Mechanistic Study on the Nuclear Modifier Gene MSS1 Mutation Suppressing Neomycin Sensitivity of the Mitochondrial 15S rRNA C1477G Mutation in Saccharomyces cerevisiae

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Abstract

The phenotypic manifestation of mitochondrial DNA (mtDNA) mutations can be modulated by nuclear genes and environmental factors. However, neither the interaction among these factors nor their underlying mechanisms are well understood. The yeast Saccharomyces cerevisiae mtDNA 15S rRNA C1477G mutation (P^R) corresponds to the human 12S rRNA A1555G mutation. Here we report that a nuclear modifier gene mss1 mutation suppresses the neomycin-sensitivity phenotype of a yeast C1477G mutant in fermentable YPD medium. Functional assays show that the mitochondrial function of the yeast C1477G mutant was impaired severely in YPD medium with neomycin. Moreover, the mss1 mutation led to a significant increase in the steady-state level of HAP5 (heme activated protein), which greatly up-regulated the expression of glycolytic transcription factors RAP1, GCR1, and GCR2 and thus stimulated glycolysis. Furthermore, the high expression of the key glycolytic enzyme genes HXK2, PFK1 and PYK1 indicated that enhanced glycolysis not only compensated for the ATP reduction from oxidative phosphorylation (OXPHOS) in mitochondria, but also ensured the growth of the mss1(P^R) mutant in YPD medium with neomycin. This study advances our understanding of the phenotypic manifestation of mtDNA mutations.

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Introduction

The phenotypic expression of mitochondrial DNA (mtDNA) mutations can be modulated by nuclear genes and environmental factors [1–5]. In the yeast Saccharomyces cerevisiae the mtDNA 15S rRNA C1477G mutation corresponds to the human mtDNA 12S rRNA A1555G mutation [6–9]. In this ideal model of the C1477G mutant, we and others have shown that the presence of additional mutations in the nuclear modifier genes mss1, or MTO1, or MTO2 causes the double mutant to exhibit a respiratory-deficient phenotype [8,10–14]. In addition, it has been shown that the yeast C1477G mutant is resistant to the aminoglycoside neomycin and paromomycin in a non-fermentable YPG medium. This mutation has therefore also been called P^R or P^R^1554 (paromomycin resistance) mutation [15,16]. Together, these studies identify the essential roles of both nuclear modifier genes and environmental factors in mediating the phenotypic manifestation of the yeast mtDNA C1477G mutation.

To date, the interaction among mtDNAs, nuclear genes and environmental factors remains poorly understood. Recently we demonstrated that the yeast C1477G mutant was sensitive to aminoglycoside neomycin in a fermentable YPD medium, and that the nuclear modifier gene mto2 mutation suppressed the C1477G mutant’s phenotypic expression [17]. In Saccharomyces cerevisiae, the products of MTO2 and MSS1 are involved in the biosynthesis of the hypermodified nucleoside 5-methyl-amino-methyl-2-thio-uridine (mm^5^U^3^4) in the wobble position of mitochondrial tRNAs [10,19]. MTO2p is responsible for 2-thiolation of the U34 nucleotide, while MSS1p is involved in the C5 modification of 5-carboxymethylaminomethyluridine (cmcm^5^U^3^4) [19,20]. One aim of this study was to examine whether the nuclear modifier gene MSS1 modulates the phenotypic manifestation of the yeast C1477G mutant in a YPD medium with neomycin.

The primary limiting factor for growth and reproduction of all biological systems is energy. The energy of cellular bioenergetic systems originates mainly from OXPHOS and glycolysis [21]. Organisms can adjust their metabolism to ensure energy supply and facilitate their growth or survival. A good example is mitochondrial defects can be compensated by an increase of glycolysis in cells for ATP production [22–25]. However, the underlying mechanisms of this metabolic shift (mitochondrial to glycolytic transcription factors RAP1, GCR1, and GCR2, and thus stimulated glycolysis. Furthermore, the high expression of the key glycolytic enzyme genes HXK2, PFK1 and PYK1 indicated that enhanced glycolysis not only compensated for the ATP reduction from oxidative phosphorylation (OXPHOS) in mitochondria, but also ensured the growth of the mss1(P^R) mutant in YPD medium with neomycin. This study advances our understanding of the phenotypic manifestation of mtDNA mutations.

