The requirements and tools to balance differences for the content of the training include general chemistry 35%, general chemistry plus haematology 65%, and flexibility in the remaining 35%, preferably including microbiology and genetics/IVF, work experience, or accredited courses, or relevant exams of national training program, or traineeships.

To have the Common Platform adopted after presenting the platform to the European Commission, the Member States should be consulted, discussing the platform within the Group of Experts. Next a set of draft measures should be submitted to the « Article 54 Committee », and adoption of a European Commission decision should be obtained, which then should be followed-up.

Proposed directive on Services

Health care is presently excluded from the proposed directive on Services in the internal market. European Health Ministers have recently participated in a first round table discussion on Health Services. A broad public consultation was launched that focuses on how to ensure legal certainty for cross-border healthcare under EU law, and to support co-operation between the health systems of the Member States. The Commission will detail proposals in 2007. The objective is to seek views on how a clear legal framework can be ensured within which patients and health care professionals have the chance to move freely in Europe, while at the same time fostering sustainable health care systems. Very recently proposals were launched to re-include health care in the proposed Services directive.

References


Mitochondrial medicine

L.G.J. NIJTMANS¹ and J.A.M. SMEITINK²

Do human disorders as diverse as diabetes, migraine, premature menopause, parkinsonism, blindness, deafness, cancer, cardiomyopathy and encephalopathy have a common denominator? The answer is yes, the above described disease states are just examples of the broad spectrum of clinical signs and symptoms associated with defects in the mitochondrial oxidative phosphorylation (OXPHOS) system. The reason for this large difference in pathology is not clear. Why do certain mutations in the mitochondrial OXPHOS complex II on the one hand lead to Leigh syndrome whereas on the other hand mutations in the same enzyme lead to hereditary paraganglioma? In this review we will not and cannot provide good answers to these questions, however we will highlight two of the aspects of mitochondria which can contribute to the clinical outcome of a defect in this organelle. Firstly, the great complexity of the OXPHOS system requires many genes for its assembly. Secondly, defects in this system will not only lead to decreased ATP production but also execute different cellular metabolic consequences.

Many proteins involved in OXPHOS biosynthesis

In textbooks, mitochondria are often depicted as oval shaped organelles, which are the “power plants” of the cell. In these organelles the main production of ATP, the free currency of energy in a cell, is produced by the OXPHOS system (1). This system comprises five complexes (complex I-V) (Fig. 1) embedded in the mitochondrial inner membrane which are build from numerous peptides, called subunits. These subunits are encoded by both the mitochondrial and nuclear genome. For instance complex I, the largest complex of the OXPHOS system contains 45 subunits of which 7 are encoded by the mitochondrial

Nijmegen Centre for Mitochondrial Disorders, Department of Pediatrics, Radboud University Nijmegen Medical Centre, Nijmegen

Correspondence: dr. L.G.J. Nijtmans, Nijmegen Center for Mitochondrial Disorders, 656 Laboratorium Kindergeneeskunde, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen

E-mail: l.nijtmans@ckz.umcn.nl

genome. In addition the complexes also contain prosthetic groups such as heme, iron-sulphur clusters, flavomononucleotide, copper, zinc and magnesium ions. To assemble these complexes many steps are required and consequently many things can go wrong as illustrated in Figure 2 and Table 1 (2).

Mitochondrial DNA

Part of the subunits of the OXPHOS-system are encoded by the mitochondrial genome. This circular DNA molecule of approximately 16 kilobases is present in multiple copies in a cell. Since the first disease causing mutations were found in 1988, many point mutations and rearrangements have been described to be associated with human disease. Because many laboratories in the world nowadays sequence the whole mitochondrial DNA in the case of a suspected mitochondrial defect, still many new mutations are found. Because mitochondrial DNA is only inherited via the mother the common mode of inheritance of mtDNA disorders is maternal. Nevertheless already in 1989 patients with an autosomal dominant myopathy with lesions in the mitochondrial DNA were described. This means that there are also nuclear mutations possible which result in a defect in the proper maintenance of mitochondrial DNA. At present many nuclear gene mutations have been elucidated, which cause such mitochondrial lesions. These mutations are in genes which are directly involved in mtDNA replication such as polymerase gamma and Twinkle (a helicase) or in genes which are involved in the maintenance of the mitochondrial dNTP pool, the building blocks of the mitochondrial DNA.

