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Invited Minireview

Revolution in mitochondrial medicine

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Abstract A revolution in chemical pathology occurred about 40 years ago with the discovery of a patient with mitochondrial dysfunction. The field of mitochondrial medicine has experienced explosive growth during the last decade. More than 50 mtDNA mutations and several nuclear gene mutations have been identified in affected patients. The recent development of animal models will continue the revolution in mitochondrial medicine by facilitating in depth studies of the molecular pathogenesis and development of novel drug and gene therapy strategies for mitochondrial dysfunction. As we enter the next millennium, we can expect mitochondrial medicine to remain a dynamic and rapidly developing field.

Key words: Mitochondrial dysfunction; Nuclear gene mutation; Ageing; Animal model

1. Introduction

Mitochondrial dysfunction is increasingly recognized as an important cause of human pathology [1–3]. The discovery of the first patient with mitochondrial disease in 1962 started a revolution in chemical pathology [4]. During the next decades, large numbers of patients with biochemical and morphological evidence of respiratory chain dysfunction were described. In 1988, a critical break-through toward our understanding of mitochondrial diseases came with the discovery of pathogenic deletions and point mutations in mtDNA [5,6]. Clinical phenotypes correlated with the presence of specific mtDNA mutations, and important insights into the pathogenetic mechanisms were obtained. Now, there are more than 50 disease-causing base substitution mutations and hundreds of mtDNA rearrangements known [3,7–9]. This brief review focuses on recent important advances in this rapidly developing area of mitochondrial medicine, e.g. nuclear gene mutations causing mitochondrial diseases in man, mitochondrial involvement in ageing and common human diseases and mitochondrial dysfunction in animal models.

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Abbreviations: ANT, adenine nucleotide translocator; CoQ, coenzyme Q; COX, cytochrome *c* oxidase; mtDNA, mitochondrial DNA; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SOD, superoxide dismutase; Tfam, mitochondrial transcription factor A

2. Mitochondria and mitochondrial DNA

The ancestors of mitochondria were probably ancient bacteria that lived as intracellular parasites in primitive eukaryotic cells [10]. Several rounds of reductive evolution deleted or transposed most of the original bacterial genome to the nucleus, and transformed the intracellular parasite into an organelle heavily dependent on the nucleus [10].

Mitochondria may constitute a dynamic cellular network. A mitochondrion is composed of four compartments, i.e. the outer membrane, the intermembrane space, the folded inner membrane and the matrix. The respiratory chain is embedded in the inner membrane of the mitochondrion. It produces ATP through the process of oxidative phosphorylation. The respiratory chain consists of ~100 different proteins distributed in five respiratory chain enzyme complexes: complex I (NADH dehydrogenase), complex II (SDH), complex III (cytochrome *c* reductase), complex IV (COX) and complex V (ATP synthase). Complexes I–IV constitute the electron transport chain that generates a proton gradient across the inner membrane used by complex V to drive ATP synthesis.

Nass and Nass [11] discovered mtDNA in 1963. The complete sequences of mouse [12] and human mtDNA [13] were published in 1981, pioneering subsequent achievements in genome sequencing. Mammalian mtDNA is a circular molecule of ~16 kb that is present in thousands of copies per cell [14]. Usually, all mtDNA copies are identical, referred to as homoplasmy. However, in some instances two or more mtDNA types may coexist within the same cell, referred to as heteroplasmy. Inheritance of mtDNA is strictly maternal, and a female harboring a heteroplasmic mtDNA mutation may transmit widely varying levels of mutated mtDNA to her offspring.

The displacement (D-) loop region is the only long non-coding region in mtDNA. It contains the promoters for transcription of both mtDNA strands and the initiation site for mtDNA replication [14]. Transcription produces polycistronic transcripts corresponding to both mtDNA strands. Processing of the primary transcripts releases the 13 mRNAs, 22 tRNAs and two rRNAs. All 13 proteins encoded by mtDNA are key components of the respiratory chain. Thus, mtDNA expression is critical for maintaining oxidative phosphorylation. The vast majority of the ~1000 different mitochondrial proteins are encoded by nuclear genes. This includes all proteins needed for mtDNA replication and transcription and for mitochondrial translation, the majority of the respiratory chain subunits, all proteins constituting the protein import machinery, and those performing other metabolic functions in mitochondria. Hence, mutations of nuclear genes may affect a wide variety of mitochondrial functions.

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3. Nuclear gene mutations causing mitochondrial dysfunction

The vast majority of the ~100 respiratory chain subunits are encoded by nuclear genes, and mutations in them can affect any of the five respiratory chain enzyme complexes (Table 1). An amino acid substitution of the flavoprotein subunit of SDH was found in two siblings with necrotizing encephalopathy (Leigh syndrome) and complex II deficiency [15]. Duplication within a nuclear gene encoding the 18 kDa protein of complex I was observed in a child with severe encephalopathy and complex I deficiency [16]. Mutations in a nuclear gene (*NDUFV1*) encoding a 51 kDa complex I subunit were found in children with leukodystrophy and myoclonus epilepsy [17]. A subgroup of patients with Leigh syndrome has COX deficiency and harbors mutations in the *SURF1* gene [18,19]. Studies of yeast mutants demonstrated that the *SURF1* homologue of yeast (*SHY1*) is required for respiration, and may regulate assembly or stability of the COX holoenzyme [20].

