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Review

Mouse models of oxidative phosphorylation defects: Powerful tools to study the pathobiology of mitochondrial diseases

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ABSTRACT

Defects in the oxidative phosphorylation system (OXPHOS) are responsible for a group of extremely heterogeneous and pleiotropic pathologies commonly known as mitochondrial diseases. Although many mutations have been found to be responsible for OXPHOS defects, their pathogenetic mechanisms are still poorly understood. An important contribution to investigate the in vivo function of several mitochondrial proteins and their role in mitochondrial dysfunction, has been provided by mouse models. Thanks to their genetic and physiologic similarity to humans, mouse models represent a powerful tool to investigate the impact of pathological mutations on metabolic pathways. In this review we discuss the main mouse models of mitochondrial disease developed, focusing on the ones that directly affect the OXPHOS system.

1. Introduction

Mitochondrial disorders are a group of clinically heterogeneous diseases and metabolic syndromes resulting from a dysfunction in the oxidative phosphorylation (OXPHOS) system, the terminal component of energy metabolism in the mitochondria of eukariotic cells. The estimated prevalence for mitochondrial disorders is 1:5000 [1,2], placing them among the most common genetically inherited diseases. The majority of the cellular energy is produced in the form of ATP by the OXPHOS, through the oxidation of organic substrates. The OXPHOS requires the finely coordinated action of five multi-heteromeric enzyme complexes embedded in the inner mitochondrial membrane and of two mobile electron carriers, ubiquinone (Q) and cytochrome *c*. Reducing equivalents, in the form of NADH and $FADH_2$, mostly coming from glycolysis and the Krebs cycle, enter into the mitochondrial respiratory chain by complex I and complex II respectively. From there, through sequential redox reactions, the electrons are translocated to ubiquinone, complex III, cytochrome *c*, complex IV and finally reducing molecular oxygen generating two molecules of water. The electron flow through the respiratory chain is coupled to an active proton translocation across the inner mitochondrial membrane, generated mostly by complexes I, III and IV. The influx of the protons back to the mitochondrial matrix through complex V (ATP synthase) allows the phosphorylation of ADP into ATP [3,4].

The biogenesis of the mitochondrial respiratory chain is extremely complex as it is under the control of both the mitochondrial and the nuclear DNAs (respectively mtDNA and nDNA). Only 13, of the approximately 85 polypeptides, are encoded by mtDNA all of which are integral subunits of the respiratory chain complexes. The rest of the polypeptides are synthesized in the cytoplasm and targeted either co- or post-translationally to the mitochondria [5,6]. Because of this unique and intricate genetics, it is often difficult to identify the etiology of mitochondrial disorders [7]. Moreover, the limited availability of human samples as well as the paucity of large consanguineous families suitable for linkage analyses, further complicates diagnosis. For all these reasons, animal models are powerful tools, not only to better understand the pathophysiology of mitochondrial disorders, but also to develop effective therapies. In this review we will focus on several mouse models of OXPHOS defects that have been created to date, grouping them in three main categories according to the function of the genes that have been manipulated (Table 1).

2. Mouse models for the study of nuclear-coded components of the OXPHOS complexes

In contrast to the high number and well characterized mutations detected in mtDNA (over 150, www.Mitomap.org), the number of mutations affecting nuclear genes that have a pathogenic role in mitochondrial diseases is relatively small. To better characterize the role that some proteins might have in the assembly/stability or in the function of the respiratory complexes, some investigators have developed useful mouse models of OXPHOS dysfunction. Unfortunately, to date, the only complex that has been extensively studied by

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Table 1
Mouse models of OXPHOS defects

Group	Modified gene	Genetics	Tissue affected	Refs.
Nuclear-encoded components of the OXPHOS complexes	COXVIa	KO	Heart	[16]
	Cox10	cd-KO	Muscle	[25]
			Liver	[30]
			Brain	[33]
	Cyt *c*	KO	Testes	[54]
	Surf1	KO	–	[39]
Proteins involved in the interaction and stability of mtDNA	Tfam	cd-KO (rec)	Heart	[79,80]
			Skeletal muscle	[83]
			Pancreatic endocrine tissue	[84]
			Brain	[87,90,91]
	Pol γ	KI (dom)	multiple	[112,113,169]
	Ant1	KO (rec)	Skeletal muscle, heart	[137]
			Extraocular muscle	[145]
	Twinkle	Transgenic (dom)	Skeletal muscle, brain	[131]
	MTERF3	cd-KO	Heart	[99]
mtDNA defects	16S rRNA	ES cybrid	Skeletal muscle, heart	[158]
	Δ mtDNA	Mito inject	Skeletal muscle, heart, kidney	[159]
	ND6ins/ COI	ES cybrid	Skeletal muscle, heart	[162]

cd-KO, KI, knockin conditional knockout; dom, dominant; rec, recessive; ES. embryonic stem cell; mito inject, mitochondrial injection.

mouse models is complex IV. Several attempts to create a mouse model for OXPHOS deficiencies have failed due to the embryonic lethality, so the majority of the studies have been conducted in conditional knockout (KO) mice.

