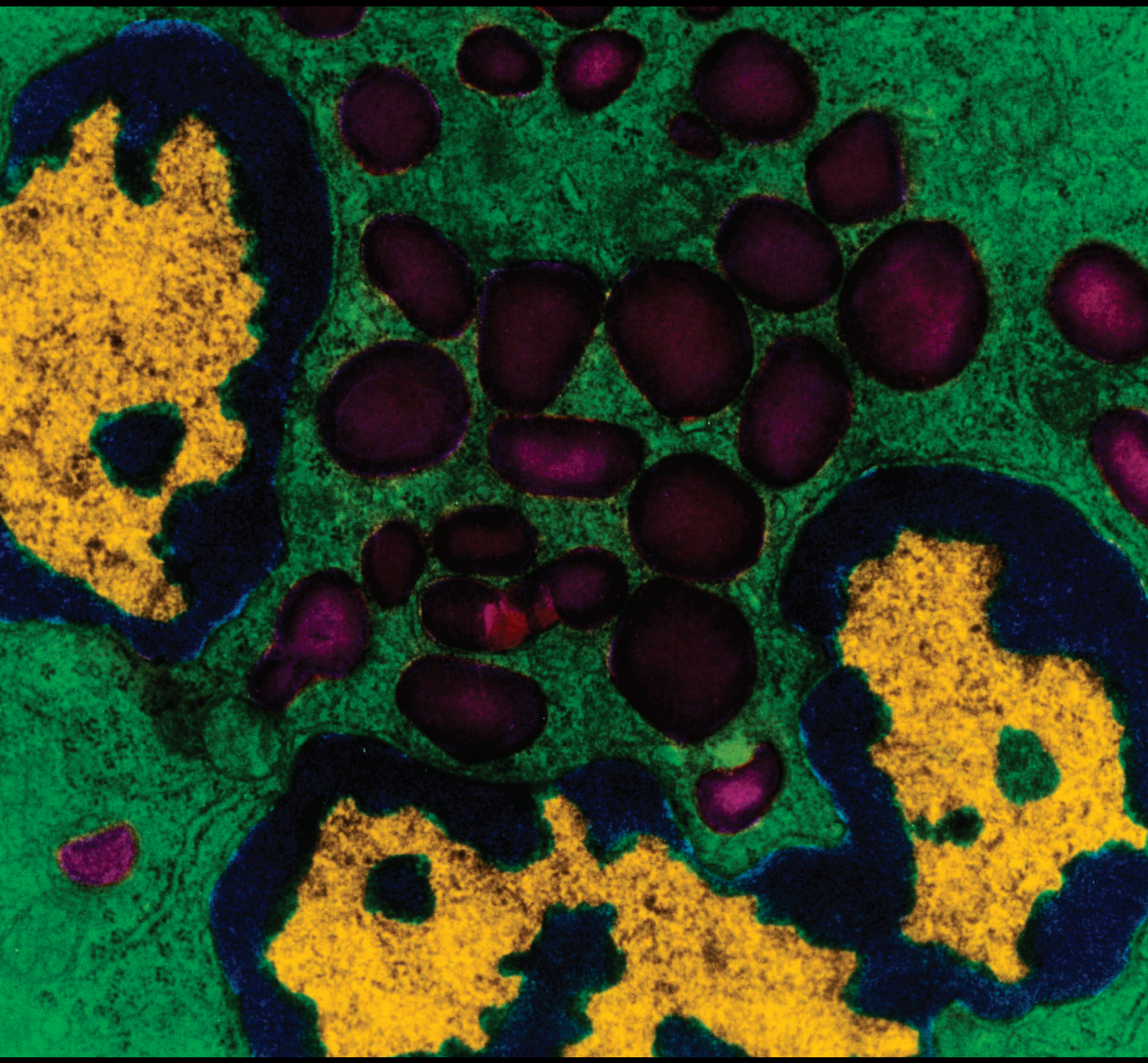
Mediators of Inflammation

Inflammation in Cachexia

Guest Editors: M. Seelaender, A. Laviano, S. Busquets, G. P. Püschel, T. Margaria, and M. L. Batista Jr.



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Editorial Board

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Editorial

Inflammation in Cachexia

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Cachexia is a complex wasting syndrome associated with a marked detrimental effect upon life quality and survival in patients with cancer, chronic obstructive pulmonary disease (COPD), chronic heart failure, AIDS, and chronic kidney disease, among other conditions. Its prevalence is of around 5 to 15% in cardiac patients at end stage, rising up to 30%, in COPD and chronic kidney disease patients, and to 80%, in patients with advanced cancer. Cachexia symptoms include pronounced weight loss, due to both lean and fat mass wasting: anorexia, malabsorption, nausea, asthenia, neuroendocrine changes, immune system function impairment, and disruption of energy metabolism. Despite its unquestionable relevance to the poorer outcome of treatment in disease and its high prevalence among patients, the syndrome is still underdiagnosed and seldom treated. Part of the difficulty in treating cachexia relies on the fact that, in the clinical setting, the syndrome is recognised solely in its most advanced stages, when therapy available to the present day is not able to fully reverse its symptoms. Therefore, scientists and clinicians should focus on identifying early changes, as to intervene in a precocious manner.

The aetiology of cachexia has not been fully unveiled, yet it appears that chronic systemic inflammation is present in the vast majority of patients. The aim of the present special issue is to address the importance of systemic inflammation in cachexia, in regard to its consequences and to its possible role in providing early markers for the diagnosis of the syndrome.

Cancer cachexia-related neuroinflammation is discussed by A. Molfino et al., as the authors propose a conceptual framework in which the hypothalamus transduces the peripheral challenge represented by the presence of the tumour into catabolic signals, as a result of central inflammation. A contribution by N. Inácio Pinto et al. examines the role of inflammatory signalling factors involved in the communication among the peripheral tissue, tumour microenvironment, and the central nervous system. Another view of such interactions is provided by J. M. Argiles et al., who bring similar emphasis on the conversation among different body compartments and organs in cancer cachexia. The authors comment on the significance of tissues other than the skeletal muscle in the mechanisms underlying the syndrome, proposing that the latter suffers wasting as a consequence of systemic inflammatory changes. Adding information on the role of inflammatory factors on muscle wasting, D. Costamagna et al. discuss the molecular mechanisms involved in muscle homeostasis disruption and mass loss.

The quest for markers of the initiation of cachexia is also debated: M. Ebadi and V. C. Mazurak propose the adoption of adipose tissue-derived factors as indicators of early

inflammatory alterations that induce fat mass wasting in the syndrome. R. Camargo et al. review the potential of microRNAs in the regulation of cancer-cachexia systemic inflammation and put forward the possibility that these molecules may serve as diagnostic tools. Finally, the article by D. Watt et al. presents the convenience and adequacy of employing prognostic scores that include systemic inflammation assessment as a valuable means for cachexia diagnosis.

Taken together, the issue provides insights on the importance of detecting early signs of inflammatory changes in patients and examines the mechanisms that act in concert, inducing cachexia symptoms.

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Review Article

Nonmuscle Tissues Contribution to Cancer Cachexia

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Cachexia is a syndrome associated with cancer, characterized by body weight loss, muscle and adipose tissue wasting, and inflammation, being often associated with anorexia. In spite of the fact that muscle tissue represents more than 40% of body weight and seems to be the main tissue involved in the wasting that occurs during cachexia, recent developments suggest that tissues/organs such as adipose (both brown and white), brain, liver, gut, and heart are directly involved in the cachectic process and may be responsible for muscle wasting. This suggests that cachexia is indeed a multiorgan syndrome. Bearing all this in mind, the aim of the present review is to examine the impact of nonmuscle tissues in cancer cachexia.

1. Cachexia as an Energy-Wasting Syndrome

Cachexia, from the Greek: "*kakos*" and "*hexis*," meaning "bad condition," is a multiorgan syndrome associated with cancer and other systemic diseases such as sepsis and renal failure and characterized by at least 5% body weight loss due to muscle and adipose tissue wasting and inflammation [1]. Abnormalities associated with cachexia include alterations in carbohydrate, lipid, and protein metabolism [2]. Cancer cachexia has been characterized as a syndrome associated with loss of muscle with or without loss of fat mass. Other disorders associated with cachexia are anorexia, inflammation, insulin resistance, and increased muscle protein [2]. Another defining characteristic is that cachexia cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment [3]. Thus, it can be concluded that cachexia is caused by an energy imbalance which is the result of both decreased food intake, due to marked anorexia, and increased energy expenditure caused by a highly hypermetabolic state. Blum et al. [4] in a recent meta-analysis of cancer cachexia conclude that current data support a modular concept of cancer cachexia with a variable combination of reduced nutritional intake and catabolic/hypermetabolic changes.

Cachexia occurs in the majority of terminal cancer patients and is responsible for the deaths of 22% of cancer patients [5]. Importantly, survival of cancer patient suffering from different types of neoplasias is dependent on the amount of weight loss [6]. Therefore, cachexia represents an important factor in the treatment of a cancer patient, affecting not only survival, but also the efficacy of anticancer treatment, quality of life, and medical costs. Thus there is a strong pressure to better understand the mechanisms that drive cachexia in order to offer cancer patients more effective care.

Here we will discuss the impact of nonmuscle tissues, only the ones that have a certain role; that is, kidney and lung do not seem to have a role in cancer cachexia. Indeed recent developments suggest that tissues/organs such as adipose (both brown and white), brain, liver, gut, and heart are directly involved in the cachectic process and may be responsible for muscle wasting. This suggests that cachexia is indeed a multiorgan syndrome (Figure 1).

2. Brain

Although a recent study involving 1853 cancer patients [7] did not find common genetic causes in appetite loss in

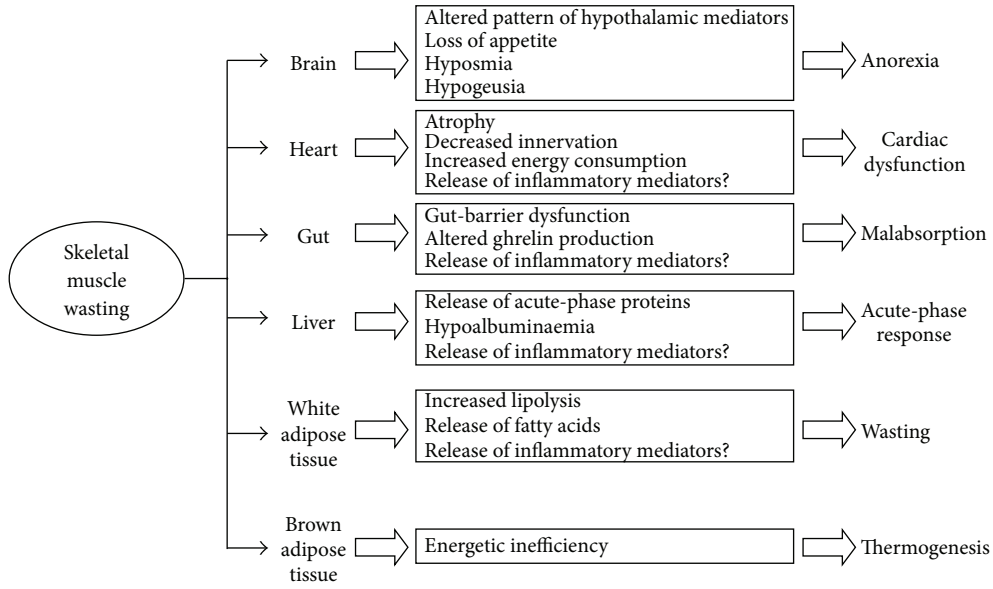

Figure 1: Interactions between different tissues/organs and skeletal muscle in the development of wasting associated with cancer cachexia. The events and metabolic alterations that take place in different tissues/organs during cancer cachexia may be related with the loss of muscle tissue. Indeed, muscle wasting may be influenced by the liver, inflammatory response, and by adipose tissues, particularly white fat. Brown adipose tissue could partially account for the energy inefficiency associated with hypermetabolism in the cancer patient. Brain, basically by modulation of appetite, also contributes to muscle wasting. The gut may be responsible for both malabsorption and changes in ghrelin production and, finally, the heart could also be a source of inflammatory mediators contributing to further muscle wasting.

cancer patients, cytokines, neuroendocrine changes, and tumour mediators are the main signals involved in appetite depression in cachexia. Additional factors contributing to the anorectic state are altered taste perception, therapy-induced side effects [8], depressed motor activity, possible mechanical interference on the gastrointestinal tract, and, of course, psychological factors [9]. Indeed, patients with cachexia often experience psychological distress as a result of the uncertainties of the disease, its diagnosis, its treatment, and its anticipated and final outcome [9]. This psychological state, which often involves depression, is bound to affect food intake. Both the limbic system and the brain stem participate in the regulation of appetite and energy balance. Thus, morphologically defined regions of the hypothalamus, the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the dorsomedial nucleus (DMH), the ventromedial nucleus (VMH), the lateral hypothalamic area (LHA), and the perifornical area (PFA), appear to play a major role in the regulation of body weight. There are two primary neuron types within the ARC that integrate signals of nutritional status and influence energy homeostasis: a subpopulation of neurons in the medial ARC expresses the orexigenic neuropeptides (neuropeptide Y (NPY) and agouti-related peptide (AgRP)). More laterally there is a second subpopulation that inhibits food intake via the expression of cocaine- and amphetamine-regulated transcript (CART) and proopiomelanocortin (POMC), which is processed to melanocyte stimulating hormone (MSH). Specific neuropeptides are involved in the signalling of the neuronal circuits within these regions of the hypothalamus, for instance, corticotrophin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), NPY, brain-derived neurotrophic factor (BDNF), orexin, and melanin-concentrating hormone (MCH). The brain stem also plays an important role in the regulation of energy balance. Reciprocal connections are present, in an extensive way, between the hypothalamus and brain stem, particularly at the level of the *nucleus tractus solitarii* (NTS). This nucleus is in close anatomical proximity to the area postrema, a circumventricular organ that has an incomplete blood brain barrier. Like the ARC, the NTS is therefore located in an ideal place to respond to peripheral circulating signals but in addition also receives vagal afferent signals from the gastrointestinal tract and afferents from the glossopharyngeal nerves.

Therefore, brain mediators involved in the control of food intake, appetite, satiation, taste, and smell of food, are responsible for the anorexia of the cancer patient, making the brain a main organ responsible for one of the components of the altered energy balance in cancer patients [10] (Figure 1). Although anorexia represents a very important factor in the development of cachexia, it has to be pointed out that in many cases the use of total parenteral nutrition does not stop the loss of body weight [11]. In addition to anorexia, the hypothalamus via the melanocortin system may contribute to muscle wasting via neuronal output, as suggested by different animal studies [12, 13].

3. Gut

Gut-barrier dysfunction is a syndrome characterized by both breakdown and leakage of the gut epithelial barrier, leading to systemic inflammation due to the entry of bacterial cell

wall components (endotoxin or lipopolysaccharide), or intact bacteria into the circulation. Gut-barrier dysfunction is often observed during the course of cancer cachexia [14] (Figure 1) and is partially connected to radiochemotherapy treatment. Additionally, tumour growth or macrophage infiltration at the level of the intestinal wall may affect gastrointestinal permeability, either locally or throughout the intestine via alterations in epithelial tight junctions [15]. From this point of view, tight junction proteins, such as ZO-1 and occludin, show decreased expression in tumour-rich regions of the intestine and colon in humans [16]. Decreases in tight junction proteins would increase permeability and allow passage of large molecules such as lipopolysaccharide (LPS) into the lymphatic circulation. Changes in mucin secretion and profiles in gastrointestinal carcinomas may become a source of inflammation in the course of cancer cachexia [17]. Indeed, gut-barrier dysfunction may lead to endotoxemia and, therefore, increased inflammation in cancer patients [14].

In addition to the gut-barrier dysfunction syndrome, recent studies support a role for gut microbiota in cancer cachexia. Indeed, decreased levels of bacteria, which have immunomodulating properties, are decreased during experimental cancer cachexia [18]. The existence of a gut-microbiota-skeletal muscle axis has been reported [19]; in fact, gut microbiota generates metabolites that can reach skeletal muscle and influence energy expenditure in the muscle cells [20].

Other aspects related with the gut may be beneficial for cachectic patients. Ghrelin is the first identified circulating hunger hormone that influences body weight regulation via a vagal pathway [21]; it is a gastric hormone initially identified in the rat stomach, in 1999, as an endogenous ligand for the growth hormone secretagogue receptor (GHSR) [21]. Under fasting conditions, the stomach, through endocrine cells located in the *antrum*, secretes ghrelin into the bloodstream. The hormone acts as a "hunger" mediator that signals the gastrointestinal fuel status from the periphery to the central nervous system in order to stimulate food intake and to adjust energy balance through decreasing energy expenditure [22]. Ghrelin also binds to the GHSR1a splice-variant that is enriched in the hypothalamus, as well as other brain regions. In the hypothalamus, at the level of the ARC, ghrelin contributes to enhanced food intake by activating orexigenic mediators such as NPY, gamma-aminoisobutyrate (GABA), and AgRP, and inhibiting anorexigenic mediators such as POMC, MSH, and CART [23]. The GHSR1a receptor is also present on vagal afferents [24] and, therefore, there is also strong evidence that ghrelin has a peripheral effect on satiety by affecting the mechanosensitivity of upper gastrointestinal vagal afferents, making them less sensitive to distension which can result in overeating. Ghrelin also has potent effects on fat storage. Ghrelin activates white adipocytes [25] while doing the opposite to brown adipocytes, therefore contributing to decreased energy expenditure [26]. These effects are related with the capacity of the hormone to stimulate growth hormone (GH) release from the anterior pituitary [27]. In addition, ghrelin increases insulin-like growth factor 1 (IGF-1) by stimulating its own receptor. These two factors are major signals being related with the regulation of energy homeostasis. The effects of ghrelin on GH and IGF-1 may be linked to the capacity of the hormone to prevent increases in protein degradation, through different components of the proteasome such as MuRF1 and MAFbx, at the level of skeletal muscle. This is particularly relevant to cancer cachexia since muscle wasting occurs mainly through activation of the ubiquitin-dependent proteolytic system. Ghrelin levels are elevated in cancer cachectic patients with neuroendocrine [28], gastric [29, 30] and lung [31] tumours. These elevated levels could represent a counterregulatory mechanism to fight anorexia associated with tumour growth. It is, in fact, an endocrine response to the so-called "ghrelin resistance" found in cancer patients. Indeed, this is, in part, the reason for the high doses of ghrelin used in clinical studies to counteract anorexia in cancer. In clinical practice the use of ghrelin and ghrelin agonists has led to promising results in cancer cachectic patients. Ghrelin treatment improves physical performance and muscle force indicating that the peptide is the best candidate for muscle wasting treatment either alone or in combination with other drugs or nutritional strategies [32–37]. Therefore, future research is needed to search for the optimal combination. Cancer cachexia is a multiorgan syndrome affecting not only skeletal muscle but also adipose tissues, heart, intestine, kidney, and liver. In fact, the final cause of death in cachectic cancer patients is, apart from the primary tumour itself, either sudden death (heart arrhythmias, hypoventilation), thromboembolic events (platelet aggregation), cardiorenal alterations (kidney dysfunction), or compromised immune function (immunosuppression). Ghrelin has a beneficial effect in all of the referred tissues. The only concern in treating cachectic cancer patients relates to the fact that ghrelin may contribute to tumour cell proliferation [38]. Indeed, ghrelin may increase the levels of growth factors, such as GH and IGF-1 that stimulate tumour growth. Additionally, ghrelin itself may have mitogenic potential. As far as we know, no *in vivo* data has examined the differences in tumour growth following ghrelin or GHS treatment. Long-term, large-scale clinical trials are required to determine whether ghrelin treatment promotes tumour growth.

In addition to the role of the gut ghrelin in the control of food intake, other mediators are bound to be involved in the anorexia associated with the cachexia syndrome. From this point of view, melanocortin-4 receptor [39] or prostaglandins [40, 41] may work independently or alongside ghrelin.

