Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
Dissertations-Kurzfassung

Identifying novel peroxisome-associated proteins in mouse liver and pig heart by implementing different quantitative proteomics approaches

Autor: Öznur Singin
Institut / Klinik: Mannheim Center for Translational Neuroscience (MCTN). Neuroanatomy
Doktorvater: Prof. Dr. Ch. Schultz

Peroxisomes are involved in a variety of metabolic functions such as the degradation of very long-chain fatty acids, D-amino acids and purins or the synthesis of ether lipids, PUFAs and bile acids. Accordingly, the absence of single peroxisomal enzymes or the whole organelle leads to severe inherited peroxisome disorders, which often lead to death during early childhood. Despite their obvious significance for human health, peroxisomes are still the least characterized subcellular compartment. To increase the knowledge on peroxisome-related functions and to complement the peroxisomal proteome, this thesis focused on identifying new potential peroxisome-associated candidate proteins in the liver and heart by conducting two different quantitative MS-based approaches in the two tissues.

The proteomic survey performed in mouse liver – the tissue, in which peroxisomes are best characterized – aimed to identify novel, low abundant peroxisome-associated proteins using a quantitative SWATH-MS approach. To this end, peroxisomes were isolated from two different prefractions with a differing organelle composition produced by step-wise differential centrifugation – the so-called light mitochondrial and fluffy layer fractions. The comparison of the protein enrichment lists from both respective peroxisome fractions generated by SWATH MS, revealed a nearly identical set of proteins, which was therefore considered to be candidates of a *bona fide* peroxisomal proteome. In order to validate the proteomics data, the peroxisomal targeting and/or localization was investigated for the five selected novel peroxisomal candidate proteins HTATIP2, PAFAH2, SAR1b, PDCD6 and OCIAD1. Confocal immunofluorescence analysis of myc-tagged HATIP2 and PAFAH2 variants confirmed their peroxisomal localization. In contrast, a direct localization of endogenous or overexpressed SAR1b at peroxisomes could not be observed. However, the reticular ER-signal pattern of SAR1b regularly enclosed peroxisomes suggesting a close membrane association between a SAR1b-positive ER subcompartment and peroxisomes. Likewise, the results of the PDCD6 overexpression analysis, suggested that the protein is not a true peroxisomal constituent, but like SAR1b localizes focally to ER exit sites, which were often found in in apposition with peroxisomes. These findings suggested a potential function for both SAR1b and PDCD6 in so-called peroxisome-wrappER membrane contacts, which were recently described to play a role in the regulation of liver lipoprotein export. The last candidate OCIAD1 localized to mitochondria and peroxisomes Of note, OCIAD1 overexpression induced mitochondrial fractionation and reduction of peroxisome abundance, which suggests that OCIAD1 might play a role in coordinating peroxisomal and mitochondrial metabolic activities.

Cardiomyocytes primarily rely on fatty acid β-oxidation for ATP production. Despite the important role of peroxisomes in lipid metabolism, their significance in cardiomyocytes is still poorly understood. Hence, in order to provide fundamental data on the heart-specific peroxisomal proteome, an MS-based organellar profiling approach was developed in this thesis.

To this end, a linear density gradient was invented to gradually distribute distinct organelle species in different gradient zones. This density gradient technique was subsequently combined with a quantitative HyperLOPIT MS analysis of 6 consecutive gradient fractions. The distribution of known organelle marker proteins was used to generate characteristic separation profiles for peroxisomes, mitochondria, the ER and lyso-/endosomes. To validate if the gradient profiles would allow to predict a potential peroxisomal localization of hitherto unknown proteins, two potential candidates, SERHL2 and KLHL41, with a potential peroxisomal targeting sequence were selected for overexpression experiments. Matching with the shape of their gradient profiles, the localization of SERHL2 was confirmed as peroxisomal, while

KLHL41 showed an ER-like distribution. These results confirm the suitability of the organelle profiling approach as a tool to identify potential peroxisomal proteins in cardiomyocytes. In this respect, the proteomics data will provide the basis for an AI-driven bioinformatics analysis, which will be used to associate further uncharacterized proteins to the peroxisomal cluster.
In summary, this thesis introduced two alternative quantitative organelle proteomics approaches for heart and liver, which both successfully allowed to expand the list of peroxisome-localized proteins. However, it also demonstrates the importance of combining proteomic analysis with supplementary subcellular localization experiments to validate organelle-specific localization of the identified protein candidates.