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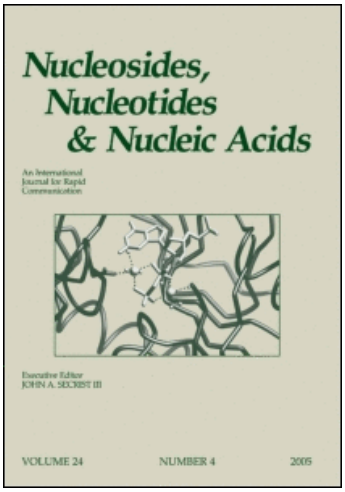

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Thymidine Phosphorylase Deficiency Causes MNGIE: An Autosomal Recessive Mitochondrial Disorder

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Thymidine Phosphorylase Deficiency Causes MNGIE: An Autosomal Recessive Mitochondrial Disorder

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ABSTRACT

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disorder caused by mutations in the gene encoding thymidine phosphorylase (TP). The disease is characterized clinically by impaired eye movements, gastrointestinal dysmotility, cachexia, peripheral neuropathy, myopathy, and leukoencephalopathy. Molecular genetic studies of MNGIE patients' tissues have revealed multiple deletions, depletion, and site-specific point mutations of mitochondrial DNA. TP is a cytosolic enzyme required for nucleoside homeostasis. In MNGIE, TP activity is severely reduced and consequently levels of thymidine and deoxyuridine in plasma are dramatically elevated. We have hypothesized that the increased levels of intracellular thymidine and deoxyuridine cause imbalances of mitochondrial nucleotide pools that, in turn, lead to the mtDNA abnormalities. MNGIE was the first molecularly characterized genetic disorder caused by abnormal mitochondrial nucleoside/nucleotide

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metabolism. Future studies are likely to reveal further insight into this expanding group of diseases.

Key Words: Mitochondria; Mitochondrial DNA; MNGIE; Thymidine phosphorylase; Thymidine and deoxyuridine.

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INTRODUCTION

Mitochondria are unique mammalian organelles because they are the products of two genomes: nuclear DNA (nDNA) and mitochondrial DNA (mtDNA), a 16.6 kilobase circular molecule. MtDNA encodes only 37 genes: 13 polypeptides, two ribosomal and 22 transfer RNAs that are required for normal mitochondrial respiratory chain enzyme activities.[1] In addition, nDNA encodes at least 74 polypeptide subunits of the mitochondrial respiratory chain enzymes, as well as all of the machinery required for the maintenance and replication of mtDNA. Because of the dual genetic control of mitochondria, mutations in either genome can cause mitochondrial diseases.

In 1988, the first mutations of mtDNA were associated with human diseases; large-scale deletions of mtDNA were identified in patients with progressive external ophthalmoplegia (PEO, impaired eye movements) and soon thereafter, a mtDNA point mutation was linked to another eye disease, Leber hereditary optic neuropathy (LHON), a maternally inherited form of vision loss.[2–4] More recently, mutations of nDNA have been identified as causes of mitochondrial diseases with impaired respiratory chain activities.[5] Among these autosomal disorders of mitochondria are the defects of intergenomic communication. These diseases are due to primary mutations of nDNA that cause quantitative (depletion) or qualitative (multiple deletions and point mutations) abnormalities of mtDNA.[6,7] Mitochondrial NeuroGastroIntestinal Encephalomyopathy (MNGIE), an autosomal recessive disorder of intergenomic communication is due to mutations in the gene encoding thymidine phosphorylase (TP).[8,9]

MATERIAL AND METHODS

The chromosomal locus and causative gene for MNGIE were identified by molecular genetic linkage studies and candidate gene sequencing.[8,9] TP gene mutations were detected by DNA sequencing.[9] TP activity was measured in buffy coat using a fixed-time spectrophotometric assay.[9] Plasma thymidine (deoxythymidine or dThd) and deoxyuridine (dUrd) were assessed using high-performance liquid chromatography with an ultraviolet detector.[10,11] MtDNA abnormalities were detected by Southern blot, DNA sequencing, and restriction fragment length polymorphism analyses.[7,9] Patients were evaluated clinically by neurologists.[12–14]

