Functional characterization of the mitochondrial 12S rRNA C1494T mutation associated with aminoglycoside-induced and non-syndromic hearing loss

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ABSTRACT

In this study, we report the biochemical characterization of the deafness-associated mitochondrial 12S rRNA C1494T mutation using 27 cybrid cell lines constructed by transferring mitochondria from 9 lymphoblastoid cell lines derived from a Chinese family into human mitochondrial DNA (mtDNA)-less (ρ°) cells. Six cybrids derived from two asymptomatic members, and nine cybrids derived from three symptomatic members of the Chinese family carrying the C1494T mutation exhibited $\sim$38 and 43% decrease in the rate of mitochondrial protein labeling, respectively, compared with twelve cybrids derived from four Chinese control individuals. These defects are apparently a primary contributor to significant reductions in the rate of overall respiratory capacity or the rate of malate/glutamate promoted respiration, or succinate/G3P-promoted respiration, or TMPD/ascorbate-promoted respiration in mutant cybrid cell lines derived from either symptomatic or asymptomatic individuals. Furthermore, the very significant/nearly identical increase in the ratio of doubling times in DMDM medium in the presence/absence of high concentration of paromomycin was observed in symptomatic or asymptomatic cybrid cell lines carrying the C1494T mutation as compared with the average rate in control cell lines. These observations provide the direct biochemical evidences that the C1494T mutation is a pathogenic mtDNA mutation associated with aminoglycoside-induced and non-syndromic hearing loss. In addition, these data provide the first biochemical evidence that nuclear background plays a critical role in the phenotypic manifestation of non-syndromic hearing loss and aminoglycoside toxicity associated with the C1494T mutation.

INTRODUCTION

Mutations in mitochondrial DNA (mtDNA) have been found to be associated with both syndromic and non-syndromic forms of sensorineural hearing impairment (1,2). The mitochondrial tRNA$^{\text{Ser(UCN)}}$ appears to be a hot spot for mtDNA mutations associated with non-syndromic hearing loss. Four deafness-associated mutations, A7445G (3,4), 7472insC (5,6), T7510C (7,8) and T7511C (9,10), have been identified in this gene. The mitochondrial 12S rRNA is another hot spot for mtDNA mutations associated with aminoglycoside induced and non-syndromic hearing loss. Of those, the homoplasmic A1555G mutation in the highly conserved decoding site of this rRNA has been associated with aminoglycoside-induced and non-syndromic hearing loss in many families of different ethnic backgrounds (11–18). In addition, mutations at position 961 of the 12S rRNA have been implicated to be associated with aminoglycoside-induced and non-syndromic hearing loss (19–22). Furthermore, the T1095C mutation in the same rRNA has also been shown to be associated with hearing impairment (23,24). Most recently, the homoplasmic C1494T mutation in the highly conserved decoding site of this rRNA has been associated with both aminoglycoside-induced and non-syndromic hearing loss in a large Chinese family (25).
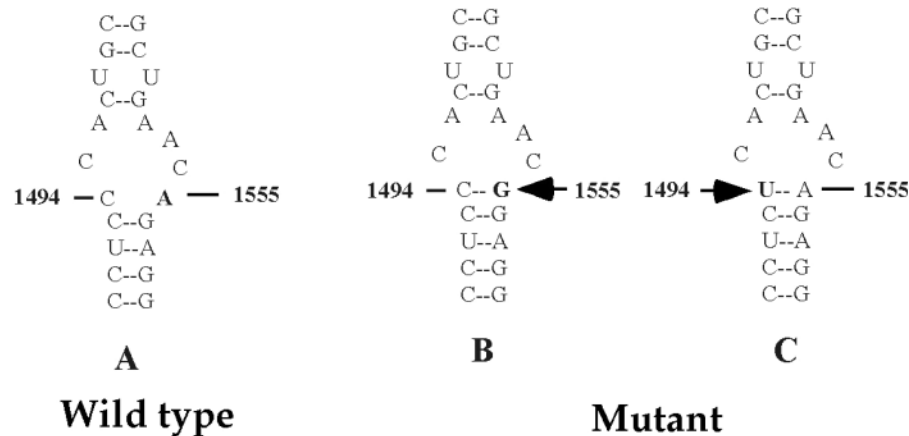
Figure 1. The location of the C1494T mutation in the decoding region of small ribosomal RNAs. The A-site of human mitochondrial 12S rRNA is shown as the wild type version (**A**) and the version containing the A1555G mutation (**B**) and C1494T mutation (**C**), respectively. The sites for the A1555G or C1494T mutation are indicated by arrows.

