Inflammation and myosteatosis in pancreatic cancer cachexia

Summary

Cancer cachexia is a multifactorial and devastating syndrome that is associated with poor survival of patients. The common features of cachexia include significant loss of body weight (both skeletal muscle mass and fat mass), elevated systemic inflammation, anorexia, nausea, and fatigue. Another aspect of cachexia that is strongly predictive of patient survival is myosteatosis, which refers to the accumulation of ectopic fat within muscle tissue. Cancer cachexia affects 50%-80% of cancer patients and directly causes 20% of cancer-associated deaths. Among cancer patients, cachexia is highly prevalent in those with pancreatic cancer (up to 80%), followed by patients with gastro-oesophageal cancer, head and neck cancers, and lung cancer. Despite significant advances in cancer treatment in the past decade, no practical guide for early diagnosis of pre-cachexia and no effective treatment for cancer cachexia has been developed and implemented in clinical practice. Studies in mouse models of pancreatic cancer cachexia have shown that several proinflammatory cytokines such as IL-6, TNF-α, and IL-α promote muscle wasting. However, the neutralization of a single cytokine is unlikely to be effective against cancer-associated cachexia in patients, and more insight into the role of the respective branches of the immune system in cachexia progression is required to develop an effective treatment. Furthermore, the mechanisms leading to the development of myosteatosis remain poorly characterized. To address these issues, complement system activation, the role of neutrophil-derived lipocalin, and myosteatosis were studied in the context of pancreatic cancer cachexia.

The complement system was discovered more than 100 years ago. It is an ancient key component of the innate immune system, playing a vital role in host defense against infection. Several studies have shown that aberrant complement system activation is also associated with inflammatory conditions such as rheumatoid arthritis, renal diseases, chronic neurodegenerative diseases, as well as cancer [1-4]. In the context of cancer cachexia, the complement system has received little attention. It is well described that systemic inflammation is a hallmark of cancer cachexia and the complement system has been shown to contribute to metabolic inflammation. In **Chapter 2**, we hypothesized that systemic inflammation in patients with cancer cachexia was associated with complement activation. The levels of circulating complement factors including C1q, mannose-binding lectin (MBL), C3a, and terminal complement complex (TCC) were determined in pancreatic cancer patients

without cachexia and in cachectic patients with or without systemic inflammation (as defined by a CRP levels >10 mg/mL). We observed that systemic C3a levels were higher in cachectic patients with inflammation as compared to patients without inflammation or without cachexia. Accordingly, TCC concentrations gradually increased in these patient groups. C3a and TCC concentrations were strongly correlated. Although concentrations of C1q and mannose-binding lectin did not differ between groups, C1q levels were correlated with both C3a and TCC concentrations. Altogether, in this study, we revealed that systemic inflammation in patients with cancer cachexia is associated with the activation of key effector complement factors. Moreover, the correlations between C1q and C3a/TCC suggested that the classical complement pathway could play a role in complement activation in patients with pancreatic cancer cachexia.

In **Chapter 3**, we explored the link between neutrophil activation and cachexia, focusing on neutrophil-released LCN-2, and we assessed whether LCN-2 levels were associated with appetite and nutritional status of patients with pancreatic cancer. A set of circulating neutrophil activation markers including calprotectin, myeloperoxidase (MPO), elastase, and bactericidal/permeability-increasing protein (BPI) was determined in PDAC patients in relation to cachexia and LCN-2 levels. Our results showed that cachectic patients with high systemic LCN-2 levels had higher concentrations of calprotectin, myeloperoxidase, and elastase than non-cachectic patients or cachectic patients with low LCN-2 levels. Systemic inflammation (defined by the CRP/albumin ratio) was also higher in cachectic patients with high LCN-2 levels as compared to non-cachectic patients or cachectic patients with low LCN-2 levels. Spearman correlation analysis revealed a significantly positive correlation between systemic LCN-2 levels and those of neutrophil activation markers calprotectin, myeloperoxidase, elastase, and BPI. Given that complement factors such as C3a and C5a have the ability to trigger and amplify neutrophil activation, and since we reported complement system activation in pancreatic cancer patients with cachexia (Chapter 1), we further extended the correlation analysis between complement factors and neutrophil activation markers in our study cohort. Importantly, we observed a positive correlation among these variables. This suggests that complement activation may underlie neutrophil activation in pancreatic cancer cachexia.

To investigate whether LCN-2 was associated with appetite, we compared systemic LCN-2 levels in pancreatic cancer patients with normal food intake and reduced food intake as assessed by validated questionnaires. No significant difference in systemic LCN-2 between the groups was observed. However, borderline significantly (p=0.00578) elevated LCN-2 levels were observed in severely malnourished PDAC patients. In conclusion, different from the recently reported effect of appetite suppression by LCN-2 in a mouse model of pancreatic cancer cachexia, the results of our study do not support that systemic LCN-2 contributes to cachexia by suppressing appetite in pancreatic cancer patients. In contrast, our data do support that LCN-2 is released by activated neutrophils in these patients.