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glycolytic) are far from understood. In yeast, the HAP (heme activated protein) complex Hap2/3/4/5p is involved with the respiratory metabolism [26]. The overexpression of Hap4p in aerobic glucose-grown cultures has been reported to positively affect the balance between respiration and glycolysis and towards respiration [27–28]. However, at low specific growth rate deletion of HAP4 had little or no effect compared with the wild-type strain on glucose [33]. Genome-wide genetic analysis has revealed that HAP5 has a genetic interaction with the glycolytic transcription factor gene GCR1 [34]. GCR1 has been demonstrated to play a central role in the regulation of glycolysis among the three glycolytic transcription factors RAP1, GCR1 and GCR2 [35]. Whether HAP5 is involved with the mitochondrial to glycolytic shift remains poorly understood.

Here we show that the nuclear modifier gene mss1 mutation suppresses the neomycin-sensitivity phenotype of the yeast C1477G mutant. We also reveal that glycolysis increasingly compensates for the mitochondrial dysfunction of the mss1[P50] mutant and insures its growth, in a similar manner to that of the mss1[PS] mutant [17]. Moreover, we found that the mss1[P50] strain expressed a significantly high level of HAP5. On the basis of the above evidence, yeast strains with disruption or overexpression of HAP5 were constructed. These strains were characterized by examining growth properties and the gene expression of the glycolytic transcription factors. Thus we identified that in the mss1[P50] mutant the increase of glycolysis could be mediated by HAP5.

Materials and Methods

Yeast strains and culture conditions

The original strains were of the W303-1B strain (MSS1[P50], α, ade2-1, his3-15,1-leu2-3,112, trp1-1, ura3-1) [12] and the M12-54 strain (MSS1[P50], a, ilv5, trp2 [p5, P154]) [11]. The mss1[P50] (α, ade2-1, his3-15,1-leu2-3,112, trp1-1, ura3-1, mss1::HIS3) and mss1[P50] (α, his3-1-15,1-leu2-3,112, ura3-1, mss1::HIS3 [P154]) strains were constructed by modification as previously described [8,12,20]. Standard procedures were used for crossing and selecting diploids, including sporulation and the dissection tetrads [8,12,20]. Similarly, the ΔHAP5/MSS1(P50) (α ade2-1, his3-1-15,1-leu2-3,112, trp1-1, ura3-1, mss1::HIS3), ΔHAP5/MSS1(P50) (a ilv5, trp2, hap5::ILV5 [p5, P154]), and the ΔHAP5/mss1[P50] (α ade2-1, his3-1-15,1-leu2-3,112, trp1-1, ura3-1, mss1::HIS3, hap5::URA3) strains were constructed from the M12-54 strain. The pDB20-ILV5 plasmid was constructed by inserting the full-coding region of ILV5 into pDB20-HAP5 plasmid.

All yeast strains were cultured on YPD medium at 30°C overnight until they reached the exponential growth phase. The cultures were harvested and diluted serially to spot on the YPD plates containing neomycin, or neomycin with 2-DG. The plates were incubated at 30°C for 3 days and photographs were taken.

For the growth curve analysis, strains were cultured in liquid YPD both in the absence and presence of 30 μg/ml neomycin and with a starting cell density of 0.01 OD600. The growth conditions were monitored by measuring the OD600 value every two hours for the first 24 hours.

