The Ubiquitin/Proteasome System in Cancer Cachexia

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Introduction

Cancer cachexia (CC) is probably the most debilitating and life-threatening paraneoplastic syndrome. It is characterised by weight loss, anorexia, asthaenia, loss of skeletal muscle protein, depletion of lipid stores, and severe metabolic alterations. CC syndrome is present in about 50% of cancer patients, especially those with tumours of the gastrointestinal tract and lung, and less frequently in those with haematological malignancies and other solid neoplasms, such as breast and thyroid cancer. The majority of terminally ill cancer patients experiences CC, which accounts for about 20% of cancer deaths. This figure translates into approximately 2000000 deaths per year worldwide [1].

The predominant phenotypic feature of CC is the steadily progressive depletion of muscle mass, which is not substantially reversible with any of the currently available nutritional, metabolic, or pharmacological approaches [2].

Muscle depletion reflects an imbalance between the rates of protein synthesis and breakdown. Studies carried out in experimental models as well as in human cancer have shown that muscle atrophy may result from increased degradation, reduced synthesis, or both. However, hypercatabolism of muscle protein, in particular of the myofibrillar proteins actin and myosin, is the most prominent feature, while changes in protein synthesis seem to occur less frequently. Intracellular protein degradation in skeletal muscle depends on several proteolytic systems, namely, the acidic lysosomal, the calcium-dependent, and the ATP-ubiquitin-dependent pathways [3–5].

This chapter will focus on the role played by the ATP-dependent ubiquitin/proteasome pathway in the pathogenesis of muscle wasting of CC.

The Ubiquitin-Proteasome System

Proteins degraded by the ubiquitin/proteasome system are first conjugated to multiple molecules of ubiquitin, a 76-amino acid, 8.5-kDa residue that is highly conserved and present in the cytoplasm of all eukaryotic cells [6]. Ubiquitinated proteins are degraded by the proteolytic 26s proteasome, the catalytic core of which is the 20s proteasome, a barrel-shaped particle consisting of four stacked rings with seven subunits in each ring. This complex and tightly regulated process takes place through different steps (Fig. 1): (1) Ubiquitin is activated in the presence of ATP by ubiquitin-activating enzyme (E1). (2) Activated ubiquitin is transferred from E1 to ubiquitin-conjugating enzyme (E2). (3) The carboxyl group of the activated ubiquitin is coupled to the amino-groups of lysines in the protein substrates by ubiquitin-protein ligase (E3). Reiteration of the ubiquitin-conjugation reactions creates a chain of five or more ubiquitins linked to each other and then to the protein substrate. (4) The ubiquitinated proteins are unfolded by a 19s complex located on the end of the 20s core proteasome. (5) The unfolded proteins are transported into the central chamber of the 20s core proteasome, where the proteins are cleaved by proteolytic sites located on subunits in the inner rings. The proteins are cut progressively into small peptides of six to twelve amino acids that are subsequently released and rapidly hydrolysed to amino acids by cytosolic exopeptidases. (6) The release of ubiquitin from the substrate protein makes ubiquitin available for recycling in the proteolytic pathway [6].

Experiments using fluorogenic peptide substrates and inhibitors have defined five activities for the 20s proteasome: (1) a chymotrypsin-like (CTL) activity that cleaves after large hydrophobic

