Human Mitochondrial Leucyl-tRNA Synthetase Corrects Mitochondrial Dysfunctions Due to the tRNA{Leu(UUR)} A3243G Mutation, Associated with Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Symptoms and Diabetes

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Human Mitochondrial Leucyl-tRNA Synthetase Corrects Mitochondrial Dysfunctions Due to the tRNA-Leu(UUR) A3243G Mutation, Associated with Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Symptoms and Diabetes†

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Mutations in mitochondrial tRNA genes are associated with a wide spectrum of human diseases. In particular, the tRNA-Leu(UUR) A3243G mutation causes mitochondrial encephalomyopathy, lactic acidosis, and stroke-like symptoms (MELAS) and 2% of cases of type 2 diabetes. The primary defect in this mutation was an inefficient aminoacylation of the tRNA-Leu(UUR). In the present study, we have investigated the molecular mechanism of the A3243G mutation and whether the overexpression of human mitochondrial leucyl-tRNA synthetase (LARS2) in the cytoplasmic hybrid (cybrid) cells carrying the A3243G mutation corrects the mitochondrial dysfunctions. Human LARS2 localizes exclusively to mitochondria, and LARS2 is expressed ubiquitously but most abundantly in tissues with high metabolic rates. We showed that the alteration of aminoacylation tRNA-Leu(UUR) caused by the A3243G mutation led to mitochondrial translational defects and thereby reduced the aminoacylated efficiencies of tRNA-Leu(UUR) as well as tRNA-Ala and tRNA-Met. We demonstrated that the transfer of human mitochondrial leucyl-tRNA synthetase into the cybrid cells carrying the A3243G mutation improved the efficiency of aminoacylation and stability of mitochondrial tRNAs and then increased the rates of mitochondrial translation and respiration, consequently correcting the mitochondrial dysfunction. These findings provide new insights into the molecular mechanism of maternally inherited diseases and a step toward therapeutic interventions for these disorders.

Mutations in mitochondrial DNA (mtDNA) have been associated with a wide spectrum of clinical abnormalities, including neuromuscular disorders, heart failure, diabetes, and hearing and visual loss (2, 20, 28, 47). More than 50% of these mtDNA mutations are located in the 22 mitochondrial tRNA genes, including tRNA-Leu(UUR) (2). In particular, the A3243G mutation in the tRNA-Leu(UUR) gene causes mitochondrial encephalomyopathy, lactic acidosis, and stroke-like symptoms (MELAS) (14). This mtDNA mutation is also one of the most important causes of maternally inherited diabetes and deafness (35, 37, 45). The primary defect in this mutation was an inefficient aminoacylation of the tRNA-Leu(UUR) (1, 5, 9, 24, 38, 50). This mutation also affected the processing of the longer mitochondrial RNA precursors (22, 24, 41) and the base posttranscriptional modification of the tRNA-Leu(UUR) (19, 26, 50). In cytoplasmic hybrids (cybrids) harboring the nearly homoplasmic A3243G mutation, the level of aminoacylated tRNA-Leu(UUR) was reduced approximately 70% to 75% (5, 9, 12, 50). The deficient aminoacylation of tRNA-Leu(UUR) mainly contributed to a shortage of tRNA-Leu(UUR) (20), thereby causing the reduced rate of mitochondrial protein synthesis and respiration defects (5, 21, 24, 51).

The formation of aminoacylated mitochondrial tRNA-Leu(UUR) was catalyzed by the human mitochondrial leucyl-tRNA synthetase (LARS2) belonging to class I of aminoacyl-tRNA synthetases (3, 27, 43). This evolutionarily conservative tRNA synthetase, encoded by the nuclear gene LARS2 at chromosome 3p21.3, was composed of 903 amino acids with a mitochondrial signal sequence (3, 18, 27). In the yeast Saccharomyces cerevisiae, mitochondrial defects due to the yeast counterpart of the human tRNA-Leu(UUR) A3243G mutation were rescued by overexpression of the translation elongation factor EFTu (11) and mitochondrial leucyl-tRNA synthetase (6). Thus, it is anticipated that the overexpression of human LARS2 in the cybrid cells carrying the A3243G mutation would improve the efficiency of aminoacylation of tRNA-Leu(UUR), enhance the stability of tRNA, and then increase the rates of mitochondrial translation and respiration, consequently correcting the mitochondrial dysfunction. To test this hypothesis, stable transfectants were constructed by transferring a human LARS2 cDNA into a cybrid cell line carrying the nearly homoplasmic A3243G mutation and an isogenic control cybrid cell line harboring the homoplasmic wild-type version of tRNA-Leu(UUR). These stable transfectants were analyzed for the aminoacylation capacity of tRNAs, the stability of the tRNA-Leu(UUR), and the rates of mitochondrial translation and respiration as well as RNA processing. Furthermore, human LARS2 was further characterized by examining the gene expression in different tissues and subcellular locations.