Oxygen consumption assay

The oxygen consumption rate (OCR) measurements were carried out as described elsewhere with minor modifications [22]. Yeast cells were cultured overnight at 30°C in a YPD medium until they reached the exponential growth phase. The cell density was adjusted and a 100 μl medium containing cells was seeded in precoated Poly-D Lysine (50 μg/ml) XF 38-well microplates (Seahorse Bioscience) at 4×10^3 cells per well and then spun down. Following incubations at 30°C for 30 min, the oxygen consumption rate was measured according to the manufacturer’s manual on a Seahorse XF96 Extracellular Flux Analyzer in the absence or presence of 30 μg/ml neomycin.

Northern blotting analysis

Total cellular RNA was obtained from yeast cultures (2.0×10^7 cells) using a TRizol Reagent (Invitrogen) according to the manufacturer’s instructions. Equal amounts (10 μg) of total RNA were separated by electrophoresis through 1.5% formaldehyde denaturing agarose gel, transferred onto a positively charged membrane (Roche Applied Science) and hybridized with a DIG-labeled HXK2-specific antisense RNA probe. The blot was then stripped with stripping buffer (50% formamide, 50 mM Tris/HCl, pH7.5, 5% SDS) and hybridized using PFK1 and PKI1 probes, respectively. Finally, the blot was hybridized with a nuclear encoded ACT1 probe as an internal control [38]. The probes were synthesized from the corresponding plasmid linearized by restriction enzymes using a DIG RNA labeling kit (Roche Applied Science). The plasmids used for preparing the probes were constructed by PCR-amplifying fragments of HXK2 (positions 24094-24972), PFK1 (positions 971431-970785), PKI1 (positions 71983-73069), and ACT1 (positions 54696-54187), and then cloning the fragments into the pCRII-TOPO vector carrying the SP6 and T7 promoters (Invitrogen).

Quantitative real-time RT-PCR assay

After the total cellular RNA was isolated, as mentioned above, the RNase-free DNase (Takara Bio Inc., Japan) was added to eliminate DNA contamination according to the manufacturer’s instructions. One micromgram total RNA was used for the first-strand cDNA synthesis using a PrimeScript RT reagent Kit (Takara). 100 ng cDNA for each sample was used to analyze gene expression with SYBR Premix EX Taq (Takara). The PCR primers specific for each gene are listed below (5’-3’): HXK2-F, TGCGGCTGGACTCAAAACCCTCACT; HXK2-R, CGATGATACCAACGGACTTACCT; PFK1-F, TGTCTGAAAGCAAAGAAGGTTAAG; PFK1-R, GAATCTCAGACTGACTGCATGG; PKI1-F, CCCATCCGGCCACAGAAACCC; PKI1-R, TCTTACACAGGGATGACCTT; RAP1-F, GTGTAACAAACTACGCGCTTCG; RAP1-R, ACTTACACGCCGTGGTCTACTAAT; GCR1-F, CAGGAATAGTGCGGACCCCAATG; GCR1-R, TAGAAAAGCTCTGCGGAAATGATG; GCR2-F, TGGATGAGGAAAGGGTGG; GCR2-R, ACTTCATATTGGGTGTATTCGC; HAP5-F, HAP5-R, CGATGATACCAACGGACTTACCT.
TTGGTCAGGGATTGGTGGG, HAP5-R, CGGATTCTCG-CAAATGGTAAG; and ACT1-F, TACTCTTTCTCCAC-CACTGCTGA, ACT1-R, CTTGACCATCTGGAAGTTCG.

Statistical analysis

All experiments were repeated at least three independent times and the representative data was presented as means ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed to determine the significance between groups. *P<0.05, **P<0.01.

Results

Growth properties of yeast strains

The growth properties of yeast strains were determined on a YPD medium in the absence or presence of neomycin (Fig. 1). All of the four strains, MSS1(P^S), mss1(P^S), MSS1(P^R) and mss1(P^R), grew well on YPD agar plates and no significant difference in growth was observed. On the YPD plates with 300 μg/ml neomycin, the growth of MSS1(P^R), the strain carrying the mtDNA C1477G mutation alone, was remarkably suppressed. Interestingly, the growth of the double mutant strain mss1(P^R) changed slightly (Fig. 1A).

