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Review

Prevention and treatment of cancer cachexia: New insights into an old problem

Maurizio Muscaritoli[a,*,1], *Maurizio Bossola*[b,1], *Zaira Aversa*[a], *Rocco Bellantone*[b], *Filippo Rossi Fanelli*[a]

[a]*Department of Clinical Medicine, University 'La Sapienza', Viale dell'Universita 37, 00185 Rome, Italy*
[b]*Istituto di Clinica Chirurgica, Catholic University, Rome, Italy*

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ABSTRACT

Cancer cachexia (CC) is a multifactorial paraneoplastic syndrome characterized by anorexia, body weight loss, loss of adipose tissue and skeletal muscle, accounting for at least 20% of deaths in neoplastic patients. CC significantly impairs quality of life and response to anti-neoplastic therapies, increasing morbidity and mortality of cancer patients. Muscle wasting is the most important phenotypic feature of CC and the principal cause of function impairment, fatigue and respiratory complications, mainly related to a hyperactivation of muscle proteolytic pathways. Most therapeutic strategies to CC have proven to be only partially effective .The inhibition of catabolic processes in muscle has been attempted pharmacologically with encouraging results in animal models. However, data in the clinical setting are still scanty and contradictory. Stimulation of muscle anabolism could represent a promising and valid therapeutic alternative for cancer-related muscle wasting. This goal may be currently achieved with the conventional, short-acting and adverse side effect-rich anabolic steroids. Insulin-like growth factor-1 (IGF-1) plays a critical role in muscle homeostasis, hypertrophy and regeneration. IGF-1 overexpression at the muscular level by gene therapy reverses muscle hypotrophy secondary to catabolic conditions and induces muscle hypertrophy increasing muscle mass and strength. This allows the speculation that this approach could also prove effective in modulating cancer-induced muscle wasting, while avoiding the potentially hazardous side effects of systemic IGF-1 administration. The present review will focus on the potential biochemical and molecular targets of CC therapy, and will define the rationale for a novel, gene therapy-based approach.

1. Introduction

1.1. *Prevalence and relevance of cancer cachexia*

It is estimated that approximately 2 million people die annually worldwide due to the consequences of cancer-related cachexia [1,2]. This debilitating and life-threatening paraneoplastic syndrome is present in about 50% of cancer patients, its prevalence being higher in patients with tumours of the gastrointestinal tract and the lung, than in those with other solid neoplasms, such as breast and thyroid cancer, and haematological malignancies. The clinical features which characterize

* *Corresponding author*: Tel.: +39 06 4997 2016; fax: +39 06 446 1341.
E-mail address: maurizio.muscaritoli@uniroma1.it (M. Muscaritoli).
[1] Maurizio Muscaritoli and Maurizio Bossola contributed equally to this work.
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cancer cachexia are progressive weight loss, anorexia, metabolic alterations, asthenia, depletion of lipid stores, and severe loss of skeletal muscle protein.

Cachexia is present in most terminally ill cancer patients, accounting for about 20% of all cancer deaths [1,2]. As a consequence, cachexia has long been considered a very late and yet ineluctable event in the natural history of cancer patients, leading clinicians to include its treatment among terminal palliative cares. However, the most recent findings on the pathophysiology of this syndrome should definitely modify such a misconception. Indeed, some 80% of upper gastrointestinal tract- and 60% of lung-cancer patients upon diagnosis have already experienced some degree of weight loss [1,2], while several of the metabolic, biochemical and molecular alterations currently believed to be responsible for the phenotypic features of cachexia are already present upon first cancer diagnosis, even in the absence of significant body weight loss [3,4]. Thus, the consolidated view is that cancer cachexia should be regarded to as an 'early phenomenon' [4].

The relevance of cancer cachexia in negatively affecting, not only patients' mortality, but also surgical risk, response to first- and second-line chemo-/radiotherapy, and not lastly quality of life [1,2] has progressively emerged during the recent years. Unfortunately, the predominant feature of cancer cachexia, i.e., the steadily progressive loss of muscle mass and function, has been shown to be only minimally reversible with the currently available nutritional, metabolic or pharmacological tools [1,2]. Consequently, the development of early and effective interventions aimed at preventing rather than reversing the metabolic perturbations ultimately leading to muscle wasting and cachexia is now perceived as a mandatory need by the scientific community, and is fostering the countinuous effort of basic and clinical research.

2. Pathogenic mechanisms

The pathogenesis of CC is multifactorial and includes reduced food intake, alterations in energy and substrate metabolism in the host and accelerated fat and muscle loss [5] (Fig. 1).

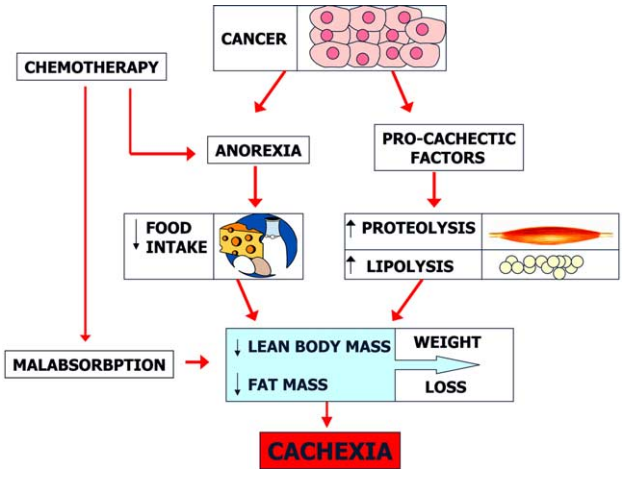

Fig. 1 – The pathogenesis of cancer cachexia is multifactorial.