MATERIALS AND METHODS

Cell lines and culture conditions. 143B.TK− is a human osteosarcoma-derived cell line (ATCC CRL 8303). The 43B cybrid cell line carrying the nearly homoplasmic A3243G mutation in the tRNA-Leu(UUR) gene and the isogenic HSI cybrid cell line carrying the homoplasmic wild-type version of the tRNA-Leu(UUR) was catalyzed by the human mitochondrial leucyl-tRNA synthetase (LARS2) belonging to class I of aminoacyl-tRNA synthetases (3, 27, 43). This evolutionarily conservative tRNA synthetase, encoded by the nuclear gene LARS2 at chromosome 3p21.3, was composed of 903 amino acids with a mitochondrial signal sequence (3, 18, 27). In the yeast Saccharomyces cerevisiae, mitochondrial defects due to the yeast counterpart of the human tRNA-Leu(UUR) A3243G mutation were rescued by overexpression of the translation elongation factor EFTu (11) and mitochondrial leucyl-tRNA synthetase (6). Thus, it is anticipated that the overexpression of human LARS2 in the cybrid cells carrying the A3243G mutation would improve the efficiency of aminoacylation of tRNA-Leu(UUR), enhance the stability of tRNA, and then increase the rates of mitochondrial translation and respiration, consequently correcting the mitochondrial dysfunction. To test this hypothesis, stable transfectants were constructed by transferring a human LARS2 cDNA into a cybrid cell line carrying the nearly homoplasmic A3243G mutation and an isogenic control cybrid cell line harboring the homoplasmic wild-type version of tRNA-Leu(UUR). These stable transfectants were analyzed for the aminoacylation capacity of tRNAs, the stability of the tRNA-Leu(UUR), and the rates of mitochondrial translation and respiration as well as RNA processing. Furthermore, human LARS2 was further characterized by examining the gene expression in different tissues and subcellular locations.
gene were isolated by transfer mitochondria from myoblasts of the same MELAS subject carrying the heteroplasmic A3243G mutation into human mtDNA-less p206 cells (51). The 143B.TK-cells and cybrid cells were grown in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (FBS). The stable transfectants were grown in DMEM supplemented with 10% FBS and 15 ng/m of phloerycin (Zeoctin).

Isolation of human LARS2 cDNA. To construct the plasmid pLARS2 containing the entire coding region of LARS2 cDNA, reverse transcription-PCR (RT-PCR) was performed by using Taq DNA polymerase (Promega) and total RNA isolated from 143B cells as template, with the primers 5'-ATGGCTCTCTGGTGGCAGAG (nucleotides nt 186 to 205) and 5'-TGTGTTTCTCTTCTGCCCG (nt 2980 to 2998) (GenBank accession no. NM_015340) (3). The predominant PCR product was purified by agarose gel electrophoresis and subsequently cloned into a PCR 2.1-Topo vector (Invitrogen). Nucleotide sequencing was done using a Dye Terminator cycle sequencing kit (Perkin-Elmer) and an ABI Prism 3700 genetic analyzer.

Northern blot analysis of LARS2 expression. A 12-h lane human multiple tissue RNA blot (Clontech) containing 2 μg poly(A)+ RNA/lane was used for this study. An 1.12-kb LARS2 cDNA fragment corresponding to the nucleotide positions 1309 to 2432 (3) was random prime labeled with [32P]dATP and hybridized with the RNA blots according to the manufacturer’s instructions. Membranes were then washed to a final stringency of 0.1× SSC (0.15 M NaCl plus 0.015 M sodium citrate), 0.1% sodium dodecyl sulfate (SDS) at 65°C for 40 min. As an internal control, the human RNA blots were stripped and rehybridized with [32P]dATP-labeled human actin β subunit cDNA as a control.