Figure 1. Growth properties of yeast strains. (A) Series dilutions of the four yeast strains MSS1(P^S), mss1(P^S), MSS1(P^R) and mss1(P^R) were spotted on YPD plates in the absence (−) or presence (+) of 300 μg/ml neomycin and incubated at 30 °C. The experiment was performed three times with similar results. (B–E) Growth curves of the four yeast strains. Cells from MSS1(P^S), mss1(P^S), MSS1(P^R) and mss1(P^R) strains were cultured in the absence or presence of 30 μg/ml neomycin-supplemented YPD medium and the cell density was determined at different times. Error bars represent standard error from three independent determinations.

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confirmed in a liquid YPD medium without or with 30 μg/ml neomycin (Fig. 1B–E), which is the 90% minimal inhibitory concentration for the MSS1(Pk) strain [17]. When cultured in a YPD liquid medium with neomycin for 16 hours, the growth of the MSS1(Pk) strain was severely inhibited and its OD₆₆₀ value was only about 0.193, whereas the mss1(Pk) strain’s OD₆₆₀ value reached about 1.701. Meanwhile, the OD₆₆₀ values for the MSS1(Ps) and mss1(Ps) strains were about 1.987 and 1.910, respectively.

This data indicates that the yeast carrying the mtDNA C1477G mutation (Pk) was sensitive to neomycin, while the nuclear modifier gene mss1 deletion suppressed this phenotypic manifestation.

Mitochondrial respiratory rates
The oxygen consumption rates (OCRs) of relative strains were measured to determine the yeast mitochondrial function in vivo (Fig. 2). The OCR level of the MSS1(Pk) strain decreased significantly (P<0.01) from 1.153 fMoles/min/cell in YPD medium to 0.467 fMoles/min/cell in neomycin-supplemented YPD medium. This represents a reduction of about 59.5%. The OCR of the mss1(Pk) strain was very low both in the YPD medium (0.184 fMoles/min/cell) and in neomycin-supplemented YPD medium (0.179 fMoles/min/cell). This data indicates that the interaction between neomycin with the mitochondrial 13S rRNA C1477G mutation (Pk), or between the mss1 deletion with the mitochondrial 15S rRNA C1477G mutation can lead to a mitochondrial respiratory defect. The nuclear modifier gene mss1 deletion may suppress the aminoglycoside-sensitivity of the mtDNA C1477G mutation through inducing other energy compromising pathways, rather than by improving mitochondrial respiration.

Effect of 2-DG on the growth properties of yeast strains
2-DG, as a glucose analogue, competitively inhibits hexokinase and blocks the glycolytic pathway [39]. The double mutant mss1(Pk) strain cannot grow on a YPD medium supplemented with 2.5 mM 2-DG, or 2.5 mM 2DG and 300 μg/ml neomycin, and incubated at 30°C. The experiment was performed three times with similar results. doi:10.1371/journal.pone.0090336.g002

Transcriptional expression of key glycolytic enzyme genes
Northern blots were used to evaluate the transcript abundance for the three key glycolytic enzymes HXK2, PFK1, and PKAI. In a YPD medium, the transcriptional expression of HXK2 and PFK1 in the mss1(Pk) strain was significantly higher than that of MSS1(Pk) strain. In the YPD with neomycin medium, the transcript abundance of all three key glycolytic enzyme genes elevated in the mss1(Pk) double mutant. The PPK1, PKAI and HXK2 transcript expression, increased by about 101.3% (P<0.01), 143.5% (P<0.01) and 12.3% (P<0.05) as compared with that in MSS1(Pk) strain, respectively (Fig. 4B–D). To more clearly and quantitatively confirm the changes of HXK2, PFK1, and PKAI expression, we performed the additional experiments by RT-qPCR analysis. In YPD medium, quantitative RT-PCR showed a significant increase of HXK2 and PFK1 mRNA in mss1(Pk) strain compared with that of MSS1(Pk) strain. In the YPD with neomycin medium, the transcriptional level of PPK1, PKAI and HXK2 in mss1(Pk) strain significantly increased in contrast to that of MSS1(Pk) strain, respectively. This was consistent with Northern blot assay and was shown in Fig. S1 A-C. These data suggests that the nuclear modifier gene mss1 mutation can lead to an increase of glycolytic capacity for a strain carrying the mtDNA C1477G mutation.

