Lung cancer cachexia

IMPACT

Relevance for the cancer cachexia research community

Lung cancer is the second most commonly diagnosed cancer and accounts for 18% of total cancer deaths, which makes lung cancer the leading cause of cancer-related deaths worldwide [1]. The overall prognosis of lung cancer patients is extremely poor due to the difficulty of diagnosing at early-stage and the resistance to current therapies [2], and is further adversely affected by cachexia [3-5]. Approximately 80% of the lung cancer patients are at risk of developing cancer cachexia [6], which is a wasting syndrome characterized by progressive weight loss resulting from depletion of skeletal muscle mass with the loss of fat mass [7].

Cachexia is a poor prognostic factor and has major adverse clinical implications. It adversely influences performance, and quality of life and number of hospitalizations in lung cancer patients [8, 9]. In addition, decreased response to antitumor therapies has been reported in cachectic lung cancer patients. Effective treatment of cachexia has a direct impact on patient's overall health status and survival and increases the effectiveness of costly antitumor therapies [10-12]. Nevertheless, there is no FDA approved cachexia treatment and no standard of care [13]. To a large extent, this is attributable to the lack of screening tools and limited understanding of the underlying mechanisms, as a result of the complexity of the syndrome and the challenge of performing clinical trials in this fragile patient population. In cancer cachexia, studies using patients-derived muscle biopsies can provide valuable new insights in the underlying metabolism and pathways, yet are very scarce. Moreover, research in patients to study the longitudinal dynamics of the wasting process in relation to the underlying molecular mechanisms with the currently available tools, is even more demanding. Therefore, the development of relevant preclinical animal models and novel methodologies to enable such studies are required. The research described in this thesis provides new insights that contribute to unravelling the underlying mechanisms and dynamics of lung cancer cachexia, and reports on the development and validation of a novel murine model that better replicates the etiology of the human disease, and provides proof of concept for the application of nutritional interventions to target cancer cachexia.

To maximize exposure and impact for follow up in research and implementation, all the data described in this thesis is/will be published in peer reviewed journals and presented at international conferences within the field of cancer cachexia, laboratory animal sciences, nutrition and respiratory diseases.

New leads for therapeutic interventions

We aimed to identify the role of miRNAs in the regulation of muscle atrophy and specifically cachexia. We proposed to name these miRNAs 'atromiRs' (Chapter 2 [14]). Remarkably, our review revealed that the involvement of miRNAs in cachexia was hardly addressed and data from patient-derived muscle biopsies was scarce. Subsequently, we profiled 754 unique miRNAs in quadriceps muscle biopsies of NSCLC patients with cachexia in comparison to healthy controls, and used an elegant bioinformatics approach followed by confirmation in a larger cohort, which also included non-cachectic NSCLC patients. We were the first to identify differentially expressed miRNAs putatively involved in lung cancer cachexia (Chapter 3 [15]). These findings are very promising and need to be validated in an external cohort. These findings have provided leads for the development of new drugs (miRNA-based therapeutics) that affect muscle wasting directly in the skeletal muscle, and also for the development of biomarkers for early detection and intervention.

In addition to the miRNA profiling, RNA sequencing was performed on the same set of muscle biopsies (Chapter 7). The majority of the differentially expressed genes were downregulated, and pathway enrichment analysis showed that these were primarily associated with mitochondrial and metabolic processes. Dysfunctional mitochondria have been implicated in lower muscle strength and muscle wasting in experimental cancer cachexia models, however, yet poorly described in human cancer cachexia [16]. As mitochondria-targeted drugs may serve as promising therapy for mitochondrial myopathies [17], such therapies could benefit muscle wasting associated with cancer cachexia by improving mitochondrial function and positively affecting the whole body metabolome.

Overall, the observations in the muscle biopsies of this well-characterized group of NSCLC patients with cachexia not only provides new insights in lung cancer-associated muscle wasting, but may serve as a unique reference data set to evaluate the relevance of findings obtained in studies using experimental models.