Subcellular localization of human LARS2. The coding region of LARS2 cDNA lacking its natural stop codon was obtained by PCR using pLARS2 cDNA as the template. Primers 5’-CTCGAGCTCTTTCTGGTGGCAGAG (nt 186 to 205) and 5’-GGAGCTTGTGAGCCGCGAG (nt 2890 to 2891) were used for the PCR amplification. PCR products were digested with EcoRI and cloned into pBluescript II KS (+) (Promega). After sequence determination, the inserts were subcloned into pEGFP-N1 (Clontech) to generate the pEGFP-N1-LARS2. Resultant constructs were transfected into 143B cells using the SuperFect transfection reagent (Qiagen, Inc.) according to the manufacturer’s protocol. Immunofluorescence analysis was performed as detailed elsewhere (29, 30, 49).

Construction of stable transfectants. The insert of pLARS2 was subcloned into pcDNA3.1/Zeo(-) (Invitrogen). The resultant constructs or vector only were transfected into 43B and HSI cell lines using the SuperFect transfection reagent (Qiagen, Inc.) according to the manufacturer’s protocol. The stable transfectants were isolated by culturing cells in DMEM supplemented with 15% FBS for 4 weeks. The expression level of the plasmid pLARS2 was determined with a YSI 5300 oxygraph (Yellow Springs Instruments) on samples of 5 × 106 cells in 1.5 ml of special DMEM lacking glucose and supplemented with 10% dialyzed FBS (23).

RESULTS

Expression analysis and subcellular localization of human LARS2. To investigate the tissue-specific expression, a LARS2 cDNA probe was hybridized with RNA blots of multiple human tissues. Figure 1A illustrates that LARS2 was ubiquitously expressed, but that there were significant variations in the steady-state LARS2 mRNA levels among tissues. Compared to a high-level expression of LARS2 in the tissues with a high metabolic rate such as skeletal muscle, heart, and kidney, the expression levels of LARS2 in small intestine, spleen, thymus, and leukocytes were very low, while LARS2 was expressed moderately in the brain, colon, placenta, liver, spleen, and lung.

To determine the cellular localization of human LARS2, pEGFP-N1-LARS2 expressing the LARS2-green fluorescent protein (GFP) fusion protein was transfected into the 143B cell line. Figure 1B shows that the immunofluorescence pattern of transfected 143B cells was double labeled with a monoclonal antibody specific for the GFP and mouse monoclonal antibody to COXI, a subunit of cytochrome c oxidase (COX) complex in the mitochondrial inner membrane. A typical mitochondrial staining pattern was observed with both antibodies, and superimposition of two panels showed complete overlap of the two patterns, demonstrating that human LARS2 localizes exclusively to mitochondria. This suggested that this protein, like other nucleus-encoding mitochondrial proteins such as MTO1, GTPBP3, and TRMU (29, 30, 49), was synthesized on cytoplasmic ribosomes and was imported into and then functioned in the mitochondrion.

Construction of stable transfectants expressing the human LARS2. A 2.8-kb human LARS2 cDNA expressed in a pcDNA3.1 vector or the vector only was transfected into the 43B hybrid cell line carrying the A3243G mutation and the isogenic HSI cell line with the wild-type version of tRNA Leu UUR (5). The HSI cell line carrying the homoplasmic wild-type version of the tRNA Leu UUR gene was isogenic with its respective mutant 43B cell line, as the wild-type and mutated mtDNA were derived from the same heteroplasmic patient cells (5, 51). These stable transfectants were isolated by culturing cells in DMEM supplemented with 15 ng/ml of phloerycin and 10% FBS for 4 weeks. The expression level of the LARS2 cDNA in...
resultant stable transfectants was examined by a Northern blot analysis, as shown in Fig. 2A. Here, the levels of exogenous LARS2 mRNA (2.8 kb) harboring only the coding region were more than 5-fold higher than those of endogenous LARS2 mRNA (4.2 kb) representing full-length mRNA in transfec-
tants 43B-LARS2 and HSI-LARS2. To test if the transfer of LARS2 may change the proportion of mutated mtDNA mol-
ecules and mtDNA copy number in transfectant lines carrying the A3243G mutation, the presence and degree of the A3243G mutation in the transfectants 43B-LARS2, expressing the LARS2 cDNA, and 43B-V, carrying the vector only, were comparable with those of the parental cybrid cell line 43B carrying the A3243G mutation. Furthermore, an analysis of the mtDNA copy numbers of these 43B- and HSI-
derived transfectants expressing the human LARS2 and HSI-LARS2, and HSI-V) were then used for further characteriza-
tion.