Transcriptional expression of glycolytic transcription factors
The transcripts of glycolytic transcription factors RAP1, GCR1 and GCR2 were analyzed by quantitative RT-PCR. No matter whether they were in YPD medium or YPD medium with neomycin, the expression levels of RAP1, GCR1 and GCR2 in the mss1(Pk) strain remained significantly higher than that of the MSS1(Pk) strain. On the other hand, with the exception of GCR2, neomycin did not increase the expression levels in the mss1(Pk) strain. Neomycin simply decreased the levels of RAP1 and GCR1 in the MSS1(Pk) strain (Fig. 5). This data indicates that the nuclear mss1 and mitochondrial C1477G mutations act synergistically in increasing the basal levels of RAP1, GCR1 and GCR2.
Gene expression of HAP5

The transcript level of HAP5 in the yeast strains was determined by quantitative RT-PCR. The HAP5 transcription level of the mss1(PR) strain increased significantly compared with that of MSS1(PR) by about 233.6% (P < 0.01) in the YPD medium, and 232.9% (P < 0.01) in the YPD with neomycin medium (Fig. 6). This data indicates that there was a positive correlation between the transcriptional expression of HAP5 with that of glycolytic genes.

Effects of HAP5 on the neomycin sensitivity of mss1(PR) mutant

On neomycin-supplemented YPD plates, the overexpression of HAP5 in the mss1(PR) strain resulted in a reduced sensitivity to neomycin compared to the mss1(PR) strain (Fig. 7A). Moreover, after the cells were cultured in a neomycin-supplemented YPD medium for 16 h, the cell density of the mss1(PR) strain with the overexpression of HAP5 increased significantly (P < 0.01) by about 40%, in contrast to that of the mss1(PR) strain (OD600 value 2.304 versus 1.702) (Fig. 7C). These results indicate that HAP5 has a positive effect on the glycolysis of the mss1(PR) strain in neomycin-supplemented medium. In contrast to the MSS1(PR) strain, the knockout of HAP5 in the MSS1(PR) strain further enhanced its neomycin sensitivity (Fig. 7B), where the cell density decreased significantly (P < 0.01) and reduced by about 57.9% in neomycin-supplemented YPD medium after 16 h of culture (OD600 value 0.190 versus 0.08) (Fig. 7C). Meanwhile, the strain with the MSS1, mtDNA C1477G and HAP5 mutations was not viable from the haploid mss1(PR) strain. These results suggest that the knockout of HAP5 has a negative effect on the glycolysis of yeast strains with the mtDNA C1477G mutation. Collectively, the above results indicate that the elevated glycolysis of the mss1(PR) strain in neomycin-supplemented YPD medium could be mediated by HAP5.

Gene expression of glycolytic transcription factors in generated HAP5 yeast strains