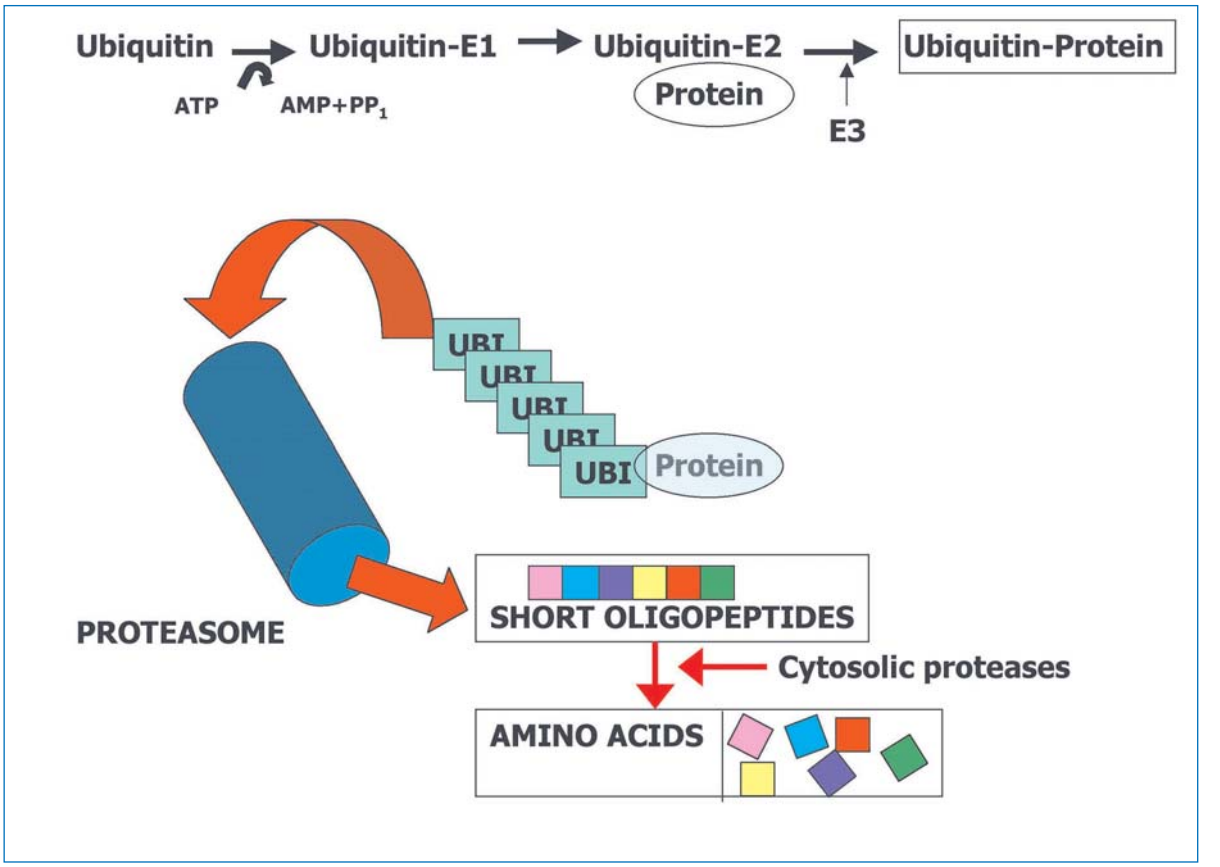

Fig. 1. The ATP-dependent ubiquitin-proteasome pathway. See text for details. *UBI*, ubiquitin molecule; *E1*, ubiquitin-activating enzyme; *E2*, ubiquitin-conjugating enzyme; *E3*, ubiquitin ligase

residues; (2) a trypsin-like (TL) activity that cleaves after basic residues; (3) a post-glutamyl hydrolase (PGP) activity that cleaves after acidic residues; (4) an activity that cleaves preferentially after branched-chain amino acids (BrAAP activity); (5) and an activity that cleaves after small neutral amino acids (SNAAP activity).

E3 ubiquitin-protein ligases are currently believed to be the key component of the conjugation apparatus that confers high specificity to the system. The several hundreds of intracytoplasmic ubiquitin-ligating enzymes, commonly designated as E3s, can be broadly divided into two categories: HECT (homologous to E6-AP C-terminus) domain-containing E3s, and RING (really important new gene)-finger-containing E3s [7]. A critical role in activating proteolysis during atrophy has been ascertained for only three of them, namely, E3[α] and ligases encoded by the genes muscle ring-finger protein-1 (MuRF-1) and muscle atrophy F-box protein (MAFbx), also called atrogin-1 [7] (see below).

