REVIEW

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Molecular mechanisms of cancer cachexia-related loss of skeletal muscle mass: data analysis from preclinical and clinical studies

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Abstract

Cancer cachexia is a systemic hypoanabolic and catabolic syndrome that diminishes the quality of life of cancer patients, decreases the efficiency of therapeutic strategies and ultimately contributes to decrease their lifespan. The depletion of skeletal muscle compartment, which represents the primary site of protein loss during cancer cachexia, is of very poor prognostic in cancer patients. In this review, we provide an extensive and comparative analysis of the molecular mechanisms involved in the regulation of skeletal muscle mass in human cachectic cancer patients and in animal models of cancer cachexia. We summarize data from preclinical and clinical studies investigating how the protein turnover is regulated in cachectic skeletal muscle and question to what extent the transcriptional and translational capacities, as well as the proteolytic capacity (ubiquitin–proteasome system, autophagy–lysosome system and calpains) of skeletal muscle are involved in the cachectic syndrome in human and animals. We also wonder how regulatory mechanisms such as insulin/IGF1–AKT–mTOR pathway, endoplasmic reticulum stress and unfolded protein response, oxidative stress, inflammation (cytokines and downstream IL1ß/TNFα–NF-κB and IL6–JAK–STAT3 pathways), TGF-ß signalling pathways (myostatin/activin A-SMAD2/3 and BMP-SMAD1/5/8 pathways), as well as glucocorticoid signalling, modulate skeletal muscle proteostasis in cachectic cancer patients and animals. Finally, a brief description of the effects of various therapeutic strategies in preclinical models is also provided. Differences in the molecular and biochemical responses of skeletal muscle to cancer cachexia between human and animals (protein turnover rates, regulation of ubiquitin-proteasome system and myostatin/activin A-SMAD2/3 signalling pathways) are highlighted and discussed. Identifying the various and intertwined mechanisms that are deregulated during cancer cachexia and understanding why they are decontrolled will provide therapeutic targets for the treatment of skeletal muscle wasting in cancer patients.

Keywords Autophagy–lysosome; Cancer cachexia; Glucocorticoids; Inflammation; Myostatin; Oxidative stress; Proteostasis; Skeletal muscle; Ubiquitin–proteasome

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Introduction

Cancer cachexia is a systemic hypoanabolic and catabolic syndrome characterized by a progressive unintentional loss of body mass that cannot be reversed by nutritional support.[1] The prevalence of cachexia is quite variable, reaching up to 70% in pancreatic cancer patients.[2] Skeletal muscle represents the primary site of protein loss during cancer cachexia.[3] Image analysis indicates that muscle depletion varies from 7 to 30%,[4,5,S1–S6] (references S1 to S215 are listed as a supplementary reference list) the depletion worsening with the severity of the disease.[5] Another noteworthy feature is muscle weakness, which often may precede muscle loss[S7,S8] and which aggravates with cachexia.[S7] Besides muscle, the syndrome can also affect other tissues including bone, heart, liver and/or adipose tissue.[6] Overall, cancer cachexia increases the risk of surgical complications[7,S9] and chemotherapy toxicity[S10–S13] and lead to functional impairments, respiratory complications and fatigue that markedly reduce patients' quality of life and ultimately patients' survival. It has been estimated that in 2013, 15.8 subjects per 10 000 of the total population in European Union suffered from cancer cachexia.[8]

Over the past 15 years, considerable progress has been made in elucidating the molecular pathways involved in muscle mass loss, thus providing outstanding information about the pathophysiological mechanisms involved in cancer cachexia. However, our current knowledge comes mainly from animal models. Even though the number of human studies exploring the biological mechanisms of muscle wasting is increasing, human studies remain scarce, thus raising the question of the translatability of animal findings to human clinical research. This is a critical point as therapeutic targets may differ between human and animals. Surprisingly, a detailed comparative analysis between cachectic human patients and cachectic cancer animals is missing.

This review provides a comprehensive analysis of the molecular mechanisms involved in cancer cachexia-associated muscle mass loss in preclinical and clinical studies. Article database of the US National Library of Medicine (PubMed) were searched using 'cancer cachexia' AND (muscle or 'skeletal muscle') AND specific terms (corresponding to the studied scientific area of each section). Any additional relevant literature was obtained from the reference lists of the published papers. This methodology has been applied for each section of the current review. Preclinical and clinical studies dealing with cancer, but for which cachexia was not characterized or demonstrated, were not included.

Protein synthesis and degradation rates

Little information is presently available on muscle protein turnover in cachectic cancer patients (*Figure* 1). A decline in muscle protein synthesis rate has been reported in cachectic cancer patients compared with healthy subjects.[9,10]

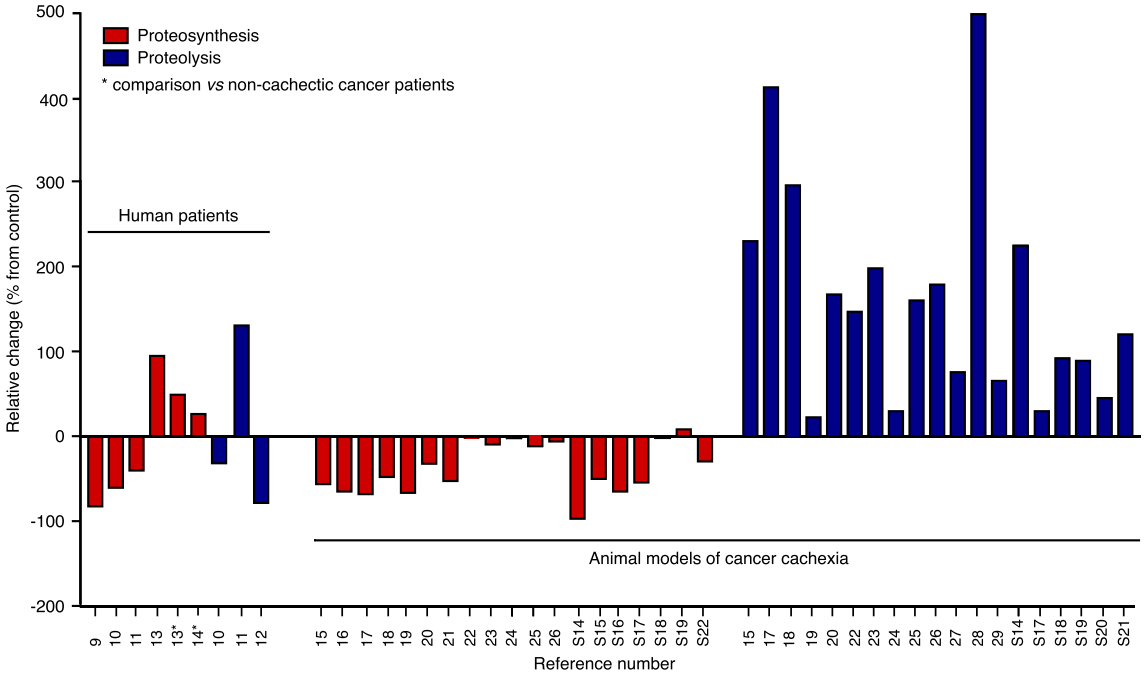

Figure 1 Relative variation in protein synthesis and degradation rates in clinical and preclinical studies. Variations have been calculated from data reported in quoted references. All data are expressed relative to controls or non-cachectic cancer patients (indicated by an asterisk).

Colorectal cancer patients with lower leg muscle mass than control subjects display unchanged muscle protein synthesis and a trend towards increased muscle protein breakdown, but reduced postprandial muscle protein synthesis,[11] which overall may confer a net catabolic status. Surprisingly, a lower protein degradation rate has been reported in weight-losing cancer patients than in healthy controls.[12] This was also accompanied by a blunted anabolic response to feeding.[12] Higher protein synthesis rates have even been documented in skeletal muscle of cachectic cancer patients,[13,14] suggesting the existence of a compensatory mechanism that may bridle the magnitude of muscle mass loss.

Animal studies clearly show a reduction in muscle protein synthesis[15–21,S14–S17] and an increase in protein breakdown,[15,17–20,22–29,S14,S17–S21] (*Figure* 1), the extent of the variation being associated with the severity of cachexia.[17,18,S16] Of note, unchanged muscle protein synthesis rates have also been frequently reported in animal experiments.[22–26,S18,S19,S22]

Therefore, currently available human data on muscle protein turnover in cachectic cancer patients are very limited and sometimes contradictory, even if a reduction in protein synthesis emerges as a mechanism that contributes to a net catabolic status. In animal models, a decrease in protein synthesis and an increase in protein degradation lower muscle mass. It is important to remind that cancer cachexia develops much more slowly in patients compared with animal models of cancer cachexia. Thus, it would be expected that protein degradation rate would be less easily identified as increased in human, compared with animals. More sensitive methods applied to the kinetic analysis of protein turnover during disease progression would be therefore necessary to provide a clear picture of skeletal muscle protein turnover in cachectic cancer patients.