4. Liver

The liver plays a key role in regulating whole-body metabolism. Claude Bernard introduced the idea that the liver is the "glucostat" of the organism, by regulating glucose production and levels in mammals. Indeed, the liver can be regarded as the central node of supply and utilization of fuel by the tissues, the direction and flux of which are mediated by the endocrine system [42].

During catabolic conditions, opposing patterns of protein metabolism are observed between skeletal muscle and liver. While skeletal muscle is under negative nitrogen balance, mainly due to enhanced protein degradation, the liver exhibits important changes in the patterns of protein synthesis such as increased production of acute-phase proteins [42]. The enhanced muscle proteolysis drives a large release of amino acids from skeletal muscle, such as alanine and glutamine [43]. While glutamine is taken up by tumour cells to sustain both the energy and nitrogen demands of the growing mass, alanine is mainly channeled to the liver for both gluconeogenesis and protein synthesis [43]. Indeed, liver fractions from tumour-bearing animals show increased production of acute-phase proteins including C-reactive protein (CRP), serum amyloid A (SAA), α1-antitrypsin, fibrinogen, and complement factors B and C3 and a decrease in the synthesis of transferrin and albumin, leading to hypoalbuminemia [44]. An acute-phase response is also observed in cancer patients. In addition, in cancer patients with advanced cancer and during the fasting state, the total albumin synthesis rate is unchanged, compared with controls, despite much lower albumin concentrations [45]. Although the function of these proteins is far from being clear, it is known that CRP contributes to the activation of complement factors, enhancement of phagocytosis, and regulation of cell immunity; α1-acid glycoprotein inhibits platelet aggregation and phagocytosis and may be involved in spacing collagen fibres; haptoglobin binds to and clears haemoglobin from plasma; α1-antitrypsin and α2-macroglobulin regulate serine-proteases; and ceruloplasmin is probably involved in copper transport. Recently, a link between SAA, in synergy with interleukin-6 (IL-6), and activation of muscle proteolysis has been described [46] (Figure 2).

During cancer, the patient's inflammatory response (Figure 1) is linked to weight loss and poor performance status. Indeed, many inflammatory mediators are able to influence different metabolic pathways related to cachexia [2, 43]. The liver is an important contributor to the inflammation observed in cancer. Indeed, CRP seems to be a very important prognostic parameter [47–49].

The liver can also contribute to energy inefficiency. Indeed, Dumas et al. found that the efficiency of oxidative phosphorylation in liver mitochondria was decreased in a rat model of peritoneal carcinosis, suggesting that this may also contribute to hypermetabolism, elevated energy expenditure, in cancer-bearing states [50]. These alterations were associated with the content and fatty acid composition of cardiolipins [51]. Indeed, the phospholipid composition and especially cardiolipins are crucial for the mitochondrial energy metabolism. Indeed, cardiolipin is known to provide essential structural and functional support to several proteins involved in oxidative phosphorylation.

Moreover, it has been described that a higher number of CD68 immunoreactive macrophages have been found in liver cross sections of patients with pancreatic cancer and cachexia, suggesting that a crucial interaction between the tumor, peripheral blood mononuclear cells (PBMCs), and the liver, may play a central role in the development and regulation of cachexia [52].

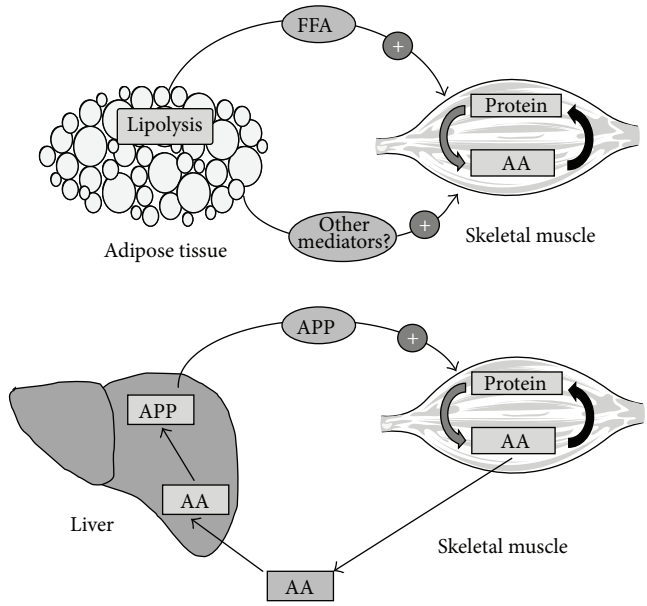

Figure 2: Examples of cross talk between adipose tissue/liver and skeletal muscle during cancer cachexia. Adipose tissue releases some factor(s), possibly fatty acids, that seem to be essential to activate muscle proteolysis. Indeed, recent evidences, using knockout deficient mice, suggest that blocking lipolysis in white fat results in an amelioration of muscle wasting. Similarly, liver acute-phase proteins (APP), such as serum amyloid A (SAA), could participate, alone or in synergy with cytokines, in activating muscle wasting by enhancing protein degradation.

5. Heart

Cancer is associated with severe heart alterations. Indeed, tumours implanted in experimental animals result in a decrease of the heart weight [53, 54], accompanied by functional cardiac changes, similar to those found in congestive heart failure. According to Schünemann et al. "cancer fatigue syndrome reflects clinically non-overt heart failure," clearly attributing a main role for heart abnormalities in the fatigue of cancer patient [55]. Tian et al. suggested that cardiac alterations in a mouse cancer cachexia model include marked fibrosis, disrupted myocardial ultrastructure, and altered composition of contractile proteins, such as troponin I and Myosin Heavy Chain-α (MHC-α) [56]. Similarly, Mühlfeld et al. using the well-established cachectic tumour rodent model Lewis lung carcinoma observed changes in heart innervation with the total number of axons in the left ventricle being reduced as a consequence of tumour burden [57]. This altered innervation was associated with a reduced expression of nerve growth factor [57]. The impairment of heart function observed in tumour-bearing animals seems to be specifically related to cardiac remodelling. Indeed, Tian et al. using the mouse C26 tumour model showed increased cardiac B-type natriuretic peptide (BNP) and c-fos expression together with decreased Peroxisome Proliferator Activator Receptor-α (PPAR-α) and its responsive gene Carnitine Palmitoyl Acyl Transferase-1β (CPT-1β) and a switch from "adult"

isoforms (MHC-α, GLUT4) to "foetal" isoforms (MHC-β, GLUT1) [58]. The heart atrophy seems to be to some extent related with increased cardiac muscle proteolysis, since protein ubiquitination and expression of MuRF-1 and atrogin-1 are elevated [58]. However, Cosper and Leinwand suggest that the cardiac proteolysis is rather caused by increased autophagy [59], in a converse manner to what happens in skeletal muscle. Interestingly, inhibition of NF-κB protects against tumour-induced cardiac atrophy, at least in experimental animals [60]. Cardiac atrophy in experimental cancer cachexia has recently been related with a high affinity activin type 2 receptor (ActRII) that mediates the signalling by a subset of Transforming Growth Factor-Beta (TGF-β) family ligands including myostatin, activin, and GDF11. Blocking pharmacologically this receptor reverses cancer-induced atrophy of the heart [61].

In addition to cardiac atrophy, Drott and Lundholm observed an increase in oxygen consumption, most likely related with the anaemia that very often is present in cancer patients, in the heart of an experimental cancer rodent model [62]. Important ultrastructural changes were also observed, such as an increase in the ratio of myofibrils/mitochondria and sarcomeric alterations, similar to those observed during cardiac failure. The increased oxygen consumption can to some extent be associated with increased energy expenditure, thus making the heart an additional organ involved in generating energy inefficiently (Figure 1). Indeed, heart rate seems to be elevated in cancer patients [63]. In fact, this parameter seems to be a very effective measure of cancer death risk. The mechanisms that could explain the association between heart rate and cancer mortality are unclear. Heart rate increase might be a marker of chronic stress and anxiety, which represent a natural consequence of the disease.

6. Adipose Tissues

6.1. White Adipose Tissue (WAT) and Muscle Wasting. Previous studies have emphasized the cross talk between adipose tissue and skeletal muscle. Indeed, signals released from both tissues, that is, Tumour Necrosis Factor-α (TNF-α), IL-6, and interleukin-15 (IL-15), may participate in a reciprocal manner in the regulation of adipose and muscle tissue mass [64, 65]. Irisin, a protein produced both in skeletal muscle and adipose tissue in response to exercise, is able to stimulate browning of adipose tissue [66]. The release of irisin seems to be promoted by the transcriptional coactivator PPAR-α coactivator-1 alpha (PGC-1α) [67]. Very interestingly, the blockade of myostatin drives browning of adipose tissue through activation of the PGC-1α-irisin pathway [68]. Alterations in the balance of the signals could well be associated possibly with obesity, diabetes, or cachexia [64, 65]. Indeed, the adipocyte releases TNF-α and other cytokines that have a direct effect on muscle metabolism. Similarly, skeletal muscle releases IL-6, IL-15, and other signals that interfere with fat metabolism [64, 65]. Loss of fat mass is a key feature of cancer cachexia and the mechanism that drives this is multifactorial. On the one hand, lipolysis is activated in the adipocyte, which reduces its cellular volume [69]. Lipolysis may be favoured by a dramatic decrease in perilipin [70], a protein that acts as a protective coating from different lipases. The intense lipolysis is accompanied by changes in the expression of genes that regulate energy turnover, cytoskeleton, and extracellular matrix, suggesting high tissue remodelling. Altogether, this results in not only a net loss of the triglyceride depot, but also a change in the phenotype of the fat cell. On the other hand, fat depletion associated with cancer is linked with a decrease uptake of VLDL and chylomicron triacylglycerol due to a decrease in lipoprotein lipase (LPL) activity [2]. The increased fat removal is accompanied by a decrease in the rate of *de novo* lipogenesis in the adipocyte [2]. Interestingly, during cachexia a concomitant inhibition of adipogenesis takes place, possibly triggered by PPAR-α [71].

Both hormonal changes, insulin resistance and hyperglucagonemia, and release of proinflammatory cytokines seem to be responsible for the changes in adipocyte metabolism. In addition, both in experimental animals and humans, a zinc-α2 glycoprotein (ZAG) has been associated with the increased lipolytic rate [72]. ZAG, released by the tumour, seems to be the mediator able to activate the triacylglycerol lipase responsible for the increased lipolysis associated with cancer cachexia.

Das et al. found that genetic ablation of adipose triglyceride lipase (ATGL) in the mouse resulted in a prevention of increased lipolysis and, therefore, reduction in WAT, associated with tumour burden [73]. Interestingly, ablation of hormone-sensitive lipase (HSL) leads to similar but less marked effects [73]. Interestingly, the lipolytic ablation resulted in a preservation of skeletal muscle mass, suggesting that the breakdown of fat precedes that of skeletal muscle proteins and implicating that some signal(s) generated during the breakdown of adipocyte triacylglycerols may actually activate muscle proteolysis (Figure 2). The ablation of the mentioned lipase was also associated with a lack of activation of the main proteolytic system involved in muscle wasting during cancer, which is that of the ubiquitin-proteasome pathway [73]. In addition to the changes related to adipose tissue itself, infiltration of adipose tissue in skeletal muscle could contribute to wasting in this tissue. From this point of view Stephens et al. [74] have reported increased presence of intramyocellular lipid droplets in *rectus abdominis* muscle of cancer patients, which seems to be related to body weight loss in these patients.

6.2. Brown Adipose Tissue (BAT) and Energetic Inefficiency. The metabolic energetic inefficiency, linked to hypermetabolism, found in the cancer patient, seems to be responsible, together with the reduced food intake, for the negative energy balance found in the patient [1]. Hypermetabolism seems to be related with inflammation [43]. Many futile cycles are responsible for hypermetabolism, including increased Cori Cycle activity between the liver and the tumour [75], liver glycolysis/gluconeogenesis [76], muscle protein synthesis/degradation [77], and adipose tissue triacylglycerol recycling [78]. An alternative mechanism contributing to hypermetabolism is the mitochondrial uncoupling proteins, originally described in BAT. Until quite recently, BAT has

been considered as a thermogenic organ in rodents. By burning fat, BAT provides fatty acids which are further oxidized but, instead of serving for mitochondrial ATP synthesis, the energy associated with the oxidative process is released as heat due to the existence in the inner mitochondrial membrane of uncoupling proteins that permeabilize? the mitochondrial membrane to the electrochemical H^+ gradient that drives ATP synthesis [79]. In 2009, Virtanen et al. showed that BAT was present in adult humans in the upper back, in the neck, between the collarbone and shoulder, and also along the spine, suggesting also a function for BAT in humans [80]. Recent data suggest the existence of two different types of brown adipose tissue cells: in addition to the "classical" uncoupling protein 1, UCP1 positive, derived from a myf-5 cellular lineage, so-called "beige" adipose cell exists with very low UCP1 expression and is derived from a non-myf-5 cellular lineage. Beige cells have their own gene pattern expression, different from both white and brown cells, and respond preferably to irisin [81]. Until now, no information is available concerning a possible role of these cells in cancer cachexia but certainly the topic deserves future attention.

Since BAT plays a key role in thermogenesis and energy balance, it may potentially contribute to the physiologic perturbations associated with cachexia (Figure 1). Several reports in experimental animals already point out a clear activation of BAT during cancer cachexia. Recently, Tsoli et al. have demonstrated increased BAT thermogenesis in cachectic tumour-bearing mice due to increased UCP1 or lipid oxidation (CPT-1α and peroxisomal bifunctional enzyme (PBE), one of the four enzymes of the peroxisomal beta-oxidation pathway) [82]. The changes observed seem to be related to an activation of STAT-3, possibly via IL-6. Unfortunately, no information is available on the role of brown fat in human cancer cachexia; therefore future research on this aspect is strongly encouraged.

In addition, there is another aspect to bear into consideration related to patients with cancer. Since the observation of the existence of BAT in adult humans, a population of cells, within WAT, which has the same characteristics as brown adipocytes, have been described [83, 84]. These cells are known as BRITE (brown into white) and seem to appear in WAT under certain conditions that seem to involve the COX-2 prostaglandin pathway. Both BAT and BRITE cells are more sensitive to insulin than WAT ones; therefore they consume glucose at a higher rate. Since both insulin and IGF-1 have been reported to fuel some tumours, having more or less BAT may affect the overall systemic insulin sensitivity and thereby have an indirect influence of tumour progression.

7. Conclusions and Future Directions

Since human skeletal muscle represents almost 50% of body weight, research on cancer wasting has for a long time been mainly devoted to this skeletal tissue. However, cancer cachexia is indeed a multiorgan syndrome affecting many types of cells, including adipose tissues, heart, liver, gastrointestinal tract, and brain. It has been recently reported that mediators released in nonmuscle tissues, during the cachexia syndrome, may actually be directly responsible for the activation of the metabolic alterations, such as increased protein degradation [85, 86], apoptosis [87, 88], and altered regeneration [89], leading to skeletal muscle wasting.

The implications of this are important since, on the one hand, the metabolic alterations affecting all cellular types may be very relevant to the understanding of the cachexia syndrome and, secondly, the development of new therapeutic approaches may benefit from this knowledge: for instance, it may be relevant to interfering with lipolysis or with acute-phase protein synthesis to block muscle proteolysis. Therefore, future studies on this field are needed and should concentrate on unrevealing the different mediators released by nonmuscle tissues that may influence muscle metabolism and, therefore, wasting. Furthermore, another important aspect that future research should contemplate is to establish the chronological involvement of the different organs/tissues in cancer cachexia.

Disclosure

Each author has participated sufficiently, intellectually, or practically in the work to take public responsibility for the content of the paper, including the conception, design, and data interpretation. All authors have read and approved the final paper.

Conflict of Interests

All authors of this research have no conflict of interests related with employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other sources of funding.

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Review Article

Potential Biomarkers of Fat Loss as a Feature of Cancer Cachexia

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Fat loss is associated with shorter survival and reduced quality of life in cancer patients. Effective intervention for fat loss in cachexia requires identification of the condition using prognostic biomarkers for early detection and prevention of further depletion. No biomarkers of fat mass alterations have been defined for application to the neoplastic state. Several inflammatory cytokines have been implicated in mediating fat loss associated with cachexia; however, plasma levels may not relate to adipose atrophy. Zinc-α2-glycoprotein may be a local catabolic mediator within adipose tissue rather than serving as a plasma biomarker of fat loss. Plasma glycerol and leptin associate with adipose tissue atrophy and mass, respectively; however, no study has evaluated their potential as a prognostic biomarker of cachexia-associated fat loss. This review confirms the need for further studies to identify valid prognostic biomarkers to identify loss of fat based on changes in plasma levels of biomarkers.

1. Introduction

Cancer cachexia is associated with increased mortality and morbidity in cancer patients [1]. By international consensus, cancer cachexia is proposed to be "a multifactorial syndrome defined by an ongoing loss of skeletal muscle mass with or without loss of fat mass that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment" [2]. A recent review [3] reported elevated lipolysis to be the major reason for fat loss in cancer cachexia [4, 5] although the underlying mechanisms are undefined. As cancer progresses, the majority of patients experience loss of fat. Fat loss precedes muscle loss, associates with shorter survival [6, 7], and is variable with respect to timing and intensity in various cancer populations [3]. Therefore, identification and validation of markers of fat loss are crucial not only for a better understanding of mechanisms, but also to identify fat losing cancer patients who will subsequently develop cachexia. Effective management of cancer cachexia is restricted to early identification of the syndrome; therefore, biomarkers are vital for development of appropriate therapeutic interventions to achieve better outcomes for individual cancer patients.

Adipose tissue (AT) is an active secretory organ, composed mainly of adipocytes and nonadipocyte cells such as inflammatory cells, immune cells, preadipocytes, and fibroblasts [8]. Adipokines are proteins synthesized and secreted from adipocytes which act both locally and distally, contributing to whole body lipid metabolism [9, 10]. In pathophysiological conditions like cancer, macrophage infiltration into AT increases [11, 12], leading to alterations in adipokine production affecting adipose tissue mass and function. Local adipokines produced by AT, circulating cytokines, and lipid mobilizing factors are collectively involved in adipose atrophy in cancer cachexia [13, 14]. Considering adipose tissue as a metabolically active organ as well as the relationship between fat loss and shorter survival in cancer, early identification of fat losing patients may increase the opportunity for therapeutic management of cachexia.

Biomarkers can be applied to represent tissue alterations under both physiological and pathological conditions [15]. A biomarker is "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease." [16]. Biomarkers indicate normal biologic processes, pathogenic processes, or pharmacological responses to a treatment [15].

Biomarkers in the oncology setting, identified using high-throughput sequencing, gene expression arrays, and mass spectroscopy [17], are classified into prognostic, predictive, and pharmacodynamic categories [18–20]. Prognostic biomarkers provide information about likely outcome of a disease, regardless of treatment, whilst predictive biomarkers assess the effect of a particular treatment. Pharmacodynamic biomarkers assess drug treatment effects on a tumour [18–20]. Ideal biomarkers are easily accessible, available, specific and sensitive, noninvasive, inexpensive, consistent, safe, and easy quantifiable in a biological fluid or clinical sample. Biomarkers are consistent across genders and ethnic groups. Levels of the biomarker should not overlap between controls and patients while significantly relating to the outcome of interest using appropriate statistical analysis [18].

While it seems important to identify a prognostic biomarker of cancer cachexia-associated fat loss, no ideal clinical biomarker has been defined yet, which demonstrates a need to identify and subsequently validate potential biomarkers in independent studies. Studies focusing on adipose tissue have identified leptin, free fatty acids (FFAs), and glycerol in plasma as indicators of fat alterations in health and diseases. On the other hand, adipokines including inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) [21] as well as Zinc-α2-glycoprotein (ZAG) [22] have also been associated with weight and fat loss in cancer. Therefore, circulating levels of these factors may represent new noninvasive prognostic biomarker of adipose atrophy and targets in the detection and management of fat loss in cancer.