The Role of the Ubiquitin/Proteasome Pathway in Experimental Cancer Cachexia

The first evidence that the ubiquitin/proteasome pathway plays a key role in muscle atrophy came from the observation that, while inhibition of calpain proteases and lysosomal proteases is responsible for the 10–20% reduction in intracellular proteolysis, ATP depletion produces much higher degrees of protein breakdown inhibition [8]. The availability of drugs specifically inhibiting proteasome proteolytic activities (i.e. proteasome inhibitors, such as lactacystin, peptide aldehydes, vinyl sulfones, and dipeptide boronic acid analogs)

allowed for in vivo inhibition of proteolysis in experimental models of diabetes, acidosis, sepsis, and denervation atrophy. The results further confirmed the role of the ubiquitin/proteasome pathway in physiological and pathological muscle protein degradation [9–11].

Upregulation of components of the ATP-ubiquitin-dependent pathway has also been reported in experimental models of CC. In 1994, Llovera et al. [12] showed a 500% increase of ubiquitin gene expression in the muscle of rats bearing the fast-growing, cachexia-inducing AH-130 ascites hepatoma. Similarly, Temparis et al. [13] showed that mRNA levels for ubiquitin, 14-kDa E2, and proteasome subunits C8-C9 increased in the tibialis anterior muscles and correlated with the enhancement of energy-requiring proteolysis in Yoshida-sarcoma-bearing rats. Costelli et al. [14] described a 650% increase in the mRNA for 2.4-kb ubiquitin, and a 130% increase in the mRNA for 1.2-kb ubiquitin in the gastrocnemius muscles of rats bearing the Yoshida hepatoma.

Animal models have also shown that muscle proteasomal activity, as measured by the cleavage of specific fluorogenic substrates, is significantly increased in CC. Indeed, Costelli et al. [15] demonstrated that at least CTL activity is significantly increased (+246 ± 39% with respect to controls) in the gastrocnemius muscle of AH-130 ascites-hepatoma-bearing rats, giving the first demonstration that the previously documented modulations of mRNA expression [12–14] are indeed reflective of increased proteolytic activity. Those findings further confirmed the suggestion that muscle wasting in CC is, at least in part, a cytokine-driven phenomenon and that pro-inflammatory cytokines participate in the hyperactivation of intracellular systems involved in protein degradation. In fact, anticytokine treatment with pentoxifylline (a drug that inhibits TNF-α synthesis) and/or with suramin (an anti-protozoal drug blocking the peripheral actions of several cytokines, including interleukin-6 and TNF-α) effectively reduced muscle protein loss by down-regulating the activity of the ubiquitin/proteasome system (Fig. 2) and calpains [15].

More recently, much interest has been devoted to the role of two ubiquitin ligases specifically expressed in striated muscle, namely, atrogin-1/MAFbx and MuRF-1, in the pathogenesis of muscle atrophy [16]. Overexpression of these ligases was initially demonstrated in denervation/disuse-induced muscle atrophy [16], sepsis [17, 18], and during starvation. [19]. Subsequently, using cDNA microarrays, Lecker et al. [20] showed that a common set of genes, termed atrogins, were induced in muscles of fasted mice and in rats with cancer cachexia, streptozotocin-induced diabetes