As shown in Figure 1, the C1494T mutation is located in the decoding region of the small ribosomal subunit (26), highly conserved from bacteria to mammals (27). The same region is important for the action of aminoglycosides (28,29). In fact, the C1494T mutation is expected to form a novel 1494U–A1555 base pair, which is in the same position as the 1494C–G1555 pair created by the A1555G mutation, at the highly conserved A-site of 12S rRNA (25). In fact, the new U–A or G-C pair in 12S rRNA created by the C1494T or A1555G transition facilitates the binding of aminoglycosides (30,31), accounting for the fact that the exposure to aminoglycosides can induce or worsen hearing loss in individuals carrying these mutations (11,25,32). In the absence of aminoglycosides, matrilineal relatives in this Chinese family carrying the C1494T mutation exhibited non-syndromic sensorineural hearing loss with a wide range of the age of onset and severity, ranging from severe hearing impairment, to moderate hearing impairment, to mild hearing impairment, to completely normal hearing (25). To elucidate pathogenetic mechanism of the C1494T mutation and the role of nuclear background in the phenotypic expression of this mutation, cybrid cell lines were constructed by transferring mitochondria from lymphoblastoid cell lines derived from three symptomatic individuals and two asymptomatic individuals in this Chinese family and from four controls into human mtDNA-less (ρ°) cells (33,34). These cybrid cell lines were first examined for the presence and degree of the C1494T mutation. These cell lines were then assessed for the effects of the C1494T mutation on the rate of mitochondrial protein synthesis, the endogenous respiration and substrate-dependent respiration, and the growth properties after treatment with aminoglycoside.

MATERIALS AND METHODS

Cell lines and culture conditions

A total of nine human immortalized lymphoblastoid cell lines derived from the members of the Chinese family [two asymptomatic members (III-14 and III-16) and three symptomatic members (III-18, IV-21 and IV-28) and four genetically unrelated Chinese control subjects (A3, A6, A7 and A8) (25) were grown in RPMI 1640 medium (Invitrogen), supplemented with 10% fetal bovine serum (FBS). The bromodeoxyuridine (BrdU)-resistant osteosarcoma 143B.TK$^-$ and cybrid cell line F7H derived from an Arab–Israeli family carrying the A1555G mutation (35) as grown in DMEM, supplemented with 100 μg of BrdU per ml and 5% FBS. The mtDNA-less ρ°206 cell line, derived from 143B.TK$^-$ (33), was grown under the same conditions as the parental line, except for the addition of 50 μg of uridine per ml. All cybrid cell lines constructed with enucleated lymphoblastoid cell lines were maintained in the same medium as the 143.TK$^-$ cell line.

To test the various cell lines for sensitivity to paromomycin, samples of 10^5 cells were plated and then grown on 10 cm Petri dishes in 10 ml of DMEM (Invitrogen), supplemented with 10% FBS, in the presence or absence of 2 mg of the antibiotics per ml. The population doubling time (DT) of the cell lines in DMEM medium, supplemented with 10% dialyzed FBS, was determined from the growth curves or by using the formula (36): $DT = (t - t_0)\log2/(\log N - \log N_0)$, where t and t_0 are the times at which the cells were counted, and N and N_0 are the cell numbers at times t and t_0, respectively.

Mitochondria-mediated ρ°206 cell transformation

Immortalized lymphoblastoid cell lines derived from five matrilineal relatives [two asymptomatic members (III-14 and III-16) and three symptomatic members (III-18, IV-21 and IV-28)] of the Chinese family and four genetically unrelated control individuals were used for the generation of cybrid cell lines (25). Transformation by cytoplasts of mtDNA-less ρ°206 cells was performed as described previously (33–35,37,38).

Mitochondrial DNA analysis

The analysis of the presence and level of the C1494T mutation in the mitochondrial 12S rRNA was carried out as described previously (25). The quantification of mtDNA copy numbers from different cybrids was performed by slot-blot hybridization as detailed previously (36).

Analysis of mitochondrial protein synthesis

Pulse-labeling of the cell lines for 30 min with [^{35}S]methionine–[^{35}S]cysteine in methionine-free DMEM in the presence of emetine, electrophoretic analysis of the translation products, and quantification of radioactivity in the whole-electrophoretic patterns or in individual well-resolved bands was carried out as detailed previously (36,37,39,40).

O_2 consumption measurements

Rates of O_2 consumption in intact cells were determined with a YSI 5300 oxygraph (Yellow Springs Instruments) on samples of 5×10^6 cells in 1.5 ml of special DMEM-glucose lacking glucose, and supplemented with 10% dialyzed FBS (33). Polargraphic analysis of digitonin-permeabilized cells, using different respiratory substrates and inhibitors, to test the activity of the various respiratory complexes was carried out as detailed previously (41).

