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Characterisation of myocardial lipid droplet protein/perilipin-5 in normal and diseased human tissues

Promotionsfach: Pathologie

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Lipid droplets (LDs), ubiquitous intracellular storage compartments of neutral lipids, are reconsidered as highly dynamic cell organelles with various functions. They are composed of a core of triacylglycerol and cholesterols esters surrounded by a phospholipid monolayer in which several proteins are embedded, including the structural proteins of the so called PAT-family. Proteomic studies indicate that at least one PAT-member is consistently found associated with LDs and from mouse models and cultured cells it is well known that loss of one or several PAT-proteins severely impairs LD-formation, -storage, and -utilisation. In mammalian cells, five constituents of the PAT-family of proteins have been identified, namely perilipin, adipophilin, TIP47, S3-12, and MLDP (according to the novel nomenclature perilipin 1-5). MLDP ("myocardial lipid droplet-protein", perilipin 5) is reported to be expressed in highly oxidative tissues such as heart, red muscle, and liver during fasting.

Yet, experiments undertaken so far were mainly conducted in murine tissues and cell cultures, whereas little was known about MLDP expression in normal human tissues as well as in human diseases.

Therefore, MLDP expression pattern in human tissues was investigated using protein biochemistry, including ESI-MS analysis, immunofluorescence microscopy, and immunohistochemistry as well as molecular biological techniques. In the frame of this dissertation, novel human-specific polyclonal MLDP antibodies were produced and established.

In human tissues, MLDP mRNA and protein showed a wider expression than hitherto reported from analyses in the mice. Higher concentrations of MLDP at LDs were detected in mitochondrial-rich cells such as striated myocytes of heart and skeletal muscle, hepatocytes, but also in brown adipocytes and parietal cells of gastric corpus mucosa. Additionally, low amounts of MLDP were detected in smooth muscle, steroidogenic tissues, kidney, prostate gland and severeal epithelia of the gastrointestinal tract. By immunoblot, antibodies against the C-terminus (commercially available), N-terminus and a possible central loop structure (own production), besides a band of the calculated size of ~ 56 kDa, lower bands of ~ 40 kDa and 28 kDa were detected in certain tissues such as skeletal muscle, possibly representing tissue-specific shorter MLDP variants. By density gradient centrifugation, immunohistochemistry, and

immunofluorescence, MLDP was found in two different subcellular localisations, in the cytoplasm and at LDs. By ESI-MS analysis of immunoprecipitates of human skeletal muscle, phosphorylated MLDP peptides were identified, and the cytoskeletal protein plectin was determined as a possible binding partner. Using confocal laser scanning microscopy, MLDP and the other PAT-proteins, adipophilin and TIP47 were differentially localised in cardiomyocytes and skeletal muscle cells. A differential localisation of MLDP, adipophilin, TIP47, and perilipin was also observed in steatotic hepatocytes. These data indicate a differential expression of PAT-proteins in striated myocytes and hepatocytes suggesting a differential role in LD-biogenesis and –storage. Recent publications have assumed that by regulation of hydrolytic activity of ATGL and mitochondrial fatty acid oxidation, MLDP may have a specific function as mediator in the channelling of fatty acids between LDs and mitochondria, a phenotype not seen in other PAT-proteins.

In diseases associated with LD-accumulation, like in cardiomyopathies and microvesicular liver steatosis, MLDP was frequently observed in association with larger and more numerous LDs. Also in rhabdomyosarcoma and hepatocellular carcinoma cell lines treated with oleic acid supplemented medium, MLDP mRNA and protein was induced *in vitro.* By analysis of MLDP distribution in certain tumour types using immunohistochemistry of tissue arrays, MLDP was expressed in certain tumours like in leiomyosarcoma, rhabdomyosarcoma and liposarcoma, as well as in clear cell renal cell and hepatocellular carcinoma. Expression of MLDP in tumours may reflect their cellular origin, as well as an enhanced LD-accumulation in certain tumour types, additionally an oxidative phenotype may be discussed.

To conclude, the result of this dissertation suggest MLDP as a helpful marker to study LD-accumulation in certain types of cells, particularly mitochondria-rich highly oxidative cells, as well as a diagnostic marker for neoplastic and non-neoplastic diseases associated with LD-accumulation.