FIG. 1. Subcellular localization and gene expression analysis of hu-
man LARS2. (A) Multiple-tissue Northern blot analysis of LARS2
expression. A human 12-lane multiple-tissue blot (Clontech) contain-
ing 2 μg of poly(A)+ RNA per lane was hybridized with a 32P-labeled
DNA probe according to the manufacturer’s protocol. The blot was then stripped and rehy-
bridized with 32P-labeled human β-actin probe as a control. (B) Sub-
cellular localization of human LARS2 in 143B cells. Cells were tran-
siently transfected with a LARS2 cDNA fused with GFP or pEGFP.
The fusion protein was visualized by indirect immunofluorescence
using antibodies to mouse COX1 and to GFP. A merged image is
shown on the right.

FIG. 2. (A) Construction of stable transfectants. Expression anal-
ysis of human LARS2 in the transfectants. Equal amounts (5 μg) of
total mitochondrial RNA from mutant cell lines and control cell lines
were electrophoresed through a 1.8% agarose-formaldehyde gel,
transferred onto a positively charged membrane, and hybridized first
with a DIG-labeled LARS2-specific RNA probe. After the blot was
stripped, it was hybridized with a human β-actin probe as a control.
43B is a cybrid cell line carrying a nearly homoplasmic A3243G mu-
tation, the HSI cell line is an isogenic wild-type cybrid line derived
from the same individuals as those for 43B-V and HSI-V are trans-
fectant lines with vector only, and 43B-LARS2 and HSI-LARS2 are
transfectant lines expressing the human LARS2. The 4.2-kb band and
2.8-kb bands correspond to the length of endogenous and exogenous
LARS2 mRNA, respectively. (B) Quantification of the A3243G mu-
tation in the tRNA Leu(UUR) gene in transfectants and parental cybrids.
PCR products around the A3243G mutation were digested with ApaI
and analyzed by electrophoresis in a 6% polyacrylamide gel stained
with ethidium bromide. The A3243G mutation creates the site for
restriction enzyme Apal (14). Transfectants and their parental cybrid
cell lines are indicated.

Aminoacylation capacity of mitochondrial tRNAs. We first
investigated the effects of the transfer of human LARS2 on the
aminoacylation deficiency of tRNA Leu(UUR) in transfectants
43B-LARS2 and 43B-V and their parental mutant cybrid cell line
43B as well as transfectants HSI-LARS2 and HSI-V and
their parental wild-type cybrid cell line HSI in vivo. Total
mitochondrial RNA was isolated from cell lines under acidic con-
ditions to preserve the aminoacyl-tRNA linkage (46). The
aminoacylated tRNAs were separated from nonaminoacylated
tRNA species on acidic denaturing polyacrylamide-urea gels and
then electroblotted and hybridized with specific probes for
tRNA Leu(UUR) as well as tRNA Leu(UUR), tRNA Leu(UUR),
tRNA Ser(UCN), and tRNA Ala, respectively. To further distin-
guish nonaminoacylated tRNA from aminoacylated tRNA,
samples of mitochondrial tRNAs were deacylated by being
heated for 10 min at 60°C at pH 8.3 and then run in parallel
Notably, the efficiencies of aminoacylated tRNA\textsubscript{Met} and tRNA\textsubscript{Ala}, in addition to those of tRNA\textsubscript{Leu(UUR)}, as shown in Fig. 3, were markedly reduced in mutant 43B cells, relative to wild-type HSI cells. In particular, the proportions of aminoacylated tRNAs in the 43B cells were 24.9%, 61.8%, 66.3%, 38.7%, 71.2%, and 56.1% in the tRNALeu(UUR), tRNALeu(CUN), tRNA\textsubscript{Lys}, tRNA\textsubscript{Met}, tRNA\textsubscript{Ser(UCN)}, and tRNA\textsubscript{Ala}, respectively, while 64.1% of tRNA\textsubscript{Leu(UUR)}, 66.4% of tRNA\textsubscript{Leu(CUN)}, 73.1% of tRNA\textsubscript{Lys}, 66.1% of tRNA\textsubscript{Ser(UCN)}, and 73% of tRNA\textsubscript{Ala} were aminoacylated in the isogenic HSI cells. In contrast, the aminoacylated levels of these six tRNAs in the 43B-V cells and HSI-V cells carrying only the vector as well as HSI-LARS2 cells expressing the LARS2 cDNA only were comparable with those of 43B cells and HSI cells, respectively. Strikingly, the expression of LARS2 in the 43B cybrids led to an ~100% increase in the efficiency of aminoacylation of tRNA\textsubscript{Leu(UUR)} (from 24.9% to 50.5%), but the level was still below that of controls (64.1%). Furthermore, the efficiencies of aminoacylation of tRNA\textsubscript{Leu(CUN)}, tRNA\textsubscript{Lys}, tRNA\textsubscript{Met}, tRNA\textsubscript{Ser(UCN)}, and tRNA\textsubscript{Ala} in the 43B-LARS2 cell line expressing LARS2 were 106.6%, 111.6%, 133.3%, 98.5%, and 118% of those in parental 43B cell line, respectively. These suggested that the deficiencies of aminoacylation of tRNAs were partially restored by the overexpression of human LARS2 in the 43B cells.

