Cardiolipin metabolism in Barth syndrome
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Cardiolipin (CL) is a phospholipid which is primarily localized in the mitochondrial inner membrane. In contrast to other phospholipids, CL has four acyl chains (in humans mostly linoleic acid) providing unique structural properties. Primary synthesis of CL occurs at the mitochondrial inner membrane and is comprised of condensation of one molecule phosphatidylglycerol and one molecule CDP-diacylglycerol by the enzyme CL synthase. Based on previous research, it was assumed that the high structural uniformity is not achieved by specificity of the CL synthase enzyme, but is derived from remodeling of the acyl chains after the primary, immature CL molecule is synthesized. As shown in chapter 3, we identified the human CL synthase and characterized its enzymatic properties. Based on the observed lack of substrate specificity we concluded that additional acyl chain remodeling indeed is required. One of the main candidates to fulfill this function is tafazzin. Mutations in the tafazzin gene, of which multiple splice variants exist, were identified as the cause of the rare X-linked recessive disorder Barth syndrome (BTHS), which is clinically characterized by (cardio-)myopathy, neutropenia and abnormal growth. Based on sequence homology it was hypothesized that tafazzin functions in phospholipid acyl chain remodeling and the finding of CL abnormalities in cells from BTHS patients substantiated this idea. It was shown that in BTHS cells CL levels are lower, monolyso-CL (MLCL; i.e. CL lacking one acyl chain) levels are markedly increased and the CL acyl chains have a reduced degree of unsaturation due to reduced incorporation of linoleic acid in CL (chapter 4). For the diagnostic analysis of BTHS, we developed a novel HPLC-mass spectrometry method based on the measurement of CL and MLCL, and we introduced the MLCL/CL ratio as a powerful diagnostic parameter for BTHS. Using this method, we identified a previously undiagnosed BTHS patient, suggesting that BTHS is underdiagnosed (chapter 4).

To determine the functional role of the various tafazzin variants we overexpressed these isoforms in a tafazzin-deficient yeast mutant and showed that only the tafazzin variant lacking exon5 was able to fully restore the aberrant growth of the mutant as well as the abnormal CL profile (chapter 5). Full-length tafazzin (i.e. containing exon5) only partially restored the CL profile and did not restore growth, whereas all the other variants showed no functionality whatsoever (chapter 5).

As shown in chapter 6, expression of the tafazzin variants in human (BTHS) cells also showed that tafazzin lacking exon5 is likely the most relevant variant with respect to CL remodeling. The enigmatic role of the tafazzins in the CL acyl chain remodeling was further investigated by mRNA analysis in different tissues and cell types (chapter 6). We tried to link the tafazzin mRNA expression profile to the CL profile of various human tissues but found no clear correlation. In contrast to what was expected based on the affected tissues of BTHS patients, tafazzin is not more abundant in heart and muscle when compared to other tissues. To our surprise, we also found that the (semi-)active tafazzin variants, i.e. full-length tafazzin and tafazzin lacking exon5, were not more abundant than the other mRNA transcripts (chapter 6). The effect of the tafazzin mutations in BTHS cells on the mRNA levels was investigated by comparing the mRNA distribution of the tafazzin variants in control and BTHS fibroblasts. In two out of twelve patients tafazzin mRNA was hardly
detectable, suggesting that the mutations of these patients result in reduced mRNA stability or increased degradation. Alternatively, transcription might be reduced due to promoter mutations. In the fibroblasts of other patients, the tafazzin mRNA content was slightly increased as was the relative abundance of the active tafazzin variants, suggesting that a compensatory mechanism results in increased transcription and preferential splicing to favor the expression of the functionally relevant tafazzin variants (chapter 6).

The sequence of events, leading from mutation in the tafazzin gene to the (variable) clinical phenotype of BTHS patients is still poorly understood. In order to get more insight in these processes we performed proteome analysis comparing wild type and tafazzin-deficient yeast mitochondria (chapter 7). We identified several proteins that were differentially expressed, including proteins involved in mitochondrial biogenesis, protein import, DNA stability and stress defense. The proteins are candidate modifiers of the BTHS pathogenesis and could be the missing link in the genotype-phenotype relation. Further research on these modifiers, especially in human BTHS cells and tissues, will be essential to clarify this contribution and will be a major step towards understanding BTHS and provide better treatment strategies for BTHS.