Computer analysis

Variance analysis was performed by the Student's *t*-test contained in the Microsoft Excel program for Macintosh (version 5), entering individual replicate values. Correlation analysis was performed using the curve fitting in CA-Cricket Graph III(tm) program for Macintosh (version 1.5.2).

RESULTS

Construction of cybrid cell lines

Lymphoblastoid cells derived from four control individuals (A3, A6, A7 and A8), aged between 20 and 40 years, and from two asymptomatic members (III-14 and III-16) and three symptomatic members (III-18, IV-21 and IV-28) of the Chinese family (25) were enucleated, and subsequently fused to a large excess of mtDNA-less human ρ°206 cells, derived from the BrdU-resistant 143B.TK$^-$ cell line (33). Cybrid clones were isolated by growing the fusion mixtures in selective DMEM medium, containing BrdU and lacking uridine (34,35,37,38). Between 25 and 45 days after fusion, 10–15 presumptive mitochondrial cybrids derived from each of the different donor cell lines were isolated, and subsequently analyzed for the presence and level of the C1494T mutation (25). The results confirmed the absence of the C1494T mutation in control cybrids and its presence in homoplasmy in mutant cybrids. An analysis of the mtDNA content of these cybrids by slot-blot hybridization was performed using the DIG-labeled mt12S rRNA, and nuclear 28S rRNA probes for normalization purposes (36,37). Three cybrids derived from each donor lymphoblastoid cell line with similar mtDNA copy number were used for the biochemical characterization described below.

Mitochondrial protein synthesis defect in the cybrid cell lines

To examine whether a defect in mitochondrial translation occurred in cybrids carrying the C1494T mutation, cells from three cybrids derived from each donor of four control individuals and each donor of two asymptomatic and three symptomatic members of the Chinese family were labeled for 30 min with [^{35}S]methionine–[^{35}S]cysteine in methionine-free regular DMEM medium in the presence of 100 µg/ml of emetine, to inhibit cytosolic protein synthesis (39). Figure 2 shows typical electrophoretic patterns of the mitochondrial translation products of the mutant and control cybrids. Patterns of the mtDNA-encoded polypeptides of the mutation-carrying cybrids were qualitatively identical, in terms of electrophoretic mobility of the various polypeptides, to those of the control cybrids and of 143B.TK$^-$ cells (Figure 2A and B). However, cybrids carrying the C1494T mutation showed a clear tendency to a decrease in the total rate of labeling of the mitochondrial translation products, relative to those of control cybrids.

Figure 3 shows a quantification of the results of a large number of labeling experiments and electrophoretic runs, which were carried out by densitometric analysis of appropriate exposures of the fluorograms and normalization to data obtained for the 143B.TK$^-$ sample included in each gel. It appears that the overall rate of labeling of the mitochondrial translation products in mutant cybrids derived from symptomatic individuals was decreased by 37–48%, with an average of 43%, relative to the mean value measured in the control cell lines ($P = 0.0006$). Similarly, the reduction in the rate of labeling of the same products in the mutant cybrids derived from asymptomatic individuals ranged from 34 to 43%, with an average of 38% ($P = 0.0197$). It is noteworthy that the decreased levels of mitochondrial protein labeling in cybrid cells carrying the C1494T mutation are comparable with ∼35% average reduction in the rate of labeling of the same products observed in F7H, a cybrid cell line carrying the A1555G mutation.

Respiration defects in the cybrid cell lines

The endogenous respiration rates of three cybrids derived from each of four control individuals and each of two asymptomatic and three symptomatic members of the Chinese family carrying the C1494T mutation were measured by determining the O_2 consumption rate in intact cells, as described previously (33). As shown in Figure 4, the rate of total O_2 consumption in the cybrids derived from three symptomatic individuals exhibited a variable decrease, ranging between ∼35 and 47%, with an average reduction of ∼41% relative to the mean value measured in the control cell lines ($P = 0.0017$). Cybrids derived from two asymptomatic individuals also revealed a variable decrease in O_2 consumption rate, ranging between 33 and 37%, when compared to the mean control value, with an average reduction of ∼35% ($P = 0.0095$). The variations in the overall respiration rate among the individual mutant cybrids derived from symptomatic members of the Chinese family, and, similarly, among the mutant cybrids derived from asymptomatic members, as compared with the individual control cybrids, showed a very significant correlation with the corresponding variations in the overall rate of labeling of mitochondrial protein synthesis ($r = 0.98$, $P < 0.001$; $r = 0.99$, $P < 0.001$, respectively).