One of the major obstacles to identify reliable biomarkers of fat loss in cancer cachexia is variation between studies in how fat loss is assessed. Body mass index (BMI) is frequently used as a clinically accessible measure of human body composition. However, as BMI does not distinguish between fat and fat-free mass, its utility in the settings of fat loss in cancer cachexia is limited [23]. Various methods including bioelectrical impedance analysis (BIA), dual-energy X-ray absorptiometry (DEXA), magnetic resonance imaging (MRI), and computed tomography (CT) scan analysis [24] have been applied to assess body composition in cancer population. CT image analysis, as the gold standard for body composition assessment in cancer patients, has an ability to discriminate and precisely quantify different adipose tissue depots. Many patients have repeated scans over the cancer trajectory enabling assessments in the same individual over time. Application of body composition assessment in the cancer setting has focussed primarily on lean body mass. The studies that do exist reveal loss of adipose tissue as cancer progress [25, 26]. However, further studies are required to establish the timeline and pattern of fat mass alterations in different adipose tissue depots during cancer progression [3]. Moreover, the majority of studies assessing fat mass focus on gastrointestinal cancer patients; there remains a gap in knowledge related to other malignant tumours. Finally, timing of CT scans differs between patients and scans may not be available over a specific time points demonstrating the need for other important prognostic biomarkers of fat loss. Overall, gaps remain related to the association between fat mass alterations assessed by CT scans and circulating markers of fat loss. This article reviews current knowledge around potential prognostic biomarkers of fat loss in cancer which may identify fat-losing cancer patients who would benefit from early therapeutic interventions to improve outcome of cancer patients. Possibilities and potential to apply these markers as prognostic biomarkers of fat loss will be discussed.

2. Inflammatory Cytokines

Serum levels of cytokines associate with clinical features of cancer cachexia such as weight loss; however, no study has specifically assessed the association between serum cytokines and the extent of fat loss in cancer patients. Inflammatory cytokines, such as IL-6 and TNF-α, are produced by tumours and by nonfat cells residing in AT [21] in addition to adipocytes. Plasma levels of inflammatory cytokines are elevated in cachexia [27] and are thought to promote adipose atrophy in animal and human models of cachexia [13]. Pathways of adipose tissue metabolism evoked by IL-6 and TNF-α include inhibition of lipoprotein lipase mRNA expression and activity which prevents fat cells from taking up fatty acids from lipoproteins [28, 29]. These cytokines stimulate hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) activity [30, 31], leading to elevated lipolysis. TNF-α has been reported to prevent preadipocyte differentiation [32] and inhibit expression of lipogenic transcription factors [33]. Collectively, these alterations would result in fat loss.

Serum TNF-α levels negatively correlate with body weight and BMI in pancreatic cancer patients [34]. Tumour presence has been associated with elevated serum IL-6 and TNF-α in mice bearing the Lewis lung carcinoma or B16 melanoma cells compared to controls [35]. In humans, data regarding the role of TNF-α in cancer-associated wasting are controversial. Measuring TNF-α in plasma is challenging due to short half-life and transient nature. Further, the sensitivity of assays used to measure plasma TNF-α is variable, making comparisons between studies limited [36]. On the other hand, TNF-R1 and TNF-R2 (soluble TNF-α membrane receptors) have been applied as serum markers of TNF-α activity due to their longer half-life and greater stability [37].

A comprehensive review of clinical factors associated with cachexia [38] showed little evidence for the association between serum TNF-α and weight loss in cancer, while several studies report an association of plasma IL-6 but not TNF-α with cachexia-associated wasting rather than cancer per se. Serum IL-6 levels were higher in fat losing gastrointestinal cachectic cancer patients compared to weight stable and noncancer controls. However, no changes in mRNA expression or secretion of IL-6 and TNF-α from SAT were observed [4]. This finding was confirmed in another study showing that circulating IL-6 levels were higher in weight losing non-small-cell lung carcinoma patients compared to weight stable cancer patients [39].

Adipose atrophy has been associated with elevated IL-6 signalling in a preclinical model of cancer cachexia [40]. In patients with gastrointestinal cancer, plasma IL-6 levels significantly correlated with the presence of tumour and

increased with each progressive stage of cancer [41]. IL-6 has been reported to be involved in early stages of cachexia [42, 43] and a study conducted in patients with mixed tumor types showed IL-6 levels gradually increased during early stages of cachexia followed by rapid increase prior to death [44]. In contrast, a study in 61 patients with advanced cancer showed no correlation between IL-6, TNF-α, and weight loss [45]. Although circulating IL-6 levels were higher in cachectic mice compared to controls [46], IL-6 receptors deficient (IL-6-R-KO) mice were partially protected so other cytokines may involve in cachexia-associated wasting. Moreover, a study published in 2012 reported that other cytokines, such as IL-1β but not IL-6, may be better indicator of cachexia features such as weight loss and body composition alterations [43].

Collectively, evidence would suggest that inflammatory cytokines are involved in AT depletion in cancer [13, 36, 42]; however, plasma concentrations may represent the presence of a tumour rather than cachexia-associated adipose atrophy per se [41]. Future studies are required to assess changes in adipose tissue depots, both visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) over the disease trajectory using validated body composition assessment tools and correlating those to changes in circulating cytokines. Given that there could be various sources of cytokines contributing to plasma levels, the transient nature of cytokines, as well as the cost associated with cytokine measures, the application of plasma measures of cytokines as biomarkers of adipose tissue atrophy in the clinical study is likely limited. Moreover, the ability of cytokines to evoke cancer cachexia depends on tumour type and the complex response within a network of mediators, rather than a single cytokine [47, 48]. Major gaps remain regarding the association between plasma cytokine levels and fat loss, clinical ranges of abnormal measures, and method sensitivity.

3. Leptin

Leptin is an adipokine, produced mainly by adipocytes [49]. Leptin regulates body weight by activating the anorexigenic neuropeptides and inhibiting the orexigenic neurons such neuropeptide Y (NPY) [50, 51]. Besides body weight and fat mass regulation, leptin is involved in immune function and inflammation [52]. Normally, a lower plasma concentration of leptin is associated with higher NPY secretion; however, NPY pathways have been reported to be dysfunctional in anorectic tumour-bearing rats [53]. Many factors influence leptin synthesis and secretion in adipocytes such as insulin, TNF-α, glucocorticoids, reproductive hormones, and prostaglandins [54, 55]. In humans, the main factor influencing plasma leptin concentration is adipose tissue mass.

A higher concentration of serum leptin in obese individuals is associated with increased fat mass and cell size [10]. Serum leptin is considered to be an accurate, reliable, and highly correlated measure of total body fat [56]. In healthy subjects [57], elderly adults [58], and obesity [52], plasma leptin levels have been shown to be a precise measure of adiposity. A relationship between low fat mass and low plasma leptin levels has also been reported in cancer patients [59–67]. Advanced gastrointestinal and lung cancer patients experiencing cachexia-associated adipose atrophy exhibited hypoleptinemia [67–69]. On the other hand, breast and gynaecological cancer patients exhibited elevated plasma leptin levels that related to the elevated levels of sex hormones and receptors, rather than cachexia per se [70].

Circulating leptin concentrations have been used as an indicator of fat mass; however further studies are required to examine changes in leptin concentrations that occur throughout the disease trajectory and relative to body fat mass alterations. Longitudinal studies that employ a precise measure of body fat would enable determination of whether changes in plasma levels of leptin change proportional to fat mass alterations. An added level of complexity is that leptin is secreted by both VAT and SAT, with SAT contributing the majority of leptin to plasma due to its larger contribution to overall body mass [65]. Therefore, measures of changes in leptin concentrations over time do not currently represent the type of fat being lost or gained.

Comparison between studies is limited by different assay sensitivities and how leptin values are reported as total, free, or bound leptin. Further, factors such as the type of cancer, BMI, and sex and age influence serum leptin concentration, as reported in adolescents [71], also need to be considered in study interpretation. Low leptin concentrations could be considered a result, not a cause of cachexia, which significantly relates to adipose atrophy and low fat mass in cachexia.

4. Plasma Glycerol

Studies indicate elevated lipolysis to be the main cause of fat loss in cancer [4, 5, 72–75]. During AT lipolysis, FFAs and glycerol molecules are produced by the action of lipolytic enzymes such as ATGL and HSL, which hydrolyze stored triglyceride [30]. Adipose atrophy has been associated with elevated activity of ATGL and HSL in human and animal models of cancer [35, 40, 46]. Elevated lipolysis produces higher plasma glycerol in cachectic cancer patients compared to healthy subjects [74] or weight-stable controls [5]. Lipolytic activity was assessed in 13 cachectic and 14 weight-stable cancer patients by assessing circulating glycerol levels (μmol/L/Kg body fat) as an indicator of in vivo lipolysis. Cachexia was defined as >5% weight loss over 3 months or >10% within the previous 6 months. Body fat mass, assessed using BIA, showed lower body fat (% and kg) in the cachectic group compared to weight stable patients. Elevated levels of plasma glycerol, FFAs, and higher expression of genes involved in energy turnover pathways and oxidative phosphorylation revealed increased lipid mobilization from subcutaneous adipose tissue in the cachectic group [72]. These results support those of Agustsson et al. [76] who showed plasma glycerol and FFAs to be higher in newly diagnosed gastrointestinal cancer patients with cachexia who had low body fat mass (kg), assessed using CT images, compared to the weight-stable group. Higher plasma glycerol and FFAs in the cachectic group positively correlated with

percent weight loss and negatively correlated with visceral adipose tissue area [76].

Plasma glycerol values in cancer cachectic patients have been reported as μmol/L [77] or μmol/L/Kg body fat [4, 5, 72, 76]. Interestingly, studies focusing on lipolytic activity in cancer cachexia report a narrow range of plasma glycerol for cachectic patients between studies [4, 5, 72, 76], strengthening its use a potential biomarker. Plasma glycerol has been reported as 6.2 ± 2.7 [5], 6.9 ± 1.3 [76], 7.0 ± 4.3 [72], and 9.8 ± 2 [4] (μmol/L/Kg body fat) in cachectic patients compared to weight stable cancer patients reported at 3.1 ± 0.7 [5], 3.9 ± 0.6 [76], 3.4 ± 1.6 [72], and 3.3 ± 0.3 [4] (μmol/L/Kg body fat). Postabsorptive whole body lipolytic rate, assessed by glycerol infusion technique, revealed basal levels of plasma glycerol to be higher in a cancer group compared to controls. While lipolytic rates were similar, glycerol clearance rate varied between the two groups and contributed to higher glycerol levels. Although preillness weight loss ranged from 0 to 20% in cancer patients, the same results were obtained when data was corrected for body weight [78].

Despite the use of plasma glycerol as an index of whole-body lipolysis, caution should be exercised when considering the results of these studies. Lipolysis results in the release of fatty acids and glycerol from adipose tissue, with glycerol being a better index of lipolysis as FFAs liberated by lipolysis may be reesterified within adipose tissue [79]. AT has very low glycerol kinase activity [80], and glycerol released by lipolysis enters into the bloodstream. However, lipolytic activity is not specific to adipose tissue and occurs also from intermuscular triglyceride stores and plasma lipoproteins [79]. Glycerol concentration may indicate that lipolysis occurs in SAT as glycerol released from visceral adipose tissue lipolysis enters the liver via the portal vein [81]. Therefore, plasma concentrations of glycerol reflect the balance between glycerol release by lipolysis (predominantly adipose tissue) and clearance of glycerol by liver [79] and should be interpreted with caution.

5. Zinc-α2-glycoprotein

ZAG is a protein discovered in human plasma [82] that has been associated with presence of several types of carcinomas such as breast, prostate, and lung [83–85]. Elevated serum ZAG, as a routine and reliable measurement, may apply to early diagnosis of cachectic cancer patients with adipose atrophy [86]. ZAG has been considered as an adipokine involved in lipid metabolism in adipose tissue [87, 88]. Both in vivo and in vitro studies have shown that increased ZAG expression in adipose tissue is associated with increased lipolysis and subsequent fat and weight loss [89, 90]. The exact mechanism by which ZAG participates in fat loss in cancer is not known. ZAG may induce lipolysis through activation of β-adrenoreceptors [89, 91] and elevated HSL activity [92, 93]. Although the mechanism behind ZAG regulation in AT is still unknown, glucocorticoids have been suggested to stimulate ZAG expression in AT [94]. Increased plasma cortisol levels in cachectic tumor bearing mice [93] and in cancer patients [95] have been associated with higher AT ZAG expression and elevated lipolysis. This implies that, in cachexia, glucocorticoids may induce lipolytic activity through an increase in ZAG expression [94, 96].

There is discrepancy in the association between circulating ZAG levels and weight or fat loss in various conditions. Data on serum ZAG levels in obesity are inconsistent, being reported as either increased [97] or decreased [98] which positively and negatively correlated, respectively, with BMI. Elevated serum ZAG levels have been observed in chronic heart failure and haemodialysis patients suggesting ZAG to be a marker of fat catabolism [22]. In contrast, two studies in cancer patients [77, 92] demonstrated that plasma ZAG levels may not be a good biomarker of cachexia-associated features such as weight and fat loss. Twenty-five GI cancer patients underwent curative abdominal surgery and were categorized as cachectic or weight stable. Cachexia was defined as unintentional weight loss of more than 5% during the previous 6 months. mRNA and protein levels of ZAG in subcutaneous adipose tissue were higher in cachectic cancer patients compared to weight-stable cancer patients which significantly correlated with fasting serum glycerol levels and weight loss. In this study, however, there was no significant difference in circulating ZAG levels between cachectic and weight stable cancer patients. Production of ZAG by tumours and nonadipose tissue, such as the liver, may also affect ZAG plasma levels [92]. This result is consistent with Rydén et al. [77] who report that ZAG is a locally produced factor, promoting AT lipolysis, but not secreted predominately to circulation [77]. Therefore, circulating levels of ZAG are not likely to relate to fat loss in cancer cachectic patients but instead may mediate local lipid mobilising action in adipose tissue.

6. Conclusion

Patients with advanced cancer frequently suffer weight and fat loss. Accelerated loss of adipose tissue is associated with shorter survival, reduced quality of life, and decreased muscle mass during cancer progression [6]. Due to the role of adipose tissue in mediating human metabolism, identification of prognostic biomarkers of fat loss in cancer may help to identify fat losing cancer patients for early therapeutic interventions, improved survival, and prevention of muscle atrophy in cancer patients.

No studies in cancer have identified a prognostic biomarker of fat mass alterations nor have the sensitivity, specificity, and reproducibility of potential indicators been assessed in the neoplastic state. Inconsistency in the literature may be due to varying sensitivity of assays used to measure plasma levels of mediators, heterogeneity of patient populations and treatment, and various body composition assessment methods. Inflammatory cytokines appear to be mediators of cachexia-associated features such as fat loss [13, 36, 42]; however, they do not fulfill several components of biomarker criteria. Relationship between circulating cytokines and degree of fat loss in cancer has not been assessed. ZAG in plasma has been suggested to indicate the presence of some type of tumours, and in AT, ZAG can act locally to modulate lipolysis. Literature regarding the potential of plasma ZAG to be a biomarker of fat loss during

the development of cancer cachexia is inconsistent. Enhanced adipose tissue ZAG expression in cancer cachexia suggests that ZAG could be a local catabolic mediator within the tissue rather than being a biomarker of fat loss [77]. Therefore, the ability of ZAG to be applied as a marker of lipid utilization in cachexia syndrome and to indirectly represent fat loss is limited.

Plasma glycerol and leptin may have potential to be considered as biomarkers of lipolysis and fat mass, respectively; however, no study has defined a confirmed range and optimal cut-off points for these markers. It is not clear whether a single biomarker or combination may have the most prognostic value, as no study has assessed various combinations in a cancer population. Measuring changes in fat mass over time concurrent with circulating levels of biomarkers of fat mass would provide valuable information about application of proposed fat loss biomarkers throughout the disease trajectory. These studies would help establish valid criteria to identify loss of whole body fat mass based on changes in plasma levels of these specific biomarkers.

Alterations in fat mass and composition between visceral and subcutaneous depots are divergent and vary over the cancer trajectory. The proportional reduction of each fat depot may be a consideration when establishing biomarkers. For example, it remains to be determined whether decreased leptin levels indicate the loss of visceral or subcutaneous adipose tissue in cancer. Future studies should consider the metabolic differences between these depots in determining specific biomarkers.

Although many of the proposed biomarkers are economical, easy, and quick to quantify in plasma, further steps such as comparison of plasma levels in healthy, weight stable, and weight losing cancer patients as well as their correlation with various degrees of fat loss assessed by CT images should be considered in determining capacity for application of a prognostic biomarker of fat loss in cancer. Proper study design, combined with extensive testing, and quantitative measurement of large numbers of proteins in body fluids using advanced techniques [99] as well as statistical validation of prognostic biomarkers [100] are important factors in identification of fat loss biomarkers. This review confirms the need for further studies to (1) assess how alterations in fat mass is reflected in measurable biomarkers, (2) minimize variations that may confound establishment of a biomarker, and (3) increase specificity and sensitivity of methods to detect biomarkers in samples at minimum levels or in repeated measures.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Cancer as a Proinflammatory Environment: Metastasis and Cachexia

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The development of the syndrome of cancer cachexia and that of metastasis are related with a poor prognostic for cancer patients. They are considered multifactorial processes associated with a proinflammatory environment, to which tumour microenvironment and other tissues from the tumour bearing individuals contribute. The aim of the present review is to address the role of ghrelin, myostatin, leptin, HIF, IL-6, TNF-α, and ANGPTL-4 in the regulation of energy balance, tumour development, and tumoural cell invasion. Hypoxia induced factor plays a prominent role in tumour macro- and microenvironment, by modulating the release of proinflammatory cytokines.

1. Introduction

The malignancy grade of a tumour is fundamentally associated with both the development of metastatic lesions, a complex process that involves the propagation of cancer cells from the primary site to distant organs, and cancer-associated cachexia, which is dependent on factors produced by either the tumour microenvironment (tumour cells and surrounding tissues) or by the macroenvironment (comprising tumour released substances in the circulation and the secretion of proinflammatory factors by other tissues).

Cancer cachexia is an inflammatory condition, as is obesity. An excess of adipose tissue depots is associated with an increased risk of the incidence of some types of cancer and increased morbidity and mortality in cancer patients. Some factors such as insulin-like growth factors, leptin, and steroid hormones seem to connect obesity and cancer, once they contribute to the development of the chronic inflammatory state [1]. The increased estrogen synthesis associated with obesity in postmenopausal women augments the risk of development of postmenopausal breast and endometrial cancer. The constant presence of a high concentration of insulin in blood related to body fat excess and the increase of bioactive fractions of insulin, such as growth factor 1, via the downregulation of levels of insulin like growth factor-binding proteins 1 and 2, contribute to increased risk of cancer development [2, 3]. Adipose stromal cells in WAT may present a role in cancer development and progression, promoting changes in the production of adipokines. Proinflammatory cytokines are involved in cancer-inducing mechanisms, while adiponectin could have anticancer effects [3, 4]. The inflammatory tumour microenvironment is amplified by infiltrating macrophages, further increasing the production of cytokines, prostaglandins, angiogenic factors, and metastasis [4].

The details of the multi-step process of metastasis have been described by others [5, 6]. Here, we will provide only a brief synopsis. In the primary tumour microenvironment, epithelial cancer cells are surrounded by pericytes, various extracellular matrix (ECM) proteins, and cancer-associated fibroblasts (CAF), which include fibroblasts, macrophages, endothelial cells, lymphocytes, and granulocytes. This tumour microenvironment produces various factors, such as TNFα, TGFβ, Wnt, and HIF-1α, all of which stimulate a transient epithelial-mesenchymal transition (EMT) to promote cancer progression, invasion, and metastasis. EMT is a phenomenon wherein cells loose

epithelial properties and gain mesenchymal properties, causing the loss of cell-cell contact and cell polarity. EMT increases cell migratory and invasive abilities, which are associated with the first step of cancer metastasis.

Cytokines such as TNFα and interleukins, which are produced by both tumour cells and surrounding cells trigger NF-κB signalling [4], consequently causing the release of TGF-β, an inducer of EMT [7, 8].

For the last decade, white adipose tissue has been considered an important endocrine organ producing several humoral factors including leptin, adiponectin, TNF-α, IL-6, zinc-a2 glycoprotein (ZAG), IL-10, plasminogen activator inhibitor-1, and visfatin. These adipokines participate in the regulation of energy balance and a several physiological processes, including insulin sensitivity and the inflammatory response [9].