Gene expression capacity during cancer cachexia

Transcriptional and translational capacities are essential for skeletal muscle homoeostasis. The transcriptional capacity is determined by the efficiency of the transcriptional machinery and the number of myonuclei, whereas the translational capacity depends on the ribosomal content and the global cellular RNA pool that is available to sustain the synthesis of myofibrillar protein.

Myonuclear death

Although not systematically observed,[S23] hallmarks of nuclear death by apoptosis have been reported in skeletal muscle of cachectic cancer patients (PARP and DNA fragmentation,[S24] increased BAX pro-apoptotic factor mRNA level,[S25] increased p53 phosphorylation,[30] activation of caspase-8 and caspase-9[30]). Animal studies also show the presence of TUNEL-positive nuclei,[27,31,S20,S26–S28] an increase in PARP cleavage[S29] and DNA fragmentation[32,S30–S32] in cachectic skeletal muscle. Increases in BAX pro-apoptotic-to-BCL2 anti-apoptotic protein and mRNA ratios[S30,S33–S35] and BAX mRNA level[S36] as well as increased expression[S33,S34] and cleavage[S35] of caspase-3, along with increased caspase-1, caspase-3, caspase-6, caspase-7, caspase-8 and caspase-9 activities,[32,S29,S37] have also been described. Accordingly, transcriptomic studies highlight heightened expression of apoptosis genes in muscle of cachectic cancer mice.[33,34]

As skeletal muscle is a very heterogeneous tissue, where approximately half of its nuclei reside outside of muscle fibres,[S38] it is essential to distinguish myonuclei from those of neighbouring mononuclear cells. Whereas most of studies presented above did not report the localization of nuclei,[27,30,S20,S26,S28] some animal studies detected TUNEL-positive nuclei outside (mononucleated cells)[31,S27] and inside[S27] the muscle fibre, suggesting the existence of myonuclear death. The multinucleated nature of the muscle fibre also implies that the death of a single myonucleus does not mean the destruction of the entire fibre. Accordingly, the whole number of fibres is maintained in skeletal muscle of human cachectic patients[S3,S39] and cancer mice.[31] Therefore, myonuclear death during cancer cachexia may trigger individual nuclei decay segmentally along the fibre, which may, over an extended period of time, locally weaken the transcriptional capacity of the fibre and contribute to atrophy and muscle dysfunction.

Muscle fibre microenvironment

Pioneering studies at the beginning of the 20th century[35] and later[S40] reported an increased number of nuclei in the vicinity of the sarcolemma. More recently, inflammatory cells,[36] macrophages and fibro-adipogenic progenitors cells[37] have been identified in muscle of cachectic cancer patients. A higher number of activated satellite cells,[38] activated stem cells,[38] undifferentiated cells[S41,S42] and inflammatory cells[27,39,40,S20,S26,S28] have also been detailed in muscle of cachectic cancer mice.

The presence of inflammatory cells and macrophages, together with an augmented cytokine content (see below), suggests the persistence of unresolved inflammation in cachectic skeletal muscle, which may alter the properties of myogenic precursors.[S43] It has thus been shown that although progenitor cells do commit to the myogenic programme, they do not completely differentiate.[38] Muscle frailty may also cause localized episodes of muscle regeneration, as exhibited in cachectic cancer patients[37,S44] and in cancer mice,[27,39,S20,S28] and also illustrated by the presence of centralized

myonuclei.[27,37,39,S20,S28,S44] Furthermore, skeletal muscle of cachectic cancer mice regenerates less efficiently after freeze clamping-induced[38,S45] or cardiotoxin-induced[S46] muscle injury. Therefore, cancer cachexia is associated with an altered regenerative capacity of skeletal muscle that could lead to a reduction in the renewal of myonuclei by myogenic precursor cells, whose activity would be further impaired by unresolved inflammation, thus ultimately contributing to decrease the transcriptional capacity of the fibre.

Ribosomal content

Reductions in skeletal muscle rRNA level,[16,20] 28S rRNA level[S47] and ribosomal S6 protein content[41] have been reported in skeletal muscle of tumour-bearing animals, along with defective transcriptional activity of polymerase I,[S48] the enzyme responsible for ribosomal gene transcription and a drop in ribosomal gene transcription.[42] Ribophagy (selective ribosome degradation by the autophagy–lysosome pathway) would be also increased.[42] Consequently, ribosomal gene transcription and protein machineries appear to be compromised in muscle during cancer cachexia. Clearly, the mechanisms regulating skeletal muscle ribosomal content warrants further research to determine the functional relevance of the translational capacity in controlling muscle wasting during cancer cachexia.

Proteolytic capacity during cancer cachexia

Ubiquitin–proteasome system

Skeletal muscle proteins targeted for degradation are marked by the 26S proteasome complex through an ATP-dependent ubiquitination process (*Figure* 2). The covalent attachment of a chain of ubiquitin molecules to the substrate protein involves a three-step enzymatic cascade driven by E1 (ubiquitin-activating enzymes), E2 (ubiquitin-conjugating enzymes) and E3 (ubiquitin-ligase enzymes). Ubiquitinated proteins are then docked to the proteasome for degradation.[S49] The activation of the ubiquitin–proteasome system has largely been inferred from increased expression of the E3 ligases, MuRF1 (*TRIM63*) and MAFbx/Atrogin1 (*FBXO32*) in multiple atrophy conditions.[S50] MuRF1 targets sarcomeric proteins (actin, myosin heavy chain, troponin) for degradation,[S51–S53] whereas MAFbx targets MyoD and the translational enhancer EIF3F for degradation.[S54,S55]

Although some studies show increased transcript and protein levels of MuRF1,[36,43,S37,S56,S57] and MAFbx,[36,S37,S57] in skeletal muscle of cachectic cancer patients, a majority of investigations report unchanged expression of MuRF1,[11,44–50,S37,S58] and MAFbx,[11,43–45,47–50,S37,S58] in muscle of cachectic cancer patients compared with non-cachectic patients or healthy subjects (*Figure* 2). A decrease in MAFbx expression has even been documented.[46] By contrast, increased ubiquitin mRNA[51,52,S59,S60] and protein[37,52] levels, ubiquitinated proteins[36,S56] and mRNA and protein levels of proteasome subunits[11,53] have been frequently reported in skeletal muscle of cancer patients, even if some discrepancies still persist.[36,50,54,S61] The activity of the ubiquitin–proteasome system also correlates with the severity of body mass loss.[S59] Finally, transcriptomic analyses provide divergent results with either increased,[S62] unchanged[S58] or decreased[45] expression of genes related to the ubiquitin–proteasome system in muscle of cachectic cancer patients.

Animal models consistently highlight an augmentation in the mRNA level of MAFbx,[18,21,41,43,44,55–77,S17,S26,S33,S34,S57,S63–S87] and MuRF1,[18,21,41,43,44,56–62,64,65,67–69,71–76,78,79,S17,S26,S33,S34,S57,S64–S68,S70–S83,S85,S87–S90] in skeletal muscle of cachectic cancer animals (*Figure* 2). This has also been confirmed at the protein level.[18,80–83,S20,S42,S57,S69,S82,S85,S91–S94] Ubiquitin mRNA[23,24,32,56,59,62,73,75,84,85,S17–S19,S57,S71,S72,S95–S98] and protein[82,S86] levels, the content of ubiquitinated proteins[18,21,27,29,59,75,76,82,83,S20,S26,S66,S67,S86,S99] and the transcript[18,20,24,28,32,62,73,85,S17–S20,S26,S95,S96] and protein[S100,S101] levels of several proteasome subunits are also increased in muscle of cachectic cancer mice. Transcriptomic studies also feature an up-regulation of genes related to the ubiquitin–proteasome system in muscle of cachectic cancer animals.[33,34,55,73,78,86–88,S70,S72,S92,S102,S103] Consistent with these observations, proteasome enzyme activity is elevated in muscle of cachectic cancer mice.[28,89,S75,S100,S104]

Regardless of a lesser number of investigations in human cancer patients, the analysis of MuRF1 and MAFbx expression presented above suggests that the ubiquitin–proteasome system does not contribute to muscle mass loss in cancer patients, which is in line with the observation that there is currently no clear evidence of an increase in muscle proteolysis rate in cachectic cancer patients. This would thus give prominence to a major interspecies difference. However, a careful examination of the data reveals that whereas the picture is effectively contrasted when looking at MuRF1 and MAFbx, a much more nuanced picture appears when looking at ubiquitin mRNA level, proteasome subunit mRNA level, ubiquitinated proteins and ubiquitin–proteasome activity (*Figure* 2). Therefore, when considering all markers of the ubiquitin-proteasome system, and not solely MuRF1 and MAFbx, currently available data rather indicate that the ubiquitin–proteasome system would be activated in human cachectic cancer patients. One may also consider the function of other E3 ligases such as MUSA1/Fbxo30[S105] and SMART/Fbxo21,[S106] all required for skeletal muscle atrophy. Although one study reports a decrease in MUSA1 transcript level in muscle of tumour-bearing mice,[S66] other studies show that MUSA1[21,43,71,73] and SMART[43] transcript levels are increased.