2.1. *Anorexia and reduced food intake*

A significant number of cancer bearing patients experiences a substantial reduction in nutrient intake which certainly contributes to weight loss [6]. Insufficient energy and protein availability may well be the intuitive consequence of cancer and/or anti-neoplastic treatments and include mechanical obstruction in the gastrointestinal tract, mucositis, vomiting, malabsorption, pain, and depression [7]. Besides these contributing factors, a prominent role is played by anorexia, i.e., the decreased desire to eat secondary to the presence of cancer that is often the presenting symptom and capable of initiating the development of weight loss. The pathogenesis of cancer-related anorexia is complex and multifactorial and implies a disruption of the central and peripheral messages physiologically regulating eating behaviour [8]. A comprehensive review of the pathophysiology and therapy of cancer anorexia has been recently published [9].

2.2. *Altered energy and substrate metabolism*

Together with reduced food intake, increased resting energy expenditure (REE), most likely secondary to the imbalance between pro-inflammatory (Tumour Necrosis Factor-α [TNF-α], Interleukin-1 [IL-1], Interleukin-6 [IL-6], Interferon-γ [IFN-γ]) and anti-inflammatory (IL-4, IL-12, IL-15) cytokines, has been long considered an essential factor in the pathogenesis of cancer cachexia [10]. However, this view has been recently reconsidered based on the observation that the metabolic response to cancer may be extremely heterogeneous, some patients showing hypermetabolism, others being frankly hypometabolic [11,12]. Indeed, in patients with overt cachexia and asthenia in spite of increased REE, total energy expenditure may be reduced as a consequence of reduced physical activity [13]. Glucose intolerance, insulin resistance, increased gluconeogenesis from amino acids and lactate, increased fat oxidation and reduced lipogenesis are the prominent disturbances in energy substrate metabolism [3]. Protein metabolism is also affected, whole body protein turnover being progressively increased in the majority of advanced cancer patients, with consequent further increased in energy demand [14].

Loss of fat mass is a prominent feature of CC: with losses of body fat of up to 85% when the total body weight loss reaches 30% [15]. Increased lipid mobilization in cancer cachexia can be attributed to a tumour catabolic factor named Lipid Mobilizing Factor (LMF) which acts directly on adipose tissue with the release of free fatty acids (FFA) and glycerol in a manner similar to that of lipolytic hormones [16]. This factor is a 43-kDa product showing identity in amino acid sequence, electophoretic mobility and immunoreactivity with human plasma Zn-α2-glicoprotein (ZAG) [17] and can be purified from the urine of cancer patients with weight loss and from MAC16 tumour bearing mice [18].

It has been demonstrated that LMF can increase the sensitivity to the lipolytic stimuli through a variation in the G protein expression by increasing the stimulatory G protein (Gαs) and by inhibiting the inhibitory one (Gαi) [19]. Lipid mobilizing Factor stimulates triglyceride hydrolysis in adipocytes by stimulation of adenylate cyclase in a GTP-dependent process

[20]. Besides its role in fat mobilisation, LMF can also up-regulate uncoupling protein (UCP) 1, 2 and 3 in brown adipose tissue and UCP-2 in skeletal muscle and liver [21]. UCPs are a family of mitochondrial membrane proteins that mediate proton leakage and decrease the coupling of respiration to ADP phosphorylation, resulting in the generation of heat instead of ATP [22]. A role for UCP up-regulation in tissue wasting of cancer cachexia is suggested by the observation that in mice bearing the MAC 16 colon adenocarcinoma with a 24% body weight loss, UCP1 in brown adipose tissue and UCP-2 and 3 in skeletal muscle are increased [23].

2.3. *Muscle wasting*

The steadily progressive loss of muscle mass is by far the most prominent phenotypic feature of CC, causing major functional impairment (Fig. 2). Under normal conditions, muscle mass reflects the balance between the rates of muscle protein synthesis and breakdown. As clearly suggested by studies performed in experimental and human cancer, muscle atrophy may result from increased protein degradation, reduced protein synthesis, or both [24,25].

Muscle hypercatabolism is thought to depend on the hyperactivation of the calcium-dependent and the ATP-ubiquitin-dependent proteolytic pathways [24,25]. Activation of calcium-dependent proteases (calpains), which has been demonstrated in the muscle of tumour bearing rats seems to be essential for the initial degradation of myofibrillar proteins to release actin and myosin [26], which will render them available to further degradative steps [26,27]. The cytokine-dependent hyperactivation of calpains and its role in increased protein degradation of cancer-related muscle cachexia is confirmed by the observation that pharmacological cytokine blockade effectively reduces calpain activity, concomitantly preventing muscle depletion in rats bearing the cachexia-inducing AH-130 ascites hepatoma [28].

