1 Abstract

The sterol element binding proteins (SREBPs) are transcription factors, which regulate the carbohydrate- and lipid metabolism in a cholesterol dependent manner. Thus, they play an important role for the development of the different cardiovascular risk factors related to alterations in metabolism, like insulin resistance and disorders of lipid metabolism. In our group it could be shown for the first time that SREBPs, besides the regulation by the cholesterol content, are substrates for MAP kinase cascades and that the transactivation can be increased by phosphorylation. In order to elucidate the physiological and clinical role of SREBP-1 isoforms and the phosphorylation especially in liver, transgenic mice were generated, which express tissue specific the N-terminal (NT) domain from one of the two isoforms, SREBP-1a or -1c, or one of the different mutated SREBP constructs.

For proteomics we selected the peroxisomes, because they are beside mitochondria the most influential organelle of cellular lipid metabolism. The peroxisomal proteins catalyse on the one hand important steps in the biosynthesis of complex lipids and on the other hand the peroxisomal β-oxidation takes a key position for the catabolism of most diverse partly toxic lipids. Thus, they possibly might represent a link between cellular lipid metabolism, insulin sensivity and energy balance. Therfore, the proteom of liver peroxisomes were investigated in three different trangenic mice genotypes, which express SREBP-1a NT, SREBP-1c NT or SREBP-1a ΔP under the control of the albumin promoter and C57Bl6 mice for control.

The respective transgen and its exclusive expression in liver could be detected at the genomic and protein level. In the following, the phenotype development of these mouse lines were observed under controlled conditions over 12 weeks, i.e. 7th to 19th week of life. At the age of 18 weeks, the Alb-SREBP-1a and the Alb-SREBP-1c mice showed post mortem, clear differences in the quantity and distribution of the fatty tissue as well as an enlarged liver caused by lipid accumulation in comparisons to the wild type (C57Bl6). However, the SREBP-1a ΔP mice did not show this observed phenotype. If one regards the energy efficiency, a benchmark for the conversion of taken up energy into the increase of body weight, Alb-SREBP-1c mice showed an increase by 58% compared to wild type mice and in contrast to mice, expressing the two SREBP-1a constructs.

For the analyses peroxisomal protein patterns a method was established to purify the peroxisomes from mouse liver. The quality of preparation and degree of enrichment were documented at the functional level with enzyme assays, at the protein level by western blots and at the morphologic level by using electron microscopy.

The proteomics of isolated liver peroxisomes from the different genotypes were carried out using the Fluorescence Difference Gel Electrophoresis (DIGE™)-technology. With this technology, a valid analysis with high sensitivity for protein profiling was selected, so that

also small differences of abundance could be detected. After the 2D-electrophoresis, the abundance of more than 1600 protein spots of the peroxisomal protein profile pH4-9 was compared between the diffrent genotypes by using the Proteomweaver software.

The protein spots which showed differences in abundance in one of the genotype comparsions were picked in triplicate from different gels and analyzed by an UltraFlex TOF/TOF mass spectrometer. The received peptide mass fingerprint (PMF) spectra were adjusted on the fly with the SwissProt database (http://ca.expasy.org/sprot/). For every protein spot a change in abundance was considered only, if it was confirmed in two gels or supplied by positive ms/ms spectra. Therfore, the efficiency of identification was about 40%.

Interestingly, the clear phenotype of the Alb-SREBP-1a mice, regarding the fat distribution and the enlarged liver, did not correlate with a high number of abundance alterations in the peroxisomal protein pattern; because only 24 changed protein spots were detected. The Alb-SREBP-1c mice exhibited clearly more abundance alterations in the comparison to the wild type and although the two isoforms differ only by around 24 amino acids, 83 abundance alterations were detected in direct comparison with Alb-SREBP-1a.

By switching off the three main phosphorylation sites of SREBP-1a the fundamental role of the phosphorylation became clear, because in the peroxisomal proteom of the Alb-SREBP-1a ΔP mice more than 200 abundance alterations were registered in the direct comparsion to Alb-SREBP-1a. These changes might realte to the observed phenotype.

In conclusion, there is clear evidence that the transcription factor SREBP-1 affects not only the well-known lipogenic metabolic pathways, but also fat-catabolizing pathways. This influence might be mediated by activation of an unkown factor X or a signaling cascade which again represses fat-catabolizing metabolic pathways. It is intersting to note, that the regulation of this factor via SREBP-1 appears to be dependent on the phosphorylation status.