EDITORIAL

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Cancer cachexia—when proteasomal inhibition is not enough

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Cachexia is a life threatening syndrome associated with several diseases, such as end-stage heart failure, end-stage renal disease, chronic obstructive pulmonary disease, chronic inflammation (i.e. rheumatoid arthritis), acquired immune deficiency syndrome, and cancer.[1,2] Cachexia is found in 31–87% of cancer patients especially in advanced disease stages.[3] It is characterized by progressive weight loss, metabolic alterations, fatigue, and persistent reduction of body cell mass in response to a malignant tumour.[2,4–6] The incidence of cachexia in cancer patients is dependent on the type and site of the tumour. While patients with non-Hodgkin's lymphoma, breast cancer, and sarcomas show low incidences, rates up to 83% in pancreatic cancer patients, and over 85% in patients with gastric cancer have been found. Additionally, around 60% of small-cell and non-small-cell lung cancer patients develop cachexia.[7–9] Cancer cachexia affects the function of several organs such as muscle, adipose tissue, liver, brain, immune system, and heart, collectively decreasing patients' quality of life and worsening their prognosis. Therefore, cachexia must be considered as a true multi-organ syndrome.[10] Because cancer cachexia leads to a decrease in physical performance and quality of life,[11] and is associated with poor survival (accounting for more than 20% of cancer deaths,[7,12–15]) it is of major clinical relevance. Even more so since cachectic patients show lower response rates to chemotherapy[7] and a reduced tolerance to anticancer treatment.[16] Despite its importance, weight loss in cancer patients is rarely recognized, assessed,[17] or treated actively.[18,19] Thus, cancer cachexia represents an important underappreciated clinical syndrome.

Muscle wasting is a major constituent of cancer cachexia

Cancer cachexia involves similar losses of muscle and adipose tissue and on a simplistic way, one could assume that this assures survival of the general organism. However, this response differs from starvation induced cachexia where the majority of weight loss is from adipose rather than muscle tissue.[5,20,21] In cancer cachexia, skeletal muscle wasting cannot be reversed by nutritional intervention arguing for cancer-specific signals that are involved in its pathogenesis. The loss of muscle mass is accompanied by decreased strength, which is responsible for most of the cancer cachexia-associated symptoms, and increased mortality and morbidity of patients.[22,23] Wasting not only involves skeletal but also chest, diaphragm, and cardiac muscle leading to fatigue and respiratory complications.[3] In fact, the majority of cancer deaths are related to respiratory[24] or cardiac failure.[25] Therefore, treatments capable to slow down, stop or even reverse muscle wasting in cancer cachexia may be beneficial for cancer patients in terms of physical independence, quality of life, tolerance to and sensitivity to anticancer treatments, and possibly reduce morbidity and mortality. However, despite its clinical importance and the foreseen impact for patients, the pathophysiology of cachexia-associated muscle wasting is still poorly understood preventing the development of specific therapies. Because maintenance of skeletal muscle mass and function is assured by a well-regulated balance of protein synthesis and degradation, a disturbed protein homeostasis with increased degradation and or decreased synthesis is a

major contributor to muscle wasting in cancer.[26] Increased protein degradation is caused by an elevated activity of muscular protein degrading systems predominantly the ubiquitin-proteasome system (UPS), which plays an important role in cancer patients experiencing weight loss.[8]

Protein degradation is important for skeletal muscle wasting in cancer

The UPS is the main protein degrading system in eukaryotic cells.[27] It mediates the degradation of misfolded and mutated proteins as well as many proteins involved in the regulation of development, differentiation, cell proliferation, signal transduction, and apoptosis.[28–30] Activation of the UPS in skeletal muscle leads to degradation of structural and contractile proteins, most notably myosin heavy chain,[31,32] resulting in atrophy and decreased muscle function.[33] Proteins targeted for proteasomal degradation are ubiquitinated by a hierarchical-ordered series of enzymes, including the ubiquitin-activating enzyme E1, ubiquitin-conjugating enzyme E2, and the ubiquitin E3 ligases, which allow the targeted proteins to be recognized by the 26S proteasome, a multi-subunit protease complex composed of the 20S catalytic core and 19S regulatory particles.[28,29,34] Substrate specificity of the UPS is assured by E3 ligases and substrate adaptor proteins.[35,36] In many models of cachexia, including cancer, UPS activation is thought to mediate muscle atrophy.[37] During tumour cachexia, the UPS is up-regulated in skeletal muscle with an increased expression of the E3 ligase muscle-specific RING-finger 1 (MuRF1) and the substrate adaptor protein FBXO32/Atrogin-1.[38] In a rat model of cachexia, induced by the Yoshida ascites hepatoma cells, UPS up-regulation was associated with increased Fbxo32/Atrogin-1 gene expression[39] and increased protein ubiquitination.[39] This UPS activation in tumour-bearing mice is mediated by an increased activity of the transcription factor nuclear factor kB (NF-kB), which stimulates MuRF1 expression and muscle wasting.[40]