Besides cytokines, a host-derived factor has been also causally involved in increased muscle degradation of cancer cachexia: Proteolysis Inducing Factor (PIF), a 24-kDa protein factor was isolated by Todorov and colleagues in the urine of weight-losing cancer patients [29], irrespective of tumour type (breast, ovarian, pancreatic, colorectal cancer) [30]. Administration of PIF in mice causes an important loss of body weight and muscle [31]. The major mechanism through which PIF is believed to induce catabolism in skeletal muscle is the up-regulation of the ATP-ubiquitin-dependent proteolytic pathway as demonstrated in mice gastrocnemius exposed to PIF [32].

The ATP-ubiquitin-dependent protein degradation is made up of two main steps: first, through an enzymatic cascade (ubiquitin-activating, ubiquitin-conjugating and ubiquitin-ligating enzymes), multiple ubiquitin molecules are covalently attached to the protein substrate; then the poly-ubiquitinated protein is degraded by the 26S proteasome complex, whose catalytic core, the 20S proteasome, is characterized by five peptidase activities, namely the trypsin-like (TL), chymotrypsin-like (CTL), peptidyl-glutamyl peptidase (PGP), branched-chain amino acid-preferring, and small neutral amino acid-preferring activities [33] (Fig. 3). Up-regulation of components of the ATP-ubiquitin-dependent pathway has been reported in experimental models of wasting conditions such as sepsis, trauma, burns, renal failure, acidosis, and cancer [34–38]. In the skeletal muscle of rats bearing the Yoshida AH-130 ascites hepatoma, overexpression of ubiquitin mRNA closely parallels the enhancement of protein degradation [38], while treatment with anti-TNF-α antibodies or clenbuterol, reduces muscle ubiquitin mRNA levels [39,40]. Consistent with experimental observations, recent studies have shown that ubiquitin mRNA expression was markedly and significantly increased in muscle biopsies obtained preoperatively in 20 patients undergoing surgery for gastric cancer [4]. A further confirmatory evidence of the involvement of the

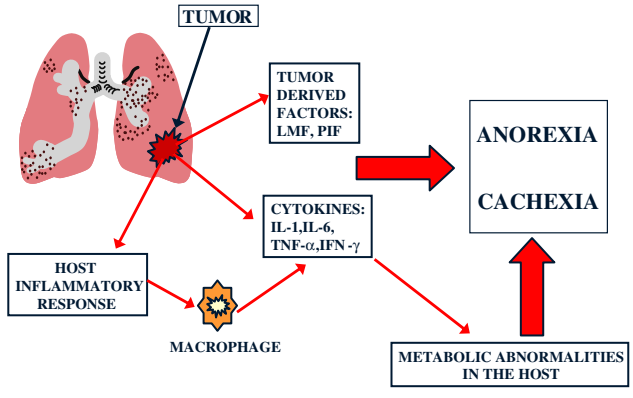

Fig. 2 – Tumor- and host-derived humoral mediators of cancer-related anorexia and cachexia. Abbreviations: IL-1, interleukin-1; IL-6, interleukin-6; TNF-a, tumor necrosis factor-a; IFN-c, interferon-c; LMF, lipid-mobilizing factor; PIF, proteolysis-inducing factor.

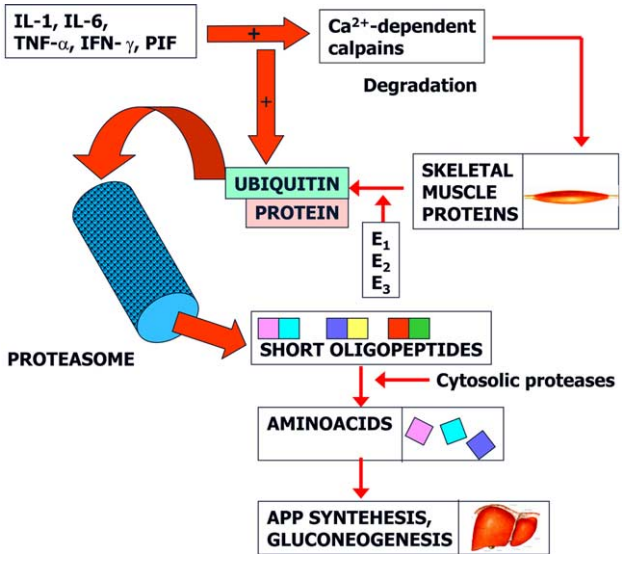

Fig. 3 – A schematic representation of the mechanisms underlying muscle proteolysis in cancer cachexia. Amino acids derived from muscle protein degradation are re-utilized in the liver, mainly for acute-phase proteins (APP) synthesis and gluconeogenesis. Abbreviations: E1, ubiquitin activating enzyme; E2, ubiquitin conjugating enzyme; E3, ubiquitin ligase.

ATP-ubiquitin-dependent proteolysis in cancer-related muscle degradation has been provided by the finding that proteasome activity is significantly increased in the muscle of gastric cancer patients (5-fold increase in CTL activity, and a 2-fold increase for both the TL and PGP ones) and that these changes are paralleled by concomitant overexpression of muscle ubiquitin mRNA [41]. The observation that both ubiquitin mRNA overexpression and increased proteasome proteolytic activities occurred even in patients with insignificant or no weight loss, strongly supports the concept that the causative mechanisms of cancer cachexia are operating early during the clinical course of human neoplastic disease, underscoring the need for early preventive/therapeutic interventions [41,42].

