状はなかった．

1）L-アルギニン投与による脳卒中様臨床症状の改善

脳卒中様発作症状を発現している MELAS 患者 22 例に L-アルギニンを静脈内投与し，各急性期症状の改善率を検討した．頭痛は，L-アルギニン投与前に 22 例全例で高度であったが，投与 30 分後には 14 例(64%)が改善し，投与後 24 時間には全例改善した．臨床的障害(Clinical disability)は，投与前には中等度 15 例，高度 7 例であったが，投与終了 15 分後には 3 例(14%)が改善し，投与 24 時間には全例改善した．嘔気は，投与前 8 例にみられていたが，投与終了 30 分後には 6 例(75%)が消失し，24 時間後には全例消失した．嘔吐は，投与前 10 例にみられていたが，投与終了 30 分後には 7 例(70%)が消失し，24 時間には全例消失した．一過性失明は，投与前 7 例にみられたが，投与終了 15 分後にはそのうち 3 例(43%)が消失し，投与終了 30 分後に 1 例再発して改善は 2 例(29%)となったが，24 時間後には全例消失した．半身痙攣は，投与前 5 例見られたが，投与終了 15 分後には 2 例(40%)，投与終了 30 分後には 3 例(60%)が消失し，24 時間後には全例消失した．意識障害は，投与前 1 例に見られたが，投与 30 分後には消失した．閃輝暗点は，投与前 9 例に見られたが，投与終了 30 分後には 6 例(67%)が，24 時間後には 8 例(89%)が消失し，残りの 1 例は 3 日目には消失した[7]．

2）L-アルギニン製剤投与後の血中 L-アルギニン，L-シトルリン，乳酸，ピルビン酸，NOx，c-GMP，ADMA 濃度の推移

MELAS 患者における L-アルギニン投与前，投与終了後 15 分，30 分，24 時間の各血中濃度推移を検討した[7]．

（1）血中 L-アルギニンおよび L-シトルリン濃度の推移

投与前の血中 L-アルギニン濃度においては，正常値(108.1±27.6 μmol/l)に比べ約 1/2 (46.99±13.01 μmol/l)に低下していたが，投与 15 分後には，最大血中濃度(9,542.27±1,169.91 μmol/l)を示し，その後徐々に低下し 24 時間後には，ほぼ正常値(92.41±15.85 μmol/l)となった．血中 L-シトルリンにおいては，正常値(34.6±8.8 μmol/l)とほぼ同じであった投与前値(21.27±9.27 μmol/l)が，L-アルギニンの最大血中濃度より少し遅れて投与 30 分後に最大血中濃度(41.54±13.04 μmol/l)を示した．

（2）乳酸，ピルビン酸，L/P 比の推移

ピルビン酸は，L-アルギニン投与後，徐々に低下し投与 24 時間後には有意に低下した(0.17±0.03 μmol/l)．乳酸は，L-アルギニン投与後に上昇し投与 30 分後には最大血中濃度(7.28±0.55 μmol/l)を示したが，投与 24 時間後には，有意に低下した(3.35±0.49 μmol/l)．L/P 値は，乳酸およびピルビン酸の変動に伴い，投与 30 分後に最大血中濃度(28.02±8.15)を示し，投与 24 時間後には，ほぼ投与前の値(19.16±4.41)となった．

（3）血中 NOx，c-GMP，ADMA 濃度の推移

NOx は，投与後 30 分に有意に上昇し(55.88±20.40 μmol/l)，24 時間後も高い血中濃度(29.06±9.68 μmol/l)を示した．c-GMP は，投与後 30 分に有意に上昇し(0.86±0.16 μmol/l)した．ADMA は，L-アルギニン投与後 15 分，30 分と有意に上昇し，投与 30 分後に最大血中濃度(0.87±0.11 μmol/l)を示したが，投与 24 時間後には投与前の値以下まで低下した(図 3)．