We then detected the expression of glycolytic transcription factors by quantitative RT-PCR in the generated HAP5 yeast strains.
strains. As shown in Fig. 8B and C, in contrast to the MSS1\(^{(PR)}\) strain, the knockout of HAP5 in the MSS1\(^{(P)}\) strain led to the expression of the glycolytic transcription factors GCR1 and GCR2 being decreased significantly (P<0.01) in a YPD medium by about 70.4% and 34.3%, respectively. This suggests that HAP5 is important for the expression and regulation of glycolytic transcription factors in the MSS1\(^{(P)}\) strain. In addition, the expressions of RAP1, GCR1 and GCR2 were lower in the MSS1\(^{(P)}\) and A/hAP5/MSS1\(^{(P)}\) strains compared to the other yeast strains in a neomycin-supplemented YPD medium (Fig. 8D–F). Compared to that of the mss1\(^{(P)}\) strain, the overexpression of HAP5 in the mss1\(^{(P)}\) strain elevated significantly the expressions of RAP1, GCR1 and GCR2 by about 60.3%, 34.7% and 11.2% in neomycin-supplemented YPD medium (Fig. 8D–F), respectively, as well as that of RAP1 and GCR2 by about 6.7% and 19.5% in YPD medium (Fig. 8A and C), respectively. This data suggests that HAP5 can up-regulate the expression of the glycolytic transcription factors RAP1, GCR1, and GCR2 and stimulate the glycolysis of the mss1\(^{(P)}\) strain in a neomycin-supplemented medium.

Discussion

mss1 mutation suppressed the neomycin sensitivity of yeast C1477G mutant due to glycolysis increase

Both of our recent works ([17] and present study) clearly showed that in neomycin-supplemented YPD medium, the greatly impaired mitochondrial function of the yeast C1477G mutant was a major cause for its neomycin-sensitivity phenotypic expression [Fig. 2, 3]. The mitochondrial function of the mss1\(^{(P)}\) strain was further impaired when compared with the MSS1\(^{(P)}\) strain in neomycin-supplemented YPD medium (Fig. 2), suggesting in mss1\(^{(P)}\) less ATP was produced from the mitochondria. Previous studies have demonstrated that in yeast the transcription factors RAP1, GCR1 and GCR2 are central to glycolytic gene regulation, and that they act synergistically to allow a high-level of gene expression and promotion of glycolysis [35,40–42]. In the glycolytic pathway, hexokinase (HXK) is involved in the first regulatory step, phosphofructokinase (PFK) in the catalyzing rate-limiting step and pyruvate kinase (PYK) in the final step. These three enzymes catalyze their corresponding irreversible reactions [43,44]. The up-regulation of HXX2, PFK1 and PK1 genes can promote glycolysis in yeast [43,45]. Our results showed that the expression of those three glycolytic transcription factors had significantly increased in the mss1\(^{(P)}\) strain, in contrast to MSS1\(^{(P)}\), no matter whether in YPD or YPD plus neomycin medium (Fig. 5). This was consistent with the expressions of HXX2, PFK1 and PK1 (Fig. 4), acting to enhance glycolysis for energy supply. Moreover, the growth of the mss1\(^{(P)}\) strain could be completely inhibited by 2-DG or a 2-DG and neomycin mixture (Fig. 3). This firmly supported the understanding that the strain depends on the glycolytic pathway to supply energy. Therefore, the nuclear modifier gene mss1 mutation suppressed the neomycin-sensitivity of the mtDNA C1477G mutant was mainly through a stimulation of glycolysis.

Figure 5. Gene expression of glycolytic transcription factors. Yeast cells were cultured in the absence or presence of 30 µg/ml neomycin for 16 h and harvested. Then RNA was extracted for quantitative RT-PCR analyses. The relative gene expression of RAP1 (A), GCR1 (B) and GCR2 (C) was normalized to the average content per cell of ACT1. Values are expressed as percentages of the average values for the wild-type strain MSS1\(^{(P)}\). Samples from at least three independent cultures of RNA content and ACT1 for each strain were used in the calculations. *P<0.05, **P<0.01. doi:10.1371/journal.pone.0090336.g005

Figure 6. HAP5 gene expression. Yeast cells were cultured in the absence or presence of 30 µg/ml neomycin for 16 h and harvested. RNA was then extracted for quantitative RT-PCR analyses. The relative gene expression of HAP5 was normalized to the average content per cell of ACT1. Values are expressed as percentages of the average values for the wild-type strain MSS1\(^{(P)}\). Samples from at least three independent cultures of RNA content and ACT1 for each strain were used in the calculations. *P<0.05, **P<0.01. doi:10.1371/journal.pone.0090336.g006
HAP5 mediated glycolysis increase of the mss1(P8) mutant

In this study, the strains with the disruption or overexpression of HAP5 were constructed to study the shift between respiration and glycolysis. However, we cannot get the ΔHAP5/mss1(P8) deletion strain through six more independent experiments yet, probably this strain was lethal.