RESULTS

MNGIE is a multisystem autosomal recessive disease. Clinically, the defining features are: 1) progressive external ophthalmoplegia typically with ptosis, 2) severe

gastrointestinal dysmotility, 3) cachexia, 4) peripheral neuropathy, 5) diffuse leukoencephalopathy on brain MRI, and 6) evidence of mitochondrial dysfunction (histological, biochemical, or genetic abnormalities of the mitochondria).[12,13] The age at onset ranges from 5 month to 43 years (average 18.5) and the average age of death is 37.6 years (range 26–58).[13]

Skeletal muscle biopsies of MNGIE patients have revealed neurogenic changes, ragged-red and cytochrome *c* oxidase-deficient fibers reflecting the neuropathy and mitochondrial myopathy although in at least one patient, the skeletal muscle was morphologically normal.[13,15] The peripheral neuropathy is predominantly demyelinating, but electrophysiological data have also shown evidence of axonopathy.[13,16] In the gastrointestinal system, histological studies have revealed abnormalities of both the intestinal smooth muscle and the enteric nervous system.[13,17]

In addition to the histological and biochemical evidence of mitochondrial impairment, analyses of patients' skeletal muscle mtDNA by Southern blot have revealed depletion and multiple deletions.[12,13,18] Subsequently, we identified site-specific somatic point mutations in mtDNA of several tissues and cultured fibroblasts from MNGIE patients.[7] Of the 36 mtDNA point mutations we documented, 31 (86%) were T-to-C transitions and of these, 25 were preceeded by 5′-AA sequences.[7] In addition, we identified a single-base pair deletion and a TT-to-AA mutation.[7] The abnormalities of mtDNA led us to hypothesize that the nDNA defects responsible for MNGIE impair mtDNA replication, repair, or both.

Mapping of the disease locus to chromosome 22q13.32-qter[8] enabled us to identify mutations in the gene encoding TP as the cause of MNGIE.[13] To date, we have identified 30 different mutations in 39 different families with the disease; all affected individuals have been either homozygotes or compound heterozygotes for *TP* mutations.[14] All MNGIE patients have virtually complete loss of TP activity. Figure 1 shows the distributions of enzyme activities in MNGIE, carriers and controls.[10] These results indicated that only mutations producing complete or almost complete loss of function of TP are pathogenic. Carriers of *TP* mutations have ~30% TP activity relative to healthy controls; this observation is consistent with the fact that TP is a homodimer. The

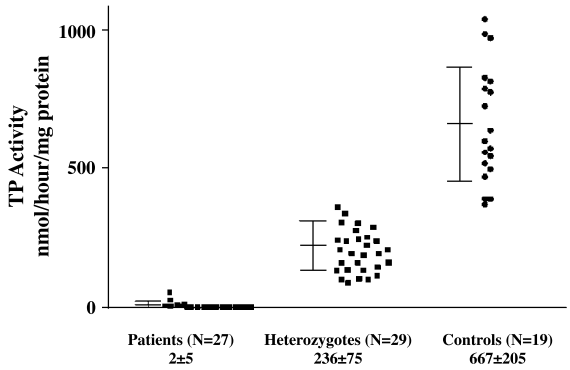

Figure 1. Thymidine phosphorylase (TP) activities in buffy cost of MNGIE patients. TP mutation carriers and controls. Lines indicate average ± standard deviation. Originally published in Ref. [10].

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Table 1. Biochemical alterations resulting from *TP* mutations in MNGIE.[a]

	Plasma			Urine
	TP activity[b] (nmol/hour/mg prot)	dThd (μM)	dUrd (μM)	Ratio of urinary elimination of dThd[c]
Controls	667 ± 205 (N = 19)	<0.05 (N = 23)	<0.05 (N = 20)	ND[d]
Carriers	236 ± 75 (N = 29)	<0.05 (N = 14)	<0.05 (N = 14)	ND[e]
MNGIE	2 ± 5 (N = 27)	8.6 ± 3.4 (N = 25)	14.2 ± 4.4 (N = 25)	20% (N = 2)

Data were originally reported in Refs. [10] and [11].
[a]Results expressed as mean ± SD.
[b]In buffy coats.
[c]Referred to urinary elimination of creatinine.
[d]Undetectable thymidine in urine of controls.
[e]Not determined.

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values of TP activity in carriers also indicate that reductions of 70% in TP activity are not pathogenic, since all heterozygotes are asymptomatic.