The continuous effort aimed at identifying potential molecular targets of the therapy of muscle wasting, has disclosed the crucial involvement of ubiquitin ligases in pathological muscle degradation. Progressive evidence is in fact accumulating suggesting that three intracytoplasmic ubiquitin-ligating enzymes, namely E3α [43] and ligases encoded by the genes *MURF-1* (muscle ring finger protein 1) and *MAFbx* (muscle atrophy F-box protein, also called Atrogin-1) [44], play a significant role in the onset of muscle atrophy. Infact, the ubiquitin conjugation rates in extracts prepared from atrophying muscles are reduced by inhibition of E3α [43], the expression of MuRF-1 and MAFbx has been shown to be increased in multiple models of skeletal muscle wasting, such as diabetes, uremia, fasting and cancer [45], and mice deficient in either MAFbx or MuRF-1 appear to be more resistant to atrophy [44].

Although increased muscle protein degradation is undisputedly the main mechanism underlying muscle wasting in cancer, the question has arisen whether perturbations in some of the pathways regulating normal muscle trophism and hypertophic response to load and/or exercise might also have a pathogenic role. Impaired anabolic response might be the consequence of a cancer-dependent reduced expression of positive regulators (i.e., MyoD), or an overexpression of negative regulators (i.e., Myostatin) of skeletal muscle growth. Indeed, Guttridge [46] have recently suggested impaired regeneration as a key pathogenetic factor for muscle depletion in cachexia. This is based on the observation that TNF-α and IFN-γ combined downregulate MyoD expression in differentiated C2C12 myotubes as well as in the mice gastrocnemius muscle in vivo, while TNF-α alone is sufficient to suppress MyoD expression and the myogenic program in differentiating C2C12 myocytes [46]. MyoD is a member of a skeletal muscle-specific family of transcription factors, also known as myogenic regulatory factors that play a determinant role in myogenesis in cooperation with other transcriptional modulators of the MEF2 family [47]. Consistent with these observations, we could demonstrate that muscle depletion is paralleled by a decrease in MyoD expression in an experimental model of TNF-α-dependent cancer cachexia [48].

Myostatin (MSTN), a member of the Transforming Growth Factors-beta (TGF-β) superfamily of secreted growth and differentiation factors is expressed almost uniquely in human skeletal muscle [49]. Following the initial observation that cattle with mutations in the MSTN gene showed a marked increase in body weight and muscle mass [50], clinical and experimental evidence has accumulated suggesting that MSTN is a negative regulator of muscle mass [49]. Indeed, a negative correlation between muscle MSTN mRNA expression and muscle mass in adult rodents under conditions of muscle hypotrophy or atrophy [51–53] and in humans with AIDS- and disuse-related muscle wasting [54,55] has been documented, while systemic overexpression of MSTN in adult mice leads to wasting [56]. The role of MSTN in the pathogenesis of cancer-related muscle wasting remains to be fully elucidated.

3. Current therapeutic strategies

Intuitively, the most effective therapeutic strategy for cancer-related cachexia would be the induction of cancer remission with surgery and/or chemo-radiotherapy. However, once a cancer is diagnosed, patients fall within a frequently long and complex therapeutic route which will expose them to the detrimental consequences of the cancer itself and of the anti-neoplastic treatments. This will progressively lead to the deterioration of their nutritional and functional status, ultimately leading to muscle wasting and cachexia [57]. To date, however, despite the several years of coordinate efforts of basic and clinical research, practice guidelines for the prevention and treatment of cancer-related muscle wasting are lacking. Indeed the 'pill for cancer cachexia' has not yet been developed, mainly because of the multifactorial pathogenesis of the syndrome.

3.1. *Prevention and correction of anorexia*

Among the causative factors of cachexia, anorexia is with no doubt the most effectively treatable [9]. In fact, the improvement of appetite, and the consequent increase of oral food intake, may be achieved with different pharmacological tools: progestagens, glucocorticoids, cannabinoids and agents capable of modulating serotonin synthesis and release at the hypothalamic level [9]. Among orexigenic agents, megestrol acetate is by far the most widely prescribed [58]. At least 15 randomized controlled studies [58] have demonstrated that this drug, at doses ranging from 160 to 1600 mg/day, significantly improves appetite, with respect to placebo. It should be underlined, however, that although the increase in appetite represents an extremely positive perception for the patients and their relatives, in most of these trials no definite improvement in global quality of life was observed [58]. Possible explanations to these results might be related to: (i) the occurrence of undesired side-effects (thromboembolism, hyperglycemia, hypertension, peripheral edema, alopecia, adrenal insufficiency); and (ii) the lack of a net improvement in lean body mass, in spite of significant weight gain, with no measurable effects on functional status [58].