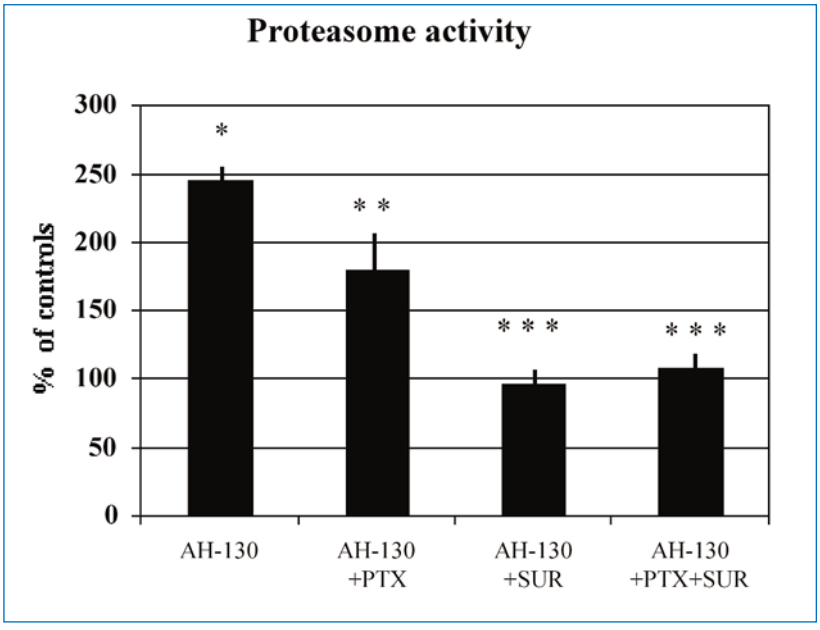

Fig. 2. Proteasome-specific activity in the gastrocnemius muscles of AH-130 ascites-hepatoma-bearing rats (*AH-130*) treated with pentoxyphilline (*PTX*), suramin (*SUR*), or both (*PTX+SUR*). Data are expressed as percent of controls ± SD. *, $p < 0.01$ vs controls; **, $p < 0.05$ vs AH-130; ***, $p < 0.01$ vs AH-130

mellitus, or uraemia. Among the strongly induced genes, some, such as ubiquitin fusion proteins, polyubiquitins, several proteasome subunits, and ubiquitin ligases, were related to protein degradation. The concept that ubiquitin ligases play a pivotal role in muscle atrophy was confirmed by the finding that mice knocked-out for atrogin genes do not develop denervation/disuse-induced muscle depletion [16].

The Role of the Ubiquitin/Proteasome Pathway in Human Cancer Cachexia

Consistent with the observations in the experimental model, Williams et al. [21] demonstrated that mRNA levels for ubiquitin were approximately three times higher in rectus abdominis muscles from patients with miscellaneous cancers than in muscles from control patients. Moreover, the muscle mRNA levels for the 20s proteasome subunits were 300-400% higher than in healthy controls.

More recently, our group confirmed that the muscle ubiquitin/proteasome system is hyperactivated in humans bearing neoplastic diseases. Indeed, ubiquitin mRNA expression was markedly and significantly increased in muscle biopsies obtained preoperatively in 20 patients undergoing surgery for gastric cancer [22]. It is of interest that ubiquitin mRNA overexpression was observed even in patients reporting weight loss of < 5% of the usual body weight (Table 1). Moreover, patients with more advanced disease (i.e., in stage III–IV) had the highest ubiquitin mRNA values.

In a subsequent study [23], Bossola et al. evaluated proteasome-specific activities in intraoperative rectus abdominis muscle biopsies obtained from 23 patients undergoing laparotomy for gastric cancer and 14 controls undergoing laparotomy for benign abdominal diseases (Table 2). The authors showed that proteasome activity is significantly increased in the muscle of gastric cancer patients (five-fold increase in CTL activity, and a two-fold increase in PGP and TL activities). A concomitant, significant overexpression of muscle ubiquitin mRNA was also observed. Higher CTL activity was associated with advanced disease stage, weight loss, and hypoalbuminaemia, in keeping with the previous observation that muscle ubiquitin mRNA levels are influenced by tumour stage [22]. CTL activity was higher in cancer patients over 50 years old, though not in controls. This difference suggests that ageing may substantially alter the response to the catabolic stimuli