**The steady-state level of mitochondrial tRNA.** To evaluate if the overexpression of LARS2 increased the stability of tRNA\textsubscript{Leu(UUR)} in transfectants carrying the A3243G mutation, we determined the steady-state level of the tRNA\textsubscript{Leu(UUR)} by isolating total mitochondrial RNAs from cell lines, separating them on a 10% polyacrylamide-7 M urea gel, and electroblotting and hybridizing with a nonradioactive DIG-labeled oligodeoxynucleotide probe specific for tRNA\textsubscript{Leu(UUR)}. After the blots were stripped, the DIG-labeled oligodeoxynucleotide probes, including tRNA\textsubscript{Leu(CUN)}, tRNA\textsubscript{Lys}, and tRNA\textsubscript{Met} as representatives of the whole H-strand transcription unit and tRNA\textsubscript{Ser(UCN)} and tRNA\textsubscript{Ala} derived from the L-strand transcription unit (16) as well as a nucleus-encoded mitochondrial 5S RNA (36), were hybridized with the same blots for normalization purposes. For comparison, the average level of each tRNA in control or mutant cell lines was normalized to the average levels in the same cell line for reference 5S RNA. As shown in Fig. 4, the average steady-state levels of tRNA\textsubscript{Leu(UUR)} in the 43B, 43B-V, 43B-LARS2, HSI-V, and HSI-LARS2 cell lines were 36%, 38%, 65%, 97%, and 98% of the isogenic HSI cybrid cell line after normalization to 5S RNA, respectively. However, the average steady-state levels of tRNA\textsubscript{Ser(UCN)}, tRNA\textsubscript{Met}, tRNA\textsubscript{Leu(UUR)}, tRNA\textsubscript{Lys}, and tRNA\textsubscript{Ala} in 43B, 43B-V, 43B-LARS2, and HSI-LARS2 cell lines were comparable with those of the wild-type HSI cybrid cell line after normalization to 5S RNA.

**Mitochondrial RNA processing.** We then tested if the transfer of human LARS2 restores the defects in mitochondrial RNA processing in the 43B cell line carrying the A3243G mutation. For this purpose, RNA transfer hybridization experiments were performed with total mitochondrial RNA from transfectants and parental cybrids using the nonradioactive DIG-labeled ND1 RNA probe (16, 30). After the blots were stripped, a set of DIG-labeled RNA probes (ND4, ND6, 12S rRNA, and 16S rRNA) were rehybridized with the same blots for normalization purposes. As shown in Fig. 5, RNA\textsubscript{19} comprising the 12S rRNA plus tRNA\textsubscript{Val} plus 16S rRNA plus tRNA\textsubscript{Leu(UUR)} plus ND1 was accumulated in the 43B cell line, confirming the defects in mitochondrial RNA processing caused by the A3243G mutation (22, 24, 41). Interestingly, the reduction in the levels of RNA19, ND1, ND4, and ND6 mRNA was observed in the 43B-LARS2 transfectant, while the
levels of 16S rRNA plus tRNA\(^{Leu(UUR)}\) plus ND1 as well as 12S rRNA appeared to be unchanged in this transfectant compared with those of the parental 43B cell line.