Table 1. Examples of gene defects affecting OXPHOS-system assembly. In between brackets the function of the protein is described.

<table>
<thead>
<tr>
<th>Mitochondrial DNA</th>
<th>Mutations in all mitochondrial DNA encoded subunits can be found (see <a href="http://www.mitomap.org/">http://www.mitomap.org/</a>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtDNA maintenance</td>
<td>Polymerase Gamma, Twinkle (mitochondrial replication)/Thymidine phosphorylase Thymidine kinase 2, Adenine Nucleotide translocator, Deoxyguanosine kinase (Maintenance of dNTP pool)</td>
</tr>
<tr>
<td>Mitochondrial translation</td>
<td>Mitochondrial tRNA mutations (see <a href="http://www.mitomap.org)/PUS1">http://www.mitomap.org)/PUS1</a> (tRNA maturation), EFG1, EFTs, EFTu (translation elongation)/MRPS16 (ribosomal protein)</td>
</tr>
<tr>
<td>Quality control</td>
<td>Paraplegin (chaperone/protease)</td>
</tr>
<tr>
<td>Mitochondrial import</td>
<td>DDP1 (import protein)</td>
</tr>
<tr>
<td>Assembly proteins</td>
<td>Surf 1(complex IV chaperone) / B17.2L (complex I chaperone)/BCS1 (complex III chaperone)</td>
</tr>
<tr>
<td>Nuclear encoded subunits</td>
<td>NDUF8, NDUFV1 (complex I subunit) / SDHA (complex I subunit)</td>
</tr>
<tr>
<td>Incorporation of additional components</td>
<td>COX10, COX15 (incorporation of heme) / SCO1, SCO2 (incorporation of copper)/TAZ (cardiolipid maturation)</td>
</tr>
</tbody>
</table>

Figure 1. The OXPHOS system. Complex I, II, III, IV, V are indicated. Electrons are transferred from NADH and FADH2 to molecular oxygen. The energy released by the oxidation is used to generate a proton gradient across the mitochondrial innermembrane, which is used by complex V to generate ATP. Dotted lines indicate the direction of the electron flow and solid arrows indicate the direction of the proton translocation. Cyt c: cytochrome c; Q: ubiquinone; QH2: ubiquinol; ims: intermembrane space. (Adapted from 1)
Mitochondrial translation, insertion and quality control

For the translation of the mitochondrial encoded subunits a proper functioning mitochondrial translation machinery is required. Also at this level many things have been shown to go possibly wrong and subsequently lead to disorders. The translation machinery includes mitochondrial encoded tRNA’s and rRNA’s. As described above defects in mitochondrial DNA also include these components. Also specific mitochondrial ribosomes, translation initiation, elongation, and termination factors are required and recently mutations in these components have been described. To assure proper insertion in the mitochondrial inner membrane, the translation of mitochondrial encoded proteins is a highly regulated process which takes place at specific sites of the mitochondrial inner membrane. For this reason the mitochondrial ribosomes need to be positioned at the right place close to the inner membrane. Specific translation regulation proteins guide this process and the inserted mitochondrial encoded subunit is subsequently checked for proper folding and insertion by a quality control machinery which contains chaperones and protease activities.

Import of nuclear DNA encoded subunits into mitochondria and assembly

Subunits encoded by the nuclear DNA are translated in the cytoplasm and contain a cleavable N-terminal target signal or an internal target sequence which ensures import into the mitochondria. This process is performed by sophisticated import machinery containing several complexes, which sort the proteins into the right mitochondrial compartment, the outer membrane, inter membrane space, inner membrane and matrix. Now the protein components are there the additional components need to be directed to or synthesised in the mitochondria and incorporated in the complexes. This process requires specific proteins to synthesise these components. Finally assembly chaperones are essential to put all the components together to obtain a well functioning enzyme complex.

Figure 2. Steps involved in the assembly of OXPHOS complexes.