Communication between tumour and host

It remains uncertain how tumour cells communicate with the host to induce cancer cachexia. The often anatomically distant locations between the tumour and the wasting musculature are suggestive for a signal released into the hosts' circulation by the tumour to cause muscle wasting in cachexia. Especially, chronic inflammation with elevated levels of circulating inflammatory cytokines is consistently observed in cachectic cancer patients.[40] Because chronic inflammation affects the function of several tissues (i.e. skeletal muscle, fat, brain, and liver) it is an important cause of cancer cachexia.[41] Among the best studied inflammatory cytokines promoting cachexia are tumour necrosis factor alpha (TNFα), interleukin-6 (IL-6),[42] interleukin-1 (IL-1), and interferon gamma.[43] These cytokines are elevated in cancer[44] and may together trigger muscle wasting, probably by increasing NF-kB or by causing the release of other cytokines.[45,46] For example, TNFα, initially named cachectin,[47] promotes anorexia[48] and skeletal muscle wasting mainly through activation of the NF-kB pathway.[49] Therefore, it has been tested if TNFα blockade is beneficial to prevent cancer cachexia. Indeed, TNFα blockade improved cachexia-associated fatigue in a small group of cancer patients.[50] However, in recent randomized controlled clinical trials anti-TNFα therapies using either the TNFα receptor–blocker etanercept in patients with incurable malignancies[51] or the TNFα-specific monoclonal antibody infliximab in patients with metastatic non-small-cell lung cancer[52] did not prevent or palliate weight loss or muscle atrophy. Instead, infliximab increased fatigue and adversely affected patients' quality of life.[52] These data suggest that targeting TNFα alone is not sufficient to prevent cachexia. IL-6 and IL-1 are up-regulated in animal models of cancer cachexia,[53] and IL-6 levels correlate with weight loss in certain human cancers.[54] Interestingly, cancer cachexia can be attenuated in an adenocarcinoma mouse model treated with anti-IL-6 antibodies.[55] However, if this strategy prevents muscle wasting in cancer patients needs to be shown.

As mentioned earlier, NF-kB is a key regulator of inflammatory responses and involved in muscle atrophy.[40,56,57] In the majority of cells, NF-kB exists in an inactive form in the cytoplasm bound to the inhibitory protein IkB. When stimulated with inflammatory cytokines (i.e. TNFα, IL-1, and IL-6), NF-kB is activated by degradation of IkB proteins. This occurs primarily via activation of IkB kinase, which phosphorylates IkB. IkB phosphorylation leads to its ubiquitination and degradation by the proteasome.[58] This allows free NF-kB to translocate to the nucleus and to stimulate the expression of its target genes.[59] Because NF-kB activation is important for muscle atrophy, many approaches were undertaken to reduce its activity. In mouse muscles, miss-expression of IkB to inhibit NF-kB reduced neurogenic atrophy in tumour-bearing mice.[40] In mice lacking IkB kinase-β, neurogenic atrophy was also attenuated.[56] Synthetic double-stranded oligodeoxynucleotides, which block NF-kB binding to promoter regions has been shown to inhibit cachexia in a mouse tumour model.[60] Importantly, proteasome inhibitors interfere with the NF-kB pathway because they inhibit IkB degradation, which in turn prevents NF-kB activation.[61] Because MuRF1 and FBXO32/Atrogin-1 are NF-kB target genes, proteasome inhibition is expected to prevent muscle atrophy by maintaining NF-kB in an inactive state, and thus preventing up-regulation of MuRF-1 and FBXO32/Atrogin-1. Indeed, the proteasome inhibitor MG132 was shown to

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attenuate immobilization-induced atrophy. Likewise, the proteasome inhibitor bortezomib has been shown to reduce neurogenic atrophy.[62]