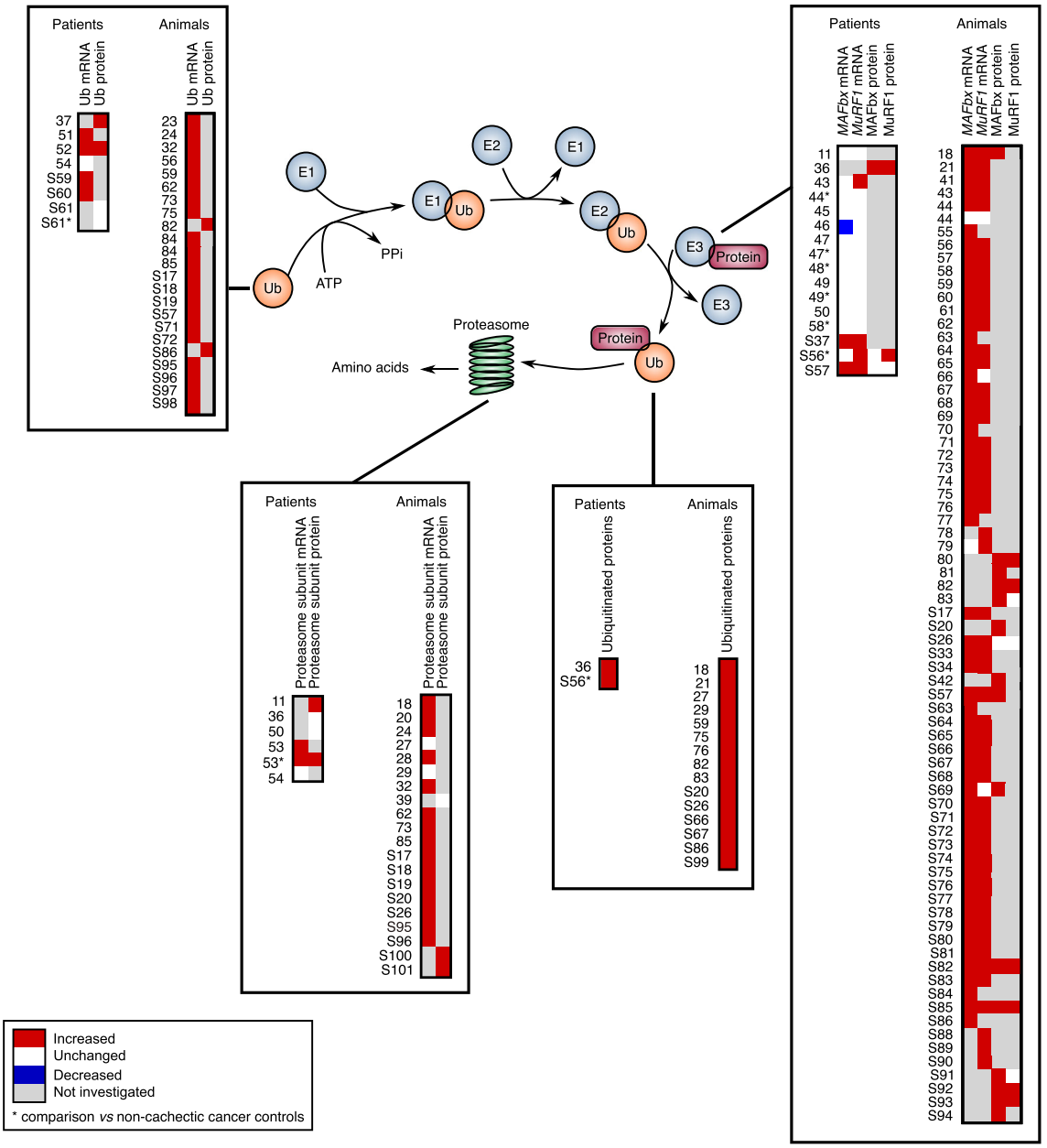

Figure 2 Comparative analysis of the transcript and protein levels of components of the ubiquitin–proteasome system in cachectic skeletal muscle of cancer patients and animals. Proteins are targeted for degradation by the 26S proteasome through covalent attachment of a chain of ubiquitin molecules. The E1 ubiquitin-activating enzyme hydrolyses ATP to bind ubiquitin. E2 ubiquitin-conjugating enzymes receive ubiquitin from E1 and brings it to the E3 ubiquitin-ligase enzymes, which catalyse the transfer of the ubiquitin from E2 to the substrate. This reaction is the rate-limiting step of the ubiquitination process. The ubiquitinated protein is then docked to the proteasome for degradation. One gene encodes the E1 enzyme, whereas one hundred genes encode the E2 enzymes and almost one thousand genes the E3 enzymes. Significant variations are reported in red (increase) or blue (decrease). Unchanged levels are reported in white.

Expression of MUSA1 is also heightened in skeletal muscle of cachectic cancer patients.[43] Expression of Fbxo31, a novel E3 ligase that is induced during denervation and fasting,[S106] is also elevated in muscle of cachectic cancer mice.[71,73] It now remains to determine which substrates are targeted for degradation by these E3 ligases and to what extent they are associated with the regulation of the ubiquitin–proteasome system. Finally, the kinetic of cancer cachexia is very different between patients and animals. In tumour-bearing mice, the subcutaneous injection of cancer cells induces a rapid and violent tumour burden in a couple of days/weeks and a fast and important depletion of muscle compartment. This allows for a well-controlled and accurate kinetic analysis of the ubiquitin–proteasome system. This is also true for genetic models

of cancer cachexia, even if they display a greater heterogeneity in disease progression and appearance of cachexia-associated symptoms. By contrast, the complexity of the clinical context in cancer patients, the difficulty of determining the onset of the tumour surge and the onset of cachexia and the lower rate of skeletal muscle depletion render the kinetic analysis of the ubiquitin–proteasome system during the cachectic syndrome particularly challenging.

Autophagy–lysosome system

The autophagy–lysosome system is responsible for eliminating long-lived proteins and large supramolecular structures, including dysfunctional mitochondria.[S49] Proteins and organelles to be degraded are engulfed during the formation of a double-membrane structure called the autophagosome, which then fuse with lysosomes allowing acidic proteolytic degradation of their contents by cathepsins. Because autophagy is constantly active to remove damaged proteins and organelles, a defect in autophagy will result in muscle functional impairment,[S107] but excessive autophagy will also contribute to muscle mass loss.[S108,S109] Therefore, a tight regulation of the autophagy–lysosome system is necessary for skeletal muscle homoeostasis.

The protein level of autophagy-related genes such as *ATG5*, *ATG7*, *Beclin1* and *LC3B* is increased in skeletal muscle of cachectic cancer patients,[30,47,68,90,S39,S56] together with the number of autophagosomes,[S56] suggesting an increase in autophagosome formation. The number of autophagosomes results from a dynamic equilibrium between autophagosome formation and autophagosome docking and fusion with lysosomes. Therefore, an accumulation of autophagosomes can be also interpreted as a default in autophagosome clearance. The multidomain protein p62/SQSTM1 is a cargo adaptor protein involved in selectively targeting protein aggregates to autophagosomes.[S110] Because p62 is constantly removed by autophagy, a rise in p62 protein content is a good marker of disturbances in autophagosome turnover. p62 protein content[68,90,S56] and p62 aggregates[37] are increased in skeletal muscle of cachectic cancer patients, an observation that is consistent with a disrupted clearance of autophagosomes and suggests that defects in lysosomal rejuvenation/biogenesis and proteolytic capacity could be involved in impaired autophagosome turnover. Nevertheless, the mRNA levels of *TFEB*,[90] a master regulator of lysosome biogenesis,[S111] and cathepsin D[54] are unchanged in muscle of cachectic cancer patients, whereas the mRNA level of cathepsin B[54] is even increased. Although very limited, these data suggest that disturbances in autophagosome turnover may primarily be due to induction of pathways that promote autophagosome formation that are not properly matched to those promoting autophagosome clearance. Of note, some studies do not report any difference in the mRNA[44,45,49] and protein[36] levels of autophagy markers nor in autophagosome number,[36] suggesting that autophagy can be also properly balanced in muscle of cachectic cancer patients.

Expression of autophagy-related genes is increased in skeletal muscle of cachectic cancer animals.[27,41,44,67,68,72,73,77,79–81,91,S26,S28,S66,S78,S88,S112] This has also been confirmed by transcriptomic analysis.[73,86,88,S70,S72] Cathepsin expression[20,32,62,69,77,79,92,S17,S81,S95] and activity[25,26,29,S95] as well as lysosomal proteolysis,[18,29,77,S95] are also increased. Together, these findings indicate that autophagy and the clearance of autophagosomes are activated in skeletal muscle of cachectic cancer mice. However, one should note that some studies mention either unchanged[24] or even decreased[77,84] cathepsin activity and unchanged lysosomal proteolysis[24,89] in muscle of cachectic cancer animals. Furthermore, an accumulation of p62 in muscle of cachectic cancer animals,[68,77,80,91,S26,S112] with an increase in autophagosome number,[68] suggests a disequilibrium between autophagosome formation and clearance.

