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Mediators of cachexia in cancer patients

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Highlights

- Systemic inflammation is a hallmark of cancer patients, the inflammatory response being the main driving force behind the metabolic alterations present in the cancer patient.
- Chemotherapy exacerbates cancer cachexia. Some chemotherapeutic agents induce both pro-cachectic cytokines and myostatin.
- The elucidation of the interactions between all the mediators involved in cancer cachexia –cytokines, chemokines, tumour factors, myostatin-- may possibly accelerate the development of novel therapeutic interventions against cancer -induced inflammation and thus, cachexia.

Running title: Cachexia mediators

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Mediators of cachexia in cancer patients

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Abstract

Alterations in amino acid and protein metabolism --particularly in skeletal muscle-- are a key feature of cancer patients that contribute to the cachexia syndrome. Thus, skeletal muscle protein turnover is characterized by an exacerbated rate of protein degradation, promoted by an activation of different proteolytic systems that include the ubiquitin-proteasome and the autophagic-lysosomal pathways. These changes are promoted by both hormonal alterations and inflammatory mediators released as a result of the systemic inflammatory response induced by the tumour. Other events, such as alterations in the rate of myogenesis/apoptosis and decreased regeneration potential also affect skeletal muscle in cancer patients. Mitochondrial dysfunction also contributes to changes in skeletal muscle metabolism and further contributes to the exacerbation of the cancer-wasting syndrome. Different inflammatory mediators --either released by the tumour or by healthy cells of the cancer patient— are responsible for the activation of these catabolic processes that take place in skeletal muscle and in other tissues/organs, such as liver or adipose tissues. Indeed, white adipose tissue is also subject to extensive wasting and "browning" of some of the white adipocytes into beige cells, therefore increasing the energetic inefficiency of the cancer patient. Recently, an interest in the role of micro-mRNAs --either free or transported into exosomes-- has been related with the events that take place in white adipose tissue during cancer cachexia.

Key words: cachexia, wasting, anorexia, weight loss, cytokines, inflammation

Cancer is an inflammatory state

Cancer is an inflammatory disease[1]. Indeed, systemic inflammation is a hallmark of cancer patients[2]. In fact, the inflammatory response is the main driving force behind the metabolic alterations present in the cancer patient. The origin of the inflammation is multiple: on one hand tumour cells may themselves release cytokines and other inflammatory mediators; on the other hand activated immune cells release cytokines and chemokines. Both the tumour and the gut mediate the activation of the immune cells. Indeed, gut barrier dysfunction and bacterial translocation is associated with cancer[3].

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Thus, the release of LPS and other bacterial toxins activates cytokine synthesis and release by immune cells. Cytokines promote the activation of transcription factors associated with wasting, both in adipose tissue and skeletal muscle, therefore leading to wasting[4]. In addition to inflammatory mediators, other molecules also contribute to the metabolic abnormalities present in the cancer patient. Tumour-derived factors, other than cytokines, have been proposed as triggers of the wasting process associated with cancer cachexia. Two of these molecules, lipid mobilizing factor (LMF) and the proteolysis-inducing factor (PIF) have been found in tumour-bearing animals and cancer patients[5]. Another interesting molecule associated with muscle wasting is myostatin. This protein is a transforming growth factor β (TGFβ) ligand, which operates through activin receptor type II B (ActRIIB)-mediated signalling[6]. Myostatin is involved in skeletal muscle wasting in different catabolic conditions, including cancer[7]. In fact, inactivation of myostatin and other TGFβ family of proteins by treatment with sActRIIB (a soluble form of ActRIIB) ablates the symptoms of cancer cachexia in tumour-bearing mice[8,9]. In fact, we now know that myostatin may be released not only by tissues such as skeletal muscle and adipose tissue (to a lesser extent) but also by cachexia-inducing tumours[10]. Interestingly, TGF-β also participates in the bone-muscle crosstalk and seems to be a cause of skeletal muscle weakness in the setting of osteolytic cancer in the bone[11].

It is clear that the elucidation of the interactions between all the mentioned molecules –cytokines, chemokines, tumour factors, myostatin-- may possibly accelerate the development of novel therapeutic interventions against cancer -induced inflammation and thus, cachexia.