In fact, cachexia is frequently accompanied by food intake reduction and increases in proinflammatory factors such as C-reactive protein, TNF-α, IL-6, and IL-1 [10].

Several reports have shown [10] that white adipose tissue (WAT) expresses and secretes proinflammatory factors in a rodent model of cancer cachexia, suggesting that these adipokines could be involved in the systemic inflammation in cancer patients.

In this review, we discuss some factors from the micro- and macroenvironment that could contribute to the development of metastasis and cancer cachexia.

2. Cancer Cachexia

Metabolic disorders that develop in anorexia and cancer cachexia are different. The weight loss in anorexia occurs mainly by the weight loss from fat, whereas cachexia involves significant weight loss from fat and muscle mass [50]. Anorectic patients respond to nutritional treatment more effectively than cachexia. In opposition, in the process of cancer cachexia, muscle mass cannot be maintained and food intake frequently cannot reverse this condition [51, 52].

Cancer cachexia is a multifactorial syndrome characterized by a persistent and involuntary loss of skeletal muscle mass and fat mass, which contributes to progressive functional damage. This condition is related to a negative protein and energy balance, caused by a variable combination of abnormal metabolism and reduced food intake; conventional nutritional support is not enough to improve it. In addition, increased lipolysis and insulin resistance and reduced physical activity are present [52].

Cachexia may have a high prevalence in patients with cancer, depending on the stage of the disease and the type of tumour. The range of cachexia in patients with advanced cancer is from 60 to 80%. Pancreatic and gastric cancers are among the cancers with the highest prevalence of cachexia, approximately 85%. Over 20% of cancer patients die from cachexia [53].

The cancer in digestive tract frequently causes undernutrition related to the obstruction of alimentary tract, constipation, maldigestion, and malabsorption. However, the cachexia present in these patients was not only associated with malnutrition, since in these patients the metabolism differs from patients with anorexia, as the former present high proteolysis associated with an increase in proinflammatory protein and cytokines, aspects that will be discussed below [54].

Cancer cachexia progression comprises a variety of phases, including precachexia, cachexia, and refractory cachexia. Refractory cachexia is more difficult to reverse and is characterized by active catabolism or the presence of factors produced by the tumour and other tissues that do not allow an improvement in weight loss. This stage of cachexia is observed in patients who have a rapid progression of cancer that conventional cancer therapy cannot affect or in patients in the terminal stages of cancer, as in the cachexia consensus published in 2011 [52].

The type and stage of cancer, cancer treatment, food intake, and systemic inflammation are factors that can influence the progression from precachexia to cachexia [52, 55].

Precachexia is considered the initial stage in which clinical and metabolic abnormalities, such as glucose intolerance and anorexia, already occur and precede an involuntary weight loss ($\leq$5%). Cachexia is identified in patients with equivalent or more than 5% of body weight loss during the previous 6 months, or a BMI below 20 kg/m^2 and a continuous weight loss of more than 2%, or continuous weight loss (<2%) and sarcopenia. For many years, the majority of researches focus on muscle proteolysis in cancer cachexia. Nowadays, both muscle and adipose tissue wasting are considered important for development of cachexia in cancer patients [52].

Several factors produced during cancer development either by the micro- or the macroenviroment promote a proinflammatory condition, which are related to alterations on the regulation of energy balance. We will discuss some factors that are potentially involved in the development of cancer cachexia and metastasis.

3. Ghrelin

Energy balance results from the amount of food intake and the total energy expenditure. The control of energy balance depends on a complex network system regulated by several peripheral and central signals [56]. Cancer patients with systemic inflammation show a decrease in food intake, and hypermetabolism has also been described [52].

Ghrelin is a hormone produced mainly by enteroendocrine cells and, to a lesser extent, in the colon, hypothalamus, pituitary, endocrine pancreas, placenta, lung, cardiomyocytes, ovaries, and testes. Additionally, several studies have demonstrated that ghrelin mRNA and protein are expressed in many cancer and tumour tissues (see review Chopin et al., 2012) [11]. Ghrelin was first reported as an important stimulator of hunger and an inhibitor of energy expenditure [57]. Many studies have been conducted to verify the relationship between the decreased appetite present in cancer patients and the hormones that stimulate hunger, such as ghrelin.

These studies found high levels of total ghrelin and acylated ghrelin in most cachectic cancer patients compared with cancer patients without cachexia and patients without

cancer. These results were demonstrated in patients with several types of cancer, such as breast, colon, and lung cancers [11].

However, interestingly, cancer cachexia patients did not show increased appetite, despite their increased ghrelin levels. It seems that, in these patients, ghrelin levels increased in an attempt to reverse the catabolic state, as a compensatory mechanism [11, 58].

Chopin et al. (2012) [11] suggested that ghrelin resistance is similar to the GH-resistant state observed in cancer cachexia patients because GH levels are elevated, whereas IGF-I levels are not.

In contrast, Fujitsuka et al. (2011) [59] evaluated mice inoculated with AH-130 ascites hepatoma cells, which cause cachexia, and found lower plasma concentrations of ghrelin and reductions in the expression of hypothalamic Y (NPY) neuropeptide, agouti-related peptide, and proopiomelanocortin (POMC) compared with animals without cancer cachexia. It appears that the reduction in ghrelin occurs due to excessive hypothalamic interactions between serotonin (5-HT) and corticotropin-releasing factor, which are stimulated by proinflammatory cytokines. In addition, tumour-bearing rats showed attenuated responses of increasing food intake when ghrelin was administered intravenously, indicating possible ghrelin resistance. This finding could partially explain why cancer cachexia patients present hypophagia despite their low levels of leptin and high levels of ghrelin.

In addition to investigations of the effects of ghrelin on energy balance, this hormone was evaluated to determine whether it could have a role in tumour growth, because ghrelin stimulates growth hormone secretion. Accordingly, Northrup et al. (2013) [60] showed that both ghrelin and anamorelin, an active agonist of the ghrelin receptor, did not cause an increase in tumours in tumour-bearing nude mice. Despite this result, the influence of ghrelin on tumour growth remains to be elucidated. As stated by Chopin et al. (2012) [11], it is controversial whether ghrelin has tumour-promoting effects or could inhibit tumourigenesis *in vivo*. Further studies are needed to better understand the effects of ghrelin on cancer cachexia development.

The administration of ghrelin as a treatment for cancer cachexia patients has been evaluated [59]. *In vitro* evidence showed that the expression of atrogenes through PI3Kβ-, mTORC2-, and p38-mediated pathways in myotubes and dexamethasone-induced muscle atrophy was inhibited by acylated and nonacylated ghrelin, suggesting that this peptide may be able to prevent muscular atrophy [12]. Additionally, ghrelin inhibits myostatin secretion, a negative regulator of skeletal muscle mass [61].

4. Myostatin

Myostatin expression is upregulated in experimental models of cancer cachexia [13, 14]. In humans, blood and muscle myostatin levels are upregulated in gastric cancer patients [62]. Myostatin, also known as growth/differentiation factor-8 (GDF-8), was described in 1997 and has been shown to be a potent negative regulator of muscle growth. This protein is expressed in muscle and other tissues, such as heart, adipose tissue, and mammary gland [63].

Active myostatin binds the activin type II B receptor (ActRIIB) and, to a lesser extent, the related ActRIIA, promoting the phosphorylation and assembly with the low-affinity type I receptor ALK- (activin receptor like-kinase-) 4 or ALK-5 [15, 64].

Myostatin has been reported as a negative regulator of skeletal muscle mass: low levels of myostatin or the knockout of the myostatin gene contributes to muscle mass growth in mice [15–17]. Additionally, the administration of soluble ActRIIB and the overexpression of activin receptor II B (ActRIIB), a dominant-negative form of the myostatin receptor, induce muscle hypertrophy, while increased levels of myostatin lead to skeletal muscular depletion [15, 17, 65].

Some reports have shown that myostatin signalling is enhanced in the skeletal muscle of tumour-bearing rats and mice [13, 14].

In this sense, it has been demonstrated that myostatin is abundantly secreted by C26 colon cancer cells, and it has been verified that the treatment of differentiated C2C12 myotubes with C26-conditioned medium promoted myotubular atrophy and enhanced the activity of the ubiquitin-proteasome pathway. Additionally, the addition of antagonists to myostatin prevented C26-conditioned medium-induced wasting in muscle cell cultures. The authors suggested that myostatin secretion by cachexia-inducing neoplasm would initiate the pathogenesis of cancer cachexia [66].

Likewise, myostatin inhibition by the administration of an ActRIIB/Fragment-crystallizable (ActRIIB/Fc) fusion protein or the ActRIIB soluble form by antisense oligonucleotides is able to prevent the development of muscle mass depletion in tumour-bearing mice [67, 68].

5. Leptin

Leptin plays a central role in the control of body weight and energy homeostasis, but it is a pleiotropic cytokine with activities in many peripheral cell types. Several studies in animals and humans have demonstrated the role of leptin in the regulation of energy homeostasis, neuroendocrine function, metabolism, immune function, and bone metabolism. Despite being produced predominantly in adipose tissue, other tissues express leptin, such as placenta, ovaries, mammary epithelium, bone marrow, and lymphoid tissues [69]. Leptin binds to receptors located throughout the central nervous system and peripheral tissues, with at least six receptor isoforms identified (LepRa-f) [70].

In the cancer context, hypoxic conditions often occur in solid tumours. Cellular hypoxia induces hypoxia-induced factor-1 (HIF-1) which activates the leptin gene promoter in human adipocytes and fibroblasts [71].

There is accumulating evidence that leptin signalling might be involved in the development of several types of cancer, such as colon cancer, mammary cancer, prostate cancer, and epithelial ovarian cancer, as well as the development of several myeloid and lymphoid leukemic cell lines. In support of these data, the expression of LR was detected in all these cancer tissues and cell lines [18, 72].

Indeed, leptin has been shown to regulate neoangiogenesis; enhance endothelial cell growth [19, 20]; suppress apoptosis through a Bcl-2-dependent mechanism [73]; act as a mitogen, transforming factor, or migration factor for many different cell types, including smooth muscle cells [74], normal and neoplastic colon cells [75, 76], and normal and malignant mammary epithelial cells [77, 78]; and induce the metastasis of breast cancer, possibly in an autocrine manner [79].

In patients with cancer cachexia, the plasma levels of leptin were lower than in patients without cachexia, which could be due to reduced fat mass in these patients [80]. Because the classic effects of leptin are stimulating α-MSH neurons and inhibiting NPY neurons, decreasing food intake, and increasing energy expenditure [56], a leptin-independent pathway of cancer cachexia has been proposed.

Along with low plasma leptin levels, animal models of cancer cachexia may also have an increased number of LRb receptors, which seems to be consistent with the severity of the body fat reduction present in this condition [81]. Moreover, hypoxia-induced factor 1 (HIF-1), which is elevated in cancer cachexia, induces an increase in LRb expression in tumour cells [82].

As stated by Garofalo and Surmacz (2006) [18], it is possible that the local leptin concentration and signalling could be involved in the stimulation of tumour progression. In addition, they suggested that the tumour and surrounding adipose tissue promote a leptin-rich environment, which could contribute to tumour development. Likewise, leptin could contribute to tumour metastasis.

6. HIF

As mentioned before, a hypoxic environment is present in different types of tumours, especially in solid tumours. To adjust to the hypoxic microenvironment, several cancer cells increase the production of hypoxia-inducible factors (HIFs). These factors are associated with increased malignancy, poor prognosis, and resistance to radiotherapy and chemotherapy [83]. However, Ranasinghe et al. (2014) [84] reported that prostate cancer cells overexpressed HIF1α even under normoxic conditions.

Hypoxia-inducible factors (HIFs) are transcriptional regulators that mediate the cellular response to low oxygen. HIFs consist of an O_2-sensitive HIF-α (HIF-1α or HIF-2α) and an O_2-insensitive HIF-1β subunit [85]. HIF-1 is the main mediator of hypoxic adaptation, but several tissues and different cell types express both HIF-1 and HIF-2 isoforms under hypoxia [86].

The accumulation of HIF-1α promotes the induction of several gene targets, such as leptin and the leptin receptor in tumour cells [87, 88], insulin-like growth factor-binding protein-1, vascular endothelial growth factor A [89], angiopoietin-2, angiopoietin-like 4, plasminogen activator inhibitor-1, glucose transporter-1, hexokinase-2, and glyceraldehyde-3-phosphate dehydrogenase [90]; it also seems to interfere with the transcription of Cdc6 and C-Myc during the regulation of the cell cycle [91].

The literature provides studies that focus on tumour cells that alter the transcriptional profiles via hypoxia-related mechanisms to modulate glycolysis, proliferation, angiogenesis, apoptosis, and metastasis, as to persist under conditions of hypoxic stress [21]. Under hypoxia the induction of glycolysis, angiogenesis, and metastasis seems to be a tumour cell adaptation to survival, which has HIF-1 as a main regulatory factor [27].

The tumour cell in hypoxia also increases the expression of macrophage chemoattractants such as VEGF, endothelins, IL-8, and endothelial monocyte activating polypeptide II (EMAP II) which promoted an increase in monocytes infiltration and macrophages accumulation, especially in tumour avascular or perinecrotic regions [28].

In addition, HIF-1 induces myeloid-derived suppressive cells (MDSC) differentiation to tumour-associated macrophages, causes a polarization of M1/M2 type with an increase of M2 in the hypoxia tumour region, and inhibits antitumour T cells, decreasing the immune response [29] (Figure 1).

Using animal models, Liao et al. (2007) [25] demonstrated that the depletion of HIF1-α did not impair mammary tumour formation, though decreasing the tumour progression and metastasis. In spite of that, Mazumdar et al. (2010) [22], employing a KRAS-driven lung tumour model, demonstrated that HIF1α deletion presents a very small effect on tumour weight and progression, whereas the loss of HIF2α actually increased tumour growth and progression.

Studies demonstrated that HIF-1 is implicated in the regulation of several genes involved on multiple key steps of metastasis, including epithelial-mesenchymal transition (EMT), invasion, extravasation, and metastatic niche formation, mostly in solid tumours (for details see review by Liu et al. (2015)) [26].

HIF also contributes for the proinflammatory macroenviroment present in several cancer patients. It is well-known that HIF-1 increases in the adipose tissue of obese individuals inducing the expression of proinflammatory adipokines such as IL-6, leptin, TNF-alpha, and angiopoietin 4, which are involved in the promotion of cachexia and metastasis [23, 24].

7. Cancer and Inflammation

The proinflammatory environment can increase the risk of cancer by providing bioactive molecules, including cytokines, growth factors, and chemokines that facilitate carcinogenesis programs and sustain cell proliferative rate, inhibit apoptosis, and stimulate angiogenesis, and extracellular matrix-modifying enzymes, such as metalloproteinases, which promote the epithelial-mesenchymal transition (EMT).

8. IL-6

There is evidence that IL-6 is implicated in promoting tumour growth metastasis and participates in the development of cancer cachexia. IL-6 is considered the prime regulator of the acute-phase response in cachectic patients.

In the 1990s, there was increasing evidence that IL-6 contributes to metastasis and that serum IL-6 levels are

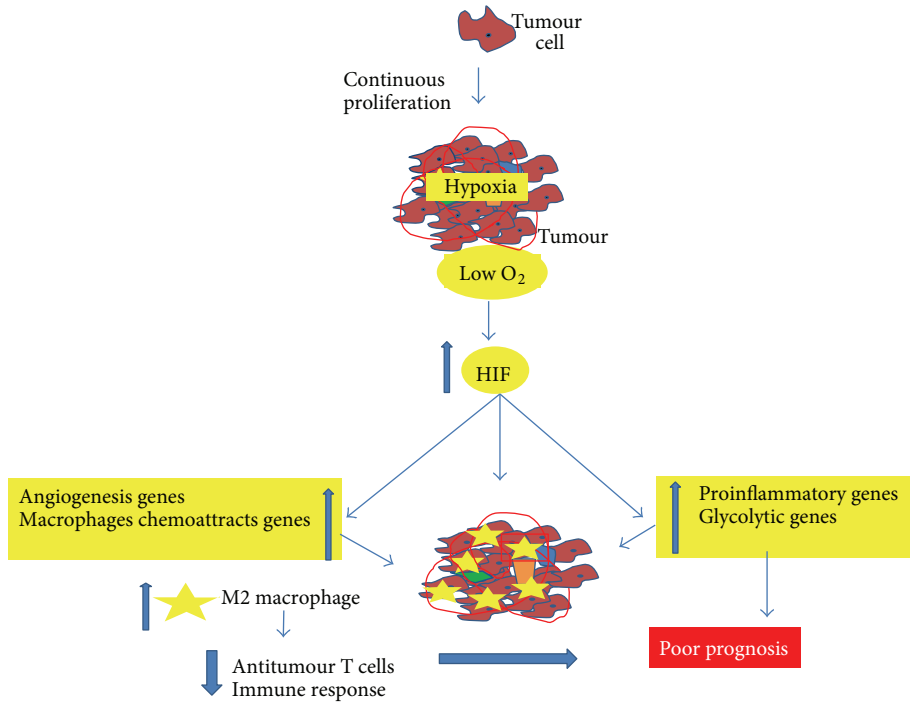

Figure 1: Schematic illustration of the effect of hypoxia on tumour gene expression, macrophage infiltration and antitumour immune response.

adverse prognostic factors for the development of metastasis in several tumour types [30, 31].

Chang et al. (2013) [32], using *in vivo* and *in vitro* experiments, analysed the effects of IL-6 on cancer development and demonstrated that there is a positive correlation between IL-6 and human mammary tumour development and metastasis, which seems dependent on STAT-3. The authors proposed the "formation of an autocrine/paracrine IL-6/JAK/STAT3 feed-forward loop, which participates in tumour proliferation, shaping of the tumour microenvironment, and metastasis."

Recently, Guyer and Macara (2015) [92] showed that IL-6 is important for inducing STAT3 in mammary epithelial cells downstream of silencing the cell polarity protein Par3, an important regulator of mammary tissue structure, which protects the development of primary tumour growth and aggressive metastatic lesions.

IL-6 signalling involves the binding of the cytokine to the membrane-bound IL-6 receptor (mIL-6r) on target tissues, which include hepatocytes, immune cells, and skeletal muscle. The activation of mIL-6r consequently promotes the downstream activation of many signalling pathways, including JAK/STAT3 and p38. Several of these pathways have also been implicated in the regulation of muscle mass loss during cancer cachexia [93].

Batista et al. (2013) [94] evaluated the correlation between adipokine tissue expression and concentrations in cachectic and noncachectic patients with or without cancer. They found that the plasma concentration of IL-6 was 11.4 times higher in the cachectic cancer group compared with the groups without cancer and with healthy weight, in addition to demonstrating a significant correlation with the presence of cancer. The interaction between cachexia and tumours increased the amount of IL-6 in subcutaneous adipose tissue and increased the IL6/IL-10 ratio, but not in visceral adipose tissue. Thus, they suggested that subcutaneous adipose tissue is associated with changes in plasma adipokines, which can play a role as markers of cachexia. However, a more specific study of the adequacy of the IL6/IL-10 ratio in the setting of cancer cachexia is necessary.

Suh et al. (2013) [95] evaluated 98 advanced cancer patients and observed that IL-6 may be a good indicator of survival time in patients with advanced cancer in later life, despite elevated IL-6 previously being considered an indicator of shorter survival in these patients. Additionally, Kim et al. (2012) [96] investigated the roles of proinflammatory cytokines in lung cancer and colorectal cancer patients with cachexia prior to treatment. They observed that patients with high levels of IL-6 showed >5% weight loss after 6 months, suggesting that IL-6 could be responsible for the induction and maintenance of cancer cachexia.

Puppa et al. (2011) [97] demonstrated in tumour-bearing mice an association among increases in gut permeability, endotoxemia, and plasma IL-6 concentration with tumour growth and cachexia development.