Proteasome inhibition as strategy to treat cancer cachexia

Several groups investigated if UPS inhibition is beneficial to prevent muscle atrophy in different mouse models. For this approach, mainly two-specific, potent and reversible proteasome inhibitors, MG132 and bortezomib, were used. Both compounds inhibit degradation of ubiquitin-conjugated IkBα resulting in suppression of the NF-kB signalling pathway.[63] In addition, bortezomib and MG132 reduce proteolysis in skeletal muscle *in vitro*[64,65] and prevented muscle mass loss in an *in vivo* rat model of skeletal muscle wasting induced by denervation and cast immobilization of the hind limb.[62,66] MG132 was shown to preserve muscle and myofiber cross-sectional area by down-regulating MuRF-1 and Fbxo32/Atrogin-1 mRNA in a mouse model of hind limb-immobilization resulting in a diminished rehabilitation period.[67] In addition, an increased proteasome activity was found in a mouse model for Laminin-deficient congenital muscular dystrophy type 1A (MDC1A). Administration of MG132 increased lifespan, enhanced locomotive activity and enlarged muscle fibre diameter in MDC1A mice.[68] Bortezomib had also beneficial effects in the MDC1A mouse model and in MDC1A patient cells.[69] To this end, it was feasible to assume that proteasomal inhibition could be useful to block muscle wasting in cancer cachexia. This hypothesis was subsequently tested by Penna *et al.* who report their findings in this issue of the *Journal.*[70] Penna *et al.* investigated if bortezomib attenuates skeletal muscle wasting in two different and well-established animal models of tumour-induced muscle wasting.[70] Cancer cachexia was induced by intraperitoneal injection of Yoshida AH-130 ascites hepatoma cells in rats and by subcutaneous inoculation of C26 carcinoma cells in mice. As expected, bortezomib reduced proteasome activity on day 7 after transplantation of AH-130 tumour cells in the skeletal muscle, which was accompanied by a decreased NF-kB DNA-binding activity indicating that animals were effectively treated. However, bortezomib administration did not prevent body weight loss and muscle wasting in the AH-130 host rats. It also did not affect MuRF1 and Fbxo32/Atrogin-1 expression. Likewise, bortezomib did not prevent body and muscle weight loss 12 days after tumour implantation in C26-bearing mice. These data together with the published body of evidence indicate that the pathophysiology of cancer cachexia possibly involves additional NF-kB- and proteasome-independent protein degrading systems, such as autophagy and calpain proteases. For example, the autophagy pathway is activated in atrophying muscle of cancer patients.[71–73] In a small cohort, lung cancer patients presented increased levels of the autophagy mediators BCL2/adenovirus E1B 19 kDa interacting protein 3 and light chain 3B, and the transcription factor FOXO1, which promotes autophagy.[71] Similarly, in another study performed on esophageal cancer patients vs. weight-stable non-cancerous control patients, autophagy was identified as the main promoter of skeletal muscle proteolysis.[74] Also calpain proteases have been proposed to initiate protein degradation during cachexia;[75,76] however, limited information concerning their role in muscle wasting is available.[39] Of note, in contrast to the data of Penna *et al.*, the proteasome inhibitor MG132 was found to attenuate weight loss and muscle atrophy, and increased spontaneous activity and survival time in a C26-tumour-induced cancer cachexia mouse model.[77] Together with other studies in which treatment with MG132 was shown to be effective in preventing muscle dystrophy,[78] disuse-induced atrophy,[67] and immobilization-mediated skeletal muscle atrophy,[79] these data indicate that MG132 could be useful to prevent cancer cachexia. However, MG132 also inhibits cathepsin and calpain proteases,[80] which might partially explain its favourable treatment effects and the differences between MG132 and bortezomib used by Penna *et al.*. Therefore, the individual contribution of specific protein degrading systems and proteases in cancer cachexia as well as the effect of their inhibition as treatment option needs to be defined. In addition, MG132 also reduced the tumour burden with a reduction in tumour volume and weight.[77] Because tumours seem to interact with the host by factors that induce muscle wasting a reduction in tumour size will also lead to their decrease, which may result in less wasting. Therefore, it is difficult to decide if proteasome inhibition by MG132 in muscle or in the tumour or both together reduced the occurrence of cancer cachexia in this mouse model. A side-by-side comparison of bortezomib and MG132 in C26-bearing mice could show if this accounts for the differences of both studies.

In addition, despite the large body of evidence supporting the UPS as a major driver of muscle atrophy in animal models investigations of UPS components or activity in patient material are non-conclusive. Studies including patients with critical illness, following bed rest, limb immobilization, COPD, and ageing have demonstrated both increased and decreased expression of MuRF1 and FBXO32/Atrogin-1.[81–84] Of note, there is no convincing evidence for increased UPS-mediated proteolysis in skeletal muscle biopsies of cachectic cancer patients.[71,85] Investigations of UPS activity in biopsies of the quadriceps muscle have shown similar levels to healthy controls in patients with lung cancer and weight loss below 10%.[85] Another study in lung cancer patients with low weight loss found no changes in UPS components.[86] In contrast, in gastric cancer patients with average weight loss of 5%, increased UPS activity was measured compared with controls.[87] These data implicate that there are certain forms of muscle atrophy and forms of cancer cachexia, respectively,

where UPS-mediated proteolysis is not the main pathway in the disease process and proteasome inhibition will therefore not be able to attenuate the atrophy response. This hypothesis is strengthened by data from non-cancer patients and animal models. For example, the UPS is activated in muscle of patients with intensive care unit-acquired weakness[81] and mice with polymicrobial sepsis.[88,89] Accordingly, mice exposed to endotoxin, a cell wall component of gram-negative bacteria, display enhanced proteolytic activity in the diaphragm leading to a reduction in muscle mass, force, and protein content. However, proteasome inhibition of endotoxin-treated mice did not prevent reductions in diaphragm-specific force generation indicating that inhibition of proteasome-mediated proteolysis alone does not prevent endotoxin-induced reductions in diaphragm force generation.[90] These data indicate that proteasome inhibition is not effective in each and every model of muscle atrophy.

