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Proteomics of human mitochondria

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ABSTRACT

Proteomics have passed through a tremendous development in the recent years by the development of ever more sensitive, fast and precise mass spectrometry methods. The dramatically increased research in the biology of mitochondria and their prominent involvement in all kinds of diseases and ageing has benefitted from mitochondrial proteomics. We here review substantial findings and progress of proteomic analyses of human cells and tissues in the recent past. One challenge for investigations of human samples is the ethically and medically founded limited access to human material. The increased sensitivity of mass spectrometry technology aids in lowering this hurdle and new approaches like generation of induced pluripotent cells from somatic cells allow to produce patient-specific cellular disease models with great potential. We describe which human sample types are accessible, review the status of the catalog of human mitochondrial proteins and discuss proteins with dual localization in mitochondria and other cellular compartments. We describe the status and developments of pertinent mass spectrometric strategies, and the use of databases and bioinformatics. Using selected illustrative examples, we draw a picture of the role of proteomic analyses for the many disease contexts from inherited disorders caused by mutation in mitochondrial proteins to complex diseases like cancer, type 2 diabetes and neurodegenerative diseases. Finally, we speculate on the future role of proteomics in research on human mitochondria and pinpoint fields where the evolving technologies will be exploited.

1. Introduction

This review aims to describe the major lines on how the research related to human mitochondria has expanded, and how it together with the fast development of proteomics has resulted in novel discoveries. We will illustrate this development and draw a picture of future directions, as well as outline what prospects mitochondrial proteomics have for the understanding of human biology and disease processes, and how it can be incorporated into mitochondrial medicine in the clinic.

The last decade has seen yet another eruption of mitochondria research, which has revealed the involvement of mitochondria in more or less all diseases — be it as primary site of the pathology or a site of cellular signaling that have a major impact on disease pathogenesis (Cherry et al., 2016; Lane et al., 2015; Nunnari and Suomalainen, 2012; Raimundo, 2014; Tocchi et al., 2015). In addition, the natural ageing process is considered to be intimately linked to mitochondrial functions and dysfunctions (Payne and Chinnery, 2015; Sun et al., 2016). Mitochondrial proteomic studies on several eukaryotic organisms have produced a wealth of data that together have contributed to define the mitochondrial proteome. The human mitochondrial proteome has been investigated mostly in connection with disease research, and several types of samples have been studied, as outlined below.

Rodent models are often used in the published research, applying proteomics to investigate disease pathology, especially for studies on tissue types unavailable from patients. Genetic animal models are mostly of the knock-out type and so far only to a small degree employ insertion of the human mutations found in patients. The results give insight into the basic functions of the respective proteins and the adaptations in tissues that together form the loss-of-function or haploinsufficiency phenotypes. Animal models exposed to various treatment regimens, for example, altered nutrition, pharmacological treatment, or physiological or psychological stressors are also serving to shed light on the response of the mitochondrial proteome to the specific treatment (Baiges et al., 2010; Henningsen et al., 2012).

The scarcity of and negative bias towards studies using certain human tissues are due to the medical and ethical issues connected with taking and using human material, resulting in very limited availability of sufficient material from human sources. Another reason is the lack of tradition in the clinic to routinely collect and freeze biopsies

Abbreviations: 2D, 2-dimensional; 2D-PAGE, 2-dimensional polyacrylamide gel electrophoresis; AIFM1, Apoptosis-inducing factor 1, mitochondrial; ANT, ADP/ATP translocase; DDA, data dependent analyses; DIA, data independent analyses; GFP, green fluorescent protein; GO, gene ontology; HK1, hexokinase 1; HK2, hexokinase 2; HPM, human proteome map; IMM, inner mitochondrial membrane; IMS, intermembrane space; IMS, intermembrane space; IP, immunoprecipitation; iPS, induced pluripotent stem; LC-MS, liquid chromatography- mass spectrometry; *m/z*, mass to charge ratio; MS, mass spectrometry; OMM, outer mitochondrial membrane; PBMC, peripheral blood mononuclear cells; PTM, post-translational modification; SRM, selected reaction monitoring; T2DM, type 2 diabetes mellitus; TCA, tricarboxylic acid; VDAC1, voltage-dependent anion-selective channel protein 1.

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for proteome analyses. Skin fibroblast cell models, tumor biopsies, blood monocytes and established human cell lines therefore dominate the picture (see Section 4). However, the constantly improving technical performance and sensitivity of mass spectrometry (MS) instruments and associated sample preparation methodology makes it more and more feasible to study small biopsy material. This will more and more enable direct investigation of the proteomes in the affected tissues in humans. We here describe the major research strategies that have been pursued, give illustrative examples as well as highlight areas with potential to harvest important new information about the role that changes in the mitochondrial proteome play in health and disease.

2. The proteome of human mitochondria

Due to the limited access to human tissues, knowledge about the human mitochondrial proteome is to a large degree based on data from more systematically and extensively determined mitochondrial proteomes of rodents and also of the well-characterized baker's yeast, the eukaryotic, unicellular *Saccharomyces cerevisiae*. The estimate for the number of proteins in human mitochondria is currently 1100–1900 (Calvo et al., 2016a; Smith and Robinson, 2016), with the majority being "core mitochondrial proteins" that are stably present in all cells, and can be detected by robust methods. In addition, a few hundred proteins are more dynamically present e.g., low-abundance proteins present in certain tissues or only present under specific circumstances, such as during pathological conditions.