Bonetto et al. (2012) [33] suggested that STAT3 is a primary mediator of muscle mass loss because STAT3 activation in skeletal muscle by elevated IL-6 family ligands appears to be necessary and sufficient to promote muscle mass loss, in addition to being a common characteristic observed *in vivo* and *in vitro* and for different types of cancer. However, it is not yet clear how the activation of

STAT3 promotes muscle atrophy. Haddad et al. (2005) [34] showed that the infusion of IL-6 in muscle reduces the phosphorylation of S6K1, which is associated with a catabolic process. S6K1 is phosphorylated and activated by mTOR, and a reduction in the phosphorylation of S6K1 is associated with the loss of the cellular capacity to synthesize proteins. Additionally, as described above, IL-6 seems to be involved in decreasing body fat and food intake by acting on both energy expenditure and NPY release control in the hypothalamus arcuate nucleus [35].

From these reports the idea that IL-6 could be a good marker to predict the evolution of cachexia associated with cancer and could be employed in treatment strategies emerged. Moreover, it was suggested that treatments that could impair the increase in IL-6 in adipose tissue could ameliorate cachexia in cancer. More studies are essential to elucidate this issue.

In this sense, it has been demonstrated that endurance exercise ameliorates cachexia-related inflammation in a rodent model, causing a systemic effect that is also associated with adipose tissue and decreases IL-6 in mesenteric adipose tissue [98, 99]. Likewise, other studies using anti-IL-6 antibodies, either *in vitro* or *in vivo*, showed improvements in cancer cachexia [100, 101].

It is important to note that although IL-6 has an important effect on the development of cachexia associated with cancer, IL-6 cannot be considered the only factor contributing to the breakdown of skeletal muscle and, consequently, to the development of cachexia [38, 102].

Some cancer patients present high serum levels of TNF-a, IL-6, and IL-1 which correlate positively with the progression of some tumours. It has been postulated that these cytokines promote anorexia in cancer by enhancing the levels of corticotrophin-releasing hormone, a central nervous anorexigenic neurotransmitter, causing a decrease in food intake [36].

Schéle et al. (2013) [35], using IL-$6^{-/-}$ and IL-$1R1^{-/-}$ mice, suggested that both endogenous IL-1 and IL-6 could suppress the expression of NPY and agouti-related protein in the hypothalamic arcuate nucleus. As stated by Trayhurn and Bing (2006) [103], IL-6 is the most interesting adipokine evolved in the regulation of energy balance. IL-6 induces weight loss [104], and it is expressed with its receptor in the neurons of the hypothalamic nuclei that regulate energy homeostasis [105]. In this sense, Wallenius et al. (2002) [37] demonstrated that the chronic ICV administration of IL-6 reduces body fat through an upregulation of energy expenditure without causing an acute-phase reaction.

9. TNF-α

Tumour necrosis factor (TNF-α) is an inflammatory mediator present in the tumour microenvironment that has been implicated in carcinogenesis, especially in the early stages, including angiogenesis and invasion, versus the progression of carcinogenesis [40]. TNF-α is the main inflammatory cytokine that induces a transcription factor, Snail, that is implicated in EMT induction and stabilization [106].

Furthermore, studies have proposed that systemic TNF-α might also be involved in the early development of some tumours. As reported by Balkwill (2006) [107], several studies using TNF-α- and TNF-R1-knockout mice and a variety of cell cultures have demonstrated the role of TNF-α in cancer development.

Recently, studies showed elevated TNF-α plasma levels to be associated with an increased risk of colorectal adenomas and the development and progression of breast tumours [108, 109].

However, the results regarding the role of TNF-α in cancer are controversial; high concentrations of this cytokine induced an antitumoural response in a murine model of sarcoma [110]. In contrast, low, sustained TNF-α production levels can induce a tumour phenotype [107].

Obesity is linked with chronic, subclinical inflammation characterized by elevated levels of circulating proinflammatory mediators produced by adipose tissue, such as leptin, TNF-alpha, and IL-6 [111]. This inflammatory condition could be involved in an increased risk of cancer development. In fact, several authors have reported a correlation between obesity and cancer development (see review Calle and Kaaks (2004)) [2].

Furthermore, TNF-α seems to be involved in the progression of cancer cachexia. As previously noted, cachexia is related, among other factors, to decreases in fat mass and skeletal muscle associated with negative protein synthesis and positive proteolysis. Muscle atrophy is characterized by a reduced cross-sectional area of myofibers accompanied by a loss of strength and a change in the composition of muscle fibre types. During the process of cancer cachexia, rapid type II fibres are affected, contributing to a higher proportion of slow fibres compared to fast fibres. In this case, catabolic factors, such as IL-6 and TNF-α, are increased, contributing to the loss of muscle mass, and it has been suggested that the tumour has a great influence on the increase in the circulation of these factors [38, 39]. In fact, it has been demonstrated that TNF-α acts more on type II fibres to stimulate apoptosis signalling [41].

Several factors, including TNF-α and IL-6, are associated with protein degradation by ubiquitination and the proteasome pathway, the most predominant pathway among the pathways of protein degradation. TNF-α is able to increase the expression of ubiquitin and to promote the accumulation of ubiquitinated proteins, contributing to the atrophy of muscle mass [112, 113]. The activation of NF-kB is a major candidate as the mediator of the cellular response of TNF-α after TNF-α stimulates the activation and nuclear translocation of NF-kB in skeletal muscle cells, which contributes to muscle catabolism [38]. The p38/MAP kinase pathway can also be stimulated by TNF-α. Both p38/MAP kinase and NF-kB upregulate the expression of genes that encode the E3 ligases MuRF1 and MAFbx, thus inhibiting protein synthesis and contributing to muscle atrophy [114].

Although the role of TNF-α during the inflammatory process in cachexia induced by cancer has been extensively studied, there is no consensus about the degree of influence of this cytokine on this inflammatory process [115]. It appears that the levels of serum TNF-α are related to cancer stage,

which reflects the size of the tumour [116]. Corroborating the result mentioned above, Kemik et al. (2012) [117] conducted a study with the aim of evaluating acute-phase proteins, cytokines, and hormones in cachectic patients with various cancers of the gastrointestinal tract. They found higher serum concentrations of several factors, including TNF-α and IL-6, in patients with oesophageal, gastric, pancreatic, colon, and rectal cancers than in controls. Thus, the authors suggested an association between proinflammatory cytokines in cachectic patients with various types of gastrointestinal cancer. However, other authors have found different results in patients with other types of cancer. Gulen et al. (2012) [118] studied 63 patients with lung cancer cachexia with advanced disease with the aim of evaluating the relationship between adipokines and systemic inflammation in patients at this stage. The authors showed the absence of a relationship between adipokines or systemic inflammation and cancer cachexia in lung cancer patients: some proinflammatory factors, such as TNF-α, did not differ from the control group.

Some authors have shown that TNF-α correlates with body mass, but others have shown no correlation between this cytokine and anorexia in patients with advanced-stage cancer [102].

Amaral et al. (2006) [42] demonstrated in rat that the administration of TNF-α in the hypothalamus promoted a decrease in 12-hour food intake by modulating the expression of neurotransmitters associated with energy balance, favoriung higher energy expenditure. Previously, Aguilera et al. (1998) [119] observed a negative correlation between NPY and TNF-α in patients with nervous anorexia.

According to Arruda et al. (2010) [120], a high concentration of TNF-α also acts in the expression of hypothalamus-modulating neurotransmitters and signal transduction pathways, thus increasing body temperature, oxygen consumption/carbon dioxide production, and energy expenditure and contributing to weight loss in cancer cachexia patients.

Busquets et al. (1998) [121] demonstrated in rat that the administration of a single intravenous injection of TNF-α increased the gene expression of uncoupling proteins 2 and 3 in skeletal muscle, suggesting this as a possible mechanism contributing to increased energy expenditure and cachexia in tumour-bearing cancers. However, weight loss in pancreatic cancer is associated with systemic inflammation and increased ubiquitin mRNA expression, but not uncoupling proteins in skeletal muscle [122].

An *in vitro* study showed that, in 3T3-L1 adipocytes, TNF-α significantly reduced the lipid accumulation and glucose uptake induced by adiponectin, and it increased lipolysis [123]. Previously, it was demonstrated in humans that the administration of TNF-alpha at low doses caused systemic lipolysis [124]. This finding also suggests a potential role of this cytokine in the control of adipose tissue depletion in cancer cachexia.

Another factor recently described to be involved in metabolic regulation is ANGPT4. This protein is associated with energy homoeostasis, wound repair, tumourigenesis, angiogenesis, and redox regulation [43].

10. Angiopoietin-Like 4 (ANGPTL4)

A new protein with similarity to members of the ANG (angiopoietin) family was identified by three independent research groups in 2000 [125, 126]. ANGPTL-4 was also named fasting-induced adipose factor (FIAF) [127].

The protein ANGPTL4 was classified as an adipokine because it was expressed predominantly in adipose tissues and liver; thus, it was believed to be involved in lipid metabolism [43, 128]. ANGPTL4 primarily showed an inhibitory effect on LPL activity [125, 129].

ANGPTL4 expression is also found in skin, intestines, kidneys, adipose tissues, liver, and a variety of tumours [125, 126, 130]. ANGPTL-4 is cleaved by proprotein convertases, releasing nANGPTL4 and the C-terminal portion of ANGPTL4 (cANGPTL4) [131, 132].

An analysis of recent studies makes it clear that the specific form of ANGPTL4 associated with the microenvironment tumour influences the clinical impact of this protein, along with other factors [44, 130, 133].

Over the past decade, there has been increasing recognition that this protein participates in numerous physiological and pathological processes [130]. ANGPTL-4 has been implicated as an important factor involved in energy homoeostasis, redox regulation, angiogenesis, the development of cancer and cachexia, and the development of metastasis and inflammation; however, the studies are still incomplete and often contradictory [43].

Galaup et al. (2006) [134] found in an experimental model that Angptl4 prevents tumour metastasis through the inhibition of vascular permeability, tumour cell motility, and invasiveness. Similarly, Yang et al. (2008) [131] demonstrated that cANGPTL4 inhibits angiogenesis. Because angiogenesis plays an important role in the progression of cancer, the authors suggested that cANGPTL4 has an antitumoural effect, thus decreasing tumour growth and metastasis.

In contrast, hypoxia stimulates the expression of ANGPTL4 in several tumour types [43, 135]. ANGPTL4 mRNA is increased in the perinecrotic areas of many human tumours [135, 136]. Moreover, elevated ANGPTL4 expression increased as tumours progressed from benign to metastatic states, thus implying a role of ANGPTL4 in tumour growth [45, 46].

The Angptl4 protein is more often expressed in colorectal cancer (CRC) tissues than that in normal tissues, and it is related to cell migration through the cytoskeletal signalling pathway [44]. The overexpression of Angptl4 promotes colon cancer cell migration through the cytoskeletal signalling pathway, while the downregulation of ANGPTL4 impairs tumour growth and metastasis [44, 46, 47].

Hypoxia, fasting, and PPARs regulate ANGPTL-4 expression [46, 137].

Inflammatory factors, such as IL-1b and IL-6 or hypoxia alone, increased ANGPTL4 protein levels, but the role of hypoxia was more significant. It was also observed that increasing tumour size and increasing degree of hypoxia in the tumour mass promoted the upregulation of ANGPTL4, especially in the hypoxic areas surrounding the necrotic area [138].

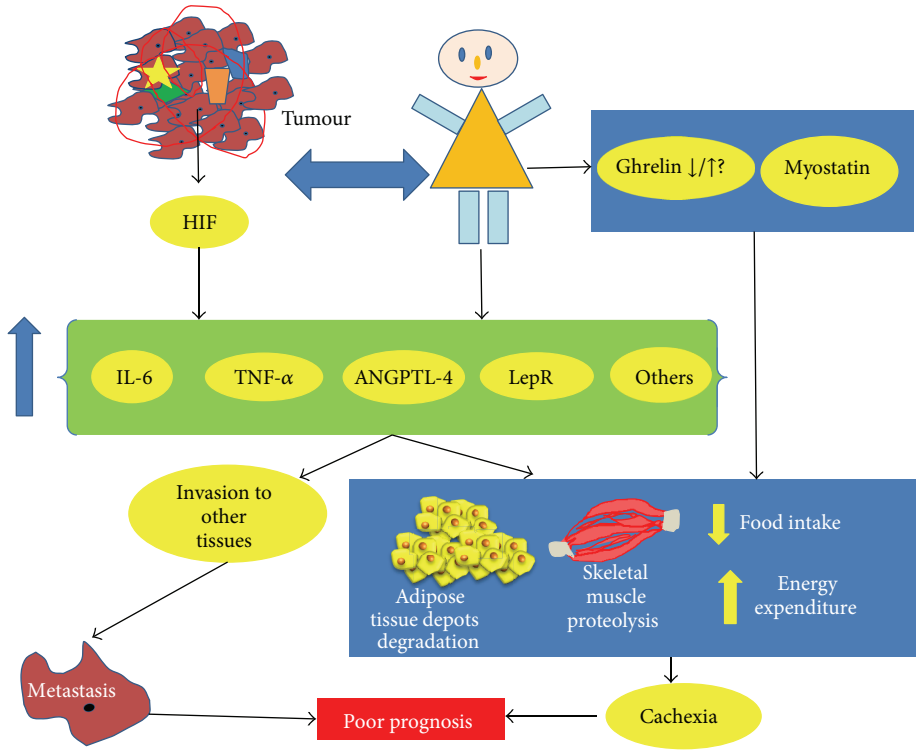

Figure 2: Schematic illustration of the cross talk between micro- and macroenvironment in the tumour-bearing patient. The importance of hypoxia and proinflammatory factors for tumour metastasis and cancer cachexia.

Studies have shown that ANGPTL4 is a target gene of PPARs, which are involved in the development of tumours [139, 140]. However, the role of PPARs in the regulation of ANGPTL-4 in tumour cells is controversial. Zhu et al. [46] (2011) did not detect a correlation between PPAR α, γ, and β and the expression of ANGPTL-4. In contrast, Girroir et al. (2008) [137] demonstrated that the addition of PPAR γ and β to tumour cell culture (MCF7 and UACC903) reduced cell proliferation and increased ANGPTL-4 gene expression.

The metabolic effects of ANGPTL-4 support the idea that this protein contributes to cancer cachexia development: fat-specific Angptl4 overexpression caused a 50% reduction in adipose tissue weight, partly by enhancing fatty acid oxidation and lipolysis [48]; the central administration of ANGPTL4 lowered food intake and body weight gain and enhanced energy expenditure [49].

11. Conclusion

The grade of malignancy of tumour is primordially associated with both the development of metastatic lesions and cancer-associated cachexia, which are multifactorial conditions depending on factors present in the micro- and macroenvironments in the tumour-bearing patient.

In this review, we point out some factors that are released and/or act in the tumour and other tissues related to the development of metastasis and cancer cachexia. Hypoxia-induced factor plays a prominent role in inducing tumour macro- and microenvironment release of proinflammatory cytokines. The cross talk among factors released by the tumour, adipose tissue, muscle, and other tissues seems to contribute to the difficulty of treating the cancer patient, once a multifactorial alteration occurs in the micro- and macroenvironment of the individual (Figure 2) (Table 1). Furthermore, there is still some controversy in the literature, related to the role of ghrelin, ANGPT4, HIF-1, and TNF-α in cancer cachexia and metastasis development, indicating the necessity of more studies.

Conflict of Interests

The authors declare no conflict of interests.

Authors' Contribution

Nelson Inácio Pinto, June Carnier, Lila M. Oyama, and Claudia M. Nascimento wrote and designed the paper; Jose Pinhata Otoch, Paulo Sergio Alcântara, and Flavio Tokeshi analyzed and reviewed the paper.

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Table 1: Summary of the effect of some factors involved in the development of cancer cachexia and metastasis.

Factor	Action	Reference
Ghrelin	↑ in cachexia cancer patients	[11]
	↓ Myostatin	[12]
Myostatin	↑ in Cancer/Cachexia	[13, 14]
	↑ Proteolysis	[15–17]
Leptin	Contribute to metastasis	[18]
	Regulate neoangiogenesis	[19, 20]
HIF	↑ Apoptosis	[21]
	↑ Angiogenesis	[21]
	↑ Proliferation tumour cells	[22]
	↑ IL-6, leptin, TNF-α, and ANGPTL-4	[23, 24]
	Contribute to metastasis	[23–26]
	↑ Glycolysis	[27]
	↑ VEGF, endothelins, IL-8, and EMAP II	[28]
	↑ M2 ↓ T cells response	[29]
IL-6	Contribute to metastasis	[30–32]
	↑ Proteolysis and atrophy muscle mass	[33, 34]
	↓ Food intake and ↑ energy expenditure	[35–37]
TNF-α	↑ Proteolysis	[38, 39]
	Contribute to angiogenesis	[40]
	Stimulate apoptosis	[41]
	↓ Food intake and ↑ energy expenditure	[42]
ANGPTL-4	Contribute to angiogenesis	[43]
	Contribute to metastasis	[43–47]
	↑ Lipolysis and fatty acid oxidation	[48]
	↓ Food intake and body weight gain	[49]

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Review Article

Role of Inflammation in Muscle Homeostasis and Myogenesis

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Skeletal muscle mass is subject to rapid changes according to growth stimuli inducing both hypertrophy, through increased protein synthesis, and hyperplasia, activating the myogenic program. Muscle wasting, characteristic of several pathological states associated with local or systemic inflammation, has been for long considered to rely on the alteration of myofiber intracellular pathways regulated by both hormones and cytokines, eventually leading to impaired anabolism and increased protein breakdown. However, there are increasing evidences that even alterations of the myogenic/regenerative program play a role in the onset of muscle wasting, even though the precise mechanisms involved are far from being fully elucidated. The comprehension of the links potentially occurring between impaired myogenesis and increased catabolism would allow the definition of effective strategies aimed at counteracting muscle wasting. The first part of this review gives an overview of skeletal muscle intracellular pathways determining fiber size, while the second part considers the cells and the regulatory pathways involved in the myogenic program. In both parts are discussed the evidences supporting the role of inflammation in impairing muscle homeostasis and myogenesis, potentially determining muscle atrophy.

1. Introduction

Skeletal muscle is the most abundant tissue in human body, except in obese patients, and is involved in several physiological functions. Indeed, glucose uptake and metabolism take place primarily in the skeletal muscle, a tissue prone to adaptation in size by means of both hypertrophy and hyperplasia. The former relies on the regulation of protein synthesis and degradation rates, while the latter involves the myogenic process that is in charge of regulating myocyte turnover as well as of supporting the rapid regeneration following injury.

The counterpart of muscle hypertrophy is muscle atrophy, even defined as sarcopenia, that naturally occurs in physiological conditions, such as aging [1]. Beyond aging, muscle wasting is a feature associated with several pathological states and chronic diseases such as immobilization following fractures or bed rest, malnutrition, cancer, CHF, CKD, COPD, burns, muscular dystrophies, AIDS, sepsis, and immune disorders [2]. Muscle depletion has important implications, exercise intolerance and inability to manage daily activities that eventually translate into poor quality of life. Most of the above mentioned pathological conditions are associated with variable degrees of local and/or systemic chronic inflammation, an element that could play a relevant role in the onset of muscle wasting [2]. Indeed, inflammation is considered one of the diagnostic hallmarks of cachexia, a wasting condition that often occurs in chronic diseases [3].

The aim of this review is to summarize the evidences supporting the role of inflammation, associated with several illnesses, in impairing muscle homeostasis and myogenesis, leading to muscle atrophy.

2. Muscle Homeostasis, Atrophy and Hypertrophy Pathways

Skeletal muscle mass represents a determinant of physical performance, and muscle size varies according to

physiological stimuli and pathological conditions that, in turn, modulate the activation state of signaling pathways involved in the control of protein turnover. Muscle atrophy occurs when the balance between protein degradation and protein synthesis is poised towards degradation, leading to the loss of myofibrillar proteins and, consequently, to reduced fiber cross section area, finally resulting in impaired contraction ability and low force generation. Muscle nitrogen balance is finely modulated by distinct agents, both intrinsic (nutrient and energy availability, mechanical stress) and extrinsic (humoral mediators: hormones and cytokines). Moreover, muscle wasting, beyond the loss of muscle mass, often determines a reduction of muscle quality, that is, specific force, as reported in patients with CHF [4] or cancer [5] or admitted to the intensive care unit [6].