Nuclear genes encode all proteins needed for maintenance and transcription of mtDNA and all proteins needed for translation of mitochondrial transcripts. Patients with autosomal recessive, mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), have multiple deletions of mtDNA in muscle [21]. Mutations in the thymidine phosphorylase gene exist in multiple MNGIE pedigrees suggesting that aberrant thymidine metabolism leads to mtDNA instability [22]. Several families with autosomal dominant progressive external ophthalmoplegia (adPEO) have been identified, and responsible genes mapped to chromosomes 10q23.3–24.3 [23] and 3p14.1–21.2 [24]. Interestingly, the distribution of the multiple mtDNA deletions is non-random in affected patients, and clonal expansion of a single type of mtDNA deletion is found in affected muscle fiber segments [25]. Several children with mtDNA depletion and fatal lactic acidosis have been described [26]. Most evidence suggests autosomal recessive inheritance, but no locus has yet been identified.

Mutations of the *DPP1* gene are found in an X-linked syndrome causing sensorineural hearing loss, dystonia, cortical blindness and other symptoms. The DPP1 protein is homologous to the yeast Tim8, Tim9, Tim10 and Tim12 proteins, which are components of the mitochondrial protein import machinery. Thus, this syndrome is probably caused by defective protein import, impairing respiratory chain function [27].

Patients with autosomal recessive spastic paraplegia have mutations in the paraplegin gene, encoding a mitochondrial protease belonging to the ATP-dependent metalloproteinase family [28]. An increased frequency of ragged-red muscle fibers is found in affected patients indicating that paraplegin deficiency impairs respiratory chain function [28].

Friedreich's ataxia is a common autosomal recessive disease caused by an intronic triplet expansion in the frataxin gene [29]. Clinical features include progressive gait and limb ataxia, dysarthria and cardiomyopathy. The function of frataxin is probably to transport iron out of the mitochondria. Mutations of the yeast frataxin homologue (*YFH1*) cause iron accumulation in mitochondria [30]. Interestingly, excessive amounts of mitochondrial iron will lead to increased formation of reactive oxygen species (ROS), which preferentially attack iron-sulfur (Fe-S) cluster containing proteins such as aconitase and the respiratory chain enzyme complexes I, II and III in affected patients [31].

4. Ageing and mitochondrial dysfunction

Several lines of correlational and genetic data implicate mitochondrial dysfunction in the naturally occurring process of ageing. In 1986, Miquel and Fleming [32] presented the 'oxygen radical-mitochondrial injury hypothesis of ageing'. It states that the raised respiration of differentiated cells will increase ROS production to levels exceeding the detoxifying defenses. This will impair the cell's capacity to regenerate mitochondria and result in progressive deterioration of ATP production. Animals with homozygous knockout of the mitochondrial isoform of SOD (MnSOD or *Sod2*) develop dilated cardiomyopathy [33], demonstrating that impaired mitochondrial defense to ROS indeed has grave consequences. Iron-sulfur containing enzymes are particularly vulnerable to ROS attacks and, as a consequence, *Sod2*−/− mice develop a deficiency of SDH and aconitase [33], similar to the biochemical phenotype of patients with Friedreich's ataxia. Treatment with a SOD mimetic chemical compound increases the life span of *Sod2*−/− mice dramatically and prevents cardiomyopathy [34]. However, these treated mutant mice develop a neurological phenotype, possibly due to failure of the

Table 1
Nuclear mutations affecting mitochondrial biogenesis

Mutated gene	Biochemical consequence	Phenotype of patients	Reference
Flavoprotein subunit of complex II	complex II deficiency	Leigh syndrome	[15]
18 kDa complex I subunit	complex I deficiency	Severe encephalopathy	[16]
51 kDa complex I subunit (NDUFV1)	complex I deficiency	leukodystrophy and myoclonus epilepsy	[17]
SURF1	complex IV deficiency, defective stability of complex IV	Leigh syndrome	[18,19]
Thymidine phosphorylase	thymidine phosphorylase deficiency, multiple mtDNA deletions	mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)	[22]
Unknown	multiple mtDNA deletions	progressive external ophthalmoplegia (adPEO)	[23,24]
Unknown	mtDNA depletion	fatal infantile myopathy	[26]
DPP1	defective mitochondrial protein import	sensorineural hearing loss, dystonia, cortical blindness	[27]
Paraplegin	metalloproteinase deficiency, defective mitochondrial protein turnover	spastic paraplegia	[28]
Frataxin	defective mitochondrial iron homeostasis, complex I, II and III deficiency	progressive gait and limb ataxia, dysarthria and cardiomyopathy	[30,31]

drug to cross the blood-brain barrier [34]. Mutations of one of the cytoplasmic SOD isoforms (CuZnSOD or *SOD1*) have been reported in familial amyotrophic lateral sclerosis, a disease with extensive loss of motor neurons [35]. Transgenic overexpression of human *SOD1* in motor neurons of *Drosophila* extends the life span of these transgenic flies by 40% [36]. These findings suggest that antioxidant defenses, at least under some circumstances, may determine cell survival and life span.