Circulating levels of dThd and dUrd were undetectable (<0.05 μM) in both healthy controls and *TP* mutation carriers, while circulating concentrations of both nucleosides are highly increased in MNGIE patients (dThd = 8.6 ± 3.4 μM, mean ± standard deviation, n = 25; dUrd = 14.2 ± 4.4 μM, n = 25) (Table 1).[10,11]

DISCUSSION

TP (EC 2.4.2.4), also known as platelet-derived endothelial cell growth factor and gliostatin,[19] catalyzes the phosphorolysis of pyrimidine deoxynucleosides to the corresponding bases and deoxyribose-1-phosphate. TP is a homodimer with a molecular weight of 90 kDa.[19,20] TP activity catalyzes the phosphorolysis of both Thd and dUrd with similar efficiencies.[21] In humans, TP is abundantly present in blood platelets and placenta and also in lymph node, spleen, liver, lung and peripheral lymphocytes, but is not expressed in skeletal muscle although this tissue is clearly affected in MNGIE.[9,20]

The absence of detectable dThd and dUrd in normal subjects strongly suggests that intracellular and extracellular levels of dThd and dUrd are largely regulated by TP. Abundant activities of TP in circulating platelets and blood cells catabolize circulating dThd and dUrd contributing to low intracellular levels of these nucleosides, particularly in tissues with negligible expression of TP such as skeletal muscle.

Loss of TP enzyme function in MNGIE causes generalized alterations of nucleoside metabolism, as summarized in Table 1. One factor contributing to the elevated levels of nucleosides is their inefficient elimination in urine. dThd is ultrafiltratable by hemodialysis[10] and presumably by the renal glomeruli, but is probably reabsorbed by

the tubules in the kidney, as indicated by the low urinary clearance of the nucleoside. Consequently, dThd and dUrd are retained in blood.

The generalized alteration of dThd and dUrd metabolism accounts for some of the phenotypic features of MNGIE. As noted above, in MNGIE skeletal muscle has abnormal mitochondria, despite the fact that TP is not expressed in skeletal muscle.[9,13] This ''muscle paradox'' can be explained by the high extracellular concentrations of dThd and dUrd which elevate the concentrations of these nucleosides in the muscle cells, either through direct incorporation of the nucleosides from extracellular space, or by precluding cellular elimination of nucleosides through plasma membrane transporters.

The accumulation of dThd and dUrd is directly linked to the lack of TP activity in MNGIE; however, the connection between this enzymatic alteration and its specific effects on mtDNA is not readily apparent.[7,12,18] Two factors that may contribute to the selective vulnerability of mtDNA relative to nDNA are: 1) the greater dependence of mtDNA replication on the thymidine salvage pathway compared to nDNA synthesis, which relies on the *de novo* pathways for nucleotide synthesis and 2) the lack of an effective mitochondrial mismatch repair system.[7,22–24]

High concentrations of dThd are likely to increase levels of thymidine triphosphate (dTTP) in mitochondria, compromising the normal maintenance of mtDNA. A factor contributing to the altered mitochondrial dNTP pools is the existence of two different forms of thymidine kinase (TK), the enzyme responsible for the phosphorylation of dThd.[25] The cytosolic form, TK1, is only expressed in proliferating cells in contrast to the mitochondrial form, TK2, which is constitutively expressed.[25] This difference could also contribute to the specific toxic effect of excess dThd on mitochondrial dTTP pool rather than on the nuclear dTTP in post-mitotic cells like muscle (Fig. 2).

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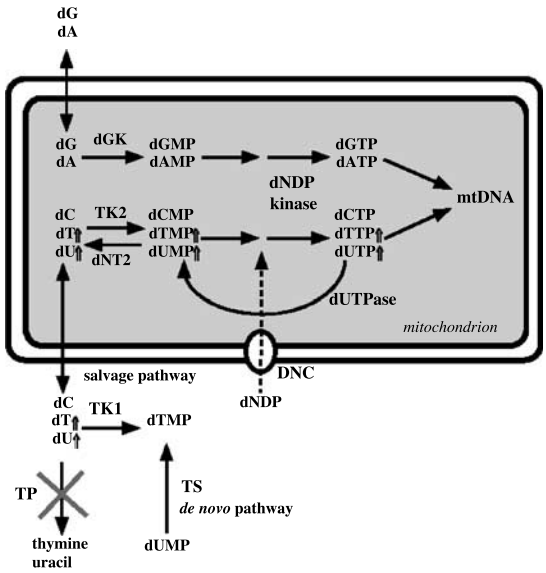

Figure 2. Biochemical alterations in MNGIE.