The E3 ligase TRAF6 mediates the conjugation of Lys63-linked polyubiquitin chains to ULK1 and BECLIN1,[S113] a post-translational modification that regulates their activity. TRAF6 expression is up-regulated in skeletal muscle of cancer patients[52] and in tumour-bearing mice.[S66,S88] Muscle-restricted ablation of *TRAF6* also preserves muscle mass in cancer mice[S88] and restores LC3B and BECLIN1 expression to control levels,[S88] suggesting an important role for TRAF6 during cancer cachexia. To what extent TRAF6 may impact the proteolytic capacity of the autophagy–lysosome system is currently unknown.

Animal studies showed marked alterations in mitochondrial function and network in cachectic muscle.[93] Therefore, the role of mitophagy in controlling mitochondriostasis needs to be questioned. The core mitophagy process is led by the kinase PINK1, which phosphorylates the E3 ligase PARKIN,[S113] whereas other proteins (BNIP3, NIX) are involved in ubiquitin-independent mitophagy.[S113] The protein level of PINK1 and PARKIN is either unchanged[90] or decreased[S114] in skeletal muscle of cachectic cancer patients, whereas the mRNA and protein levels of BNIP3 and NIX are unchanged.[90] In muscle of cachectic cancer animals, PINK1 protein level is either unchanged[94] or increased,[91] whereas that of PARKIN remains unchanged.[94] Consequently, human and animal data may suggest that mitophagy would be either unchanged or decreased. Mitochondrial fusion and fission proteins are essential in regulating organelle dynamic. The expression of fusion proteins is diminished (MFN2)[S114] or unchanged (OPA1),[30,S114] in muscle of cachectic cancer patients, whereas the mRNA level encoding the fission protein FIS1 is augmented,[30,S114] Expression of mitochondrial fusion proteins (MFN1, MFN2, OPA1) is unchanged[79,94] or reduced[95,S36] in skeletal muscle of cachectic cancer mice, whereas expression of FIS1 is unchanged[94,S78] or elevated.[94,95,S36] Therefore, the

fission of mitochondria would be favoured in cachectic muscle, which, together with unchanged or decreased mitophagy, would lead to an accumulation of dysfunctional organelles. Clearly, the data currently available suggest that our view of the mechanisms involved in mitophagy during cancer cachexia is still fragmented and needs further exploration.

Calpains

The calpain family consists of a group of 15 calcium-activated cysteine proteases.[S115] The ubiquitously expressed calpain-1 and calpain-2 as well as the muscle-specific isoform calpain-3 are expressed in skeletal muscle. Calpains have originally been described as facilitators of protein turnover by releasing myofilaments from myofibrills.[S115,S116]

In human, one study demonstrates increased calpain activity in skeletal muscle of non-weight-losing cancer patients,[S117] whereas another one does not show any difference.[S118] Although former experiments indicated that calcium-dependent proteolysis would not be involved in muscle proteolysis in cachectic cancer animals,[20,24] a more recent examination shows that calcium-dependent proteolysis is activated in tumour-bearing mice.[S21] Regarding calpain activity *per se*, investigations show unchanged[23,S75,S119] or increased activity[S33,S100,S104] in muscle of cachectic cancer mice. This last result agrees with reports showing heightened expression of calpain isoforms[24,27,32,62,S17,S21,S71,S72,S74] and decreased expression[S21,S33] and activity[S119] of calpastatin, the endogenous specific inhibitor of calpain. Therefore, considering the major impact of cancer cachexia on skeletal muscle structure and the remodelling function attributed to calpains,[S115] a deeper understanding of calpain function during cancer cachexia would be beneficial.

Insulin/IGF1–AKT–mTOR pathway

Insulin and IGF1

Insulin and IGF1 activate a cascade of phosphorylation that coordinately regulate protein synthesis and degradation (*Figure* 3). IGF1 is mainly produced by the liver under the control of pituitary-secreted growth hormone and accounts for 70–80% of serum IGF1.[S120] However, liver IGF1 only contributes approximately to 30% of adult body size,[S121] indicating that IGF1 originating from other tissues, including skeletal muscle, also contributes to the regulation of tissue growth. Among the different IGF1 isoforms expressed in skeletal muscle, IGF1-Ea, and to a lesser extent IGF1-Eb (IGF1-Ec in human), are the most powerful in increasing muscle mass and force in mice.[S122]

Data show that the driving force of the pathway is reduced in cachectic muscle. Circulating IGF1[S123] and muscle IGF1-Ec transcript level[46] are decreased in cachectic cancer patients. The circulating level of insulin,[26,83,S124] and IGF1,[56,76,83,S64] as well as muscle IGF1-Ea[56,S63] and IGF1-Eb,[18,55,S15,S94] transcript levels, and muscle IGF1 protein content[S64] are also decreased in cachectic cancer animals.

The pathway

Insulin/IGF1 signalling impinges a crucial regulatory step in the pathway, that is, the reaction catalysed by the serine/threonine protein kinase AKT. Under its phosphorylated active form, AKT indirectly activates the kinase mTOR. mTOR and then signals to the translation machinery via the activation of S6K, which controls the ribosomal protein S6, and the inhibition of 4EBP1, which hinders translation initiation by sequestering EIF4E.[S125] AKT also phosphorylates and inhibits GSK3, thus relieving GSK3-dependent inhibition of EIF2B.[S126]

AKT protein level[50,96] and the phosphorylation of GSK3,[96] mTOR[96] and S6K[50,96] are decreased in skeletal muscle of cachectic cancer patients compared with non-cachectic cancer patients, along with the phosphorylated inactive form of the translational repressor 4EBP1[50] (*Figure* 3). Transcriptomic analyses also show that the expression of genes involved in protein anabolism is down-regulated[45,S127] or altered[S62] in skeletal muscle of cachectic cancer patients. Therefore, both transcriptional and post-translational events regulate the pathway. In animal models, a large majority of studies reports an inhibition of the pathway, as shown by the decrease in the phosphorylation of AKT,[S26,S64,S65,S91] mTOR,[18,S15,S20,S91,S100,S128] S6K,[18,21,41,82,S15,S20,S66,S91,S128] S6,[21,41,82,S66,S94] and 4EBP1.[18,41,S15,S94,S100,S128] These events are also associated with weight loss,[18,S15,S128] attesting that the greater is the inhibition of the pathway, the greater is the extent of cachexia. Microarray analyses also highlight a transcriptional regulation of the pathway in skeletal muscle.[33] Consequently, evidence based on global transcript and biochemical analyses indicate that the activity of the insulin/IGF1–AKT–mTOR pathway is reduced in cachectic muscle of cancer patients and cancer animals.

However, some discrepancies exist between studies both in human and animals. Some human investigations reveal unchanged[11,30,36,49,50] or even increased AKT phosphorylation[47] in skeletal muscle of cachectic cancer patients, together with unchanged phosphorylation of GSK3,[47,50] mTOR,[47,50] S6K[11,47,50] and 4EBP1[11,47] (*Figure* 3). Similarly, AKT phosphorylation remains unchanged,[57,58,S20,S63,S66,S67], or even increased,[18,97,S94] in muscle of cachectic cancer animals. A few works also describe unchanged phosphorylation of S6K,[39,58] S6[97] and GSK3,[S68] and sometimes even increased phosphorylation of S6K,[57,S63] 4EBP1[S91] and