The systemic inflammatory response leads to muscle wasting by different mechanisms

Different altered metabolic pathways contribute to muscle wasting during cachexia[12]. Thus, skeletal muscle protein turnover is characterized by an exacerbated rate of protein degradation promoted by an activation of different proteolytic systems that include the ubiquitin-proteasome and the autophagic-lysosomal pathways[13]. Changes in the rate of myogenesis/apoptosis also determine skeletal muscle mass during cancer cachexia[14]. Indeed, a

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decreased skeletal muscle regeneration capacity is observed together with an increased rate of cell death, resulting in muscle wasting[15,16]. Mitochondrial dysfunction also results in changes in skeletal muscle metabolism and further contributes to the exacerbation of the cancer-wasting syndrome[17].

Inflammatory cytokines contribute to the activation of proteolysis. However, tumour-derived factors have also been involved[18]. The cytokines involved in muscle wasting during cancer, Tumour necrosis factor-α (TNF-α), TNF-like weak inducer of apoptosis, (TWEAK), Tumour necrosis factor receptor (TNFR), -associated factor 6 (TRAF6), Interleukin-6 (IL-6), gamma-Interferon (γ-IFN) and Leukaemia Inhibitory Factor (LIF)[19,20] act via two different intracellular pathways: the NF-kappaB and the p38 MAP kinase pathways. They are both involved in the up regulation of the expression of E3 ligases MuRF-1 and MAFbx. Inhibition of NF-kappaB, at least in rodents, results in a decreased muscle loss in tumour-bearing animals[21]. This pathway acts partly by activating the formation of NO --through induction of the iNOS— therefore increasing nitrosative stress[22] which can also activate protein degradation in skeletal muscle. These cytokines also activate the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway. During cancer, there is an induction of STAT3 phosphorylation in skeletal muscle[23]. However, interestingly, STAT3 also seems to have a role in autophagy resulting in an alteration of the beclin complex and, therefore, blocking autophagy and stopping muscle degeneration[24]. In particular, LIF -- a cytokine that can also be released by some tumours-- is also involved in promoting adipose tissue dissolution by activating lipolysis[25].

According to White et al. IL-6 suppression of mTOR activity is dependent on AMP kinase (AMPK) activation and independent of STAT signalling in myotubes[26]. AMPK ? is able to phosphorylate FOXO, therefore, stimulating its transcriptional activity (26). AMPK is also able to activate autophagy genes [27].

Sun et al. found a positive correlation between TRAF6 and ubiquitin expression in skeletal muscle of gastric cancer patients, suggesting that TRAF6 may up-regulate ubiquitin activity in cancer cachexia[28]. On the same lines, TWEAK, a cytokine belonging to the TNF-α family, is able to induce a cachectic phenotype through the induction of MuRF-1. The same cytokine is

also able to induce the autophagy system in skeletal muscle cells and this seems to be dependent on NF-kappaB activation[29]. Furthermore, caspases seem to contribute, at least in part, to the activation of NF-kappaB in response to TWEAK treatment[29]. The cytosolic release of its inhibitory IkappaB proteins allows the translocation of NF-kappaB to the nucleus and subsequent transcription of genes involved in proteolysis. Inhibition of NF-kappaB decreases muscle loss in a rat model of cancer cachexia, in part, by inhibiting the up regulation of MuRF-1[30].