In order to investigate which of the enzyme complexes of the respiratory chain was affected in the mutant cell lines, O_2 consumption measurements were carried out on digitonin-permeabilized cells, using different substrates and inhibitors (41). As shown in Figure 5, in the cybrids derived from three symptomatic individuals, the rate of malate/glutamate-driven
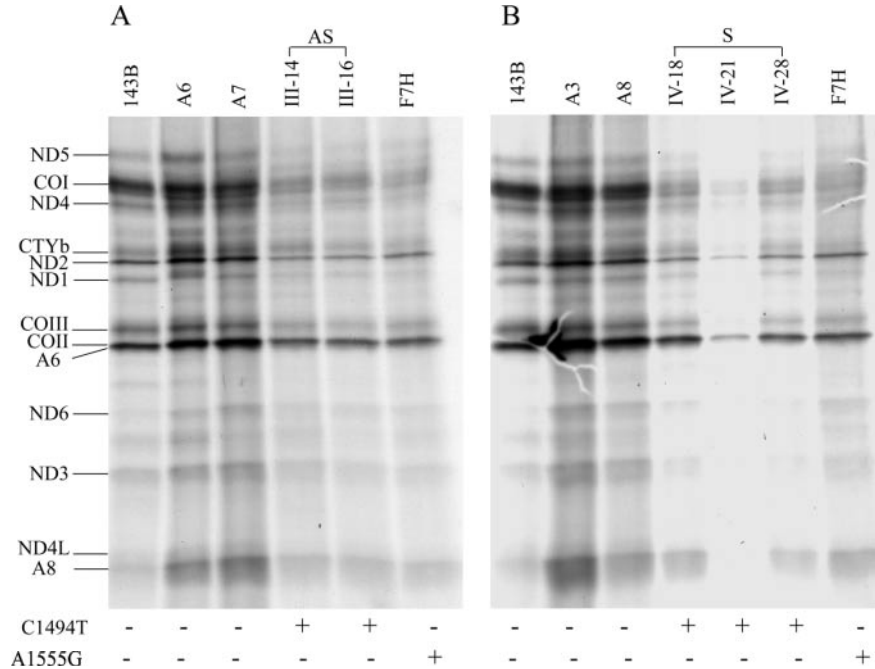
Figure 2. Electrophoretic patterns of the mitochondrial translation products of cybrids and of 143B.TK⁻ cells labeled for 30 min with [^{35}S]methionine in the presence of 100 µg/ml of emetine. Samples containing equal amounts of protein (20 µg) were run in SDS/polyacrylamide gradient gels. The two panels represent electrophoretic patterns obtained in separate gel runs, each one including the 143B.TK⁻ control for normalization purposes. COI, COII and COIII, subunits I, II and III of cytochrome *c* oxidase; ND1, ND2, ND3, ND4, ND4L, ND5 and ND6, subunits 1, 2, 3, 4, 4L, 5 and 6 of the respiratory chain NADH dehydrogenase; A6 and A8, subunits 6 and 8 of the H⁺-ATPase; and CYTb, apocytochrome *b*.
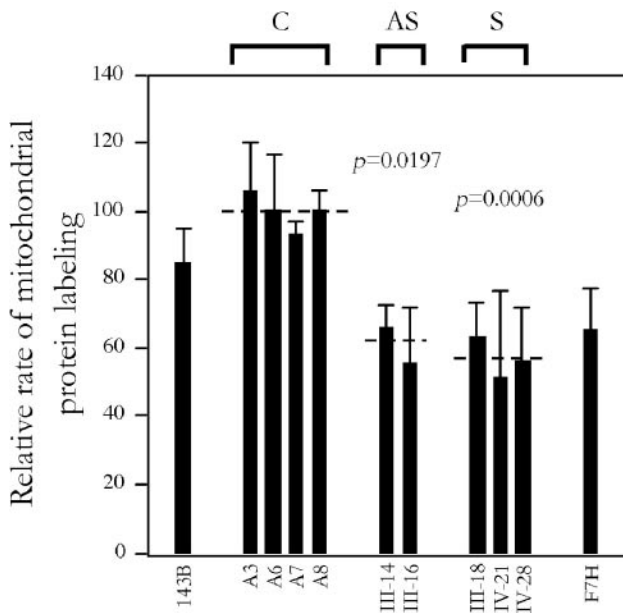