Glucocorticoids inhibit the synthesis or release of proinflammatory cytokines involved in the pathogenesis of cancer anorexia as IL-1 and TNF [59]. Administration of dexamethasone at 0.75 mg per 4 times a day causes the same degree of appetite improvement obtained with the treatment of megestrol acetate [60]. Corticosteroids have shown also the ability to control pain and to reduce asthenia and nausea even if no positive effect has been reported on body weight gaining. This evidence, associated to the side effects observed after

prolonged treatment (immunosuppression, gastrointestinal bleeding, delirium, osteoporosis and weakness) should be taken into account when considering this terapeutic regimen [61]. Cannabinoids are used for their ability to stimulate appetite; they are marijuana derivates and act either through an interaction with cytokines network [62] or through an interaction with endocannabinoid receptors situated in the brain lymbic system and in the hypotalamus or in peripheral organ systems like the intestine and the adipose tissue [63].

Dronabinol at 2.5 mg twice a day is the cannabinoid that has shown major prophagic effects. The patient-reported toxicities are: drowsiness; muddled thinking; loss of coordination; fluid retention; vomiting; and impotence among male population [64]. Among serotonin modulators cyproheptadine, an antiserotonergic compound with anti-histaminic activity, is able to increase appetite, but not body weight [65]. Ondasetron is a type-3 serotonergic receptor blocker effective in treating chemotherapy-induced nausea and vomiting with transient orexigeinic effect, but unable to increase body weight [66]. The gluconeogenesis inhibitor hydrazine sulfate (HS) was initially proposed to improve appetite and to reduce weight loss in cachectic cancer patients [67]. However, all the subsequent randomized, placebo-controlled trials failed to show any beneficial effects of HS in terms of better quality of life and improved survival [68]. It was also associated with clinically relevant undesired effects such as nausea, pruritis, dizziness, drowsiness, excitation, peripheral neuropaties [67], and fatal epatorenal failure [69].

3.2. *Nutritional intervention*

Artificial nutrition is frequently used to alleviate cancer related malnutrition; unfortunately this therapeutic procedure is unable to increase lean body mass [70]. Although substantially unable to correct advanced cachexia, artificial nutritional intervention appears opportune to prevent further deterioration of the nutritional status [71], to reduce 'caregivers anxiety' and to ameliorate patients' psychological condition [72]. The decision on which route of administration is preferred basically depends on the patient's clinical condition: if the gut is not damaged, enteral feeding is advantageous. It has in fact, less side effects, lower cost and contributes to maintain gut barrier function [73]. Total parenteral nutrition, however, still remains the standard treatment regimen for patients who are unable to eat because of mechanical obstruction of the gastrointestinal tract or are unable to absorb enterally provided nutrients, on a temporary or even permanent basis and proved to be equally beneficial in the treatment of malnutrition [74]. The role of nutritional counselling is becoming increasingly clear: a recent prospective, randomized, controlled trial in colorectal cancer patients undergoing radiotherapy, has demonstrated that individualized dietary counselling, based on regular foods and not just protein and calorie supplementation, is the most effective means of improving patients' nutritional intake, status, and quality of life [75].

3.3. *Prevention and correction of muscle degradation*

The maintenance or recovery of an adequate functional status is essentially based on the maintenance or recovery of skeletal muscle mass. On a theoretical ground, this could be achieved by either: (i) attenuating muscle protein catabolism or (ii) stimulating muscle protein anabolism.

The attenuation of muscle loss has long been attempted through an upstream approach (Fig. 4), which was mainly based on the knowledge that pro-inflammatory cytokines are among the most important humoral mediators of muscle catabolism in both experimental and human CC [10]. Thus,

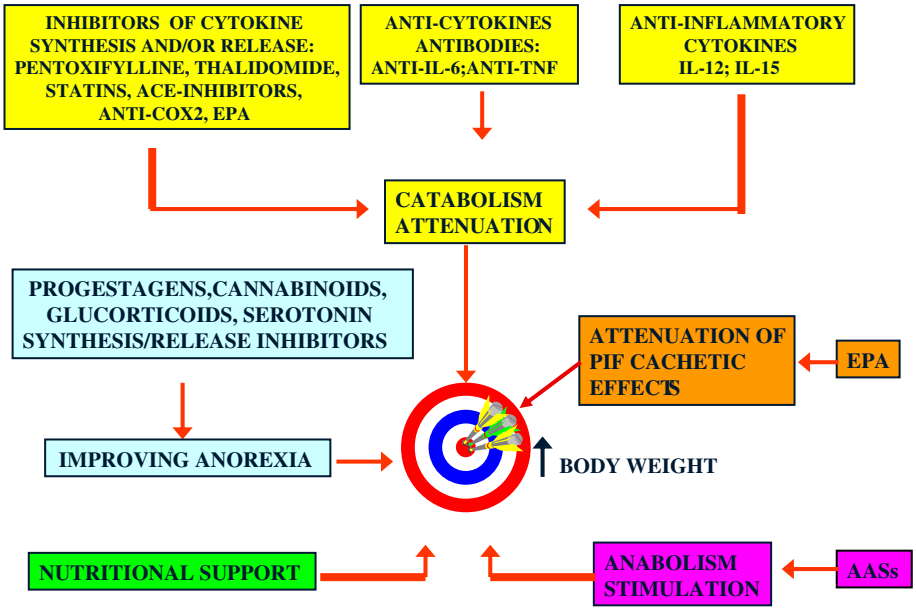

Fig. 4 – Therapeutic strategies for cancer cachexia: the "upstream approach". Abbreviations: ACE, angiotensin-converting enzyme; Anti-COX2, inhibitors of cyclo-oxygenase-2; EPA, eicosapentaenoic acid; PIF, proteolysis-inducing factor; AASs, anabolic androgen steroids.