Mitochondrial dysfunction is present in skeletal muscle during cancer cachexia. Indeed, distorted mitochondria are present in muscle during cancer cachexia[31], this being associated with loss of skeletal muscle structural integrity. In these mitochondria, ATP synthesis is decreased[31] and oxidative phosphorylation uncoupling is present[32]. Free radical species --superoxide, hydroxyl radicals, nitric oxide, peroxynitrite and hydrogen peroxide-- play a key role in modulating inflammation-driven alterations in skeletal muscle function, particularly in mitochondria[22]. Indeed, free radicals have important effects on sarcoplasmic reticulum, mitochondrial function and sarcolemmal integrity. Sarcoplasmic reticulum and mitochondria act in very close collaboration in the process of muscle contraction. Precisely, alterations in some of the proteins participating in this "tandem" --such as mitofusin-- have been described in experimental cancer cachexia[33]. Cytokines, TNF-α in particular, are associated with increased oxidative stress in skeletal muscle during cancer. In fact, the TNF-α-1031T/C polymorphism seems to be a marker of cachexia risk in head and neck cancer patients[34]. Padrao et al. using a bladder cancer rat model of muscle wasting, concluded that mitochondrial dysfunction was associated with an accumulation of both oxidized and nitrated proteins which could not be repaired by mitochondrial proteases and the mitochondrial shaping machinery[35]. This enzymatic machinery is responsible for maintaining the cellular redox state by removing oxidized or misfolded proteins[36]. Interestingly, many cytokines activate the transcriptional PGC-1α, through phosphorylation by p38 kinase, resulting in stabilization and activation of PGC-1α[36].□This causes increased respiration and expression of genes linked to mitochondrial uncoupling and energy expenditure[36]. White et al. (49) clearly demonstrated a role for IL-6 in the skeletal muscle mitochondrial abnormalities associated with tumour growth[37]. By using the Apc[Min/+] mouse

model --this contains the adenomatous polyposis coli (APC) gene required to initiate familial adenomatous polyposis (FAP)-- repressed expression of PGC-1α and mitofusin 1&2 --related to mitochondrial fission in skeletal muscle— were found while FIS1 expression --related with mitochondrial fission-- was increased[38]. All of these changes were linked to alterations in mitochondrial morphology and decreased mitochondrial content and decreased complex IV and cytochrome C proteins[39]. Interestingly in the Apc[Min/+] mouse model, IL-6 is necessary to induce muscle wasting and overexpression of the cytokine in precachectic Apc[Min/+] mice activates the development of cachexia[38]. Administration of IL-6 antibody to Apc[Min/+] mice was able to restore the alterations associated with abnormal mitochondrial function -- increased PGC-1α, complex IV and cytochrome C proteins and improved mitochondrial content[37]. The same authors demonstrated direct effects on mitochondrial parameters of IL-6 on isolated muscle cells suggesting that the cytokine mediates directly mitochondrial muscle alterations[37].

Another interesting cytokine is Interleukin-8 (IL-8). Indeed, elevated serum levels of the cytokine correlate with cancer-related cachexia[40]. In pancreatic cancer IL-8 is an indicator of cancer outcomes[40]. The genetic polymorphism of this myokine can contribute to the pathogenesis of cachexia in gastric cancer[41]. Another molecule that has been shown to be a possible biomarker of cancer cachexia is monocyte chemo attractant protein-1: (MCP-1) is associated with cachexia in cancer patients[42].

Wang et al. have recently identified the metal-ion transporter ZIP-14 as a critical mediator in cancer cachexia[43]. Indeed, the transporter is up regulated in skeletal muscle of both cancer patients and in tumour-bearing animals. The protein is induced by cytokines, TNF-α and TGF-β. This suggests a role for altered zinc homeostasis in cachectic cancer patients.

Concerning tumour factors, the so-called proteolysis-inducing factor (PIF) was identified as a circulating tumour-released mediator in mice bearing a cachexia-inducing (MAC16)[44]. Moreover, human tumours also express the so-called PIF[44]. This molecule --a 24kDa glycoprotein-- causes weight loss by inducing enhanced protein degradation without decreasing the appetite in mice. PIF was also found to be present in the urine of cachectic cancer patients while being absent from normal subjects[45]. However, this presence

has been highly debated[46]. In addition, the lipid mobilizing factor zinc-α2-glycoprotein (ZAG) actively participates in the dissolution of adipose tissue by activating lipolysis[5].

It has to be pointed out that some cytokines (IL-4, IL-10) can actually act as anti-cachectic[47]. One of them, IL-15, has been shown to have a clear anti-proteolytic[48–50] and antiapoptotic action in skeletal muscle of animals under cancer cachexia[51].