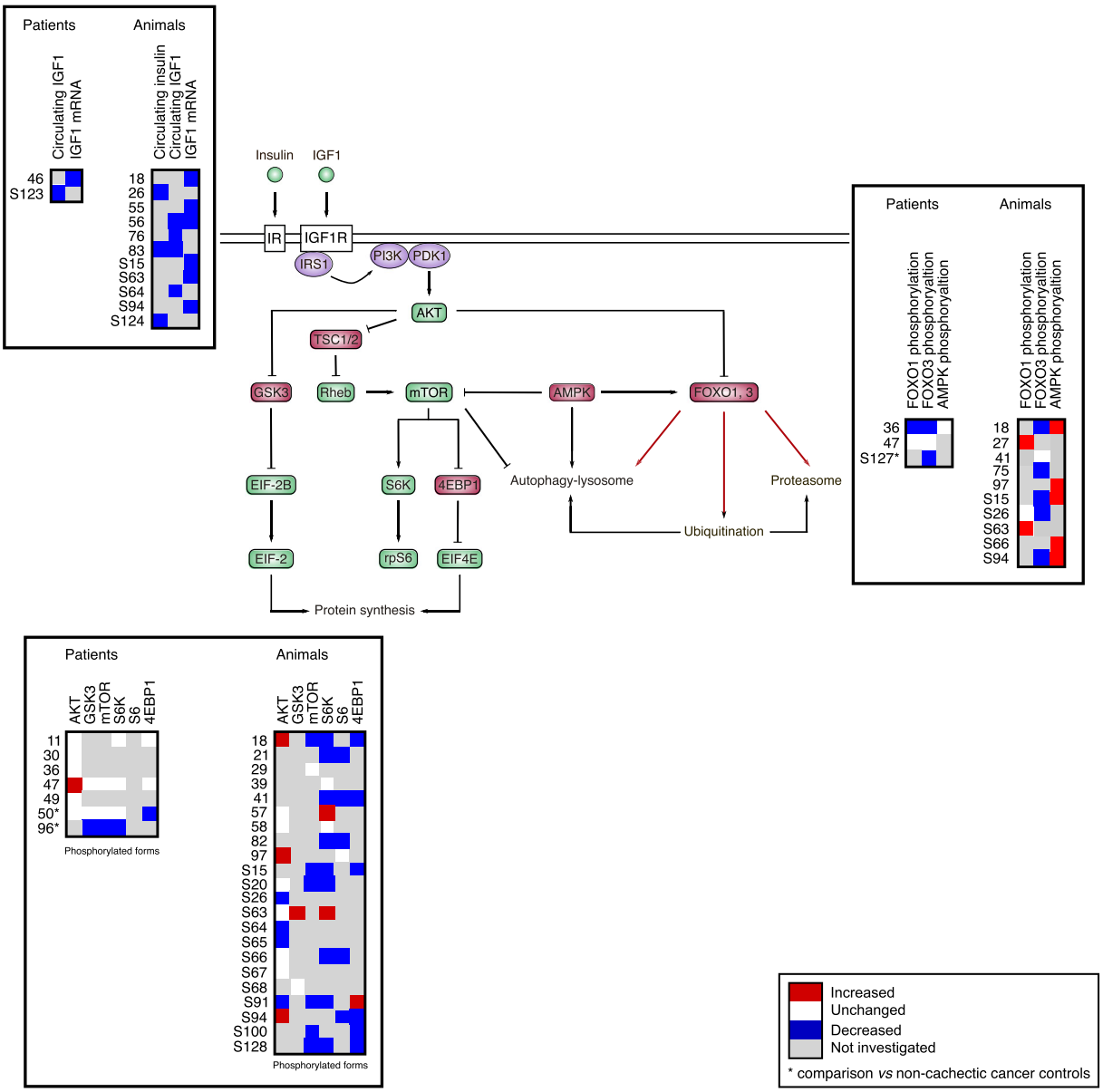

Figure 3 Comparative analysis of the regulation of the insulin/IGF1–AKT–mTOR pathway in clinical and preclinical studies. Upon receptor activation, IRS1 promotes the phosphorylation of phosphatidylinositol 4,5-bisphosphate into phosphatidylinositol 3,4,5-triphosphate at the plasma membrane by recruiting the kinase PI3K. Phospholipid phosphorylation promotes AKT recruitment and activation by PDK1. AKT positively or negatively regulates multiple targets including mTOR from the mTORC1 complex (not represented) and FOXO transcription factors. AMPK, whose activity is increased by energy stress, is another important modulator of the pathway. Black arrows indicate post-translational regulation. Red arrows indicate transcriptional regulation. Significant variations are reported in red (increase) or blue (decrease). Unchanged levels are reported in white.

GSK3.[S63] Divergences between studies are important to consider. First, an activation of the pathway, which is at first counter-intuitive, could be interpreted as a compensatory response aimed at limiting the magnitude of muscle mass loss. Second, these results also strongly suggest that the pathway can be turned on or off depending on the clinical/experimental context (cancer type, severity of the disease and progression through the disease) and that a temporal regulation of the pathway may occur during the time course of the disease. Is the insulin/IGF1–AKT–mTOR pathway constantly repressed to maintain the driving force of cancer cachexia, or are there episodes of reactivation of the pathway that succeed/alternate to episodes of repression? The data presented above hint at the second possibility would be an interesting alternative to consider. Finally, these data also suggest that regulatory influences coming not only from insulin/IGF1 but from other sources may converge to the pathway to modulate its activity.[S125] For instance, the accumulation of amino

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acids released from catabolized proteins may mitigate the down-regulation of mTOR.[S129] Conversely, AMPK, a metabolic sensor that down-regulates mTOR signaling,[S130] is activated in muscle of cachectic cancer mice[18,97,S15,S66,S94] and may thus further exacerbate mTOR inhibition.

FOXO transcription factors

AKT inhibits the FOXO family of transcription factors by phosphorylation.[S131] FOXO regulate the expression of MuRF1 and MAFbx, as well as that of genes of the autophagy-lysosome system.[S106,S108,S109,S132,S133] AKT thus allows a coordinated regulation of protein synthesis and degradation.

FOXO-related genes are up-regulated in skeletal muscle of cachectic cancer patients,[37] and the transcript levels of FOXO1 and FOXO3 negatively correlate with body mass and muscle mass.[98] The ratio of the phosphorylated-to-total forms of FOXO1[36] and FOXO3[36,96] is diminished in skeletal muscle of cachectic cancer patients (*Figure* 3), suggesting heightened FOXO nuclear translocation and transcriptional activity. Importantly, the reduction in FOXO1 and FOXO3 phosphorylation reported in Puig-Vilanova et al.[36] was accompanied by an increase in MuRF1 and MAFbx protein content, which is consistent with a FOXO-dependent regulation of these E3 ligase. Similarly, unchanged transcript level of MuRF1 and MAFbx was associated with unchanged phosphorylated-to-total forms of FOXO1 and FOXO3 in muscle of cachectic cancer patients.[47]

In animal models of cancer cachexia, FOXO3,[18,75,S15,S26,S94] phosphorylation is lowered, which agrees with increased FOXO3 nuclear localization[83] and transcriptional activity[69] reported by other studies (*Figure* 3). Furthermore, microarray analysis of FOXO-regulated transcripts[34] and motif analysis of promoter sequences[S70] identify FOXO as a transcription factor involved in muscle atrophy during cancer cachexia. This concurs with the observation showing that blocking FOXO prevents muscle fibre atrophy and spares force deficits in tumour-bearing mice.[34] Nevertheless, a promoter analysis applied to skeletal muscle of cancer patients shows that weight-loss associated genes have only fewer FOXO binding sites,[S58] highlighting that the molecular characteristics of skeletal muscle from cachectic cancer patients may be different from those of preclinical models.

Finally, some animal studies report unchanged FOXO1[S26] and FOXO3[41] phosphorylation, whereas others even report increased FOXO1[27,S63] phosphorylation and decreased FOXO1 DNA binding activity.[S63] This may be interpreted as a compensatory mechanism to limit the extent of muscle mass loss. These data also remind us that additional FOXO-regulatory mechanisms exist, including phosphorylation by FOXO-activating kinases, such as AMPK, acetylation, ubiquitination and methylation.[S131] This adds another level of complexity to the FOXO code and may dictate the transcription of different FOXO target genes.

Endoplasmic reticulum stress and unfolded protein response

The endoplasmic reticulum (ER) is an organelle involved in the folding, maturation and trafficking of newly synthesized proteins.[S134,S135] ER stress, which leads to an abnormal accumulation of unfolded or misfolded proteins, is perceived by three key transducers of the unfolded protein response (UPR). ATF6, IRE1α and PERK act as a surveillance system to relieve ER stress and regulate proteostasis.[S136] Although adaptive UPR contributes to skeletal muscle homoeostasis, its prolonged or exacerbated activation leads to muscle atrophy.[S137]

The expression of several ER stress markers (GRP78, HSP60, HSP70, calnexin and calreticulin mRNA; PDIA3 and PI3K mRNA and protein) and the UPR (ATF6, XBP1 and CHOP mRNA and protein) is up-regulated in skeletal muscle of cachectic cancer patients,[S37] as well as the ratio of the phosphorylated-to-total forms of PERK and eIF2α.[S37] Together, these data indicate the existence of an ER stress and the activation of the UPR in muscle of cachectic cancer patients. Nevertheless, supplementary investigations are needed to obtain reliable clinical data regarding the implication of ER stress and the UPR in cancer cachexia. In animal models of cancer cachexia, the induction of ER stress and all three arms of the UPR has been demonstrated.[S66] These findings were recently corroborated by several animal studies,[S138–S141] thus providing robust evidence of ER stress and UPR activation during cancer cachexia. One should note, however, that a study mentions unchanged expression of multiple markers of ER stress and the UPR.[S112] This also agrees with repressed phosphorylation of eIF2α reported in skeletal muscle of cachectic cancer rodents.[S63]

A relevant question raised by these data is the function of ER stress and UPR activation during cancer cachexia. Under experimental ER stress, PERK-mediated eIF2α phosphorylation contributes to stoppage of translation.[S142] The UPR represses the insulin/IGF1–AKT–mTOR pathway and activates autophagy.[S143] Furthermore, ATF4 transcription factor (PERK arm), whose expression is increased in cachectic skeletal muscle,[78] promotes muscle atrophy by modulating a subset of atrogenes like Gadd45α, which induces the remodelling of chromatin to repress genes involved in anabolic signalling and to activate pro-atrophy genes.[S144] During cancer cachexia, the activation of ER stress and the UPR is associated with a noticeable inhibition of the insulin/IGF1–AKT–mTOR pathway and increased AMPK activity.[S66] Therefore, an interplay between ER stress, the UPR, the insulin/IGF1–AKT–mTOR pathway, AMPK and the

expression of atrogenes may contribute to down-regulate protein synthesis and activate proteolysis. Intriguingly, the use of 4-phenylbutyrate (ER stress pan-inhibitor) or the targeted ablation of PERK in skeletal muscle aggravates the deleterious effects of cancer cachexia in tumour-bearing mice,[S66,S138] suggesting that ER stress and the UPR (PERK arm) could be also viewed as a response originally aimed at limiting the effects of cancer cachexia.

