Investigations on the role of impaired lysosomes of macrophages in disease

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Summary

The research described in this thesis combines the latest insights in lysosomal function with lysosome centred cell signalling. Novel imaging and labelling techniques are applied to provide in depth characterization of lysosome function in health and disease. An integrative approach was used to study the physiological role of the lysosome, characterizing the function of lysosomal hydrolases and signalling on a cellular level as well as within the context of tissue.

The general introduction covers the current knowledge on the composition and functions of lysosomes, with a focus on macrophages. Lysosomal hydrolases and corresponding inherited lysosomal storage disorders are introduced, with emphasis to glucocerebrosidase (GCase) and Gaucher disease. The effect of (MiT/TFE) transcriptional regulation of cellular content on lysosomes is described, as well as the role of lysosome-associated kinases in regulation of cellular metabolism with emphasis on lipid homeostasis. Excessive lipid accumulation as cause of metabolic derailment (lipotoxicity) is introduced and illustrated by inherited and acquired disorders.

Chapter 1 reports on an increase in lysosome biogenesis in cells cultured in medium containing the buffer HEPES, which is driven by the transcription factors of the Mi/TF family, TFEB, TFE3 and MITF independently of mTORC1. In macrophage-like cells, exposure to HEPES results in an enlarged vacuolar compartment and alterations in lysosomal signalling, proteolytic capacity, autophagic flux, and inflammatory signalling. This is accompanied by an increase in GCase activity and GPNMB expression, a protein that is part of the cellular response to lysosomal perturbation. Altogether, the findings show that chemical buffering agents in cell culture media can potentially confound lysosomes.

Chapter 2 describes the impact of culture conditions on the maturation of GCase as measured with specific mechanism-based probes visualizing the enzyme. The zwitterionic buffer HEPES, earlier identified as lysosome stressor, is shown to reduce the normal maturation of GCase to its final 58kDa form by means of glycan modification in lysosomes by local glycosidases. The processing of other glycosidases such as alpha-glucosidase and beta-glucuronidase is also impaired by the presence of HEPES in the culture medium. In cells grown in the presence of HEPES, GCase markedly accumulates due to impaired degradation. This phenomenon potentially has impact on diagnosis of Gaucher disease when residual GCase activity in cultured cells is assessed. In fibroblasts of Gaucher disease patients grown in the presence of HEPES, the enzyme levels may be very close to those observed in control cells grown without the buffer. It is concluded that supplementation of HEPES to cell culture medium should be treated with caution when cultured cells are used to confirm the diagnosis of Gaucher disease.

Chapter 3 describes the visualization of active GCase molecules by means of correlative light and electron microscopy (CLEM). Cyclophellitol-derived activity-based probes (ABPs) with a fluorescent reporter are employed that irreversibly bind to the catalytic pocket of GCase. By combining electron microscopy and fluorescence microscopy, the subcellular localization of active GCase molecules can visualized. This technique

confirmed that endogenous active GCase molecules reside in the electron dense lysosomes. Gaucher disease is currently treated by infusion of mannose receptor targeted, recombinant GCase. Pre-labelling of therapeutic enzyme with an ABP with a distinct fluorophore allowed the simultaneous visualization of endogenous and exogenous, therapeutic enzyme in cells expressing mannose-receptor. This method revealed the efficient delivery of recombinant GCase to lysosomal compartments that contain endogenous active enzyme in an unprecedented manner.

Chapter 4 reviews the current knowledge on glycoprotein non-metastatic protein B (GPNMB) as biomarker for macrophage-associated lysosomal storage disorders. The transmembrane glycoprotein has emerged as one of the most abundant proteins in lipid-laden macrophages accumulating in spleen of Gaucher patients. A soluble fragment of GPNMB is released from the storage cells which results in prominently elevated GPNMB levels in plasma of symptomatic Gaucher disease patients. GPNMB is also found to be markedly increased in storage cells in Niemann-Pick type C liver. The review extends the knowledge on lipid laden macrophages to other fields, including neurodegeneration and obesity, in which lipid storage by macrophages has also been reported to induce GPNMB expression.

Chapter 5 provides a molecular and biochemical characterization of the liver of mice suffering from a deficiency in the lysosomal protein NPC1, a condition that results in the lysosomal storage disorder Niemann-Pick type C. The transmembrane protein NPC1 is involved in export of cholesterol from lysosomes and its deficiency causes intralysosomal cholesterol accumulation, which is accompanied by accumulation of other lipids such as sphingomyelin and glucosylceramide. As previous reports suggested, analysis of the NPC1-deficient liver revealed a reduction in hepatic GCase protein along with reduced enzyme activity. Surprisingly, the hepatocytes of 80 weeks old NPC1-deficient liver showed a strong increase in LIMP2, the lysosomal membrane protein that transport GCase to lysosomes and that is speculated to also act as cholesterol channel in the lysosomal membrane. The upregulation of LIMP2 was not observed in cholesterol-laden Kupffer cells in the same NPC1-deficient livers. These findings might point to compensatory role for LIMP2 in cholesterol transport during deficiency of the NPC1-mediated pathway.

Chapter 6 reports on the molecular and metabolic consequences of interfering in lysosomal biogenesis of obese adipose tissue macrophages. An siRNA-based approach was employed to elucidate the role of three Mi/TFE members, MITF, TFEB and TFE3, in regulating lysosome and inflammatory gene expression in macrophages. In cultured cells, a biomarker of lysosomal stress, GPNMB, was found to be highly dependent on Mi/TFE mediated transcription. The simultaneous knock down of all three Mi/TFE members was required for optimal reduction in GPNMB response to lysosomal perturbations, pointing to redundancy among the transcription factors. Delivery vehicles called glucan encapsulated particles were loaded with siRNAs targeting MITF, TFEB and TFE3 simultaneously. These were used to selectively interfere with macrophages in the adipose tissue of obese mice. The treatment resulted in worsening of glucose tolerance and reduced production of beneficial by adipocytes. Based on these results, the Mi/TFE driven induction of gene expression in macrophages present in obese adipose tissue seems

a favourable response limiting metabolic abnormalities and not a driver of pathology.

The discussion considers the major findings made during the investigations. The findings are discussed in view of recent literature.