A new, promising pre-clinical lung cancer cachexia model

Because of challenges posed by heterogeneity, limited group sizes and the invasive nature of sample collection, most of the information regarding the underlying mechanisms of (lung) cancer cachexia and the potential therapeutic options is derived from pre-clinical models. However, the most frequently used animal models do not simulate anatomical, physiological, and immunological features key to lung cancer and associated cachexia seen in humans. These

shortcomings hamper translating experimental findings into the clinic. Therefore, we developed and characterized a novel syngeneic orthotopic lung cancer cachexia (OLCC) mouse model in which a relatively small tumor (max 0.5% of total body weight) grows in its original stroma, the lung (Chapter 7). We showed that these lung tumor-bearing mice developed cachexia approximately 3 weeks after tumor injection. The observed muscle wasting was accompanied by increased systemic inflammation and alterations in the regulation of muscle protein turnover reflecting increased proteolysis. Strikingly, comparison of muscle transcriptomic data of lung tumor-bearing mice with cachexia and NSCLC patients with cachexia revealed a remarkable overlap in processes that were affected in muscle of man and mice. Overall, this demonstrates that our model closely overlaps the human pathophysiology and biology of lung cancer cachexia, which has two important ramifications. First, these findings answer to the need for critical assessment of the external validity of an experimental model, which is an integral part of 'Refinement' of the 3R-principles that are embedded in the EU regulation on animal research [18, 19]. Timely and robust confirmation that an experimental model captures the critical aspects of a disease or condition ensures that the use of animals and associated discomfort and costs of follow-up work based on that model are not spent in vain. Secondly, it is anticipated that mechanistic insights and interventions evaluated in this model will better translate to the human setting. This will contribute to increased clinical success rates for interventions in development, and reduce costs and patient burden.

Innovative tools for automatic tumor and muscle volume quantification

Obviously, in contrast to subcutaneous growing tumors, the tumors in orthotopic lung cancer models are not visible and accessible for caliper measurements to assess their growth. Similar to the clinical setting, cone beam computed tomography (CBCT) can be used in preclinical research to detect orthotopic growing tumors in rodent models. However, analysis of the imaging data to extract quantitative information, such as tumor size and shape, is a very labor intensive and time-consuming task. In addition, the analysis is a challenging task as it requires great expertise in anatomy and CT-imaging, and is susceptible to bias. To facilitate the quantification of orthotopic growing tumors in preclinical research, we developed and validated a deep learning algorithm for automatic lung tumor segmentation and quantification on whole-body mouse CT-scans (Chapter 6 [20]). This deep learning algorithm enables fast and highly accurate tumor quantification with minimal operator involvement in data analysis.

To detect when muscle wasting starts and study the dynamics of muscle mass changes in function of experimental life expectancy, longitudinal evaluation of muscle mass is essential. However, until now, skeletal muscle mass evaluation in preclinical animal models is mostly based on assessment of muscle wet masses, or muscle fiber cross-sectional area using histological examination of postmortem tissue. Both methods require muscle dissection and, consequently, are terminal experiments. Therefore, a similar approach as used for longitudinal tumor volume assessment was applied to develop a deep learning algorithm to automatically segment and quantify muscle mass on whole-body mouse CT-scans (Chapter 5 [21]). This deep learning application enables highly accurate non-invasive longitudinal evaluation of skeletal muscle mass changes in mice.

These deep learning algorithms provide us with the unique opportunity to study the relation between tumor development (growth speed, size and location) and muscle mass over time in mice. In addition, they increase the amount of data derived from animal studies while reducing animal numbers, analytical workload and costs. The reduction of animal numbers is an integral part of 'Reduction' of the 3R-principles that are embedded in the EU regulation on animal research [18, 19]. Importantly, these algorithms can easily be implemented in other labs interested in longitudinal evaluation of muscle atrophy and/or hypertrophy, or in research related to conditions associated with volumetric changes in other tissues.

Therapeutic efficacy of nutritional intervention

It is generally acknowledged that a multidisciplinary approach is currently considered the best option to tackle cancer cachexia, in which nutritional intervention is recommended as an integral part of the multimodal therapy [22]. Adequate nutritional care is pivotal to (1) provide building blocks for tissue maintenance, (2) provide energy and (3) regulate metabolic processes (Chapter 4). In the last chapter of this thesis, a targeted dietary intervention, directed at each of these three pillars, was tested in the newly developed mouse model of lung cancer cachexia. The intervention diet delayed the onset and progression of cachexia, and resulted in prolonged maintenance of muscle function. These data are very promising and suggest that targeted dietary interventions have the potential to support patients to maintain muscle function and mass and thereby improve tolerance to cancer and anticancer therapy, increasing quality of life and survival. Considering the high external validity of our novel model, further evaluation of the therapeutic effect of an analogous human intervention diet in a clinical trial is warranted to test its efficacy to modulate cachexia, and confirm the feasibility

of integrating dietary interventions as part the treatment regimen for patients with NSCLC.