Oxidative stress

Oxidative stress (OxS) is due to an excessive production of reactive oxygen species (ROS) and reactive nitrogen species, together with impaired antioxidant defence, which results in an accumulation of oxidized and damaged proteins, organelles, membranes and DNA.[S145] In skeletal muscle, OxS reduces muscle strength and triggers atrophy.[S146]

OxS is increased in skeletal muscle of cachectic cancer patients, as shown by the elevation in total protein carbonylation[36] and malondialdehyde-protein adducts,[36,S147] and the reduced expression of genes encoding antioxidant proteins,[S25] such as superoxide dismutase 2, glutamate–cysteine ligase (involved in glutathione synthesis) and Nrf2 (a transcription factor that regulates the expression of multiple cytoprotective genes). However, an increase in the protein level and activity of the antioxidant enzymes, superoxide dismutase 1 and 2, has also been reported in skeletal muscle of cachectic cancer patients,[36] which may illustrate the induction of a compensatory mechanism to alleviate increased ROS production.

OxS is also increased in skeletal muscle of cachectic cancer mice, as indicated by higher levels of ROS,[91,94,S75,S148] lipid peroxidation[40,63,99,100,S75,S149] and protein carbonylation,[27,40,63,99,100,S20,S67,S82,S91,S150] as well as by the increase in oxidized-to-reduced glutathione ratio,[63,91,S74,S75,S112,S149,S151] the decrease in glutathione peroxidase activity[S148] and the depletion of reduced glutathione and antioxidant peptides.[S152] The expression of antioxidant enzymes has been documented to be either decreased[27,55,S20,S28,S75,S148] unchanged[27,91,94,95,99,100,S28,S67,S148] or even increased,[40,55,91,100,S28,S67,S74] suggesting that the main factor responsible for augmenting OxS in muscle of cachectic cancer rodents is an increased ROS production. Transcriptomic analyses in mice also highlight increased transcriptional response of OxS-related genes.[33,55,78] Importantly, OxS would be more significant in Type II myofibres[100] and would also precede muscle mass loss,[94] suggesting that OxS could be a precocious event. Altogether, these data indicate that cachectic skeletal muscle is subjected to a chronic OxS that mainly results from increased ROS production associated or not with lowered antioxidant defence.

This obviously raises the question of the origin of ROS and the mechanisms linking OxS to muscle wasting during cancer cachexia. The main sources of ROS in skeletal muscle are complexes I and III of the mitochondrial respiratory chain and NADH oxidases, but also ER and peroxisomes where enzymatic complexes generate ROS.[S145] Sources of ROS are probably multiple during cancer cachexia, but previously reported alterations in muscle mitochondrial metabolism of cachectic cancer mice,[79,93,101,S153] should significantly contribute to ROS production by increasing leakage of electrons and the subsequent formation of superoxide anion. OxS integrates signal transduction pathways by regulating post-translational modifications of proteins through the redox regulation of the thiol side chain of cysteine amino acids.[S154] Redox proteomic studies have revealed that several hundred proteins contain reactive and potentially modulatory cysteine residues,[S155] including transcription factors (FOXO and NF-κB) and kinases (AMPK and mTOR), which functional implication in the loss of muscle mass during cancer cachexia we have demonstrated. Furthermore, the wide spectrum of action of OxS on other biomolecules (lipid and DNA) also suggests that OxS may target other pathways that contribute to muscle wasting during cancer cachexia.

Inflammatory cytokines and downstream pathways

Circulating and skeletal muscle inflammatory cytokines

Systemic inflammation is a well-described feature of cancer cachexia. Increased circulating level of C-reactive protein (CRP) is associated with weight loss in cancer patients.[4,47,51,102,103,S4,S156,S157] The circulating levels of pro-inflammatory cytokines such as IL1ß,[S158] IL4,[104] IL6,[47,50,51,104,105,S159–S163] IL8,[47,104,105,S160,S162,S163] IL10,[105] IFNγ[104] and TNFα[104,S158,S162,S164] are also elevated in cachectic cancer patients. Similar observations have been done in mice models of cancer cachexia for the circulating levels of CRP,[S91] IL1ß,[75,S82,S91] IL6,[18,39,43,55,72,74,75,80,87,95,105,106,S8,S15,S42,S73,S82,S85,S165–S167] IL10,[55] IL11,[87] IFNγ,[55,80,S168] TNFα[22,55,75,80,87,S19,S82,S85,S91,S124,S167] and Tweak.[S91]

The shock wave of this systemic inflammatory response is largely perceived by skeletal muscle. A large majority of human studies shows increased protein contents of IL1ß,[36] IL6,[50,S169] IFNγ,[36] TNFα[S169] and Tweak[S91] in skeletal muscle of cachectic cancer patients. Only one work reports unchanged IL6 and TNFα protein levels.[36] IL1ß,[S92] IL6,[S64,S85] IFNγ[27] and TNFα[S28,S64,S85] protein levels are also augmented in muscle of cachectic cancer mice, whereas one report shows unchanged IL1ß, IL6 and TNFα protein levels.[27] One

may also consider that skeletal muscle itself can produce cytokines. However, a majority of investigations reports unchanged transcript levels (IL4,[S170] IL6[S25] and TNFα[50S25]) in muscle of cachectic cancer patients, whereas one study reports an increase in IL6 mRNA level.[50] The picture is more contrasted in animal models of cancer cachexia with a fairly equivalent number of experiments describing increased (IL1ß,[S8,S92] IL6,[21,43,58,106,S8,S74,S77,S92,S94] and TNFα[S92]) or unchanged (IL6,[95,S67,S69] TNFα[58,S67,S74] and Tweak[41]) mRNA levels in muscle of cachectic cancer mice. Therefore, these data suggest that although non-muscle tissues (host immune system, tumour cells) seem to mainly contribute to skeletal muscle inflammation, it could also be partly supported by an increase in cytokine production by skeletal muscle. Corollary, the contribution of skeletal muscle fibre, resident and/or recruited mononucleated cells in producing cytokines needs to be evaluated.

IL1ß/TNFα-NF-κB pathway

Omic studies in human[S62,S127] and animals[33,34,55,87,88,S92,S103,S171] indicate that cachectic skeletal muscle is subjected to a persistent activation of a pro-inflammatory cytokine signalling. NF-κB transcription factor relays information from various cytokines (mainly IL1ß and TNFα). Five NF-κB transcription factors [p65 (Rel A), Rel B, c-Rel, p52, p50] are expressed in skeletal muscle.[S172] Activation of cytosolic NF-κB occurs when the IκB kinase phosphorylates IκB, resulting in its ubiquitination and proteasomal degradation. This allows NF-κB to translocate into the nucleus and regulate the expression of target genes. NF-κB activation in mouse skeletal muscle overexpressing IκB kinase leads to severe muscle wasting associated with increased MuRF1 expression and ubiquitin–proteasome proteolysis.[S173] Here, we will mainly discuss about the canonical p65 pathway, whose activation is associated with pathological conditions.

The phosphorylation level of NF-κBp65 is similar in skeletal muscle of cachectic cancer patients compared with non-cachectic patients.[S39] However, mRNA level of NF-κB1 (precursor of p50)[50] and NF-κBp65 protein content[36,S169] are increased compared with healthy controls, suggesting a precocious activation of NF-κB pathway before the development of cancer cachexia. A majority of animal experiments reports an increase in the phosphorylated active form of NF-κB,[27,57,74,82,97,S8,S20,S26,S67,S94] the phosphorylated inactive form of IκB,[41] the nuclear localization of NF-κB,[S92] NF-κB DNA binding[S88,S89,S92,S101,S173] and transcriptional activity[27] in cachectic muscle. The phosphorylated active form of NF-κBp65 also negatively correlates with body mass loss and muscle force in cachectic cancer mice.[S8] Although still debated,[88] motif analysis of promoter sequences also identified NF-κB as a transcription factor involved in muscle atrophy during cancer cachexia.[S70] Of note, studies report unchanged NF-κBp65 phosphorylation[41,88,S75] and NF-κB DNA binding activity.[32,88,S98,S174]

IL6–JAK–STAT3 pathway

The JAK-STAT pathway is activated by IFNα, IFNβ, IFNγ, IL2 and IL6.[S175] Upon cytokine binding, activated JAK tyrosine kinase phosphorylates STAT proteins, which translocate into the nucleus to regulate the expression of target genes.[S176] STAT transcriptional activation contributes to muscle wasting by indirect activation of myostatin expression and expression of MAFbx and MuRF1.[S177,S178]

Limited information in patients indicate that the phosphorylation level of STAT3 is similar in cachectic compared with non-cachectic muscle.[S39] By contrast, numerous evidence of IL6–JAK–STAT3 pathway activation have been reported in preclinical models. Circulating IL6 level correlates with the development of cachexia in tumour-bearing mice.[95,S166] STAT3 activation[74,82,95,97,106–108,S8,S15,S69,S82,S94] and the expression of STAT3 target genes[87] are increased in skeletal muscle of cachectic cancer mice. The active form of STAT3 negatively correlates with body mass and muscle force,[S8] and media conditioned with serum of tumour-bearing mice activate STAT3 in C2C12 myotubes.[S178] *In silico* analysis of transcriptome data also reveals STAT3 as a transcription factor involved in muscle atrophy during cancer cachexia.[S70] Finally, hyperactivation of STAT3 in cancer mice exacerbates weight and muscle mass losses compared with control cancer mice.[109] Interestingly, recent results show that the IL6–JAK–STAT3 pathway contributes to the regulation of Noggin, an inhibitor of the anti-catabolic BMP-SMAD1/5/8 signalling pathway[43] (see below).