Figure 3. Quantification of the rates of labeling of the mitochondrial translation products, after a 30 min [^{35}S]methionine pulse, in cybrids derived from different donor lymphoblastoid cell lines. The rates of mitochondrial protein labeling, determined as detailed in Materials and Methods, are expressed as percentages of the value for 143B.TK⁻ in each gel, with error bars representing two standard errors of the mean (SE). A total of 2–3 independent labeling experiments and 2–4 electrophoretic analyses of each labeled preparation were carried out on three cybrids derived from each donor lymphoblastoid cell line. C, control; AS, asymptomatic individuals; and S, symptomatic individuals. The horizontal dashed lines represent the average value for each group, and the vertical arrows refer to two SE; *P* indicates the significance, according to the Student's *t*-test, of the differences between AS mean and C mean, and between S mean and C mean.
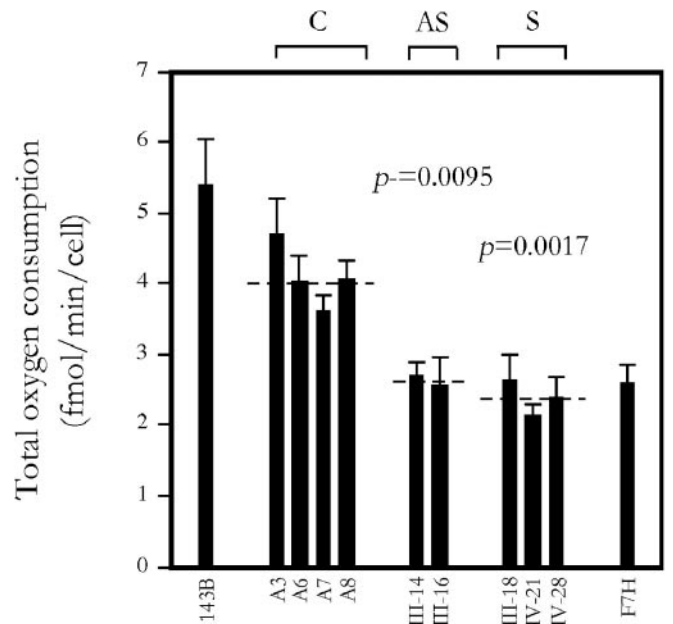
Figure 4. Average rates of endogenous O$_2$ consumption per cell measured in different cybrids are shown, with error bars representing two SE. A total of 4–6 determinations were made on each of three cybrids derived from each donor lymphoblastoid cell line. Graph details and symbols are explained in the legend to Figure 3.

respiration, which depends on the activities of NADH:ubiquinone oxidoreductase (Complex I), ubiquinol–cytochrome *c* reductase (Complex III) and cytochrome *c* oxidase (Complex IV), but usually reflects the rate-limiting activity of Complex I
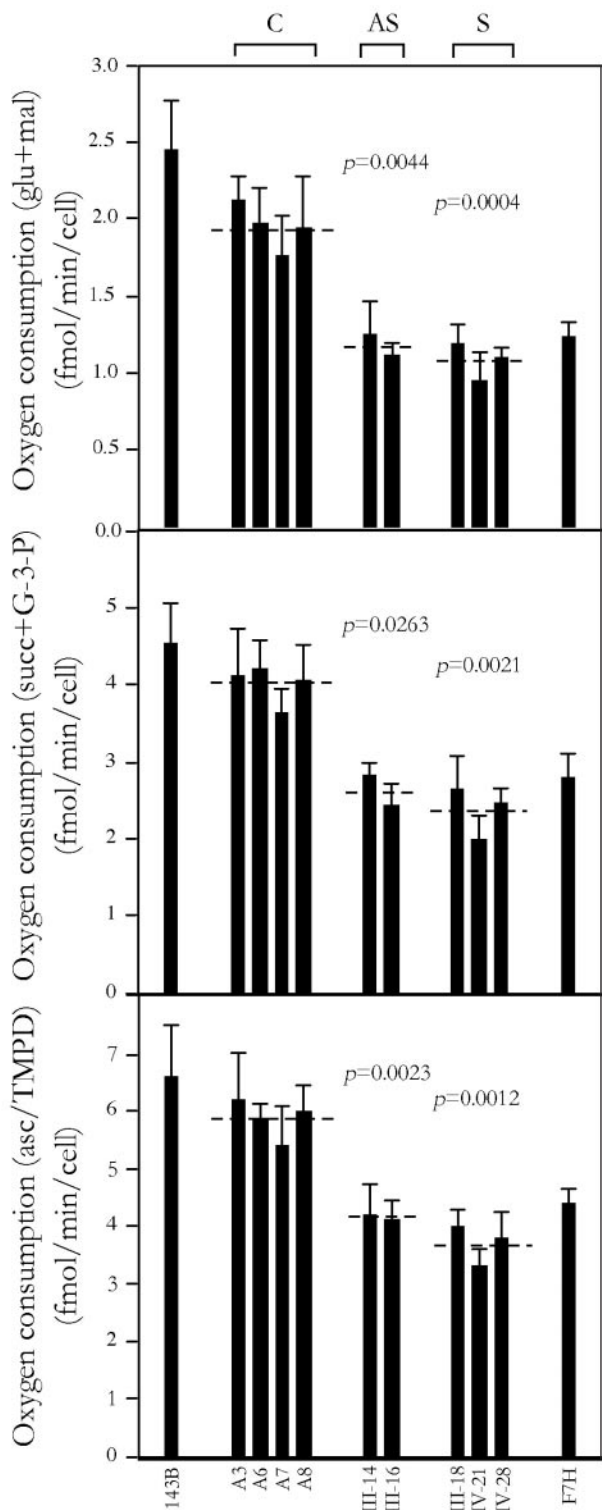
Figure 5. Polarographic analysis of O$_2$ consumption in digitonin-permeabilized cells of the various cell lines using different substrates and inhibitors. The activities of the various components of the respiratory chain were investigated by measuring on ~5 × 10^6 digitonin-permeabilized cells the respiration dependent on malate/glutamate, on succinate/G3P and on TMPD/ascorbate. A total of 2–4 determinations were made on each of the three cybrid cell lines derived from each donor lymphoblastoid cell line. Graph details and symbols are explained in the legend to Figure 3. mal/glu, malate/glutamate-dependent respiration; succ/G-3-P, succinate/G3P-dependent respiration; and asc/TMPD, TMPD/ascorbate-dependent respiration.