The role of exosomes

Extracellular vesicles (EVs), including exosomes and microvesicles, are nanovesicles involved in cellular communication, immune response, tissue repair, epigenetic regulation, and in various diseases including cancer. In cancer these EVs regulate progression, metastasis and chemoresistance. Exosomes, measuring from 30 to 100 μm, can act in an autocrine, paracrine, and endocrine manner. Recently, an interest in the role of EVs in regulating cancer cachexia has emerged. Zhang et al. reported extracellular heat-shock proteins --either released in soluble form or in exosomes--, Hsp 70 and Hsp90, to be responsible for induction of cancer cachexia in mice[52]. These proteins can also be related with the secretion of pro-cachectic cytokines together with the activation of Toll-like receptor4 (TLR4) in skeletal muscle[53]. Interestingly, Henriques et al. have shown that genetic ablation and pharmacological – Atorvastatin-- inhibition of TLR4 were able to attenuate the main clinical markers of cachexia in mice bearing Lewis lung carcinoma (LLC)[54]. Moreover, the treatment was effective in prolonging survival and attenuating tumour mass growth when compared to non-treated-tumour-bearing animals.

Chemotherapy exacerbates cancer cachexia

Cancer chemotherapeutic treatment often contributes to body weight loss and cachexia [55]. For instance, platinum-based cancer treatment induces weight loss, fatigue and inflammation in cancer patients[56]. It seems that this type of treatment induces both pro-cachectic cytokines and myostatin[56]. Interestingly recent work[57] has shown that Cachexia induced by the tumour and chemotherapy give rise to distinct alterations in energy metabolism. Indeed, cancer-induced and chemotherapy-induced cachexia are

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characterized by a number of distinct metabolic derangements, indicating that effective therapeutic treatments for cancer-induced and chemotherapy-induced cachexia must take into account the specific metabolic alterations induced by the pathological or pharmacological mediators of cachexia. Chemotherapeutic treatment affects skeletal muscle in cancer patients through effects of the drugs on mitochondrial content and/or ROS production[58].

Adipokines and adipose tissue browning

Cancer cachexia is a multi-organ syndrome involving different organs/tissues. Therefore, muscle wasting occurs together with alterations in other target tissues. Following suit, adipose tissue is also under huge wasting. Metabolically, in addition to massive lipolysis, decreased lipogenesis from glucose and impaired entry of fatty acids owing to decreased activity of lipoprotein lipase (LPL) contribute to adipose tissue wasting. In addition, a clear interplay of adipokines and myokines in cancer exists, therefore, supporting the importance of inter-organ signalling between skeletal muscle and adipose tissue[59,60]. Apart from these metabolic alterations that lead to adipose tissue dissolution, in cancer cachexia, white adipose cells acquire some of the molecular machinery that characterizes brown adipose cells. This represents a 'browning' of white cells, in which uncoupling protein 1 (UCP1) is expressed and promotes uncoupling and, consequently, heat production and energetic inefficiency. This cell conversion can be triggered by both humoral inflammatory mediators, such as interleukin-6 (IL-6)[59,61], and tumour-derived compounds, such as parathyroid-hormone-related protein (PTHRP)[62]. In addition, the lipid mobilizing factor zinc-α2-glycoprotein (ZAG) --a protein secreted by some tumours[5]-- not only induces lipolysis in adipose tissues but also causes robust browning in WAT[63].

As previously mentioned, exosomal circRNA derived from gastric tumour promotes white adipose browning by targeting the miR-133/PRDM16 pathway. Interestingly, inhibiting exosome generation and release can inhibit lipolysis and adipose tissue browning, and may be useful as a novel strategy for treating cancer cachexia[64].

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The understanding of adipose tissue dysfunction in cancer cachexia will hopefully promote the development of new therapeutic approaches to prevent or treat this wasting syndrome.

Hypothalamic inflammation

In cancer patients, functional alterations within brain areas controlling energy homeostasis contribute to the onset of anorexia, reduced food intake, and increased catabolism in both muscle and adipose tissue. Increases in proinflammatory cytokines, suggest neuroinflammation as an adaptation to tumour growth. In particular, the vagus nerve appears to be involved in conveying input signals to the hypothalamus, whereas hypothalamic serotonin appears to contribute to triggering catabolic signals[65]. The *area postrema* (AP) and the *nucleus tractus solitarii* are two important brainstem centres for the control of eating during acute sickness conditions. Recently, the tumour-derived macrophage inhibitory cytokine-1 (MIC-1) emerged as a possible mediator of cancer anorexia because lesions of these brainstem areas attenuated the anorectic effect of exogenous MIC-1 in mice[42,66]. Therefore, the AP seems to have a very important role in the cancer-induced anorexia and body weight loss suggesting a role of MIC-1 as a tumour-derived factor in cancer anorexia, via an AP-dependent action.