Protein breakdown in the skeletal muscle is mediated by two main degradation systems, the ubiquitin-proteasome and the autophagy pathways. The proteasome system preferentially targets short-lived proteins, and several reports considered the proteasome as the degradation machinery mostly involved in wasting processes of distinct origin [7].

The autophagy system is in charge of degrading long-lived proteins and organelles (mitophagy, pexophagy, etc.), and recent observations suggest that, beyond the proteasome, even autophagy plays a crucial role in muscle wasting [8]. In addition to proteasome and autophagy dependent proteolysis, intact myofilaments were postulated to undergo a preliminary cleavage in order to be released from the myofibrils for the subsequent ubiquitin dependent degradation, and such activity was proposed to be carried out by calpains [9] or by caspase-3 [10].

The observations reported above prompted the idea that protein breakdown inhibition could be the right way to prevent disease-associated muscle wasting. However, directly targeting the different proteolytic systems is unlikely an effective strategy. Indeed, proteasome inhibition proved effective only in experimental muscle unloading [11], while several reports show the detrimental effects of autophagy suppression [12–14]. Reasonably, both defective and excessive autophagy are deleterious by opposite mechanisms, namely, the lack of damaged protein/organelle removal and the exaggerated degradation, respectively. A distinct strategy in order to reduce muscle protein breakdown would be to target the muscle-specific ubiquitin ligases, since their activity represents the limiting step in determining both substrate-specificity and degradation rate. Since the beginning of the 2000s, the discovery of muscle-specific E3 enzymes and the characterization of their substrates have emerged and are still growing. The first ones, MAFbx/atrogin-1 and MuRF1/TRIM63 [15, 16], are actually the most commonly used read-out measurements for the molecular assessment of muscle protein catabolism, even though obvious limitations are implied. Few years later, TRIM32 was shown to target myofibrillar components [17], but its role seems to be primarily related with muscular dystrophies rather than wasting processes. The role of FBXO40, firstly characterized in denervation-induced atrophy in 2007 [18], was then clearly established by the identification of IRS-1 as substrate, thus defining a negative feedback on the anabolic PI3K/Akt axis [19]. Finally, FBXO30/MUSA1 was described as a BMP-regulated gene required for denervation- and fasting-mediated muscle loss [20]. However, investigations in humans are still lacking and no evidence is actually available in order to validate the use of ubiquitin ligases as therapeutic targets for muscle wasting.

Muscle protein degradation systems are modulated by a coordinated network of signaling pathways activated or suppressed by hormones and cytokines (Figure 1). On one hand, anabolic signals are activated by insulin, IGF-1, GH, and androgens, while catabolism is stimulated by a variety of proinflammatory cytokines as well as glucocorticoids and ROS. IGF-1 promotes muscle hypertrophy, while low IGF-1 circulating levels have been associated with several muscle atrophy conditions [21]. IGF-1, and similarly insulin, activates the PI3K/Akt pathway, which promotes protein synthesis through mTOR and its downstream effectors, mTORC1/2 complexes, not to mention that mTOR activation in the skeletal muscle results in autophagy inhibition [22], thus determining at the same time both protein synthesis activation and inhibition of protein degradation. Another IGF-1-mediated anticatabolic action is due to Akt phosphorylation/inhibition of the FoxO transcription factor family, determining their inability to translocate to the nucleus and promote the expression of the ubiquitin ligases atrogin-1 and MuRF1 [23] and autophagy genes [24].

Opposite to the IGF-1 pathway, one of the most relevant inducer of muscle atrophy is myostatin, a member of the TGF-β family. It signals through ACTRIIB, and recruiting the transcription factors Smad2/3 leads to increased atrogin-1 and MuRF1 mRNA levels [25]. The negative regulation of muscle mass exerted by myostatin likely relies on the suppression of Akt signaling [26].

In the complex network of signals relevant to muscle homeostasis, the BMP pathway has been recently characterized [20], showing that the downstream activation of the transcription factors Smad1/5/8 regulates a fundamental anabolic signal. In the same paper, the alternative activation of either the myostatin or the BMP pathway, both competing for Smad4 recruitment, was demonstrated. Indeed, BMP inhibition reverts the hypertrophic phenotype of myostatin-K.O. mice, suggesting that the balance between these signal cascades is crucial for the modulation of muscle mass.

Finally, few signaling pathways, less characterized in the skeletal muscle, displayed their relevance to muscle mass regulation during wasting conditions. Histone deacetylases 4 and 5 were shown to abrogate Dach2 expression that in turn lead to myogenin increase and ubiquitin ligase accumulation [27]. Overexpression of ATF4 was sufficient to induce muscle atrophy, regulating the expression of genes mainly related to cell growth suppression [28]. Similar to ATF4 action, the transcription factor and tumor suppressor p53 was able to trigger muscle atrophy through the induction of the cyclin-dependent kinase inhibitor p21 [29].

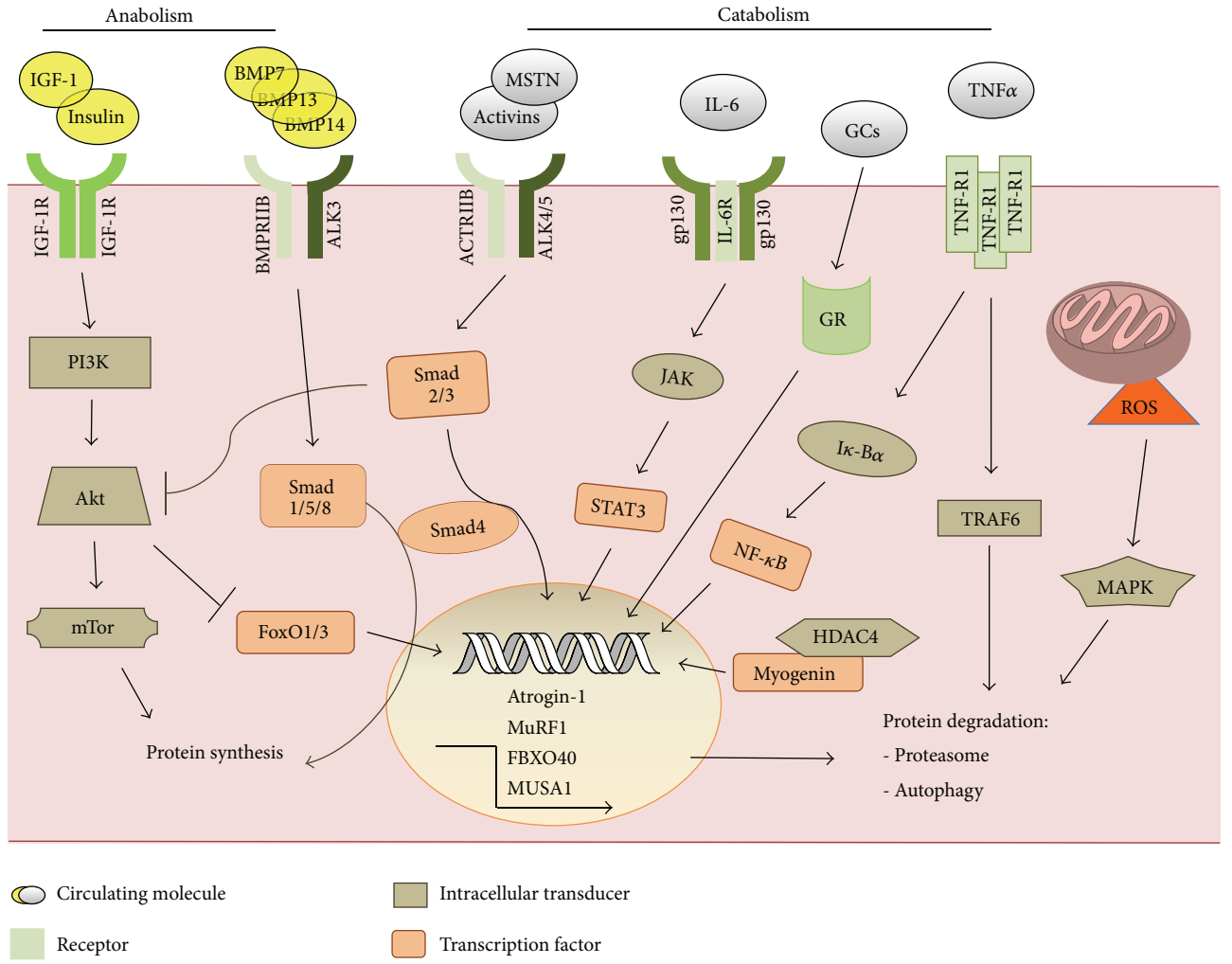

Figure 1: Humoral mediators and associated pathways drive anabolic and catabolic responses in the skeletal muscle.

3. Inflammation Triggers Protein Catabolism and Impairs the Anabolic Response in the Skeletal Muscle

Loss of muscle mass, a common feature of chronic diseases, is frequently associated with increased production of proinflammatory cytokines such as TNF-α, IL-1, IL-6, and IFN-γ. For example, patients affected by chronic renal or heart failure show increased circulating levels of TNF-α and TNF soluble receptors [30, 31]. Similarly, several proinflammatory cytokines are increased in cachectic cancer patients (reviewed by [32]). Chronic inflammation may depend on increased expression of proinflammatory mediators but also on reduced levels of anti-inflammatory factors; consistently, mice K.O. for IL-10, one of the best known anti-inflammatory cytokines, display weakness and accelerated muscle loss [33] that can be improved by treatment with the anti-inflammatory, resveratrol rich, grape seed extract [34]. In addition to cytokines, other factors are produced during the inflammatory response, such as the so-called acute phase proteins, markers of systemic inflammation. In this regard, cancer cachexia and the acute phase response appear to be correlated: the enhanced synthesis of acute phase reactants in the liver has been proposed to drive muscle protein hypercatabolism contributing to increase of the resting energy expenditure [35]. Of interest, acute phase proteins have been shown to be produced even by the skeletal muscle itself [36].

Proinflammatory cytokines, together with altered homeostasis of classical hormones, put on a complex network that results in inhibition of anabolic and/or anticatabolic signals (see above), in favor of lipolysis and proteolysis. In particular, proinflammatory cytokines are well known to impinge on muscle protein metabolism. In this regard, data obtained in both experimental models and human pathology have demonstrated that systemic inflammation is associated with reduced rates of protein synthesis paralleled by enhanced protein breakdown, both accounting for the loss of muscle mass. However, the precise mechanism by which inflammation modulates protein turnover rates is still poorly investigated. Data obtained in clinical studies show a variegated situation: both rates are markedly increased

in severely burned patients, with the balance remaining in favor of degradation [37]; synthesis rates are maintained in face of enhanced degradation rates in critically ill septic subjects [38], while in cancer patients with cachexia, protein turnover rates have been described as increased, decreased, or unchanged (reviewed in [39]).

The regulation of muscle protein metabolism by humoral factors is widely accepted. In this regard, the activity of both proteasome and lysosomes, the two proteolytic systems mainly involved in muscle depletion, is known to be affected by the hormonal and cytokine milieu. As an example, healthy animals exposed to proinflammatory cytokines such as TNF-α, IL-1, or IL-6 develop muscle wasting associated with increase of both ubiquitin expression and proteasome enzymatic activity (reviewed in [40]). Consistently, few studies demonstrated in the past that cytokines play a crucial role in the onset of muscle wasting. Indeed, muscle depletion, enhanced protein breakdown, and increased ubiquitin can be prevented by treating tumor-bearing animals with antibodies directed against IL-6, TNF-α, or IFN-γ [41–43]. Proinflammatory cytokines contribute to muscle wasting also in chronic diseases of noncancer origin. Indeed, increased circulating levels of TNF-α, IL-1, and IL-6 in sepsis appear to be correlated with disease severity and lethality. Similarly, the proinflammatory shift occurring in AIDS patients likely accounts for muscle protein hypercatabolism, a feature frequently reported in these patients before the adoption of combined antiretroviral therapy [44]. A recent report shows that muscle wasting in diabetic rats is associated with enhanced expression of TNF-α, IL-1, and IL-6 in the skeletal muscle and that such increase can be corrected by exercise training [45]. Finally, also sarcopenia and the loss of muscle quality that characterize aging are associated with high levels of proinflammatory mediators [46, 47].

The effects exerted by proinflammatory cytokines on muscle mass are mediated, partially at least, by activating the transcription factor NF-κB. The transcriptional activity is regulated by the phosphorylation and consequent degradation of the inhibitor Iκ-Bα, allowing the positive regulation of MuRF1 [48] and other atrophy related genes, including the inducible nitric oxide synthase [49]. Studies performed on experimental models suggest that the NF-κB signaling is activated in skeletal muscle during cancer cachexia, and recently modulations of this transcription factor have been observed in gastric and lung cancer patients [50, 51], as well as in experimental models [52]. Another protein related to the TNF-α cascade, TRAF6, was shown to coordinate the activation of both proteasome and autophagy [53].

Among proinflammatory cytokines, TWEAK has been shown to induce muscle wasting mainly by stimulating proteasome-dependent proteolysis [54]. TWEAK-induced downregulation of both PGC-1α expression and mitochondrial biogenesis has been proposed to mediate the effects on muscle mass. Indeed, PGC1α hyperexpression protects against TWEAK-induced effects such as NF-κB activation, increased ubiquitin ligase levels, and muscle wasting [55]. In addition, several cytokines act on the JAK/STAT pathway, and muscle STAT3 was demonstrated to induce atrogin-1 [33] and to block autophagy, leading to muscle degeneration [34].

Proinflammatory cytokines act on muscle protein metabolism not only by activating catabolic pathways, but also by downregulating the anabolic ones. As an example, lipopolysaccharide-induced muscle wasting is associated with increased circulating levels of TNF-α and IL-1 that lead to inhibition of the Akt/mTOR signal transduction pathway [56, 57]. In this regard, treatments able to restore Akt physiological activity appear to counteract TNFα-induced wasting [58, 59]. Recent observations show that cancer cachexia occurring in the Apc (Min/+) mice also depends on the inhibition of mTOR activation due to the high circulating IL-6 levels [60]. The antianabolic action of proinflammatory cytokines is partially exerted through interactions with the IGF-1-dependent signaling pathway. Indeed, TNF-α leads to serine phosphorylation of IRS-1, inhibiting its recruitment to the insulin/IGF-1 receptor. TNF-α can impinge on the insulin/IGF-1 signaling via direct interaction between the IKK complexes and IRS-1. Alternatively, TNFα-induced activation of JNK may play a role, as shown by the observation that the downregulation of the IGF-1-dependent signaling exerted by the cytokine does not occur in the presence of a JNK inhibitor (reviewed in [61]). Finally, proinflammatory cytokines may modulate anabolism also by inducing leucine-resistance, resulting in decreased mTOR phosphorylation and reduced protein synthesis [62].

4. Adult Myogenesis: Satellite Cells and Adult Stem Cells

In addition to modulations of protein synthesis and breakdown rates, several reports in the last years suggest that also alterations of the myogenic response may play a role in the maintenance of skeletal muscle mass in the adult, in both physiological and pathological states. Myogenesis is the process that guarantees the generation of myoblasts to give rise to skeletal muscle tissue. Embryonic myogenesis is definitely better understood thanks to the extensive work of developmental biologists that generate important genetic tools to establish the exact hierarchical activations of skeletal muscle transcription factors triggering the early embryonic process. In the adulthood, the situation is more complicated since those genetic tools are not sufficient to identify the major key players involved in adult myogenesis. Skeletal muscle injuries are extremely common and mainly caused by intensive muscle exercise, trauma, laceration, burns, freezing, and toxin exposure (the latter are also commonly used in experimental models of muscle regeneration). These insults result in muscle injury that determines a diffuse degeneration followed by the induction of regeneration. However, the characteristics of regeneration have been shown to differ according to the type of injury; thus a direct comparison of the results obtained in various studies is extremely difficult.

When skeletal muscle is damaged, muscular fiber degeneration is compensated by the regeneration of new fibers formed at the expense of resident myogenic satellite cells localized underneath the basal lamina of muscle fibers [63]. Each degeneration process is followed by a new regenerative

cycle. Skeletal muscle regeneration is mainly sustained by SCs [64, 65] and this is critical for chronic muscle diseases including muscular dystrophies. In MDs, dystrophic SCs share the same molecular defect and the newly formed fibers during regeneration cycle are susceptible to degeneration. With time, the reservoir of satellite cells is totally consumed and the muscle tissue is progressively replaced by connective tissue. In the adulthood, skeletal muscle represents half of the body weight, and SCs are able to maintain their functionality thanks to the generation of muscle precursor cells able to proliferate and, upon fusion, to generate new fibers. All trunk and limb muscle source cells are originated from embryonic somite source, with exception of the head muscle [66]. Pax3 precursors were identified in the embryonic dorsal aorta; they are able to give rise to both smooth and skeletal muscle cells, suggesting a common origin in the two muscle lineages [67]. Oxidative-slow muscles contain a relatively high number of SCs, up to six times more than fast-glycolytic muscles [68]. SCs can be easily isolated after enzymatic digestion and/or physical trituration [69] or by FACS for specific surface markers including CXCR4, β1-integrin, Sca-1, M-cadherin, Syndecan-4, Notch-1, and NCAM/CD56 [70–72]. It is relevant to note that SC myogenic potential seems to be diverse according to the markers considered for cell sorting, thus revealing heterogeneity in SC populations. Pax7 is a transcription factor considered as a biomarker for quiescent and proliferating SCs [73], while Jagged-1 is considered a marker of activated SCs [74]. The expression of Pax7, NCAM, and c-Met has been shown also in human SCs. Numb is an inhibitor of the Notch signaling but also a cell fate determinant and was found asymmetrically distributed in a SC subpopulation, suggesting that only a small subset of SCs retains the stem cell characteristic and undergoes asymmetric division [75]. There are still pending questions regarding SC isolation and characterization. Although encouraging results have been obtained in preclinical [76, 77] and clinical [78] studies, the use of SCs for the treatment of muscle degeneration is hampered by the inability of SCs to pass the endothelial barrier when injected systemically. Further work is needed to confirm and improve the therapeutic efficiency of SC autologous injection for skeletal muscle degeneration.

Bone marrow cells, including MSCs, blood, and muscle-derived $CD133^+$ and SP cells, have been also implicated in skeletal muscle regeneration [79]. Several studies have demonstrated that MSCs are incorporated into regenerating skeletal muscle fibers. However, in some cases, engrafted cells failed to express skeletal muscle proteins, suggesting that under standard conditions they fuse rather than differentiate to skeletal muscle potency. In other cases, results have been more encouraging. In bone marrow and peripheral blood, stem cells characterized by the expression of the CD133 antigen are present and have been shown to give rise to dystrophin-positive fibers following their intramuscular transplantation [79].

SP cells are referred to as a small subpopulation of stem cells able to exclude Hoechst 33342 dye and participate to adult myogenesis [80]. Related to the SP populations are the $CD34^+/CD45^-$ cells, known as Sk-34 cells that are apparently derived from $CD34^-/CD45^-$, named Sk-DN cells [81]. Being different from the other myogenic stem cells, they still retain the ability to differentiate into vascular cells, including pericyte, endothelial cell, and smooth muscle cells and peripheral nerve cells as Schwann and perineurial cells [82]. Interstitial Cajal-like cells or telocytes are recently discovered c-Kit cells type populating the muscle interstitium [83]. These cells own a small body (9–15 um) and a certain number of telopodes organized in network to maintain tissue homeostasis and renewal through exosome delivery.

In the interstitium among the fibers are usually present several cell types that were also showed to contribute to adult myogenesis. Whether these cells are missing or altered in pathological muscles and their origins are still heavily debated. These cell types include FAPs, $Tcf/L2^+$ cells, and $Pw1^+$ cells. Emerging evidences highlight that intramuscular adipocytes and fibrocytes are the differentiated stages of FAPs [84–86]. FAPs are isolated as $CD34^+/Sca\text{-}1^+$ [84] or as $Sca\text{-}1^+/CD140a^+$ [85] and they are able to differentiate into myoblasts. These cells mediate the ability of HDAC inhibitors to promote skeletal muscle regeneration in mdx mice, animal model for DMD [86]. The key cytokines and growth factors responsible for their paracrine positive effects are still under investigation, and more information regarding the human counterparts is necessary for translational clinical implications. In murine models were identified a particular class of fibroblasts expressing the transcription factor 7-like 2 (Tcf/L2 or Tcf4, [87]). These $Tcf4^+$ fibroblasts present in the connective tissue seem also to regulate muscle fiber generation and as such they could be directly related to FAPs. Pw1 is a zinc-finger-containing transcription factor expressed in myoblasts, and it seems as an important marker for myogenic progenitor cells since postnatal muscle growth is severely impaired in mice lacking Pw1 in myogenic lineages [88].