(41,42), was very significantly decreased, relative to the average rate in the control cell lines, by 39–51% (~44% on the average; $P = 0.0004$). Similarly, the rate of succinate/glycerol-3-phosphate (G3P)-driven respiration, which depends on the activities of Complexes III and IV, but usually reflects the rate-limiting activity of Complex III, was significantly affected, relative to the average rate in the control cell lines, by 34–50% (~41% on the average; $P = 0.0021$); furthermore, the rate of N,N,N',N'-tetramethyl-p-phenylene-diamine (TMPD)/ascorbate-driven respiration, which reflects the activity of Complex IV, exhibited a 32–44% reduction in Complex IV activity (~37% on the average; $P = 0.0012$). The corresponding rates in cybrids derived from asymptomatic individuals were also reduced relative to those from the control cybrids. These decreases were only slightly lower than those observed in the cybrids derived from symptomatic individuals, i.e. 36–43%, with ~39% on the average ($P = 0.0044$), for malate/glutamate promoted respiration; 29–39%, with ~34% on the average ($P = 0.0263$), for succinate/G3P-promoted respiration; and 28–30%, with ~29% on the average ($P = 0.0023$), for TMPD/ascorbate-promoted respiration. The variations in the rates of malate/glutamate-driven, succinate/G3P-driven and TMPD/ascorbate-driven respiration among the individual mutant cybrids derived from symptomatic subjects, as compared with the individual control cybrids, showed a significant correlation with the corresponding variations in the overall rate of labeling of mitochondrial protein synthesis ($r = 0.98$, $P < 0.001$; $r = 0.97$, $P < 0.001$; $r = 0.98$, $P < 0.001$, respectively). Similarly, the variations in the rates of malate/glutamate-driven, succinate/G3P-driven and TMPD/ascorbate-driven respiration among the individual mutant cybrids derived from asymptomatic subjects, as compared with the individual control cybrids, showed a significant correlation with the corresponding variations in the overall rate of labeling of mitochondrial protein synthesis ($r = 0.99$, $P < 0.001$; $r = 0.98$, $P < 0.001$; $r = 0.98$, $P < 0.001$, respectively).