drugs capable of inhibiting the synthesis and/or release of cytokines, (pentoxifylline, thalidomide, melatonin, statins, ACE-inhibitors and anti-COX-2); drugs and other molecules interfering with cytokine actions (anti-cytokine antibodies, suramin [SUR]); anti-inflammatory cytokines (IL-12, IL-15) have been extensively tested in experimental CC [28,76–83], with substantially positive results. Unfortunately, clinical trials testing the efficacy of this approach in human beings have provided extremely limited and disappointing results [84,85]. Moreover, what could be the consequence of chronically interfering with whole body's inflammatory (and immune) response in cancer patients remains to be fully elucidated.

The progressive knowledge of the biochemical and molecular mechanisms regulating to muscle protein degradation under physiological and pathological conditions, led to the hypothesize that a more selective, 'downstream approach' (Fig. 5), would be more promising for the prevention and treatment of cancer-related muscle wasting. In this respect, direct proteasome inhibition could prove an effective strategy and deserves proper attention. Proteasome inhibition can be achieved by pharmacological or nutritional manipulation. [49,50], Indeed, data on the efficacy of pharmacological proteasome inhibitors (namely peptide aldehydes, lactacystin/β-lactone, vinyl sulfones and dypeptide boronic acid analogs, reviewed by Kisselev and collegues [86]) in modulating disease-related muscle loss are lacking in humans and still scanty in animal models [87] and do not allow for definite conclusions. Conversely, the dietary fatty acid eicosapentaenoic acid (EPA) is the only known nutritional supplement capable of interfering with proteasome activity through different mechanisms, at least in experimental conditions [88]. Preliminary studies in humans showed that EPA supplementation at the dose of 2 g/day prevented weight loss in patients with pancreatic cancer [89]. However, the results obtained in pilot studies have not been completely confirmed in a subsequent, large multicentre study on 200 patients with cancer of the pancreas, probably because many of the patients included in the control group were already taking significant amounts of fish-oils without informing their physicians [90]. Moreover, the type of cancer patients recruited to assess efficacy of EPA (i.e., pancreatic cancer) accounted for an extremely significant rate of dropouts, which further limited the statistical power of the results [91]. Interestingly enough, in a post-hoc analysis of this trial [91] EPA supplementation was shown to increase total energy expenditure with a parallel improvement in the physical function. Another multi-institutional trial including more than 400 patients with cancer-associated wasting has demonstrated that EPA supplementation was less effective than megestrol acetate in causing a 10% weight gain, but that it was relatively comparable to megestrol acetate with respect to appetite stimulation, survival and quality of life. Combination therapy (EPA with megestrol acetate) did not add benefit [92]. Therefore, this nutritional substrate, because of its largely demonstrated anti-inflammatory properties, is likely to become an 'indispensable ingredient' in nutritional formulations for cancer patients.

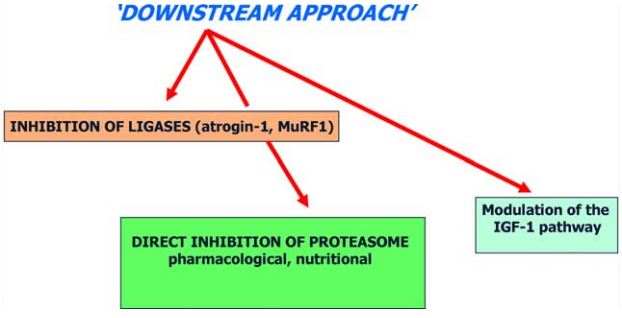

Fig. 5 – Possible 'downstream' therapeutic approaches to cancer-related muscle wasting. Abbreviations: MuRF1, muscle ring finger 1; IGF-1, insulin-like growth factor-1.

Potential targets for future 'downstream' approaches aimed at attenuating muscle catabolism are the ubiquitin ligases Atrogin-1 and MURF-1, since these enzymes are specifically expressed in skeletal muscle cells and are unlikely to be required for both growth and function of the normal muscle [44]. While pharmacological inhibitors of these enzymes are currently not available, there is evidence suggesting that Insulin-Growth Factor-1 (IGF-1) may reduce atrogin-1 and MuRF-1 expression by blocking mRNA synthesis in vitro [93], suggesting a potential therapeutic role of this already available hormone in attenuating pathological muscle proteolysis.

3.4. *Stimulation of muscle protein anabolism*

Stimulation of protein anabolism in muscle may be attempted through anabolic androgenic steroids (AASs), a large family of testosterone-related hormones which vary in terms of chemical structure, mode of action, anabolic effects, and risk of undesired side effects [94]. AASs administration induces increases in mRNA expression of skeletal muscle androgen receptor, increases the intracellular utilization of amino acids derived by protein degradation, and stimulates net muscle protein synthesis [95]. While positive effects have been documented with nandrolone decanoate and oxandrolone in burned [96], HIV-infected [97] and chronic obstructive pulmonary disease [98] patients, data on cancer patients, although undoubtedly promising, are limited [99,100] and warrant further controlled clinical trials evaluating both the long-term efficacy as well as the risk of dangerous side effects [101,102].