TGF-ß signalling pathways

Myostatin–SMAD2/3 signalling pathway

Myostatin is a TGF-ß superfamily member acting as a master negative regulator of skeletal muscle growth during embryonic and postnatal development in animals[S179,S180] and human[S181] and also in adulthood.[110] Myostatin binds to activin type IIB receptors (ActRIIB and ActRIIA) leading to the recruitment of TGF-ß Type I receptors (ALK4 and ALK5)[S182] and activation of SMAD2 and SMAD3 proteins (*Figure* 4). Once activated by phosphorylation, SMAD2/3 recruit SMAD4 to regulate the transcription of target genes.[S183] Myostatin also inhibits the insulin/IGF1–AKT–mTOR pathway.[111]

Surprisingly, myostatin circulating level is reduced in cachectic cancer patients compared with healthy controls[S184,S185] and decreased[4] or unchanged[S185] when compared with non-cachectic cancer patients (*Figure* 4). Myostatin mRNA

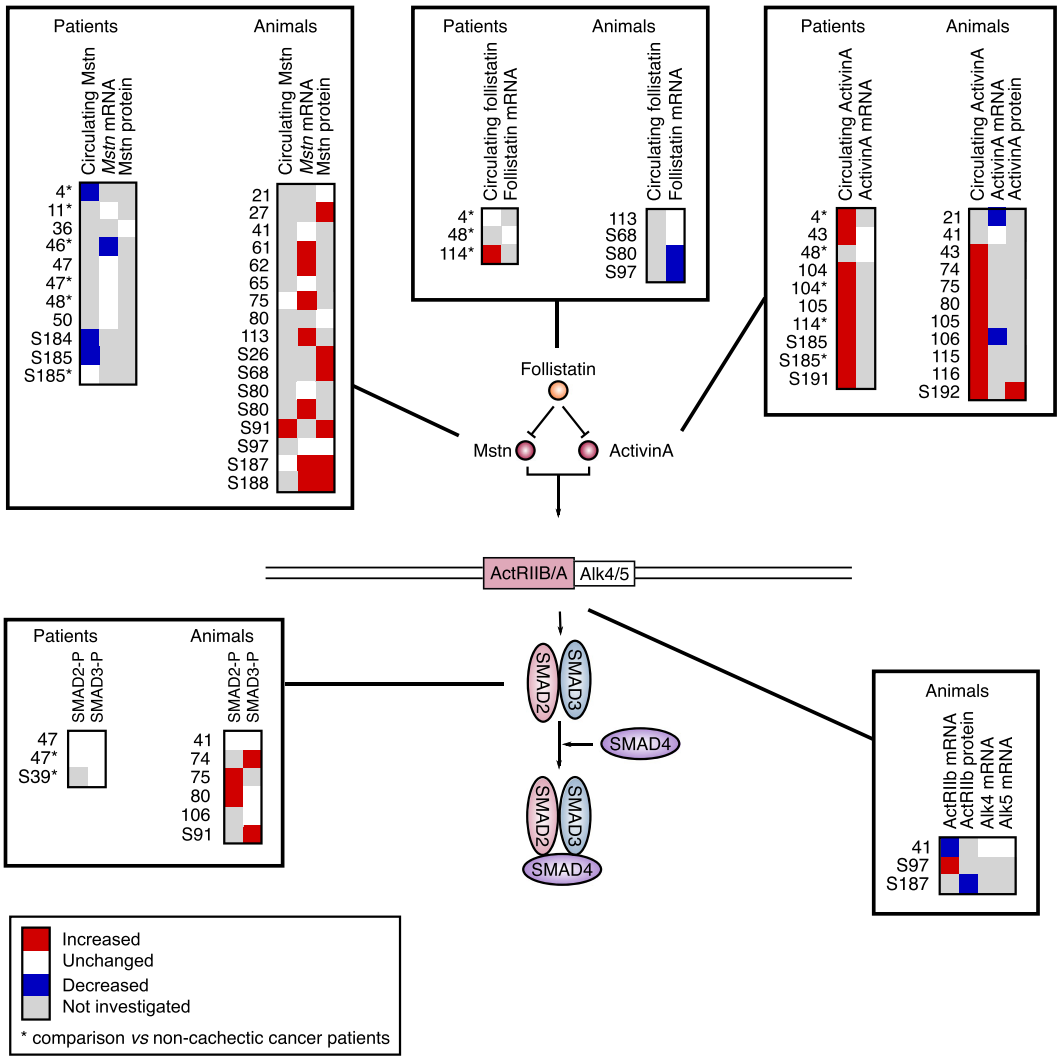

Figure 4 Comparative analysis of the regulation of myostatin/activin A signalling in cachectic skeletal muscle of cancer patients and in animal models of cancer cachexia. Myostatin and activin A bind to activin Type II receptors (ActRIIB/IIA) that activate Type I receptors (ALK4/5/7), which phosphorylate and induce SMAD2/3 to form a complex with SMAD4 and translocate into the nucleus. Myostatin and activin A binding is modulated by the inhibitory action of follistatin. Significant variations are reported in red (increase) or blue (decrease). Unchanged levels are reported in white.

level is also unchanged[11,47,48,50] or diminished[46] in skeletal muscle of cachectic cancer patients. Accordingly, myostatin protein level,[36] as well as the phosphorylation of SMAD2 and SMAD3,[47,S39] is unchanged in muscle of cachectic cancer patients. Circulating[4] and muscle transcript[48] levels of follistatin, an inhibitor of myostatin and activin signalling, are also unchanged. Therefore, these studies indicate that myostatin signalling is not activated in skeletal muscle of cachectic cancer patients, even if this conclusion must be nuanced in the light of investigations showing that genes related to TGF-ß signalling[37] are up-regulated in skeletal muscle of cachectic cancer patients and that the transcript level of ActRIIB negatively correlates with muscle mass in cancer patients.[98]

How to reconcile the fact that myostatin is a master negative regulator of muscle mass in human[S181] and the observation that myostatin expression and signalling are not activated in muscle of cachectic cancer patients? One may first consider that as myostatin is produced by skeletal muscle, myostatin circulating level can thus simply be lowered as a consequence of the reduction in muscle mass during cancer cachexia. Furthermore, the data described above do not exclude the possibility that myostatin expression had increased earlier during the disease when muscle mass had not started to decrease yet. In support of this hypothesis, a strict temporal regulation of myostatin expression has been demonstrated in mice models of atrophy.[112,S186] Kinetic analysis of its expression during the time course of cachexia would allow to answer this question.

Animal studies show that the circulating level of myostatin is either elevated[S91] or unchanged[75,S187] (*Figure* 4). Although some studies report unchanged myostatin expression

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in skeletal muscle of cachectic cancer rodents,[21,41,65,80,S80,S97] a majority of works report an increase in both myostatin mRNA[61,62,75,113,S80,S187,S188] and protein[27,S26,S68,S91,S187,S188] levels. Downstream, the mRNA level of ActRIIB[S97] and the phosphorylation of SMAD2[75,80] and SMAD3[74,S91] are also increased in muscle of cachectic cancer mice. Muscle follistatin mRNA level is either unchanged[113,S68] or decreased,[S80,S97] which is also consistent with increased myostatin activity. However, a reduction in ActRIIB mRNA level,[41] unchanged ALK4/5 mRNA level[41] and SMAD2/3 phosphorylation[41,80,106] have also been reported in tumour-bearing mice. Consequently, and even if this cannot be generalized to all studies, myostatin signalling is commonly activated during cancer cachexia in rodents.

Activin A–SMAD2/3 signalling pathway

Activin A is another TGF-ß family member that binds to ActRIIA and ActRIIB and activate SMAD2/3 signalling with comparable potencies and efficacy as myostatin does.[S189] Increasing circulating activin A levels in mice increases E3 ligase expression, inhibits the insulin/IGF1–AKT–mTOR-pathway and reduces muscle mass and function.[S190]

Activin A circulating level is consistently elevated in cachectic cancer patients,[4,43,104,105,114,S185,S191] as well as in cachectic cancer mice[43,74,75,80,105,106,115,116,S192] (*Figure* 4). By contrast, studies indicate that skeletal muscle activin A mRNA level is either decreased[21,106,S192] or unchanged,[41,43] whereas activin A protein level is increased.[S192] Therefore, circulating activin A may thus come from another source than skeletal muscle, in particular tumour cells.[21,75,116,S76,S192–S194] Importantly, while myostatin circulating level is higher in mouse than in human[S189] and is the main negative regulator of muscle mass in mouse,[S87] activin A circulating level is higher in human than in mouse.[S189] Therefore, activin A could play a more prominent role in cachectic cancer patients than myostatin.