Indeed, hypothalamic inflammation contributes to muscle and adipose tissue loss in cancer via hypothalamic IL1β-induced activation of the hypothalamic-pituitary-adrenal axis[67]. As a result glucocorticoids are released directly activating skeletal muscle protein catabolism through activation of the ubiquitin-dependent proteolytic system. In addition, hypothalamic inflammation in cancer results in a decrease in food intake in cancer by promoting changes in orexigenic and anorexigenic mediators via up regulation of serotonin availability and stimulation of its signalling pathways in hypothalamic tissues. Both reduced food intake and stimulation of tissue catabolism represents a dual mechanism by which hypothalamic inflammation contributes to the development and maintenance of anorexia and cachexia in cancer[67].

Hypothalamic activity in anorectic cancer patients is decreased as compared to non-anorectic ones, and responding differently to oral nutrition. This

suggests a central control of appetite dysregulation during cancer anorexia, before, and after oral intake[65].

The role of microRNAs

Exosomes containing microRNAs (miRNAs) and muscle-specific miRNAs (myomiRs) constitute a mechanism recently reported to be involved in cancer cachexia[68]. miRNAs transported in exosomes transport and preserve miRNAs from degradation, delivering the miRNAs to specific cell targets, making communication more efficient. Several miRNAs are known to modulate inflammatory pathways, to induce metastasis, to mediate cancer aggressiveness and even to participate in the regulation of beige/brown adipocytes differentiation. Thus breast cancer-released exosomes contained miR-155 which promotes adipocyte browning by down regulating PPARg expression[69,70]. Circular RNAs (circRNAs) are a novel family of endogenous noncoding RNAs that have been proposed to regulate gene expression in mammals. These RNAs are stable in exosomes. However, little is known about the biological role of circRNAs in exosomes. Zhang et al. showed that circRNAs in plasma exosomes have specific expression features in gastric cancer (GC), and ciRS-133 is linked with the browning of white adipose tissue (WAT) in GC patients[71].

Using anti-inflammatory therapeutic strategies

Blocking the inflammatory response may have positive effects on muscle wasting. Due to the multifactorial aspects of cachexia syndrome, any therapeutic approach based on increasing food intake has to be combined with a nutritional/pharmacological strategy to counteract the inflammatory response and prevent skeletal muscle metabolic changes. A clear research direction is, therefore, the identification of biological, immunological, chemical, genetic or behavioural non-invasive markers to be used. These markers, in addition to their utility to detect pre-cachectic subjects that will eventually suffer the full syndrome, may serve as an index of successful outcome measures in cachexia or the treatment and management of the syndrome. Additional future directions in the field should contemplate the role of the neuroendocrine system in the development of cachexia, the determination of which symptoms of cachexia are amenable to metabolic or biochemical

interventions and which symptoms require behavioural interventions in order to improve quality of life of the patients and finally to explore regimens (e.g., physical activity and nutrient supplementation) to improve dyspnoea, impaired mobility, pain, anorexia and fatigue associated with cachexia. On the lines physical activity seems to be an element to decrease inflammation[72–74], however there is insufficient evidence to determine safety and effectiveness in patients with cancer cachexia. Findings from on-going studies are awaited. Assessment of cachexia domains, ideally against international criteria, is required for future trials of exercise and supportive care interventions[75].

In conclusion, although both tumoural and humoural (mainly cytokines) factors that trigger cachexia may share common signalling pathways, therefore, it is not very likely that a single drug will block the complex processes involved in cachexia. In addition, some of the mediators proposed for the wasting syndrome also play a role in the regulation of body weight in absolutely opposite states such as obesity.

Acknowledgments

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