Mitochondrial protein synthesis. To assess if the overexpression of LARS2 enhanced the rate of overall mitochondrial protein synthesis in the transfectant carrying the A3243G mutation, samples of cultures of transfectants as well as their parental cybrid cell lines were labeled with short \([35S]\)methionine pulses in the presence of emetine to inhibit the cytosolic protein synthesis (4). As shown in Fig. 6A, the patterns of the mtDNA-encoded polypeptides of parental cybrid cell lines and their transfectants were qualitatively identical, in terms of electrophoretic mobility of the various polypeptides, to those of the isogenic control cybrid cell line HSI. However, the 43B cells exhibited a decrease in the total rate of labeling of mitochondrial translation products, relative to the control cybrid. In contrast, the overexpression of LARS2 apparently increased the total rate of labeling of mitochondrial translation products in the 43B-LARS2 transfectant line. Figure 6B illustrates a quantification of the results of three labeling experiments and three electrophoretic runs, which was carried out by Image-Quant program analysis of appropriate exposures of the fluorograms and normalization to the data obtained for the HSI sample included in each gel. The overall rates of labeling of the mitochondrial translation products in the 43B cell line and 43B-LARS2 transfectants were 32.3%, 31.1%, and 48.3%, respectively, of the mean value measured in the control HSI cell line. This translated to a 51% increase of the rate of mitochondrial translation in 43B-LARS2 cells, compared to the parental cybrid cell line 43B. In contrast, the rates of mitochondrial translation of HSI-LARS2 and HSI-V transfectants were comparable with that of the parental HSI cell line.

Analysis of respiration. To examine if the overexpression of LARS2 rescued the respiratory deficiencies caused by the A3243G mutation, the endogenous respiration rates of transfectants and their parental cybrid cell lines were measured by determining the O\(_2\) consumption rate in intact cells, as described previously (23). As can be seen in Fig. 7, the rate of total O\(_2\) consumption in 43B cell lines was 24%, relative to the mean value measured in the control HSI cell line. However, the rate of total O\(_2\) consumption in the 43B-LARS2 transfectant cell line expressing LARS2 was 142% of that of the parental 43B cell line, while the rate of total oxygen consumption
of HSI-LARS2 was 92% of that of the parental HSI cell line. However, rates for 43B-V and HSI-V transfectants were comparable with those of parental 43B and HSI cell lines. These data demonstrated that the increase of the rate of mitochondrial translation products by overexpression of LARS2 indeed enhanced the rate of respiration in the transfectant 43B-LARS2 carrying the A3243G mutation.

**DISCUSSION**

In the present study, we have further investigated the molecular pathogenesis of the MELAS-associated tRNALeu(UUR) A3243G mutation and whether the overexpression of human LARS2 in the cybrid cells carrying the A3243G mutation corrects the mitochondrial dysfunctions. The A3243G mutation changes the A to G at conventional position 14 (A14) of the mitochondrial tRNALeu(UUR) (13) and thus alters the structure and function of tRNALeu(UUR). In particular, the tRNALeu(UUR) carrying the A3243G mutation was less charged to a lesser extent by the leucyl-tRNA synthetase, thereby altering aminoacylation. Alteration in aminoacylation of tRNALeu(UUR) may result in stalling of ribosomes at leucine codons, mistranslation of UUR codons, or translational shifting (1, 21, 42). This was evidenced by the disproportionate decrease of translation in some polypeptides (42). The idea that an inefficient aminoacylation of tRNALeu(UUR) is the primary defect in MELAS was further supported by the recent isolation of a suppressor mutation in the anticodon of the mitochondrial tRNALeu(UUR) gene in an A3243G lung carcinoma cell line (8, 9). Furthermore, the A3243G mutation disturbed the processing of the tRNALeu(UUR) precursor (22, 24, 41) as well as the posttranscriptional modification of uridine at the first position of the anticodon in the tRNALeu(UUR) (19, 26, 50). The mutant tRNALeu(UUR) may be metabolically less stable and subject to turnover, thereby lowering the steady-state level of tRNA Lys, tRNAGlu, and tRNA Gln in the wobble position, not only lowered the steady-state level of tRNALeu(UUR) but also reduced the steady-state level of other tRNAs. Indeed, the mutation in TRMU, encoding a highly conserved 5-methylaminomethyl-2-thiouridylate-methyltransferase responsible for the biosynthesis of 5-taurinomethyl-2-thiouridine (tms2U) of mitochondrial tRNA^{A3}, tRNA^{Glu}, and tRNA^{Gln} in the wobble position, not only lowered the steady-state level of tRNA^{A3}, tRNA^{Glu}, and tRNA^{Gln} but also reduced the steady-state level of other