Muscle-derived stem cells are also identified in the skeletal muscle interstitium based on the expression of Flk1, Sca-1, and desmin. Since they are able to differentiate into myogenic, adipogenic, osteogenic, chondrogenic [89], and even hematopoietic [90] lineages, they are strictly associated with mesoangioblasts and Pw1 positive cells although comparative studies are still missing.

Noncanonical progenitors of mesodermal tissues were originally isolated from murine dorsal aorta and for their multipotent characteristic to give rise to mesodermal cell types *in vitro* and *in vivo* they were mesoangioblasts [91]. MABs express CD34/c-Kit/Flk-1 but are negative for NKX2.5/Myf5/Oct4 [92] and are able to give rise to multiple mesodermal lineages *in vitro* and *in vivo* [93]. In the adult muscles, MABs are usually isolated and cloned using their pericyte markers, alkaline phosphatase, Sca-1, NG2 proteoglycan, CD140a, and CD140b [94–99]. MABs are able to differentiate into myogenic, osteogenic, chondrogenic, and adipogenic lineages [97, 98]. Also human MABs display pericyte markers, as CD146/CD140b1/NG2, but are negative for hematopoietic or SC markers $CD45^-/CD34^-/CD56^-/CD144^-/Pax7^-$ [96, 100]. Since a few markers are shared between human pericytes and MSCs (CD10/CD13/CD44/CD73/CD90), the origin and the interaction between those multipotent stem cells are

still matter of debate [100, 101]. Interestingly, a comparison among MSCs, MABs, and multipotent adult progenitor cells resulted in specific differention/functional properties that can be partially converted *in vitro* by culture conditions [102]. In addition, Notch signaling seems to have a primary role in modulating the myogenic potential of MSCs and MABs [99].

Stem cell biology in adult myogenesis is a very active and fast moving field, and probably some redundancies in stem cell type and function observed here will be explained with further comparative studies. There is a clear need for more basic research to better understand the interconnected roles of stem cells in skeletal muscle regeneration and to explore the integration of signaling pathways such as Notch and BMP. This new information not only will improve our understanding of the adult myogenesis process but also in principle could reveal potential therapies for the enhancement of tissue repair in acute and chronic skeletal muscle degenerations.

5. Inflammation Interferes with the Myogenic Program

Changes in cellular composition of muscle microenvironment are crucial for metabolic modifications occurring during both acute damage with consequent muscle regeneration and chronic degeneration. Overall, the pathophysiological alterations occurring during the onset and the progression of muscle diseases highlight the complexity of the possible interactions among muscle resident/recruited cells and immunological mediators (Figure 2). The modulation that takes place during acute events, such as muscle trauma inducing regeneration after temporary atrophic conditions, is different from the one occurring during chronic events, such as long lasting inflammatory processes affecting muscle during genetic diseases (MDs or myopathies), cancer-induced muscle atrophy, and sarcopenia.

The inflammatory process during trauma or fractures is a controlled and finely regulated event that through different and defined stages can ensure a complete and efficient reconstruction of muscle fibers. The process begins with the release of chemoattractant molecules like desmin [103] but also heat shock proteins and HMGB1 among many others, responsible for local activation of the innate immune response and comprehensively recognized as damage associated molecular patterns [104]. Muscle injury is usually followed by a local increase in myeloperoxidase activity that reflects neutrophils activation. Their contribution to skeletal muscle regeneration and myofiber remodeling relies on the oxidative and proteolytic modification of damaged tissue, to allow phagocytosis of cellular and matrix debris [105]. Interestingly, targeted ablation of $CD11b^+$ cells (including neutrophils and monocytes/macrophages) reduces muscle fiber repair after muscle injury [106]. Moreover, neutrophils release proinflammatory cytokines such as TNF-α, IL-1β, IFN-γ, TGF-β1, and IL-12 that reach a peak of concentration 24 h after injury [107] and can modulate the regenerative process in skeletal muscle [108]. Recent findings show that IL-4 production mainly due to eosinophils could play a role during early stages of muscle regeneration [109]. Coherently, muscle cells lacking IL-4 or its receptor show impaired myotube formation [110].

Myogenic cells attract monocytes from the blood stream to their location next to capillaries [111]. Indeed, this privileged position could be the reason for easy access and activation through chemotactic molecules such as MCP-1, macrophage-derived chemokine, fractalkine (CXCL1), VEGF, and the urokinase system [112]. However, *in vivo* studies have reported that monocytes/macrophages recruited into skeletal muscle after injury could come from the connective tissue surrounding muscle/epimysium, where these cells accumulate before invading muscle tissue in the site of damage.

Notably, already twenty years ago, some studies reported that the activation of M1 macrophages ($CD68^{high}/CD163^-$) at days 1-2 after injury was concomitant to activation and proliferation of SCs. By contrast, M2 macrophages ($CD68^{low}/CD163^+$) reach the peak of concentration closely to the surface of regenerating myofibers just 4 days after injury, when muscle differentiation is starting [113]. Consistently, different *in vitro* studies showed that SCs increase their proliferation rates when cocultured with M1 macrophages [114], while they enhance both their fusion index and myogenin expression in the presence of M2 cells [106]. Muscle regeneration in mice induced by cardiotoxin injection was impaired after treatment with neutralizing antibodies against Macrophage Colony Stimulating Factor, a cytokine able to activate macrophages [115]. M1 to M2 transition, timing, and correct sequence have been demonstrated to be essential for both resolution of inflammation and myofiber repair. Indeed, IL-10 administration after muscle injury leads to M1 disruption and M2 promotion, resulting in reduced myofiber growth and regeneration [116]. Ablation of IL-10 *in vivo* can disturb the transition from M1 to M2 macrophages and decrease fiber growth. When cocultures of muscle cells with macrophages activated through IL-10 to the M2 phenotype are stimulated to proliferate, no effect on MyoD or myogenin expression is observed, showing that M2 macrophages promote the early, proliferative stage of myogenesis [117]. Anyway, the window where IL-10 can promote regeneration is short and if not respected can induce a delay in myogenic program [116, 118]. Moreover, macrophage-derived IL-10 seems as well able to promote skeletal muscle differentiation of MABs, vessel derived myogenic precursors, and IL-10 antibodies can effectively decrease the capabilities of these cells to participate in myofiber commitment [118].

As IL-10, other mediators such as TNF-α and IFN-γ are produced in the muscle microenvironment and play a role during muscle myogenesis. As an example, TNF-α can impair regeneration, keeping SCs in the proliferation stage and inhibiting differentiation, mainly by acting on NF-κB and MyoD [119, 120]. However, a second important role for TNF-α is related to myofiber release of activin-A through both NF-κB and p38 MAPK, activating the phosphorylation of SMAD2/3 to inhibit SC differentiation and fusion [121]. M1 macrophages produce high levels of activin-A that, *in vitro*, can act both on the further polarization of M1 macrophages and on the inhibition of anti-inflammatory IL-10 release

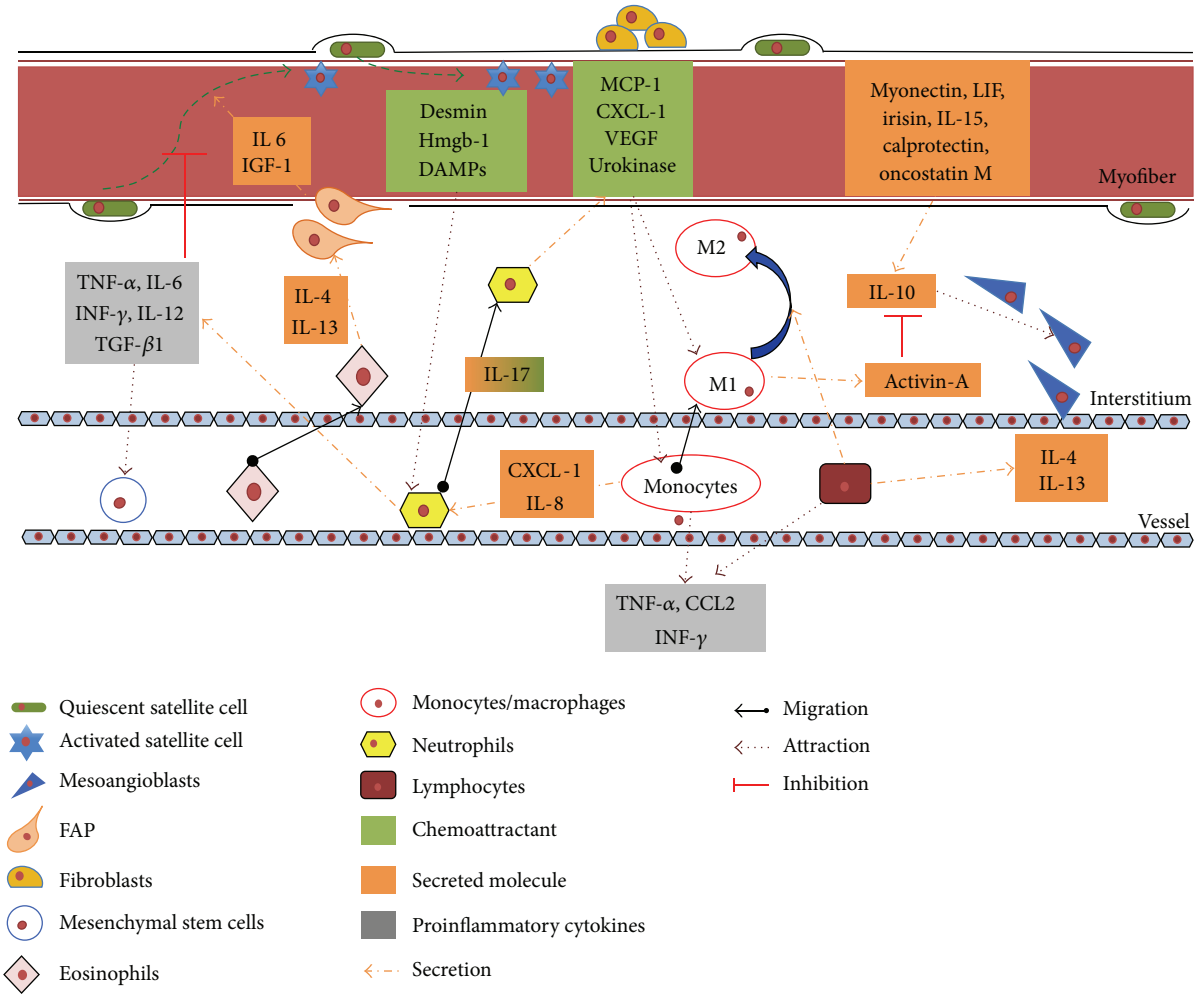

Figure 2: Secreted molecules and paracrine effects from resident and circulating cells involved in skeletal muscle inflammation.

from M2 cells [122]. Suppression of p38 MAPK signaling is usually followed by Th2 cytokine elevation (IL-10 and TGF-β), suggesting the switch in macrophage phenotype during skeletal muscles regeneration [116].

The mechanisms through which IFN-γ can directly influence muscle regeneration are still poorly understood and, so far, the knowledge is limited to some *in vitro* data, reporting a delay in proliferation, impaired fusion, and low expression of terminally differentiation markers when this cytokine is added to myoblasts cultures [123–125]. All of these data confirm the vision of skeletal muscle as an endocrine organ able to secrete specific myokines endowed with both paracrine and endocrine functions [126]. Indeed, leukemia inhibitory factor [127] and IL-15 [128] are usually mentioned among the paracrine effectors, while myokines, such as myonectin [129], IL-6 [130], irisin [131], calprotectin [132], and oncostatin M [133], once secreted from muscle tissue, especially during contraction but also in pathologic conditions, are able to induce anti-inflammatory cytokines (IL-1 receptor antagonist and IL-10 [134]).

Despite a transient and finely tuned activation of the inflammatory system required to sustain muscle repair after injury, chronic inflammation can be deleterious, driving uncontrolled wound healing and fibrosis, as well as triggering muscle wasting. For these reasons, systemic administration of immune-modulators can reveal beneficial effects, particularly in the case of anti-inflammatory drugs able to reduce M1-macrophages, as shown, for example, in DMD patients [135]. Interestingly, treatment with cyclosporin A, well-known immunosuppressant drug, was found to have multiple beneficial effects on the myopathic and mitochondrial phenotype of collagen VI a1-null mice, recovering muscle strength and, recently also, increasing $Pax7^+$ pool and stimulating the formation of new myofibers [136–138]. This effect may be exerted through an indirect regulation of the inflammatory state that occurs during muscle regeneration [139]. All of these findings have been as well supported by the increased muscle regeneration observed in Ullrich patients undergoing CsA treatment, pointing on one side to immunosuppressive drugs as potent inducer of myogenesis and on the other side underlining the involvement of immune system with myogenesis, at least for these myopathies. Administration of neutralizing antibodies against TNF-α to *mdx* mice reduced p38-dependent inflammation, increased $Pax7^+$ cells, and

impaired the growth of regenerating fibers [140], suggesting the occurrence of an epigenetic link among inflammation, activated p38 MAPK, and Pax7 expression from SCs during regeneration [140].

Further studies are needed to understand the function of SCs and their possible induction to differentiate as a means to counteract muscle atrophy. Increased Pax7 and decreased myogenin levels have been reported also in the cachectic muscle of mice bearing the C26 carcinoma and in cancer patients, opening the possibility that cancer-driven inflammation induces muscle atrophy, dysregulating SC differentiation program [52, 141, 142]. SC accumulation was demonstrated to be due to an increase in the ERK MAPK signaling able to maintain the cells in an undifferentiated state [141]. Treatment with a chemical inhibitor of ERK phosphorylation can indeed rescue the levels of Pax7 and myogenin [141]. Lately, this process of SC accumulation has been pointed as contributing to muscle atrophy and has been related to NF-κB, known transcription factor able to be activated by many proinflammatory cytokines. In the skeletal muscle of cachectic mice, NF-κB is able to sustain Pax7 expression [52], and moreover, in the same study, the authors revealed that other muscle precursors, such as MSCs, participate in muscle wasting since they cannot complete their myogenic commitment. Chronic injury and proinflammatory cytokines due to tumor progression could be responsible for differentiation program failure.

When mesenchymal progenitors are missing or are unable to display their prodifferentiation effects on SCs, these latter ones actually contribute to fibrosis in mdx mice, leading to pathogenic effects in MDs [143]. In the last years, cells expressing CD34/Sca1/PDGFRa and not related with other lineages, such as hematopoietic, endothelial, or skeletal muscle, have received more and more attention. Indeed, these cells can both differentiate *in vitro* and *in vivo* towards fibroblast and adipocytes producing aSMA and perilipin, respectively [84, 143]. Recently, suppression of fibroadipogenic phenotype of mesenchymal cells through HDAC inhibitors has been demonstrated to induce the myogenic transcriptional activity in young mice by upregulating MyoD. On the contrary, FAPs taken from old mice fail in the activation of promyogenic phenotype mainly because of HDAC inhibitor resistant [144]. These last results already suppose a different behavior of the microenvironment that is actually acting on the myogenic program of SCs in the elderly. Aging of skeletal muscle should not be underestimated when considering myogenic potential. Indeed, geriatric SCs show reduced proliferation and differentiation potential and can easily switch from a quiescent to a senescent state [145]. Some studies have already focused on the importance of the microenvironment where SCs are studied, since if old murine SCs are exposed to a young environment or growth factors, their ability to proliferate and differentiate is partly restored, suggesting the extreme importance the environment has on the single cells and on the other side the plasticity cells can have [146]. During aging, extrinsic factors can alter SC functions, starting from the niche where they lay, since fibrous connective tissue is usually increasing [147, 148]. In addition, SCs in the elderly were shown to convert to a fibrogenic lineage mainly due to humoral factors. In particular, this lineage conversion seems to depend on Wnt signaling [149]. Lately, a comparison between old and young SCs identified an increased expression in JAK-STAT pathway with aging that could avoid their differentiation in old conditions [150]. This pathway is of particular importance in chronic inflammatory conditions, since infiltration of inflammatory cells and increased circulating levels of proinflammatory cytokines (such as TNF-α) can have together a detrimental effect on skeletal muscle regeneration [151]. Moreover, this pathway has usually a pivotal role in transduction of extracellular signals from cytokines and growth factors with particular importance for inflammatory cells [152]. Interestingly, in a recent work, murine and human SCs were demonstrated to progress in their differentiation thanks to STAT3 and a fine regulation of the activation of this protein could interfere with myogenesis [153]. The authors indeed speculate that chronic degenerative stimuli could favor this prodifferentiative pathway leading to exhaustion of the SC pool. Indeed, in chronic regenerative conditions, a pharmacologic inhibition of STAT3 could have a therapeutic relevance.

6. Conclusions

Inflammation is a common trait of several pathological conditions characterized by the loss of muscle mass. During the past, a direct link was established among proinflammatory cytokines, modulation of intracellular signaling pathways, and protein breakdown. In recent years, an additional hypothesis, suggesting the impairment of the myogenic program as underlying cause of muscle atrophy, is becoming popular. In this line, the importance of a finely orchestrated balance between pro- and anti-inflammatory cytokines in regulating physiological myogenesis is a well-established concept, while data suggesting the relevance of inflammation in impaired myogenesis are growing. Inflammation likely contributes to muscle depletion by both enhancing protein breakdown and impairing myogenesis in parallel and no priority between the two processes can actually be identified. The other way round, an effective strategy aimed at counteracting muscle wasting should take into consideration not only anabolic/catabolic aspects but analogously the continuous involvement of the myogenic counterpart. In addition, the role of interstitial and circulating progenitors involved in myogenesis and paracrine effects is also critical to modulate inflammatory responses in muscle wasting conditions.

The adoption of anti-inflammatory agents for the treatment of chronic wasting diseases has been widely described [154] and is not the topic of the present review; however, the modulatory effect on inflammation exerted by exercise training deserves a short consideration. Regular, nonstrenuous exercise seems to be protective against inflammation. Indeed, combined endurance and resistance training in elder subjects resulted in decrease of proinflammatory $CD14^+/CD16^+$ monocytes and low levels of TNF-α production *in vitro* [155]. Moreover, circulating IL-10 and regulatory T-cells ($CD4^+/CD25^+/CD127^{low}$) increase in well-trained athletes

with respect to a sedentary age matched population, even in resting conditions [156]. Keeping in mind the above-mentioned anti-inflammatory effect and considering that exercise is the most physiological stimulus able to coordinate both myogenesis and muscle hypertrophy, the adoption of patient-tailored exercise protocols will potentially have impact on muscle wasting associated with distinct pathologies.

Abbreviations

CHF: Chronic heart failure
CKD: Chronic kidney disease
COPD: Chronic obstructive pulmonary disease
BMP: Bone morphogenetic protein
IGF-1: Insulin-like growth factor-1
GH: Growth hormone
ROS: Reactive oxygen species
mTOR: Mammalian target of rapamycin
TGF: Transforming growth factor
ACTRIIB: Activin receptor type IIB
iNOS: Inducible nitric oxide synthase
HDAC: Histone deacetylase
ATF4: Activating transcription factor 4
TNF-α: Tumor necrosis factor alpha
IL: Interleukin
IFN-γ: Interferon gamma
TWEAK: TNF-like weak inducer of apoptosis
PGC-1α: Peroxisome proliferator-activated receptor coactivator 1 alpha
IRS-1: Insulin receptor substrate-1
MD: Muscular dystrophy
SC: Satellite cell
MPC: Muscle precursor cells
FACS: Fluorescent-activated cell sorting
NCAM: Neuronal cell adhesion molecule
MSC: Mesenchymal stem cell
SP: Side population
FAP: Fibroadipogenic progenitor
DMD: Duchenne muscular dystrophy
MAB: Mesoangioblast
Sca-1: Stem cell antigen-1
HMGB1: High mobility group box 1
MCP-1: Macrophage-derived chemoattractant protein 1
VEGF: Vascular endothelial growth factor
ERK: Extracellular-signal regulated kinase.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

All the authors equally contributed to the present work.