Numerous reports demonstrate that deleted mtDNA accumulates in certain tissues with increasing age. This may lead to an age-related reduction in oxidative phosphorylation capacity [37]. Indeed, some studies demonstrate an age-related reduction in respiratory chain function [38], but others find no correlation between age and respiratory chain function [39]. Levels of deleted mtDNA in old individuals are low ($< 10\%$), but such low levels will not impair respiratory chain function if the deleted mtDNA is evenly distributed. However, uneven distribution resulting in accumulation of deleted mtDNA in particular cells may lead to a mosaic respiratory chain deficiency and pathology.

In vitro cell cultures of cybrids between human cells lacking mtDNA (ρ^0 cells) [40] and mitochondria from young and old subjects demonstrate respiratory chain deficiency in cybrids containing mitochondria from older subjects [41]. In contrast, other cybrid experiments show that nuclear rather than mtDNA mutations are responsible for the observed age-related reduction of oxidative phosphorylation [42].

Genetic studies suggest that respiratory chain function may regulate life span in the worm *Caenorhabditis elegans*. The *mev-1* mutant is hypersensitive to increased oxygen concentrations, and its life span is dramatically decreased in response to high oxygen [43]. The *mev-1* mutation affects a SDH subunit and severely impairs electron transport [43]. Mutations of the *clk-1* gene moderately extend life span in *C. elegans* [44] and the yeast homologue of this gene (Coq7) has been found to be involved in different metabolic pathways, e.g. CoQ synthesis [45]. There are dual roles for CoQ in vivo; it is an essential component for respiratory chain electron transport and also functions as a lipid soluble antioxidant. It is possible that the decreased CoQ synthesis in the *clk-1* mutants slows the metabolic rate in *C. elegans* and that this explains the prolonged life span [45]. It has also been speculated that the *clk-1* mutation leads to the disappearance of CoQ semiquinone, which has a prooxidant activity, and that loss of this compound explains the extended life span in mutant worms [46]. Interestingly, a *clk-1* mutation combined with a *daf-2* mutation, affecting another genetic pathway, dramatically prolongs the life span of *C. elegans* demonstrating that regulation of longevity is polygenic [45].

5. Animal models for mitochondrial diseases

The genetics of mitochondrial disorders has revealed much about the disease mechanisms. Recently, new insights into the pathophysiology of these disorders have been obtained from animal models.

An animal model for mitochondrial diseases was generated by knockout of the *Ant1* gene. Homozygous knockout animals (*Ant−/−*) are viable, but exhibit increased levels of blood lactate, mitochondrial myopathy and hypertrophic cardiomyopathy with mitochondrial proliferation [47].

Disruption of the*Tfam* gene demonstrated that this nuclear-encoded protein is necessary for mtDNA maintenance in vivo [48]. Homozygous *Tfam* knockouts die in midgestation and have a mutant phenotype with lack of heart, delayed neural development and absence of optic discs. The mutant embryos lack mtDNA, and have a severe respiratory chain deficiency with massive accumulation of morphologically abnormal mitochondria [48]. Recently, the *cre-loxP* conditional knockout strategy was used to selectively disrupt *Tfam* in heart and skeletal muscle [49]. These mutant animals develop dilated mitochondrial cardiomyopathy with biochemical, morphological and physiological features that are similar to the findings in mtDNA deletion diseases [49].

Heteroplasmic mice containing two different types of mtDNA were created by fusing cytoplasts from oocytes of one mouse strain with fertilized one-cell stage embryos from another [50]. Mating of these heteroplasmic animals demonstrates that the segregation of the two types of mtDNA is determined by random genetic drift in the female germline [50]. Directional selection with increasing age is noted in adult animals; one type of mtDNA increased in liver and kidney, whereas the other type of mtDNA increased in spleen and blood [51]. Introducing pathogenic mtDNA mutations into mice remains unaccomplished.

A novel method for producing animal models of mitochondrial myopathy, recently described by Clark et al. [52], may provide a valuable model for treatment trials. Human myoblasts containing different pathogenic mtDNA mutations were transplanted into irradiated muscle of severe combined immunodeficiency (SCID) mice. Injected myoblasts expressed human muscle markers and were also innervated.

Recent observations using animal models suggest the possibility of introducing mtDNA mutations into the animal germline. This may result in a better understanding of how mtDNA mutations affect energy production and will provide insights into the role such defects play in the pathophysiology of mitochondrial disorders.

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