The mss1(P8) strain nearly depended on glycolytic metabolism to ensure its growth. mss1(P8) expressed the high level of HAP5 and maintained consistently with the high level of glycolysis (Fig. 2 and 3). The disruption of HAP5 in mss1(P8) strain was probably lethal, while the overexpression of HAP5 in mss1(P8) increased cell growth and the levels of glycolytic transcription factors (Fig. 7 and Fig. 8), which indicated that HAP5 may play an essential role in the anaerobic respiration. In contrast, the MSS1(P8) strain mainly depended on mitochondrial respiration for its growth. MSS1(P8) expressed the low level of HAP5 and maintained consistently with the low level of glycolysis (Fig. 2 and 3). The disruption of HAP5 in

Figure 7. Growth properties of yeast strains with the overexpression or knockout of HAP5. (A) Growth properties of wild-type (WT) HAP5 yeast strains MSS1(P8), mss1(P8), MSS1(P8), mss1(P8) and their individual HAP5 overexpression (OE) strains. (B) Growth properties of WT HAP5 yeast strains MSS1(P8), mss1(P8), MSS1(P8) and their individual HAP5 knockout (KO) strains. Series dilutions of the yeast strains were spotted on YPD plates in the absence (−) or presence (+) of 300 μg/ml neomycin and incubated at 30°C. The experiment was performed three times with similar results. (C) The yeast cells were cultured in 30 μg/ml neomycin-supplemented YPD medium for 16 h and the cell density was determined. Error bars represent standard error from three independent determinations. **P<0.01. doi:10.1371/journal.pone.0090336.g007
MSS1(PS) strain decreased remarkably the expression of GCR1 and GCR2, while the overexpression of HAP5 in MSS1(PS) did not increase cell growth and the levels of glycolytic transcription factors (Fig. 7 and Fig. 8), which indicated that HAP5 did not acts as an important factor in aerobic respiration. These data suggested that HAP5 may play a key role to maintain the glycolysis.

Taken together, the comparison between the MSS1(P) and the mss1(P) strains in neomycin-supplemented medium, showed that the nuclear modifier gene mss1 mutation led to a significant increase in the steady-state level of HAP5, which greatly up-regulated the expression of glycolytic transcription factors and thus stimulated glycolysis. Glycolysis then generated sufficient ATP to compensate for the energy reduction due to the mitochondrial dysfunction. Therefore, the nuclear modifier mss1 mutation suppressed the neomycin-sensitivity of the yeast C1477G mutant. This study demonstrates that a mtDNA mutation integrates a nuclear gene with environment factors to mediate its phenotypic manifestation. These findings provide a novel insight for us to better understand the phenotypic expression of mtDNA mutations.

Supporting Information

Figure S1 Quantitative RT-PCR analysis of key glycolytic enzyme genes. Yeast cells were cultured in the absence or presence of 30 μg/ml neomycin for 16 h and harvested. RNA was then extracted for quantitative RT-PCR analyses. The relative gene expression of HAP5 (A, D), GCR1 (B, E) and GCR2 (C, F) of strains was normalized to the average content per cell of ACT1. Values are expressed as percentages of the average values for the wild-type strain MSS1(PS) in YPD medium. Samples from at least three independent cultures of RNA content were used in the calculations. *P<0.05, **P<0.01.

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Author Contributions

Conceived and designed the experiments: QZ QY. Performed the experiments: QZ WW XH X. Zhu YS ZY XQ MF X. Zhang YD SY. Analyzed the data: QZ WW QY. Contributed reagents/materials/analysis tools: QY. Wrote the paper: QZ QY.
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