Low muscle mass (sarcopenia) is one of the criteria for the diagnosis of cancer cachexia which has been well studied in the past decade. However, the mechanisms underlying poor muscle quality (myosteatosis) and its impact in the context of cachexia received comparatively less attention. In **Chapter 4**, we investigated whether intramyocellular lipid accumulation in pancreatic cancer patients is associated with inflammation and other defining aspects of cancer cachexia, and identified the types of intramyocellular lipids in patients with cancer cachexia and their distribution. Body composition was analyzed by using L3-CT scans. Rectus abdominis muscle biopsies were collected during surgery from PDAC patients for muscle morphology, lipidomics, and qPCR analyses. We observed that cachectic patients with inflammation had significantly lower muscle radiation attenuation as compared to those without inflammation or weight loss, reflecting increased lipid accumulation in the muscle of those former patients. In line with this, intramyocellular lipid content was lower in patients without cachexia as compared to those with cachexia with inflammation or without inflammation. Although the expression of muscle atrophy-related genes did not differ significantly among the studied groups, a notable leftward shift in the frequency of smaller muscle fibers was observed in cachectic patients with inflammation. Untargeted lipidomics analyses revealed alterations in intramyocellular lipid classes and species in pancreatic cancer patients without cachexia compared with cachectic patients with or without inflammation. In particular, a higher relative abundance of intramyocellular glycerophospholipids and a lower relative abundance of intramyocellular glycerolipids were found in cachectic patients with inflammation, as well as certain elevated ceramides species. In addition, genes coding for

enzymes involved in *de novo* ceramides synthesis such as SPT1/2, KDSR, Cers1-2, Cers4-6, and DEGS1 tended to show an increased expression in the skeletal muscle of cachectic patients with inflammation. We were not only able to determine the levels of intramyocellular lipid species but could also visualize the altered intramyocellular lipid species such as PC (34:1), PC(33:2), and TG (48:1) by using mass spectrometry imaging. Taken together, these findings indicate that patients with cachexia exhibit intramyocellular accumulation of specific lipid species that may be partly related to elevated ceramide synthesis.

Tumor-derived factors are known to play a key role in driving the progression of cancer cachexia. However, whether tumor-derived factors have direct actions promoting lipid accumulation in skeletal muscle during cancer cachexia remains uncertain. Therefore, in **Chapter 5**, we investigated the effect of the human pancreatic tumor organoid secretome on lipid accumulation in C2C12 myotubes. Pancreatic tumor organoids were established from six PDAC patients (three with cachexia, three without cachexia), and conditioned medium (CM) was collected from these tumor organoids. We exposed the differentiated C2C12 muscle cells to 50% CM plus fatty acids for 8 hours. Lipid accumulation in myotubes was assessed by Oil-red O staining and live cell imaging. LC-MS/MS-based lipidomics was performed to determine global lipid changes in myotubes after treatment. Lipid metabolism-related genes were analyzed by RNA sequencing. CM from pancreatic tumor organoids of cachectic patients caused significant lipid accumulation in differentiated C2C12 from 6 hours onward, which was not seen with CM from organoids of non-cachectic patients or control medium. We observed that the pancreatic tumor organoid secretome induced alterations in intramyocellular lipid species, mainly from the glycerolipids, glycerophospholipids, and sphingolipids classes. Furthermore, several genes related to lipid uptake were upregulated and genes related to fatty acid oxidation were suppressed in C2C12 myotubes after exposure to fatty acids plus CM from human pancreatic tumor organoids of cachectic patients. A trend toward decreases in mitochondrial membrane potential and key genes (PPARGC1A and NRF1) related to mitochondrial biogenesis was observed. Although the pancreatic tumor organoid secretome of cachectic patients tended to decrease myotube fiber diameter, muscle atrophy genes Foxo32 and Trim63 were not altered, and no apoptosis was observed. This result suggested that pancreatic tumor organoid secretomes do not induce muscle atrophy. GO/KEGG

enrichment pathway analyses revealed a significant pathway with biological relevance to cytokine-cytokine receptor interaction in myotubes after exposure to fatty acids plus human pancreatic tumor organoid factors. These results imply that the human pancreatic tumor organoid secretome induces lipid accumulation in C2C12 myotubes. This process may be caused by disruption of lipid metabolism pathways and mitochondria dysfunction. Our findings highlight the important role of factors directly released by pancreatic tumor cells in promoting lipid accumulation in skeletal muscle.

Finally, in **Chapter 6**, the main findings of this thesis are discussed and future perspectives are presented. Altogether, our studies highlighted the important role of inflammation and the factors released by cancer cells and immune cells during the development of cancer cachexia.

Reference

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