Functions beyond oxidative phosphorylation

Although this view of mitochondria as energy producers of the cell is certainly true, our insight in mitochondrial function has evolved during the last decades. It has become clear that mitochondria are not the sausage shaped organelles that act as standalone energy factories. Instead mitochondria form a dynamic network in the cell. This network is constantly remodelled by mitochondrial fission, fusion and movement and is thought to be influenced by the metabolic state of the cell as illustrated by the altered mitochondrial morphology observed in cells from patients with an isolated complex I deficiency (3). Mitochondria are also much better integrated within the cellular signalling network than previously assumed. During the last years, it has been recognized that mitochondria play important roles in calcium homeostasis, reactive oxygen species (ROS) production and apoptosis regulation (5,6). In addition, our recent finding highlighting a novel mitochondrial function of the cytosolic adaptor protein Ecsit, suggests a functional link between mitochondria and inflammatory responses, illustrating a broad involvement of mitochondria in cell functioning (4).

Mitochondria and calcium homeostasis

Calcium is an important intracellular signal which plays a role in the regulation of cellular processes with variable spatial and temporal dynamics. The rapid uptake of calcium by the mitochondria after a temporal elevation of cytosolic calcium is critical in this signalling process. The precise physiological function of calcium sequestration has not been elucidated however it is believed that it might play a role in the control of the rate of ATP production or might induce programmed cell death. It is not difficult to imagine that disturbances of this delicate balance of calcium homeostasis in patients with an OXPHOS deficiency can have severe consequences for the physiology of a cell.

Reactive oxygen species production

During oxidative phosphorylation electrons can escape from this process and give rise to free radicals, which can form reactive oxygen species. Although there are defences in the mitochondria which can scavenge these reactive oxygen species, disturbances in OXPHOS can significantly enhance the occurrence of such molecules in the cell. This can have serious consequences for the physiology of a cell because: firstly, ROS cause damage to biological macromolecules such as DNA, lipids, and proteins; secondly, there are indications that ROS also act as signalling molecules and play a role in regulation of energy metabolism.

Mitochondria and programmed cell death

Mitochondria play an important role in triggering the process of programmed cell death or apoptosis. The release of cytochrome c, an electron carrier of the OXPHOS system, from the mitochondria to the cytosol is one of the key processes which activates the cascade leading to apoptosis. Many extra and
intra mitochondrial signals can lead to the induction of apoptosis and how this exactly relates to OXPHOS function is not fully clear yet. However it is believed that mitochondrial signals such as calcium, ROS, membrane potential and ATP levels might play a role. Dysfunction of the OXPHOS system might sensitize cells for apoptosis resulting in premature cell death, leading to neurodegeneration, or might prevent cells to go into apoptosis, leading to cancer.

A mitochondrial link with immunity
Finally we would like to give an example of a surprising finding we recently observed, which again illustrates the integration of mitochondrial proteins in other cellular processes. While investigating a complex I assembly chaperone we discovered that this chaperone, NDUFAF1 was associated with a a cytosolic protein, Ecsit, that is known to plays a role in the Toll/IL-1 immune signal transduction cascade. Knock down experiments of Ecsit by RNA interference result in a specifically decreased complex I amount and disturbed mitochondrial function. These findings have great implications, as they support that the assembly process of complex I itself is directly connected to other cellular processes (4). This connection would allow feedback between complex I and the needs of the cell, e.g. regarding energy production or induction of apoptosis. Possibly some immune or autoimmune disorders might turn out to be caused by mitochondrial defects.

Concluding remarks
Mitochondrial OXPHOS disorders have an extremely broad clinical spectrum (for an overview see web links). Although mitochondria are necessary for ATP production and ATP is required in every part of our body, it became apparent that a decreased ATP production cannot fully explain the clinical phenotype occurring in patients. As pointed out in this review the differences in pathology of mitochondrial disorders might be explained by the many factors involved in the biosynthesis of the OXPHOS system and by the role of mitochondria in other processes. Subtle enzyme defects in oxidative phosphorylation can already have effects in important processes such as calcium homeostasis, ROS production and programmed cell death. These new insights have expanded the spectrum of mitochondrial disorders and in the future probably more disorders will turn out to be caused by mitochondrial dysfunction. Nevertheless our expanding knowledge on mitochondrial function and cellular consequences might pave the way for exploring new therapeutic approaches for these orders, for instance targeted restoration of ROS and intracellular calcium homeostasis may stabilize disease progression (4).

References

Web links
- Mitochondrial disorders overview: http://www.geneclinics.org/profiles/mt-overview/
- Nijmegen Centre for Mitochondrial Disorders: http://www.ncmd.nl