Growth properties of cybrid cell lines after treatment with aminoglycoside

To examine the role of the C1494T mutation in the effect of aminoglycoside on the cell growth, three cybrids derived from each donor of five individuals carrying the C1494T mutation and from each donor of four controls lacking the mutation were grown in DMEM medium in the presence of aminoglycoside paromomycin or in its absence for four days. As shown in Figure 6, in the presence of 2 mg paromomycin per ml, the growth rates of the mutant cybrids carrying the C1494T mutation exhibited a significant/uniform average decrease, relative to the growth rates of controls cybrids. In particular, in the mutant cybrids carrying the C1494T mutation, the ratios of DTs in the presence and absence of paromomycin in the mutant cell lines were increased by 29–33%, with an average of 30.5%, relative to the average DT ratio in control cybrids ($P < 0.0001$). This DT ratio in the C1494T mutation-carrying cells was comparable with that of cybrid cell line F7H carrying the A1555G mutation. From these data, we conclude that the C1494T mutation plays a critical role in the sensitivities to the aminoglycoside of the growth rate of the cybrid cell lines derived from matrilineal relatives of this Chinese family.
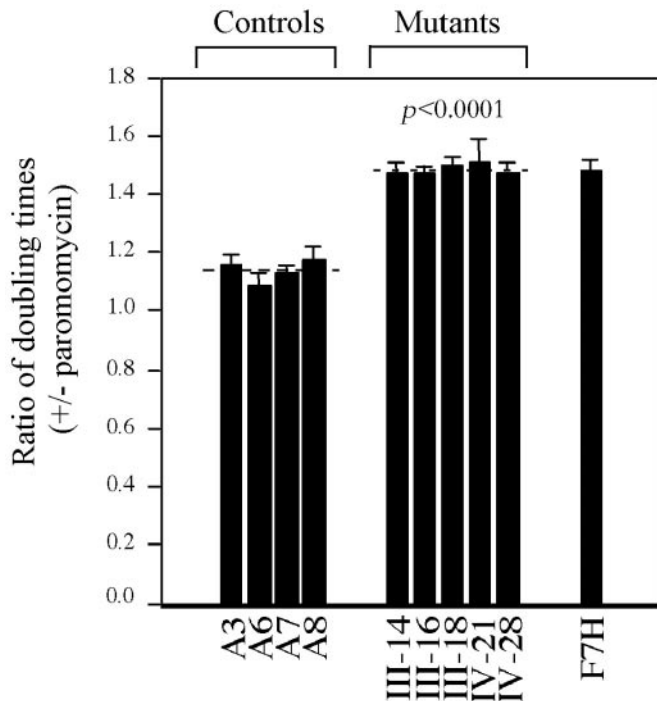
Figure 6. Growth properties of cybrid cell lines. The population DTs during 4 days of growth was determined in the DMEM medium in the presence and absence of paromomycin. The ratios of DTs in the presence and absence of 2 mg paromomycin per ml are shown. A total of 4–6 determinations were made on each of the three cybrids derived from each donor lymphoblastoid cell line. Graph details and symbols are explained in the legend to Figure 3.

DISCUSSION

In the present study, we have investigated the pathogenetic mechanism of the deafness-associated mitochondrial 12S rRNA C1494T mutation in a large Chinese family using osteosarcoma cell cybrids. Under a constant nuclear background, cybrids derived from either symptomatic or asymptomatic mitochondria donors of the Chinese family exhibited nearly identical average degrees of mitochondrial dysfunction. In particular, the very significant decrease in the rate of mitochondrial protein synthesis was observed in mutant cybrids derived from three symptomatic or two asymptomatic individuals, as compared with the average rate in control cybrids. These data strongly suggested that the C1494T mutation is the primary factor responsible for impairing mitochondrial protein synthesis. Furthermore, there was a very significant correlation between the rate of mitochondrial protein synthesis, and overall respiratory capacity or the rate of malate/glutamate promoted respiration, or succinate/G3P-promoted respiration, or TMPD/ascorbate-promoted respiration in the control and mutant cybrids derived from either symptomatic or asymptomatic individuals. Indeed, ∼40% decreased rate of mitochondrial protein synthesis caused by the C1494T mutation appears to be responsible for a significant reduction in respiration. These reduced levels in cybrids carrying the C1494T mutation are comparable with ∼35 and ∼37% average reduction in the rate of mitochondrial protein labeling observed in cybrids derived from matrilineal relatives of an Arab–Israeli family carrying the A1555G mutation (35). However, ∼50% decrease in the rate of mitochondrial protein synthesis was proposed to

be a threshold level to produce the deafness phenotype associated with the A1555G mutation (36). Thus, the reduced rate of mitochondrial protein synthesis in cybrids carrying the C1494T mutation appears to be below the proposed threshold level to support a normal respiratory phenotype (36). The threshold level supporting a normal respiratory phenotype was consistent with the evidence that a reduction of >50–60% in the copy number of the tRNA genes comprised within the 5 kb mtDNA deletion associated with chronic external ophthalmoplegia (43), or with the Pearson syndrome (44), or a 50–60% decrease in aminoacylation capacity of the mitochondrial tRNA^{Lys} associated with the MERRF-associated A8344G mutation (45) produces a dramatic decrease in the mitochondrial translation rate and respiratory capacity. In this study, the fact that mild biochemical defects detected in mutant cybrids cells may explain why only a portion of subjects with the C1494T mutation developed hearing impairment in this Chinese family (25). These data suggest that the C1494T mutation alone is not sufficient to produce the clinical phenotype and other factors including nuclear modifier genes modulate the phenotypic manifestation of this mutation.