（4）L-アルギニン投与の頭蓋内血流動態に及ぼす影響

3 名の MELAS 患者に対して，脳卒中発作時 L-アルギニン投与を行い，その前後で頭部 ^{99m}Tc-ECD-SPECT を実施した．L-アルギニン投与 1 時間後には，臨床症状の改善とともに，虚血部位の局所的脳血流(rCBF)の改善が認められた．ROI による虚血部位の血流増加率は，健常側同部位の血流比で算出し，11〜13%であった．

（5）副作用

L-アルギニン投与後に L-アルギニン投与によるものと思われる軽度の嘔気が 2 例に認められたが，経過観察により 2〜3 時間で消失し，特に問

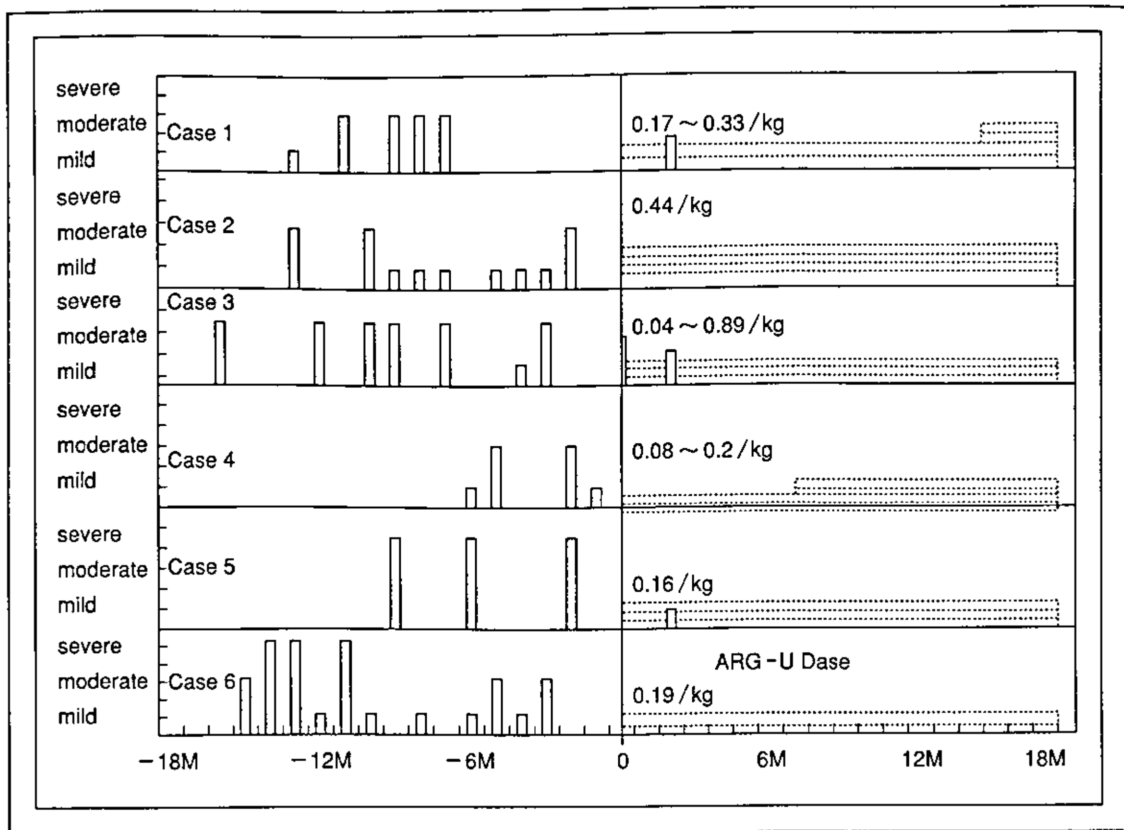

図4　L-アルギニンの脳卒中予防効果

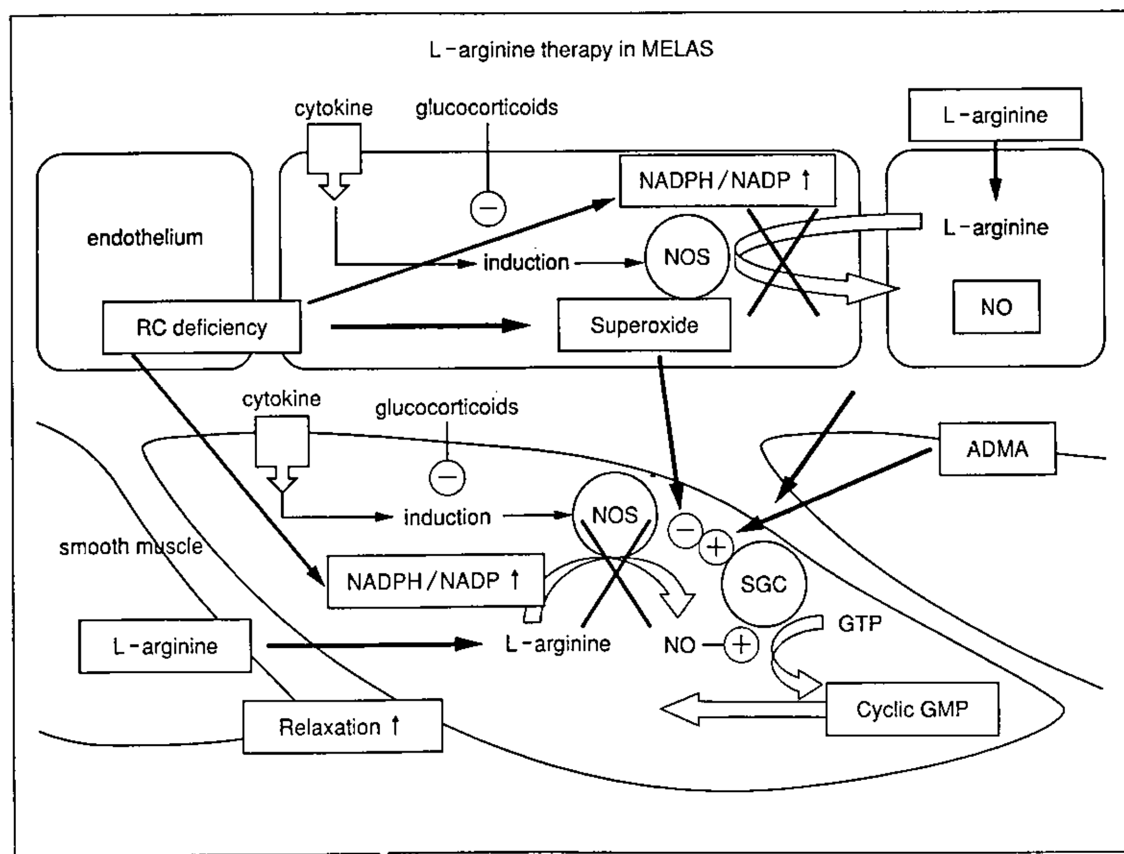

図5　MELAS 患者における血管拡張機能障害
RC deficiency：電子伝達系酵素欠損
ADMA：asymmetrical dimethyl arginine

題となるものではなかった．

6．MELAS の発作寛解期における予防効果

過去 18 か月間に頻回に脳卒中様発作を起こしている MELAS 患者 6 名に対して，L-アルギニンを内服させ，発作予防効果について検討した．その結果，患者では，発作の重症度および頻度共に有意に低下したことが判明した（図 4）[7]．

7．おわりに

MELAS 患者の脳卒中様発作発現時における NO の供与体である L-アルギニンの投与は，低下している血中 L-アルギニン濃度を上昇させ，脳の小中動脈の急性虚血性障害を著明に改善させることが多くの患者で確認され，その効果がより明確となった（図 5）．MELAS 患者に対する L-アルギニン投与は，MELAS 患者の脳卒中様発作急性期のみでなく，発作寛解期の予防にも極めて有効な治療法であると考えられる（図 5）．

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