Table 1. Muscle ubiquitin m-RNA expression and weight loss in gastric cancer patients. (Data from [22])

Group	Ubiquitin m-RNA (arbitrary units ± SD)
Controls	1162 ± 132
Gastric cancer (weight loss 0–5%)	2338 ± 929[a]
Gastric cancer (weight loss 6–10%)	2581 ± 962[a]
Gastric cancer (weight loss > 10%)	2936 ± 756[a]

[a] $p = 0.0005$ vs controls

Table 2. Muscle proteasome chymotrypsin-like (CTL) activity and weight loss in gastric cancer patients. (Data from [23])

Group	Proteasome CTL activity (nkatal × 10^{-3}/mg protein ± SD
Controls	67.5 ± 37.4
Gastric cancer (weight loss < 10%)	185.3 ± 112[a]
Gastric cancer (weight loss >1 0%)	621.6 ± 499[b]

[a] $p < 0.0001$ vs controls
[b] $p < 0.003$ vs weight loss < 10%

evoked by the tumour.

Taken together, the results of the two latter clinical studies provide a number of interesting insights into the pathogenesis of muscle wasting in human cancer. First, they suggest a crucial involvement of the ATP-dependent ubiquitin/proteasome pathway in cancer-related muscle loss in humans. Second, the observation that both ubiquitin mRNA overexpression and increased proteasome proteolytic activities occur even in patients with insignificant or no weight loss strongly supports the concept that the pathogenic mechanisms ultimately leading to the phenotypic pattern of CC operate early during the clinical course of human neoplastic disease.

In 36 patients undergoing thoracothomy for lung cancer, Jagoe et al. [24] demonstrated an increase of skeletal muscle mRNA for cathepsin B with respect to healthy controls. mRNA levels for components of the ubiquitin/proteasome pathway were also higher in lung cancer patients than in controls, although the differences did not reach statistical significance. However, it should be noted that in Jagoe's study the majority of patients were in an early disease stage, while only nine out of 36 patients had advanced cancer.

Conclusions

There is accumulating evidence, in both the experimental and clinical setting, suggesting an involvement of the ATP-dependent ubiquitin/proteasome system in the pathogenesis of muscle protein degradation in cancer-related cachexia, as well as in other types of muscle atrophy. Therefore, it is reasonable to hypothesise that single or multiple components of this finely regulated degradative pathway may provide the target of pharmacological, molecular or gene therapy.

Since hyperactivation of the ubiquitin/proteasome system has been demonstrated to be an early phenomenon, occurring even before the onset of weight loss and muscle wasting, preventive and therapeutic strategies for cancer cachexia must be adopted soon after a cancer has been diagnosed.

Based on currently available knowledge, however, it is likely that other proteolytic pathways, i.e. calpains and lysosomal proteases, also participate in the complex machinery responsible for muscle depletion in cancer - as well as in other acute and chronic diseases. The interrelationships between the ubiquitin/proteasome system and other cytosolic proteolytic pathways, however, remain to be fully elucidated.

Table 3. Ubiquitin (Ub)-proteasome pathway in human cancer cachexia

Authors [reference]	Year	Type of cancer	No. of patients	Analysis	Results
Williams et al. [21]	1999	Miscellaneous	6	Ub mRNA 20s proteasome subunitsm-RNA	300 increase 300-400% increase
Bossola et al. [22]	2001	Gastric	20	Ub mRNA	200% increase related to disease stage
Jagoe et al. [24]	2002	Lung	36	Ub mRNA E1 mRNA E2 mRNA 20s proteasome C2 subunit	Increases were not statistically significant
Bossola et al. [23]	2003	Gastric	23	Ub mRNA Proteasome-specific activities	130% increase 500% increase of CTL activity 200% increase of PGP activity 200% increase of TL activity CTL activity related to disease stage and nutritional status and age

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