In general, bortezomib is well tolerated by patients. Nevertheless, it is associated with some toxicity, such as nausea, diarrhoea, fatigue, and generalized weakness. Indeed, proteasome inhibition is a double-edged sword as the proteasome mediates degradation of a multitude of proteins involved in critical biological processes and its inhibition possibly promotes protein accumulation that may cause proteotoxic effects. Penna *et al.* described that bortezomib exerted a transient toxicity, which led to a reduced food intake in their animals.[70] Because food deprivation increases MuRF1 and Fbxo32/Atrogin-1 expression and causes muscle atrophy,[89] this side effect is important for data interpretation. Even if bortezomib would have inhibited cachexia, the treatment-associated reduced food intake could have counteracted this effect.

Finally, caution is needed when interpreting animal models of cancer cachexia to the true cachexia phenotype in patients. Penna *et al.* used well-established models of cancer cachexia. However, these models do have their limitations; the young age and rapid progression of inoculated tumour cells are only few of them. Furthermore, analysis 7 days after injection of AH-130 cells and 12 days after C26 transplantation, respectively, argues for an acute cachexia model. These points do not reflect the clinical situation in tumour patients where cachexia develops over a longer period in mainly older patients. Further studies also need to consider that cancer cachexia is a continuum with at least three stages of clinical relevance including pre-cachexia, cachexia and refractory cachexia.[91]

Cancer cachexia is a multifactorial syndrome

In conclusion, we need to realize that not a single cytokine or signalling pathway is responsible for cancer cachexia; it is rather caused by a multitude of factors and signalling pathways that we only begin to understand. Therefore, it is unlikely that treatments targeting only one aspect of the syndrome, such as the proteasome or individual cytokines, will effectively block its pathogenesis or progression. Cachexia itself is a multifactorial syndrome that might phenotypically appear similar. However, the appearance of a patient does not provide mechanistic information. The path towards the cachectic phenotype is most likely different for various tumour types. When the pathway responsible for the cachectic phenotype is uncertain, it is difficult to develop or apply the right treatment. The data from Penna *et al*. should encourage us to look into proteasome- and NF-kB-independent signalling pathways involved in cancer cachexia and identify novel targets to treat this syndrome. Further studies are needed to elucidate precise signalling pathways involved in cancer cachexia; and first steps towards this direction are already being taken.[92,93] Some of the factors increased in cancer cachexia, such as angiotensin II,[94] and the transforming growth factor beta family members myostatin[95] and activin A,[96] have already been identified. Especially, myostatin and activin A are up-regulated in patients with various types of malignancies (for a review, see[97]). Myostatin inhibits muscle growth and its overexpression promotes it.[98] Therefore, inhibition of myostatin–activin A signalling is an attractive therapeutic target for treatment of cancer-associated muscle wasting. Indeed, blockade of activin receptor IIB (ActRIIB), the receptor for activin A and myostatin and other transforming growth factor beta family members, was sufficient to prevent cachexia, increased muscle function and even prolonged survival in several cancer cachexia mouse models.[99,100] This study showed that inhibition of cachexia has direct impact on cancer death.[99] If myostatin inhibitors are beneficial as therapies for cancer, associated muscle wasting is currently being tested in clinical trials. Further mechanisms, which are involved in the pathogenesis of cancer cachexia include growth differentiation factor-15, macrophage inhibitory cytokine-1,[101,102] leukaemia inhibitory factor,[103] Fn14,[104] signal transducer and activator of transcription 3,[105] parathyroid hormone-related protein,[106] and histone deacetylases;[107] and this list is steadily increasing.[92,93] Future studies need to be performed in a tumour-specific and disease stage-dependent manner to answer the question why certain cancers are more prone to cause cachexia than others, and to identify tumour-specific differences in cachexia pathways. Information gained by those studies will be useful to develop target and disease stage-specific treatments. Although animal models are useful in this regard they may or may not reflect the situation in patients at advanced tumour stages, and we need to appreciate this limitation cautiously. Results from treatment studies on cachexia animal models that could not be successfully confirmed in patients should encourage us to intensify collaboration between clinicians and basic scientists to promote patient-based research and to tackle this life-threatening syndrome.

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Conflict of interest

None declared.

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