Finally, the possibility to induce muscle hypertrophy through myostatin inhibition via specific blocking antibodies [103], is extremely intriguing, albeit merely speculative until further knowledge will clarify the role of myostatin (if any) in cancer-related cachexia.

4. Future strategies: a role for IGF-1?

The concept that innovative therapies to counterbalance accelerated muscle loss in cancer-related cachexia are necessary and near to come is therefore based on a number of considerations: (i) the molecular mechanisms controlling muscle trophism under normal and pathological conditions are becoming increasingly clear; (ii) currently available pharmacological and nutritional strategies are only partially effective in cancer cachexia; and (iii) the ability of Insulin-like Growth Factor-1 (IGF-1) to modulate cellular and molecular mechanisms involved in muscle hypertophy is largely documented.

It is widely recognised that IGF-1 may induce hypertrophy (see Fig. 6) of skeletal muscle in an endocrine or paracrine/autocrine fashion [104–107]; and that high and constant muscle levels of IGF-1 are able to induce muscle hypertrophy has been demonstrated in several studies where IGF-1 has been locally infused, introduced in muscle with recombinant viruses, or locally hyperexpressed in transgenic mice [108–113]. To date however, the mechanisms through which IGF-1 induces muscle hypertrophy are still a matter of discussion, but could include actvation of satellite cells and/or activation of the PI(3)K/Akt/mTOR signalling cascade that is closely linked to the protein synthesis machinery [113,114,47].

Several in vitro observations indicate that IGF-1 promotes proliferation and differentiation of satellite cells, with consequent increase in myotube formation [115]. Barton-Davis and colleagues [109] demonstrated that the injection of a recombinant adeno-associated virus directing the expression of IGF-1 in differentiated muscle fibers could promote an increase in muscle mass and strength in young adult mice, preventing aging-related muscle changes in old adult mice. The authors hypothesized that these findings could be secondary, besides a general anabolic effect, to the activation of satellite cells, leading to increased rates of muscle repair. This hypothesis was supported by the appearance of central nuclei and developmental myosin expression occurring four months after viral infection and was based on the concept that satellite cell activation is essential to achieve hypertrophy, as suggested by the lack of chronic overload-induced hypertrophy in irradiated muscles [115,116].

In vitro experiments by Rommel and collegues [117] showed that hypertrophy of C2C12 myotubes induced by IGF-1 was dependent of the PI(3)K/AKT pathway, which in turn activated downstream signalling pathways (mTOR) implicated in activated protein synthesis, a key step in this process being the activation of p70-s6 kinase which in turn phosphorylates the s6 protein component of ribosomes, possibly modulating the rate of translation and protein synthesis. The role of PI(3)K/AKT/mTOR cascade in muscle hypertrophy was subsequently confirmed in vivo by Bodine and collegues [118] in a rat model of compensatory hypertophy.

The considerable body of evidence closely linking IGF-1 to muscle hypertrophy has recently led to propose this hormone as 'a medicine to induce hypertrophy' [114] and suggests that modulation of the IGF-1 pathway could represent a promising approach to the prevention/treatment of cancer-related muscle atrophy. However, both efficacy and safety of systemic IGF-1 administration in CC are still uncertain. Systemic administration of recombinant IGF-1 has proven uneffective in preventing loss of skeletal muscle mass in experimental CC [119]. Indeed, consistent with these observations, we recently showed that systemic recombinant IGF-1 administration failed to prevent muscle atrophy in rats bearing the cachexia-inducing AH-130 ascites hepatoma (personal communication).

No data are currently available on the efficacy of IGF-1 administration in cancer patients, while the effects on muscle mass in humans with wasting diseases other than cancer is controversial [120,121]. It must be recognized however, that systemic IGF-1 administration poses a number of concerns in the clinical setting, first because of its glucose-lowering effect, which restricts the dose that can be used [122] and consequently its anabolic effects. Secondly, exogenously administered IGF-1 has a short half-life [123], with consequent limited bioavailability and scarce therapeutic potential for single intravenous injections. In physiological conditions, bioavailability of unbound IGF-1 and capability of interaction with its receptor (IGF-1R) is modulated by IGF-binding proteins (IGFBP1-6) [105]. The administration of a IGF-1/IGFBP-3 binary complex may improve IGF-1 bioavailability, and attenuate loss of muscle mass in both cancer- and non-cancer related experimental cachexia, with no apparent stimulation of tumour proliferation [124–126]. However, the use of this compound to attenuate human CC deserves caution. In fact, besides the antiproliferative effects of IGFBP-3 [127], evidence exists of a link between serum concentrations of IGF-1 and increased risk of breast, prostate, colorectal and lung cancer [128]. In this respect, it should not be overlooked that IGF-1 may function as a growth factor for several different tissues, as indicated by the ubiquitous expression of IGF receptors [105]. Therefore, it is likely that IGF-1 effects could take place also in tissues other than skeletal muscle, thus promoting stimulation and survival of rapidly dividing cells (e.g., cancer cells). Taken together, all these considerations discourage the use of systemically administered IGF-1 for the prevention or correction of cancer-related muscle wasting. An alternative approach could be the induction of IGF-1 overexpression confined to the muscle through a gene therapy approach.