To date, the most comprehensive study elucidating the mitochondrial proteome of mammals combined several strategies, i.e. MS analysis of mitochondria isolated from 14 mouse tissues, large-scale GFP-fusion microscopy analysis and bioinformatics using datamining, prediction and evolutionary conservation (Pagliarini et al., 2008). The MitoCarta inventory of mitochondrial proteins obtained in this way comprised approximately 1100 proteins for both mice and humans. It also lists proteins likely to be localized on the surface of mitochondria or being partially or temporarily outside mitochondria (see also section "Dual and Conditional Localization of Proteins" below). In 2016, MitoCarta was updated to version 2.0 listing 1158 genes out of which more than two hundred were newly added (Calvo et al., 2016a). Another dedicated database for mitochondrial proteins that is curated and – at least up to now – continued is the Mitominer database (Smith and Robinson, 2016). It collects curates and annotates information on mitochondrial proteins. It is based on literature data, proteomics data (based on both LC-MS and 2D gel studies), antibody staining, other subcellular localization data, and provides a collective score for each protein's probability to have mitochondrial association. Mitominer records mitochondrial proteins from 12 organisms (Smith and Robinson, 2016). For humans there are currently (2016) 1837 proteins in the Mitominer database with experimental and/or bioinformatic indication for mitochondrial localization.

Protein annotations derived from evolutionary relationships in combination with literature data on protein function have been incorporated and made accessible through the annotation systems in the protein database Uniprot (www.uniprot.org) (Breuza et al., 2016; Li et al., 2015) and the systematic protein function Gene Ontology (GO) database (www.geneontology.org) (The-Gene-Ontology-Consortium, 2015). However, although all these abovementioned resources are continuously improved by incorporation of new information and corrections by curators, the annotation databases are still incomplete or even partially erroneous for some proteins. This requires caution and scrutiny in the interpretation of the information on, for example, protein function and mitochondrial localization. This incompleteness is very much influenced by the complexity and dynamic nature of the mitochondrial proteome, which is illustrated in the following sections by description of dual localization and tissue-specific protein expression of mitochondrial proteins.

2.1. Dual and conditional localization of proteins

The presence of mitochondria inside eukaryotic cells and their active interaction with other sub-compartments like for example the endoplasmatic reticulum, the nucleus, and peroxisomes, makes it almost impossible to obtain pure mitochondrial preparations and define the genuine mitochondrial proteome. Furthermore, many proteins have dual localization, i.e. they are present both inside and outside mitochondria under normal conditions (Kisslov et al., 2014; Yogev and Pines, 2011). In yeast as much as one third of the mitochondrial proteins have been estimated to have dual localization (Ben-Menachem et al., 2011), and dual-targeted proteins tend to be more evolutionarily conserved than other proteins (Kisslov et al., 2014), indicating their biological importance. There are many mechanisms behind how one gene can yield gene product(s) with more than one localization (Yogev and Pines, 2011). For example, a gene with one translation product may contain several targeting sequences, or a gene may have two translation products each with a specific targeting sequence. In addition, proteins may intermittently bind to the surface of mitochondria and proteins localized in the mitochondrial intermembrane space (IMS) may leak out into the cytosol under certain conditions (Fig. 1) (Petrungaro and Riemer, 2014).

The glycolytic enzyme hexokinase (both gene product HK1 and HK2) intermittently associates with mitochondria by binding to the outer mitochondrial membrane (OMM) channel protein VDAC1 (Fig. 1B) (Roberts and Miyamoto, 2015). Interaction of VDAC1 with the adenine nucleotide translocase ANT in the inner mitochondrial membrane (IMM) results in channeling of ATP from mitochondria to hexokinase, which uses it for the phosphorylation of glucose. Stimulation of glycolysis by increased hexokinase binding to VDAC1 is seen in tumor cells, and it contributes to the Warburg effect.

A classic example of how a mitochondrial protein can have a potent effect outside mitochondria is the release of cytochrome *c* from its normal localization in the IMS (Fig. 1A) and its impact on caspase-dependent apoptosis (Liu et al., 1996) (Fig. 1). Also another apoptosis-signaling protein, the caspase-independent apoptosis inducing factor (AIFM1), is released from mitochondria. Under normal conditions it functions as a NADH oxidase in the (IMS) and upon apoptotic stimuli it is released, and migrates to the nucleus where it induces chromatin condensation and DNA fragmentation followed by apoptosis (Kettwig et al., 2015).

Another example of partial localization to the IMS is the superoxide dismutase [Cu-Zn] SOD1. The SOD1 protein is mainly localized in the cytosol, however, a small proportion is present in the mitochondrial IMS (Carrì and Cozzolino, 2011). Import to and retention of SOD1 in the IMS depend on oxidative folding mediated by the MIA40/ALR redox relay system and the copper chaperone CCS (Kawamata and Manfredi, 2008, 2010; Sztolsztener et al., 2013) that mediate formation of an intramolecular disulfide bridge and incorporation of the copper ion into the active site. The IMS localized SOD1 can remove superoxide originating from electron leakage from respiratory complex III. Mutations in SOD1 promote aggregation of the protein and are accompanied by increased association with mitochondria in familial amyotrophic lateral sclerosis (Cozzolino et al., 2013).

Some mitochondrial proteins may also localize both to mitochondria and nucleus under more normal physiological conditions (Monaghan and Whitmarsh, 2015). This can be exemplified by the metabolic enzyme complex pyruvate dehydrogenase, which is an important entry point of acetyl-CoA into the tricarboxylic acid (TCA) cycle in the mitochondrial matrix, and in the nucleus it serves as the source of acetyl-CoA for histone acetylation and thus genetic regulation (Sutendra et al., 2014).

Curiously there also is the possibility that genetic variants can give a new localization for a protein, exemplified by the peroxisomal protein EHHADH which caused disease when its target sequence was altered, falsely directing it to mitochondria (Klootwijk et al., 2014).