**FIG. 6.** Analysis of rates of mitochondrial protein labeling. (A) Electrophoretic patterns of the mitochondrial translation products of different cell lines labeled for 30 min with [35S]methionine in the presence of 100 μg of emetine per ml. Samples containing equal amounts of protein (20 μg) were run in SDS-polyacrylamide gradient gels. COI, COII, and COIII, subunits I, II, and III of cytochrome c oxidase, respectively; ND1, ND2, ND3, ND4, ND4L, ND5, and ND6, subunits 1, 2, 3, 4, 4L, 5, and 6 of the respiratory-chain NADH dehydrogenase, respectively; A6 and A8, subunits 6 and 8 of the H+-ATPase, respectively; CYTB, apocytochrome b. (B) Quantification of the rates of labeling of the mitochondrial translation products. The rates of mitochondrial protein labeling in three mutant cell lines and three control cell lines, determined as described elsewhere (16, 17), are expressed as percentages of the value for HSI in each gel, with error bars indicating standard errors of the means. Three independent labeling experiments and three electrophoretic analyses of each labeled preparation were carried out on each cell line.

**FIG. 7.** Respiration assay. Average rates of total O2 consumption per cell were measured in different cell lines. Four to eight determinations were carried out for each cell line, with error bars indicating standard errors of the means.
tRNAs such as tRNA^{Leu(UUR)}, tRNA^{Ser(UCN)}, tRNA^{Met}, and tRNA^{His} (17).

In the present investigation, we have shown that the overexpression of human LARS2 in the cybrid cells carrying the A3243G mutation corrected the mitochondrial dysfunctions. In particular, the overexpression of LARS2 in the 43B cybrid cell line carrying the A3243G mutation raised the percentage of aminoacylated tRNA^{Leu(UUR)} from 24.9% to 50.5%. Despite ~100% increasing efficiency of charged tRNA^{Leu(UUR)}, the partial function of tRNA^{Leu(UUR)} in the 43B cybrid cell line would cause the level of aminoacylated tRNA^{Leu(UUR)} in 43B cell lines expressing LARS2 to be below that of control cell line HSI. The fact that overexpression of LARS2 did not elevate the level of aminoacylated tRNA^{Leu(UUR)} in cells appeared to be the maximum threshold level to maintain the normal function. On the other hand, the improvement of aminoacylation of tRNA^{Leu(UUR)} likely modulated the uncharged/charged ratios of other mitochondrial tRNAs, thereby enhancing the efficiency of aminoacylation of other tRNAs, including tRNA^{Met} and tRNA^{Ala}. Moreover, an improvement of LARS2 expression in 43B cells appeared to lead to more efficient processing of the longer RNA precursors (22, 24), evidenced by the fact that a reduced phosphorylation. However, this result was not in good agreement with the observation that the full recovery of respiration chain function occurred by means of overexpression of LARS2 in a mutant cybrid cell line carrying the A3243G mutation (39). This discrepancy may be attributed to different nuclear backgrounds, as in the case of cell lines carrying other mtDNA mutations (7, 15). In conclusion, we demonstrated that the overexpression of human mitochondrial leucyl-tRNA synthetase corrected the mitochondrial dysfunction caused by the MELAS-associated tRNA^{Leu(UUR)} A3243G mutation. These findings will provide new insights into the pathophysiology of maternally inherited diseases and a step toward therapeutic interventions for these disorders.

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