BMP–SMAD1/5/8 signalling pathway

BMP (BMP7, BMP13 and BMP14) bind to BMP Type II receptor (BMPRII), ActRIIB or ActRIIA and promote the recruitment and activation of Type I receptors BMPRIA (ALK3), BMPRIB (ALK6) or ActRIA (ALK2). This triggers the phosphorylation of SMAD1, SMAD5 and SMAD8, which, together with SMAD4, regulate the expression of target genes. As SMAD4 is shared by SMAD1/5/8 and SMAD2/3, BMP–SMAD1/5/8 and myostatin–SMAD2/3 pathways operate in parallel, and in opposition. Increased BMP-SMAD1/5/8 pathway activity in muscle induces hypertrophy,[S105,S195] whereas its inhibition causes muscle atrophy[S105,S195] and abolishes the hypertrophic phenotype of myostatin-deficient mice,[S105] indicating that this pathway is dominant over myostatin signalling.

Diminished BMP signalling and augmented expression of the BMP inhibitor Noggin are observed in skeletal muscle of cancer patients and mildly cachectic mice.[43] Importantly, both IL6 and activin A trigger the expression of Noggin,[43] a BMP–SMAD1/5/8 pathway inhibitor. BMP signalling inhibition is also associated with neuromuscular junction impairment.[43] Therefore, perturbed BMP signalling appears to be a critical pathogenic mechanism regulating muscle mass and function in cancer patients and animals.

Glucocorticoid signalling

Glucocorticoids are steroid hormones produced by the adrenal glands under the control of the hypothalamic–pituitary axis. The hypothalamus secretes CRH, which stimulates the secretion of ACTH by the anterior pituitary gland. ACTH then binds its receptor on the adrenal cortex to activate the biosynthesis and release of glucocorticoids. Glucocorticoids bind the nuclear receptor NR3C1 of target tissues to activate or inhibit the transcription of multiple target genes.[S196] Glucocorticoids are well known to exert strong catabolic effects on skeletal muscle by activating the expression of multiple genes involved in proteolysis while inhibiting those involved in proteosynthesis.[S197,S198]

Circulating glucocorticoid level is increased in cachectic cancer patients[117,S199,S200] and in cachectic cancer mice.[22,23,26,85,86,118,S124,S201–S203] Hypothalamic CRH mRNA level[67] and pituitary ACTH secretion[S204] are also increased in animals, as well as the adrenal gland mass in cachectic cancer patients[S205] and animals.[25,S204,S206] More recently, our group established that the hypothalamic–pituitary–adrenal axis was activated in cachectic cancer mice, along with increased corticosterone level in serum and muscle, and increased skeletal muscle expression of glucocorticoid-responsive genes.[119] Interestingly, the analysis of transcriptomic data also reveals an increase in the expression of multiple glucocorticoid-responsive genes in muscle of cachectic cancer mice.[33,34,44,55,73,78,86–88,S70,S72,S83,S92,S102,S103] Therefore, a neuroendocrine mechanism that involves the hypothalamic–pituitary–adrenal axis may also contribute to the transcriptional regulation of skeletal muscle catabolism.

Therapeutic perspectives

A brief description of the effects of therapeutic strategies in preclinical models is presented below.

IGF1

IGF1 treatment reduces weight loss and improves outcome in a rat model of cancer cachexia.[S207] Anamorelin is a ghrelin agonist that increases the production of growth hormone from the pituitary gland and stimulates the liver to secrete IGF1.[S208,S209] Anamorelin has proven efficacy to limit the extent of cancer cachexia in human,[S210,S211] suggesting potential positive effects on the regulation of insulin/IGF1–AKT–mTOR pathway in skeletal muscle. However, care should be taken as IGF1 may promote tumour growth, even if there was no clear trend of increased tumour progression due to anamorelin.[S210]

ER stress and the UPR

Targeted ablation of XBP1 (IRE1α arm of the UPR) in skeletal muscle reduces muscle mass loss in tumour-bearing mice,[S139] whereas targeted ablation of PERK worsened muscle wasting.[S138] Different arms of the UPR may thus be implicated differently and provide different signalling outcomes.

Oxidative stress

Administration of different antioxidants (α-tocopherol, dehydroepiandrosterone, cocktail of catechins, quercetin and vitamin C) have provided contrasted results. Some studies show a sparing effect on muscle mass loss,[S75] reduced expression of E3 ligases[63S75] and a restoration of MyoD and myogenin expression,[S75], whereas another investigation reports an acceleration of cachexia by increasing muscle atrophy and promoting tumour growth.[S76]

TNFα–NF-κB

Therapeutic strategies invalidating TNFα receptor,[S18,S19,S31] as well as the injection of anti-TNFα antibody,[22] failed to provide beneficial results. However, the pharmacological inhibition of NF-κB has proven its efficiency in cancer cachectic animals.[27] Besides, selective pharmacological inhibition of iNOS, which is a downstream effector of NF-κB pathway and is highly expressed in cachectic muscle,[120] ameliorates cancer cachexia in mouse.[120]

IL6–JAK–STAT3

Cancer mice lacking IL6[107,109,S69] do not develop cachexia. Injection of an anti-IL6 antibody[S166] or an anti-IL6 receptor antibody[18] prevents cachexia progression in tumour-bearing animals. STAT3 inhibition also reduces muscle wasting in tumour-bearing mice.[S178,S212]

Myostatin/activin A-SMAD1/3

Cancer cachexia is blocked by myostatin gene invalidation,[41] the administration of a myostatin antisense RNA,[S188] a myostatin antibody,[31] a soluble form of ActRIIB,[21,62,75,113,S17,S192] an ALK4/5 receptor antagonist,[66] and by the administration of IMB0901 (myostatin signalling inhibitor).[S93] AAV-targeted inhibition of myostatin and activin A also prevents muscle wasting in tumour-bearing mice.[S87]

BMP–SMAD1/5/8

Increasing BMP signalling in skeletal muscle of tumour-bearing mice by gene delivery or pharmacological means can prevent muscle wasting.[43]

Glucocorticoids

Ablation of adrenal glands does not attenuate cachexia[26,S213] in tumour-bearing animals. However, adrenalectomy itself induces weight loss.[S214] The steroid inhibitor RU486 shows contrasted results, with some positive effects on the attenuation of body weight loss[S202] or not.[118,S201] However, RU486 also exerts anti-progestogenic and anti-androgenic effects, which may mitigate the potential anti-catabolic effects of glucocorticoid inhibition. Muscle wasting is abrogated in muscle-specific glucocorticoid receptor knockout mice inoculated with LLC cells.[61] This spatially targeted tissue approach suggests that targeting glucocorticoids through the hypothalamic–pituitary–adrenal axis by specific molecular tools may be promising in preclinical models of cancer cachexia.

Conclusion and future directions

This analysis of the literature highlights several points and redraw the contours of some accepted ideas.

Our analysis shows the existence of species-dependent molecular and biochemical responses. This is an essential factor to consider when evaluating the relevance of preclinical data to the clinical field. This also raises the question of the preclinical models used for research that should reproduce as closely as possible the complexity of the clinical context. Important factors are the model used (syngeneic ectopic/orthotopic graft, human tumour xenograft, genetic engineered mouse), the rate of disease progression and tumour growth, the age of the animals, the presence of metastasis or not and the use of additional chemotherapy or not.[S215]

There are still uncertainties whether protein degradation rate and MuRF1 and MAFbx expression are increased in skeletal muscle of cachectic cancer patients. This remains a key issue, even though data suggest that the ubiquitin–protea-

some system would be activated in muscle of human cancer patients.

Activin A may play a more pre-eminent role in cachectic cancer patients than myostatin, the reverse being true in animals.

The regulation of the molecular mechanisms involved in cancer cachexia is not a linear process during the natural history of the disease. A temporal analysis of the mechanisms involved in muscle proteostasis should be performed in preclinical models. In cancer patients, due to the complexity of the clinical context and the impossibility to determine the onset of tumour growth, a time-course analysis of muscle atrophy and muscle function will help to more accurately determine the cachectic state of the patients throughout the course of the disease.

Emerging evidence indicate that muscle fibre microenvironment contributes to cancer cachexia. Whether or not this is quantitatively important in determining muscle wasting remains to investigate.

One major unsolved question is the influence of gender on the molecular and biological expression of cancer cachexia. This is a key issue that needs to be addressed.

Finally, although substantial progress has been made in developing therapeutic strategies in preclinical models, treatments of cancer cachexia are not available in the clinic. Considering the numerous molecular mechanisms that are coordinately regulated within a specific time frame during the disease, multi-targeted strategies could be more effective in addressing the diversity and complexity of cancer cachexia.

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Conflict of interest

A.M., Y.S.G. and D.F. declare that they have no conflict of interest.

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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