Title : Identification of new pathways driving muscle atrophy and biomarkers reflecting muscle atrophy in cancer cachexia

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- *Introduction* :

Cancer cachexia is a complex metabolic syndrome characterized by weight loss, in particular skeletal muscle. Loss of muscle mass in advanced cancer is recognized as an independent predictor of mortality and its reversal leads to prolonged survival in animal models. Therefore, maintaining muscle mass seems per se helpful in improving survival in cancer cachexia. However, reliable parameters for early diagnosis of muscle atrophy are still lacking.

- *Aims* :

We aim at highlighting new pathways driving muscle atrophy during cancer cachexia and at identifying new potential biomarkers in mice and potentially in humans. The secondary goal is to develop a signature of early cancer cachexia markers detectable in the plasma.

- *Methods and results :*

To assess muscle atrophy in cancer cachexia, an experimental mouse model was used: C26 carcinoma cells were injected in male CD2F1 mice on day 0 (Bindels *et al*, ISME Journal, 2016). Mice were sacrificed 10 days later and gastrocnemius samples were collected for protein extraction and fractionation. The sarcoplasmic fraction (SF) (=soluble proteins) and the myofibrillar fraction (MF) (=insoluble proteins) were prepared and provided for the proteomic discovery study. Differential label free proteomics was used to compare muscle peptide extracts from cachectic (n=6) and control mice (n=6). Tissue peptide extracts were analyzed on an instrumental system composed of a 2Dnano UPLC (Waters) coupled to a QExactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer (ThermoFisher Scientific). Protein identifications and relative label free quantitations (LFQ) were obtained using MaxQuant and the differential analysis was performed using Perseus (Tyanova *et al*, Nature Methods, 2016). We obtained 974 proteins identified and quantified in SF and 948 in MF. The comparison of the two groups: "*cachectic" versus "control mice*" allowed the selection of a total of 232 proteins found differentially distributed in SF and 203 in MF and being potential biomarkers. Proteins that focused our attention were the most significant ones with the highest difference between both groups. Gene Ontology, Reactome and the David tools were used and showed that the significant proteins were involved in the metabolism of lipids and carbohydrates with potential global perturbations. Many pathways were affected, as well as some structural muscle proteins, and the general inflammatory response. The most abundant proteins in cachectic muscles were involved in coagulation and complement cascades and in the insulin signaling pathway. The less abundant proteins in cachectic muscles were implicated in energy metabolism as the oxidative phosphorylation, the citrate cycle, as well as in protein degradation.

In addition, the significant proteins were searched on the Plasma Proteome Database, leading to 204/232 proteins in SF and 177/203 in MF, that were found detectable in plasma. Some of these will be targeted for further development of a blood test for detecting early cachexia in both mice and humans. The selection of the potential biomarkers is under progress with their empirical detection/ quantitation by a technique of targeted proteomics: Selected Reaction Monitoring (SRM). This SRM method development using mice (cachectic and control) and humans is ongoing for the pragmatic selection of the most relevant biomarkers accessible in the plasma matrix. While the most significant proteins of our lists appear to be linked to inflammation and probably to the inflammatory response driven in cachectic muscles, the markers involved in the SRM signature should also represent the status of the other impacted pathways and perturbed metabolisms that have been highlighted in our muscle analyses.

- Conclusions :

From the proteomic analysis of muscles showing atrophy induced by cancer cachexia in a mouse model, we highlighted some potential biomarkers which require confirmation on blood samples. The development of a targeted proteomic method using SRM should enable this confirmation on mouse plasma. Such SRM signature should also be tested on samples of patients suffering from cancer cachexia.