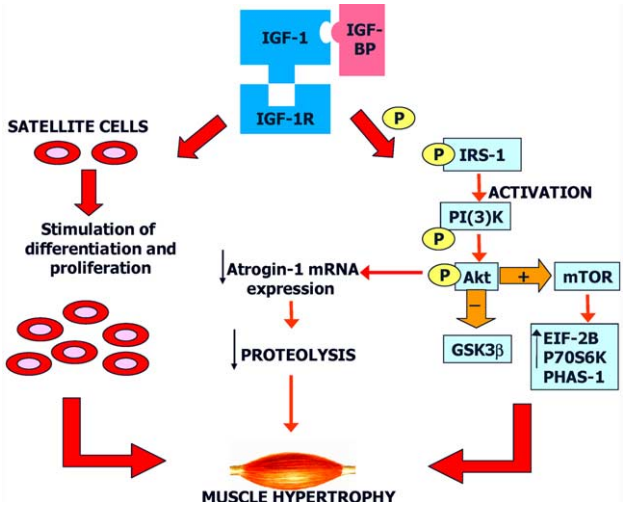

Fig. 6 – Schematic representation of the currently proposed mechanism by which IGF-1 induces muscle hypertrophy. Abbreviations: IGF-1, insulin-like growth factor-1; IGF-BP, IGF-1 binding protein(s); IRS, insulin-related substance; IGF-1R, IGF-1 receptor; P, phosphate; PI(3)K, phosphatidyl-inosytol 3-kinase; Akt, Akt kinase; GSK, glycogen-synthase kinase 3b; mTOR, mammalian target of rapamycin; Eif, eukaryotic translation initiationfactor 2B; P70S6K, P70S6 kinase; PHAS-1, phosphorylated heat- and acid-stable protein 1.

Gene therapy is defined as a therapy in which gene(s) or gene-transducer cells are introduced to the patient's body for a therapeutic or gene-marking purpose. Gene therapy has been recently revealing its promising potential for the

Table 1 – Effect of IGF-1 gene transfer in muscle

Author (ref)	Year	Model	Method	Effect
Alila [108]	1997	Rat	Plasmide enconding human IGF-1	↑ IGF-1 mRNA and protein
Barton-Davis [109]	1998	Mouse	Recombinant adeno-associated virus	↑ Muscle mass and strength
Jeschke [110]	1999	Burned rat	Liposome	↑ Gastrocnemius muscle protein content
Shiotani [111]	2001	Rat	Sendai virus	↑ Increased total and regenerating myofibers
Takahashi [112]	2003	Mouse	Electroporation	↑ IGF-1 protein, number and diameter of regenerating myofibers
Alzghoul [113]	2003	Mouse	Electroporation	Fiber hypertrophy, ↑ Muscle size, inhibition of disuse mediated muscle atrophy

treatment of many clinical disorders, including hereditary and acquired muscular diseases [129]. Gene transfer is achieved by different means, including viral, physical and chemical methods [108–113]. IGF-1 indeed appears a good candidate for gene therapy of muscle atrophy, gene transfer into muscle being followed by increase of muscle IGF-1 expression, protein content, total and regenerating myofibers, hypertrophy and prevention of disuse atrophy, at least in experimental models without elevation of circulating IGF-1 (Table 1).

Recently, the intramuscular injection of a GH-Releasing Hormone-expressing plasmid was shown to increase body weight, activity level and exercise tolerance in 17 geriatric and 5 cancer-afflicted companion dogs through an increase in IGF-1 serum levels [130]. The authors reported the absence of undesired side effects, but, unfortunately, did not provide any data on the effects on either muscle or tumour tissue, not allowing for definite conclusions about the feasibility and safety of this approach, particularly with respect to the potential long-term risks of IGF-1-induced carcinogenesis.

Besides IGF-1, other intracellular pathways involved in hypertrophy might be promising targets of gene therapy for muscle cachexia, as suggested by the recent evidence that the injection of a genetic construct designed to express a constitutively active form of Akt into the tibialis anterior muscle of adult mice is capable of inducing hypertrophy of normal fibers and to prevent denervation atrophy [118]. The most recent demonstration that systemic delivery of genes to striated muscles using adeno-associated viral vectors in dystrophin-deficient *mdx* mice may allow for the genetic manipulation of skeletal muscle [131], further strengthening the view that gene therapy will soon be considered a valid tool for the treatment of aging- or disease-associated muscle wasting [130].

5. Conclusions

Cancer cachexia still represents a frustrating condition for both the patient and his physician. Current preventive and therapeutic options are largely uneffective, and most advanced cancer patients still die of cachexia. The progressive knowledge of the cellular and molecular mechanisms leading to cancer cachexia is setting the stage for the development of more effective therapeutic strategies for cancer-related muscle wasting. The aim of future therapies will be to interfere with those downstream pathways triggered by systemic humoral mediators, by selectively acting at the muscular level. The evidence that overexpression of IGF-1 confined to muscle tissue is capable to induce hypertrophy, makes this substance a promising candidate for gene therapy of muscle wasting of cancer.

This approach could become part of an integrated intervention for cancer patients including specific anti-neoplastic regimens, together with a preventive nutritional and metabolic support.

Conflict of interest statement

None declared.

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