Our previous data indicated that the C1494T mutation led to the sensitivity to aminoglycosides (25). Strikingly, the very significant/nearly identical increase in the ratio of DTs in DMDM medium in the presence/absence of high concentration of paromomycin was observed in cybrids derived from three symptomatic and two asymptomatic individuals carrying the C1494T mutation, as compared with the average rate in control cybrids. These results were in contrast to the fact that there was a considerable variability in sensitivity to aminoglycosides in lymphoblastoid cell lines derived from two asymptomatic members and four symptomatic members of the Chinese family carrying the C1494T mutation (25). In fact, the reduced growth rate of mutant cybrids carrying the C1494T mutation in media containing paromomycin is most probably due to a worsening of mitochondrial translation and subsequent respiration defect, as in the case of the cells carrying the A1555G mutation (18,32). The observation that variable degrees of sensitivity to aminoglycosides in lymphoblastoid cell lines derived from asymptomatic and symptomatic members of the Chinese family was significantly reduced by the transferring of mitochondria from matrilineal relatives into the constant nuclear background of an mtDNA less cell line is the first direct evidence that the nuclear background plays a critical role in aminoglycoside toxicity associated with the C1494T mutation. These data also strongly indicated that sensitivity to aminoglycoside in cybrids under a constant nuclear background is fully dependent on the presence of the mitochondrial 12S rRNA C1494T mutation.

Table 1 shows the aminglycoside sensitivity and mitochondrial defects detected in the previous studies of cybrids carrying the A1555G mutation and the present work for cybrids carrying the C1494T mutation. In our previous investigation, there were almost identical decreases of mitochondrial protein labeling observed in cybrids derived from five symptomatic and from three asymptomatic individuals of the Arab–Israeli family carrying the A1555G mutation (35). In the present work, similarly, almost identical average reductions in the rate of mitochondrial protein synthesis have been found in cybrids derived from three symptomatic and from two asymptomatic individuals of the Chinese family carrying

Table 1. Mitochondrial protein labeling rate, respiration rate and DT ratio in the presence and absence of paromomycin of cybrid cell lines carrying the A1555G and C1494T mutations

| | Average percentage related to control | | | | | |
| | Cell lines carrying the A1555G mutation[a,b] | | | Cell lines carrying the C1494T mutation | | |
	AS	S	*P*	AS	S	*P*
Mitochondrial protein labeling rate	65 ± 7	63 ± 8	0.7209	61 ± 9	57 ± 6	0.5375
Total O_2 consumption rate	67 ± 4	62 ± 6	0.1677	65 ± 3	59 ± 7	0.2275
Malate/glutamate-dependent respiration rate	59 ± 11	49 ± 8	0.2013	60 ± 6	55 ± 8	0.3844
Succinate/G3P-dependent respiration rate	68 ± 4	66 ± 6	0.6599	66 ± 9	59 ± 10	0.3872
COX-dependent respiration rate	69 ± 5	68 ± 6	0.9981	70 ± 2	63 ± 7	0.1656
DT ratio (± paromomycin)	128 ± 7	127 ± 6	0.7518	129 ± 1	131 ± 2	0.1687

[a]Guan *et al.* (35).
[b]Q. Yan and M.X. Guan, unpublished data.

the C1494T mutation. A comparison of the behavior of the other parameters tested in cybrids carrying the A1555G mutation and the C1494T mutation revealed the same trend and comparable degrees of mitochondrial dysfunction and sensitivity to aminoglycosides when compared with those of controls. Therefore, our data provide the strong biochemical evidence that the C1494T mutation is the novel pathogenic mtDNA mutation associated with both aminoglycoside-induced and non-syndromic hearing loss.

In summary, our findings convincingly demonstrate the pathogenic mechanism leading to an impaired oxidative phosphorylation and aminoglycoside toxicity in cybrids carrying the C1494T mutation. A significant decrease in the rate of mitochondrial protein synthesis, produced by the C1494T mutation, is most probably a primary contributor for respiratory phenotype in mutant cells, consequently resulting in a decline in ATP production in the cochlear cells, which are essential for the hearing function. However, incomplete penetrance of hearing loss and mild biochemical defects observed in this investigation strongly indicated that the C1494T mutation itself is not sufficient to produce a hearing-impaired phenotype. Aminoglycosides modulate the phenotypic expression of the C1494T mutation. Furthermore, nuclear background plays a critical role in the phenotypic manifestation of non-syndromic hearing loss and aminoglycoside toxicity associated with the C1494T mutation.

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