2.1. Cytochrome c oxidase models

Mammalian cytochrome *c* oxidase (COX or complex IV) is composed of 13 polypeptides, 3 of which are encoded by mtDNA (*COX I*, *II*, *III*) and are part of the catalytic core of the enzyme. COX biogenesis has been extensively studied in yeast and is the result of an intricate process, implicating the cooperation of more than 20 additional nuclear encoded proteins [8]. Isolated defects of COX are the most frequent causes of Leigh syndrome (LS), which is a subacute necrotizing encephalomyelopathy. Despite the large number of proteins involved in the formation of the holoenzyme, no mutations have been found in the nuclear encoded structural components of COX, but only in proteins involved in its assembly, such as: *Surf1*, *COX10*, *COX15*, *SCO1*, *SCO2* and *LRPPRC* [9–15].

To address the question of whether structural nuclear encoded proteins could have a protective role by shielding the catalytic core of the enzyme, Radford and colleague have developed a mouse in which the nuclear encoded subunit CoxVIaH was knocked out [16]. In mammals, the CoxVIa subunit is present in two isoforms, heart (H) and liver (L) [17]. During fetal life, the L form is predominant and ubiquitously expressed whereas, after birth, the H isoform completely replaces the former one in striated muscle [18]. The *CoxVIaH* KO (*CoxVIaH*$^{-/-}$) mice displayed a reduction in COX activity to about 23% of the controls. Still, the mice were viable and showed a normal life span. Phenotypically, *CoxVIaH*$^{-/-}$ mice developed a specific myocardial diastolic dysfunction, but the pathogenic mechanisms leading to this phenotype have not been clarified. CoxVIa contains a binding site "sensor" for adenine nucleotides and the level of ATP/ADP can modulate the activity of COX [19]. However, in this model, the disruption of CoxVIa did not affect the activity of COX, as there was no difference in the levels of ATP in the heart of CoxVIaH$^{+/+}$, CoxVIaH$^{+/-}$ and CoxVIaH$^{-/-}$ mice. Conversely, a reduction in the levels of fully assembled complex has been detected in the null mice, suggesting a role for this subunit in the assembly/stability of COX rather than in its regulation.

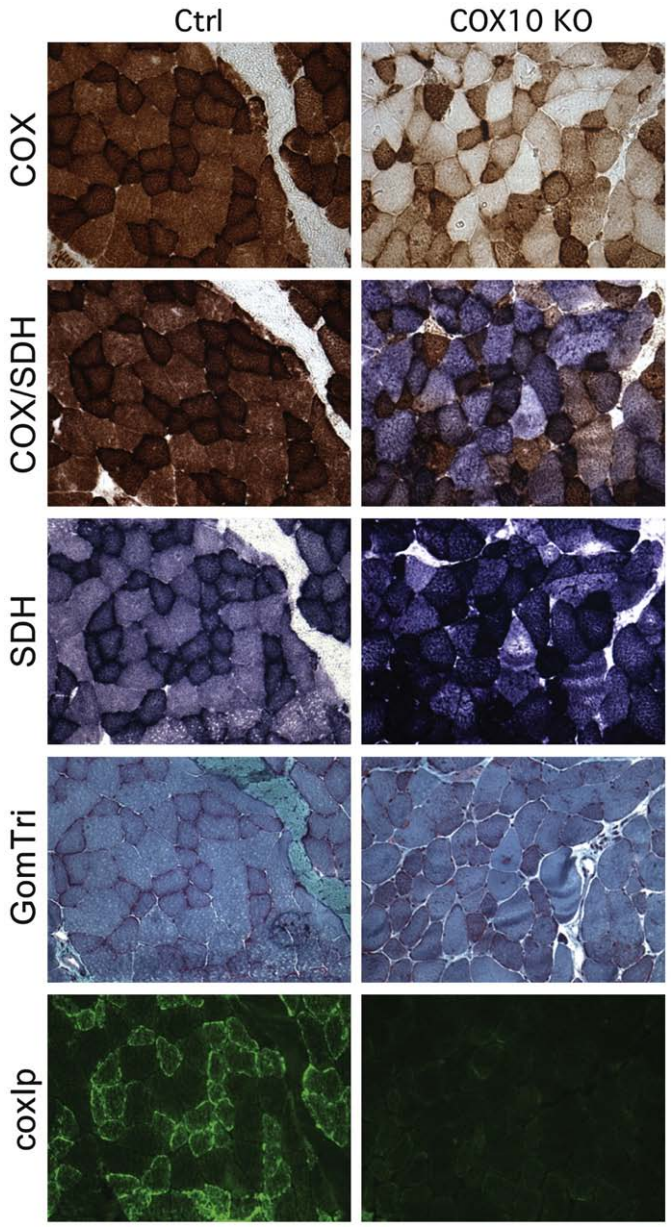

Fig. 1. Muscle pathology from *Cox10* KO (muscle conditional) mice closely resembles human clinical findings. Triceps surae muscle from 3-month-old mice was snap frozen in isopentane cooled in liquid nitrogen. Serial cross sections of 8 μm thickness were stained for cytochrome *c* oxidase activity, combined cytochrome *c* oxidase (COX) and succinate dehydrogenase (SDH) activity, succinate dehydrogenase activity, Gomori Trichrome stain and immunostained with Alexa conjugated COXI monoclonal antibody to show the a COX deficiency and mitochondrial proliferation in muscles of the Cox10 knockout mice. The non-synchronous Cre deletion of the gene reproduces the mosaic pattern observed in patients with mtDNA mutations.

Although several mutations have been described in assembly factors of COX their pathogenic role in humans is still poorly understood. An extensive study, using mouse models, has involved two of the maturation/assembly proteins involved in the biogenesis of COX, namely, COX10 and Surf1. The product of *COX10* is a protoheme: heme-*O*-farnesyl transferase, involved in the biosynthesis of heme *a*,