Lung cancer cachexia

SUMMARY

Lung cancer is a leading cause of cancer related mortality worldwide. Approximately 13.000 patients per year are diagnosed with lung cancer in the Netherlands of which more than 11.000 diagnosed with Non-Small Cell Lung Carcinoma (NSCLC). The prevalence of NSCLC increases with the age, and in combination with the improved screening methods, the number of patients diagnosed with lung cancer has increased by 22% over the past ten years. The overall prognosis for NSCLC patients is very poor with an average 5-year survival rate of 17%.

Many patients with (advanced) lung cancer suffer from cancer cachexia. Cancer cachexia is a complex multifactorial catabolic syndrome that leads to significant unintentional body weight loss, characterized by an ongoing loss of skeletal muscle mass with or without a reduction in fat mass. This loss of body weight cannot be fully reversed by conventional nutritional support. Cachexia is associated with poor clinical outcome, decreased survival and negatively influences tumor therapy, as is illustrated by increased postoperative mortality and decreased response to radiation-, chemo- and immunotherapy. Muscle wasting is an important contributing factor to muscle weakness in cachexia, which adversely affects performance status, quality of life and hospitalization risk of cancer patients. However, despite increasing efforts to improve our knowledge of cancer cachexia required for development of therapies, the management of cancer cachexia remains a major challenge for clinicians and is until now an unmet medical need.

The overall objective of the research described in this thesis is to unravel the molecular mechanisms and dynamics of lung cancer cachexia. A combination of both translational and experimental research approaches are used to study this objective.

MicroRNAs (miRNAs) are small non-coding RNAs that play a central role in post-transcriptional gene regulation. Changes in intramuscular levels of miRNAs have been implicated in muscle wasting conditions, including cancer cachexia. In **chapter 2**, we provide an overview of miRNAs directly associated with muscle atrophy, which we proposed to term “atromiRs”. The regulation and mode of action of these atromiRs is described, and the challenges to explore their potential as putative therapeutic target in cachexia are discussed.

Our work in chapter 2 reveals that the involvement of miRNAs in cancer cachexia was hardly addressed and identified that systematic analyses of differential

regulation of miRNAs in patient-derived muscle biopsies are lacking. In **chapter 3**, we profiled 754 unique miRNAs in quadriceps muscle biopsies of NSCLC patients with cachexia in comparison to healthy controls. We identified 28 differentially expressed miRNAs of which five miRNAs were upregulated and 23 were downregulated. Bioinformatics-based analysis showed that these miRNAs were involved in pathways related to the degenerative or regenerative processes of muscle tissue. We are the first to identify differentially expressed miRNAs putatively involved in lung cancer cachexia. These findings call for further studies to investigate the causality of these miRNAs in muscle atrophy and their potential as biomarker or putative therapeutic target in cachexia.

Optimal nutritional care is pivotal in treatment of cancer cachexia, and the effects of nutrients may extend beyond the provision of adequate energy intake, targeting different mechanisms or metabolic pathways that are affected or deregulated by cachexia. The evidence to support this notion, derived from nutritional intervention studies in experimental models of cancer cachexia, is systematically discussed in **chapter 4**. The combination of high quality nutrients in a multitargeted, multinutrient approach appears explicitly promising, although more studies are needed to define optimal quantities and combinations of nutrients. We also concluded that standardization in dietary design should improve, and that development of more representative experimental models is essential to translate experimental findings faster into the clinic.

Various lung tumor-bearing animal models have been used to study cancer cachexia and to study the therapeutic effect of (nutritional) interventions. However, these models do not simulate anatomical and immunological features key to lung cancer and associated muscle wasting. In **chapter 7**, we therefore developed and characterized a syngeneic, orthotopic lung cancer cachexia (OLCC) mouse model and evaluated whether it replicates systemic and muscle-specific alterations associated with human lung cancer cachexia. The OLCC mice showed significant loss of body weight and skeletal muscle mass and function, indicating the development of cachexia. The observed muscle wasting was accompanied by increased systemic inflammation and alterations in the regulation of muscle protein turnover. Finally, we showed that the muscle transcriptome of the OLCC mice reproduces key aspects specific to cachexia in lung cancer patients. The newly developed OLCC mouse model provides opportunities to advance cancer cachexia research, including understanding the etiology of lung cancer cachexia and the development and evaluation of novel intervention strategies.

Two main readout parameters are a major challenge in monitoring the development of cancer cachexia in an orthotopic mouse model: muscle mass and tumor volume. Micro-CT is an X-ray-based imaging technology for small animals that allows tissue density quantification, which makes micro-CT a good technique for longitudinal muscle mass and tumor volume assessment. To investigate muscle mass and tumor volume changes quantitatively, manual segmentation of the muscle and tumor volume on the CT reconstruction is required, which is a very time-consuming task and prone to inter- and intra-observer bias. Therefore, we developed algorithms for the standardized, automated segmentation and quantification of muscle mass (**chapter 5**) and tumor volume (**chapter 6**) on whole-body mouse CT-scan using artificial intelligence techniques. The performance of these algorithms was in good agreement with the training dataset. In addition, the algorithms increase the amount of data derived from animal studies while reducing animal numbers and analytical workload.

In **chapter 8**, we provide proof of concept for the application of nutritional interventions to target cancer cachexia. We investigated the therapeutic effect of a multitargeted, multinutrient intervention diet containing high protein, leucine, fish oil, vitamin D, galacto-oligosaccharides and fructo-oligosaccharides on the development of cachexia in the OLCC mouse model. The intervention diet delayed the onset and progression of experimental lung cancer cachexia, while attenuating loss of muscle strength, mass and alterations in protein turnover signaing, resulting in prolonged maintenance of muscle function and average time to study endpoint.

Finally, in **chapter 9**, the findings and advances described in this thesis and opportunities for future research are discussed in a broader context of four fundamental pillars in cancer cachexia research: I) Early detection and dynamics of cancer cachexia; II) Intra-cellular mechanism of muscle wasting in cancer cachexia; III) Extra-cellular mechanism/triggers of cancer cachexia; and IV) Management of cancer cachexia.