Like dThd, dUrd at high levels is also likely to affect mitochondria preferentially. During cell division, TK1 can phosphorylate dUrd, and the resultant deoxyuridine monophosphate (dUMP) can be transformed into dTMP through the *de novo* synthesis enzyme thymidylate synthase (TS), or can be further phosphorylated to dUDP and dUTP; however, dUTPase cycles dUTP back to dUMP[26] (Fig. 2). Consequently, nuclear accumulation of dUTP is unlikely due to the activity of TS and dUTPase. By contrast, mitochondria lack TS, therefore, dUTPase provides the only mechanism to prevent the accumulation of mitochondrial dUTP. In the presence of high levels of dUrd and the constitutive expression of its phosphorylating enzyme, TK2, dUTPase activity may be insufficient to prevent accumulations of dUTP in mitochondria.

As previously noted, we have identified of site-specific somatic point mutations in mtDNA in MNGIE.[7] These point mutations, almost exclusively in a residue followed by a poly-T run, can be explained as a consequence of the ''next-nucleotide effect'', which has been observed in polymerase γ (pol γ) mediated DNA replication in vitro.[27,28] High concentrations of a specific deoxynucleoside triphosphate (dXTP) produce an acceleration of pol γ when replicating poly-X sequences, because of the increased availability of the substrate dXTP. This acceleration compromises the proofreading activity of pol γ (exonuclease-3′ activity) thereby promoting mutations at the 5′-nucleotide preceding the poly-X row. In MNGIE, high concentrations of dTTP (and dUTP) are likely to promote point mutations in positions 5′ before a poly-T segment as we have observed.[7]

In contrast to the proposed specific mechanisms for generating mtDNA point mutations in MNGIE, it is more difficult to postulate mechanisms to account for the other mtDNA alterations (depletion and multiple deletions). One reason for this difficulty is the lack of information about the composition of nucleotides within mitochondria. Although it is reasonable to assume that dTTP and dUTP concentrations are increased, the actual nucleotide pool imbalances in MNGIE are not known yet and other dNTP levels could be altered. As suggested by Wang and Eriksson, decreased mitochondrial levels of dCTP may cause mtDNA depletion in MNGIE.[29] Determination of the mitochondrial concentrations of dNTPs in MNGIE will undoubtably lead to a more complete understanding of the physiopathology of this unique disease.

Over the last four years, defects in nuclear genes involved in the mitochondrial metabolism of nucleotides have been identified as causes of several other human diseases. Mutations in the gene encoding ANT1, the heart- and skeletal muscle-specific isoform of the ADP/ATP translocator present in the inner mitochondrial membrane cause adPEO with multiple mtDNA deletions.[30] In addition, mutations in the genes encoding TK2 cause a myopathic type of mtDNA depletion syndrome (MDS)[31] while a hepatocerebral form of MDS caused by mutations in dGK.[32] Mutations in the gene encoding the mitochondrial deoxynucleoside carrier (DNC) have been associated with in patients of Amish origin with a severe autosomal recessive congenital microcephaly.[33] DNC transports deoxynucleotides across the mitochondrial inner membrane,[34] therefore, defects of DNC may alter mitochondrial nucleotide pools.

Mitochondrial toxicity observed due to nucleoside analogue therapy in patients with viral infections is another example indicating that alterations of nucleoside or nucleotide metabolism can impair mtDNA replication and produce mtDNA depletion.[35–37] Inhibition of pol γ leading to mtDNA depletion has been associated with zidovudine and other nucleoside analogue therapy in HIV-infected patients[35–37] and treatment of chronic hepatitis B with the nucleoside analogue fialuridine caused severe

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toxicity in patients, with lactic acidosis, hepatic failure hepatic steatosis, skeletal and cardiac myopathy, peripheral neuropathy, and pancreatitis due to inhibition of mtDNA replication.[38,39]

MNGIE was the first inherited mitochondrial disorder to be attributed to defects in metabolism of the substrates DNA synthesis. Over the last five years, significant progress in the biochemical and molecular characterization of this disease has been achieved. In addition, mutations in several additional genes encoding factors regulating nucleosides/nucleotides have been reported as causes of mitochondrial diseases. Homeostasis of the nucleosides and nucleotides, both at the extracellular and intracellular levels, is extremely important for the mitochondrial function, and will continue to be an area of active and productive research.

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