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Review Article

Cancer Cachexia and MicroRNAs

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Cancer cachexia is a paraneoplastic syndrome compromising quality of life and survival, mainly characterized by involuntary weight loss, fatigue, and systemic inflammation. The syndrome is described as a result of tumor-host interactions characterized by an inflammatory response by the host to the presence of the tumor. Indeed, systemic inflammation is considered a pivotal feature in cachexia progression and maintenance. Cytokines are intimately related to chronic systemic inflammation and the mechanisms underlying the release of these factors are not totally elucidated, the etiology of cachexia being still not fully understood. Therefore, the understanding of cachexia-related mechanisms, as well as the establishment of markers for the syndrome, is very relevant. MicroRNAs (miRNAs) are a class of noncoding RNAs interfering with gene regulation. Different miRNA expression profiles are associated with different diseases and inflammatory processes. miRNAs modulate adipose and skeletal muscle tissue metabolism in cancer cachexia and also tumor and tissue derived inflammation. Therefore, we propose a possible role for miRNAs in the modulation of the host inflammatory response during cachexia. Moreover, the establishment of a robust body of evidence in regard to miRNAs and the mechanisms underlying cachexia is mandatory, and shall contribute to the improvement of its diagnosis and treatment.

1. Introduction

Cachexia is a wasting syndrome for which descriptions may be found as far as 2000 years ago [1], and is a consequence of cancer and other diseases, such as chronic obstructive lung disease, multiple sclerosis, congestive heart failure, tuberculosis, and AIDS, among others, with a high impact on quality of life [2]. In this review, we focus primarily on cancer cachexia, which affects approximately half of all patients with cancer. In advanced stages, this figure rises up to 80% [3, 4]. The condition compromises the responsiveness to cancer treatment and represents, per se, the direct cause of death of up to 20% of all patients [5].

The syndrome is characterized by unintentional significant reduction in body weight and, among other symptoms, reduced energy intake, fatigue, systemic inflammation, and metabolic abnormalities are frequently reported [6].

Despite the long search for etiologic factors underlying cachexia, and the fact that many scientific efforts have been devoted to its understanding, researchers agree that "we are still a long way from knowing the whole truth about the exact mechanisms behind its etiology" [7], which makes it very hard to diagnose and treat the syndrome, frustrating physicians and patients. The most widely accepted hypothesis is that cachexia would appear as the result of tumor-host

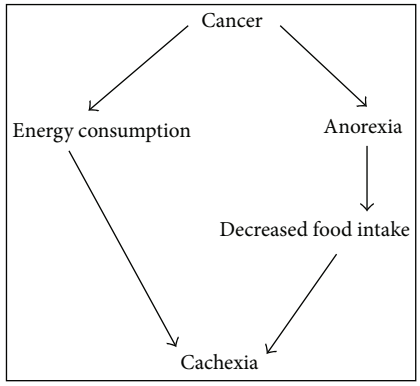

Figure 1: Traditional view of cachexia as discussed by Tisdale [8].

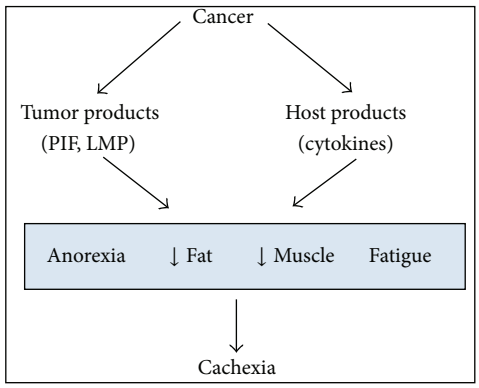

Figure 2: Emerging view of cachexia as proposed by Tisdale [8].

interactions (Figure 1) [8], being deeply related to the increase and release of proinflammatory factors (Figure 2) [8, 9].

2. Cachexia Definition and Main Symptoms

Marked weight loss is the central symptom in many of the proposed diagnostic criteria [10–12]. In 2011, a definition consensus for cachexia suggested the existence of different degrees of the syndrome. The syndrome would thus develop through three different and specific stages: precachexia, when anorexia and metabolic changes may be observed before weight loss; cachexia itself, with a weight loss $\geq 5\%$ or BMI ≤ 20 and weight loss $\geq 2\%$ or sarcopenia and weight loss $> 2\%$ and often reduced food intake and systemic inflammation; and, finally, refractory cachexia, in which survival expectance usually does not exceed three months [11].

3. Cachexia and Peripheral Tissues

In the wasting scenario in cancer cachexia neuroendocrine changes play an important part, provoking early satiety and aversion to food and leading to undernutrition [13]. These, combined with diminished food absorption and hypermetabolism, lead to a negative energy balance [14, 15] and contribute to the loss of mass, specially of adipose tissue and of skeletal muscle [16].

Nevertheless, peripheral tissues are highly affected by cachexia even before the presence of anorexia. Thus, the loss of adipose tissue and skeletal muscle mass precedes any decrease in food intake, which at the initial period of wasting can be normal or even increased [17, 18]. These tissues exhibit impaired homeostasis and altered metabolism, resulting in increased lipolysis in the adipose tissue and augmented proteolysis in the skeletal muscle. The white adipose tissue seems to be importantly adding to the inflammatory status in cachexia. Several studies showed that circulating levels of cytokines are altered in cachectic patients [19–22]. These cytokines elicit an inflammatory response in the adipose tissue, which then releases chemoattractant proteins, which in turn will recruit immune cells from the blood stream; these cells infiltrate the tissue, provoking further release of proinflammatory mediators. As a consequence, lipolysis is activated, causing adipocytes and immune cells to secrete, in a vicious cycle, proinflammatory mediators such as tumor necrosis factor (TNF-alpha), interleukin- (IL-) 1β, interferon-gamma (INF-gamma), and IL-6. These cytokines may reach other tissues through the circulation and are associated with increased muscle catabolism and reduced muscle protein synthesis. On the other hand, the high concentration of circulating free fatty acids is sensed by the liver. This organ responds by increasing the uptake of these substrates, which, ultimately, may lead to the onset of steatosis and the induction of acute phase protein secretion. These changes contribute to the systemic onset of the so-called metabolic chaos.

4. miRNAs

Recently, changes in metabolism and in aspects of the inflammatory response have been found to be modulated by miRNAs, which are small noncoding RNAs of approximately 19–25 nucleotides (nt), known to be regulatory molecules for some of the most important levels of genome function, including chromatin structure, chromosome segregation, transcription, RNA processing, RNA stability, and translation [23].

These molecules are widely found in organisms including plants, nematodes, fruit flies, and mammals and are highly conserved among evolution [24]. miRNA biogenesis involves the transcription of genomic DNA by RNA polymerase II to produce primary miRNA transcripts (pri-miRNA). In sequence, the Drosha-DGCR8 RNase complex initiates miRNA maturation through the cleavage of a stem loop into the primary transcript. This generates a 60- to 70-nucleotide-long miRNA precursor, the "pre-miRNA," characterized by the presence of an overhang of 2-3 nucleotides, still in the nucleus [25]. The newly produced pre-miRNA is then transported to the cytoplasm by exportin5 and processed to a double-stranded RNA molecule of about 19 to 25 nucleotides in length by yet another enzyme, the Dicer. Once incorporated into the effector complex miRISC (miRNA-induced silencing complex), one strand of the recently produced RNA molecule remains as a mature miRNA [26, 27], while the other strand may be either degraded, incorporated into another miRISC, or exported to the periphery by exosomes to exert its effects in a paracrine or endocrine way [28, 29].

The complex miRISC, together with the recently incorporated mature miRNA, acts directly on the mRNA to repress the translation of target genes by cleavage (perfect or near-perfect binding) or by forming a "hairpin" in the 3′UTR, through imperfect base pairing [30]. The binding site may also not be in the 3′UTR, but in the ORF or 5′UTR region of the target [31].

According to the miRBase [32], over 6,000 miRNA genes were identified in more than 223 known species, including viruses, plants, fungi, and animals. In humans, the number of miRNAs reaches up to 1500 (access in December 2014). Computational analysis estimates that more than 50% of human protein-coding genes are putatively regulated by miRNAs [33].

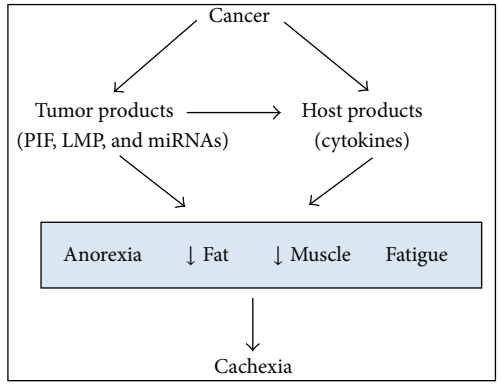

Figure 3: miRNA as part of cachexia modulation as an addition to the Tisdale proposition [8].

5. miRNAs, Peripheral Tissues, Cachexia, and Inflammation

The expression of miRNAs is highly dependent on tissue type, metabolic status, and presence of disease. Several studies describe miRNAs as important regulators of biological processes as cellular differentiation, proliferation, tissue development, and cell-type specific function and homeostasis. Nowadays, an increased number of diseases have been found to be associated with altered miRNAs expression [34–36]. Several miRNAs have been studied and confirmed as having a role in inflammatory processes in peripheral tissues such as adipose tissue and muscle [37]. Moreover, there is strong evidence that miRNAs would function as an effective system that regulates the magnitude of inflammatory responses, by displaying effects on cellular development and aspects of cellular function [38].

miRNAs-modulated pathways in skeletal muscle have been extensively studied. Several highly expressed miRNAs are described in striated muscle: the "myomiRs." miRNAs such as miR-1, miR-133a, miR133-b, miR-206, miR-208, miR208b, miR486, and miR-499 are part of this group and are associated with cell growth and differentiation, stress responsiveness, and protection against apoptosis [39, 40]. Muscle protein degradation in cachexia is mainly mediated by the ubiquitin proteasome system, which is induced through the activation of E3 ligases, atrogin-1/MAFbx, and MurF-1. The Forkhead box O (FoxO) signaling pathway participates in this process by the induction of the transcription of E3 ubiquitin ligases and has three members in skeletal muscle (FoxO1, FoxO3, and FoxO4). Muscle-specific overexpression of these proteins is described as sufficient to cause skeletal muscle atrophy in vivo; and inhibition of FoxO transcription activity prevents muscle fiber atrophy during cachexia [41]. Xu and colleagues verified that the miRNA-486 decreases FoxO1 protein expression and promotes FoxO1 phosphorylation to suppress E3 ubiquitin ligases [42], presenting an excellent candidate for future studies on the mechanisms of regulation of muscle atrophy by miRNAs in cachexia. miR-206 and miR-21 were also recently described as having a role in muscle wasting in catabolic conditions [43]. miR-21 has been already confirmed as being produced and exported from tumor cells of rodent and humans and uptaken by the skeletal muscle, in exosomes. The effect of this process is the onset of proteolysis through toll-like receptor 7 signaling, in a JNK dependent manner [44]. Moreover, the detection of aberrant miRNA expression in body fluids, that is, blood, urine, and saliva, opens ways to explore these molecules as diagnostic and prognostic tools for cancer cachexia. miRNAs are also known to play a major role in the regulation of the transcription of several genes involved with key aspects of white adipose tissue metabolism. To date, one study involving cachectic patients, white adipose tissue, and miRNA profile is available in the literature [45]. In this study, five miRNAs showed specific cachexia associated patterns of expression. miR-483-5p/-23a/-744/-99b expression is downregulated and miR-378 is upregulated in cachectic patients. miR-378 is strongly involved with catecholamine-stimulated lipolysis in adipocytes and modulates the expression of key lipolytic proteins such as LIPE, PLIN1, and PNPLa2. No information is available in the literature about miRNAs expression and the modulation of inflammation in the white adipose tissue in specific wasting conditions. However, Xie et al., 2009 [46], demonstrated that a chronic inflammatory environment characterized by high cytokine concentration may, per se, change miRNA pattern expression in the white adipose tissue, both in cultured differentiated adipocytes and in rodent models. Potential miRNA candidates for studies regarding adipose tissue inflammation and cachexia would be miR-155, miR-146a, miR-21, and miR-9, whose expression is induced by the activation of innate immune system through toll-like receptors [47].

6. Conclusion

Considering that miRNAs are known to regulate the expression of genes involved in several types of diseases as cancer and autoimmune disorders [34, 35] and play a pivotal role in the regulation of inflammatory responses, the study of miRNAs in cachexia is a promising field of research, and patients could benefit not only from the development of new targets for treatments, but also from earlier diagnosis. Chronic inflammation in cancer cachexia is a highly complex biological process. The discovery of noncoding RNAs and the improvement of molecular biology techniques have changed

the concept that inflammation could be understood and explained by the study of signaling pathways and by the contribution of specific proteins. Knowledge on the regulation of gene and protein expression has changed profoundly, and miRNAs are nowadays established as pivotal components of the signaling networks that modulate inflammatory processes [48], leading to wasting conditions such as cachexia. Based on such evidence, we propose that miRNAs participation in the onset and maintenance of cachexia should be added to the study of the syndrome (Figure 3).

Conflict of Interests

The authors declare no conflict of interests.

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Review Article

Contribution of Neuroinflammation to the Pathogenesis of Cancer Cachexia

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Inflammation characterizes the course of acute and chronic diseases and is largely responsible for the metabolic and behavioral changes occurring during the clinical journey of patients. Robust data indicate that, during cancer, functional modifications within brain areas regulating energy homeostasis contribute to the onset of anorexia, reduced food intake, and increased catabolism of muscle mass and adipose tissue. In particular, functional changes are associated with increased hypothalamic concentration of proinflammatory cytokines, which suggests that neuroinflammation may represent the adaptive response of the brain to peripheral challenges, including tumor growth. Within this conceptual framework, the vagus nerve appears to be involved in conveying alert signals to the hypothalamus, whereas hypothalamic serotonin appears to contribute to triggering catabolic signals.

1. Introduction

Metabolic changes due to tumour growth profoundly impact nutritional status [1]. Anorexia and reduced food intake are frequently the presenting symptoms of several types of cancer [1, 2]. Although anorexia and reduced food intake largely contribute to weight loss of cancer patients, wasting cannot be accounted for by inadequate eating only. Indeed, cancer-induced derangement of protein, carbohydrate, and lipid metabolism magnifies the impact of anorexia on nutritional status and also reduces the efficacy of nutritional interventions [2].

Tumor-associated changes of energy and macronutrient metabolism, together with behavioral changes (i.e., anorexia and reduced food intake), negatively influence patients' quality of life and increase their morbidity and mortality [3]. Inflammation plays a major role in the pathogenesis of metabolic and behavioral abnormalities during disease. Consequently, inflammatory markers are frequently used as predictors not only of metabolic abnormalities but of clinical outcome as well. As an example, high circulating levels of C-reactive protein (CRP) are frequently observed in cancer patients with cachexia. Thus, CRP levels, in combination with reduced food intake and weight loss, could be used as a clinical marker of cancer cachexia. Moreover, CRP might be directly involved in cancer-related wasting since it has been shown to exacerbate tissue injury of ischemic necrosis in heart attack and stroke [4]. Therefore, a potential role for CRP in inflammatory conditions such as cancer could be speculated, in which increased CRP production leads to binding of CRP to exposed ligands in damaged cells, thereby increasing tissue injury [5]. Systemic inflammation is also correlated with increased proteasome-mediated proteolysis in skeletal muscle of cancer patients [6].

Cancer anorexia also appears to be significantly influenced by increased inflammatory status, as demonstrated by increased brain levels of proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNFα) in experimental models of cancer anorexia [7–11]. In fact, blockade of circulating TNF or inhibition of intrahypothalamic interleukin-1 receptors enhances food intake in animal models of cancer anorexia [12, 13]. Proinflammatory cytokines alter brain neurochemistry by enhancing the release of neurotransmitters able to influence neuronal anorexigenic pathways such as serotonin [14]. Further supporting the role of increased inflammatory response in mediating the onset of anorexia, Jatoi et al. showed in a prospective, controlled, randomized trial that the percentage of cancer patients

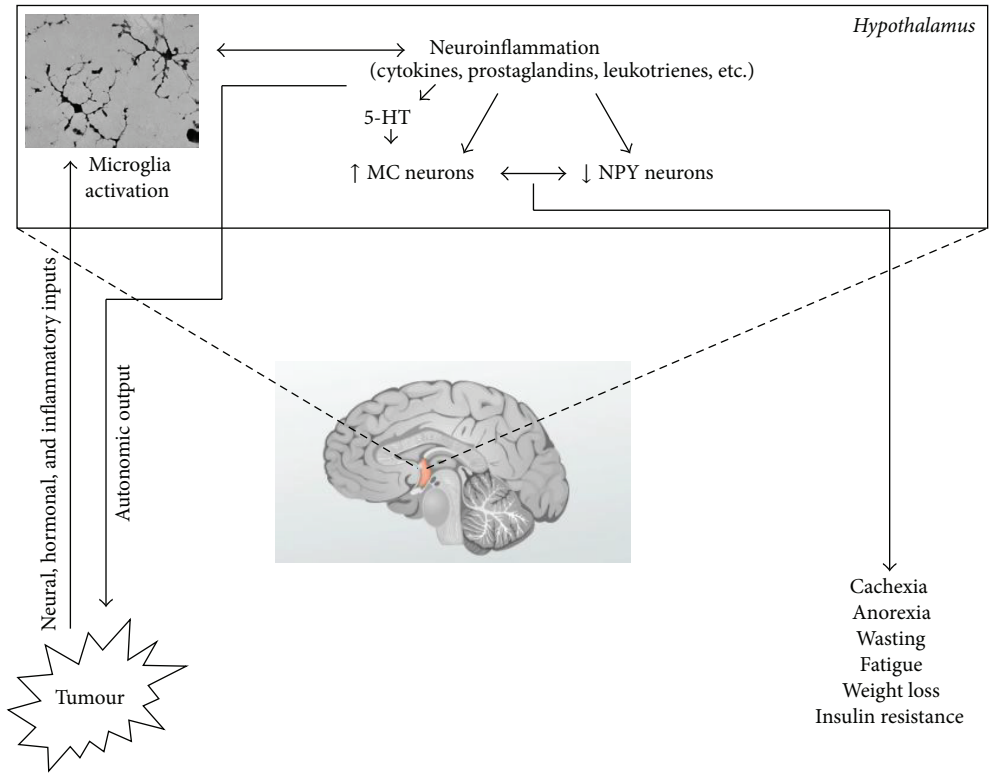

Figure 1: The growing tumor is sensed by the brain via neural, humoral, and inflammatory input. These signals activate the behavioural and metabolic response to stress by activating microglia cells, although it cannot be excluded that signals from peripheral tissues directly influence the activity of hypothalamic neurons, at least in the initial phase of the response to stress. Microglia activation triggers and perpetuates neuroinflammation, which is characterized by the release of inflammatory mediators within the hypothalamic areas. In the arcuate nucleus, inflammatory response hyperactivates catabolic neurons, that is, melanocortin (MC) neurons, which in turn contribute to the inhibition of prophagic neurons, that is, neuropeptide Y (NPY) neurons. Disruption of the physiological balance between the activity of MC and NPY neurons yields to the behavioural and metabolic consequences of cachexia. Experimental data also suggest that neuroinflammation may contribute to tumour growth and aggressiveness by modulating the peripheral immune response through autonomic output.

with appetite improvement was similar following eicosapentaenoic acid (EPA) supplementation or megestrol acetate intake, a potent appetite enhancer [15]. EPA is an omega-3 fatty acid whose biological effects include the modulation of inflammatory response. By competing with omega-6 fatty acids, EPA is degraded by cellular lipoxygenase and cyclooxygenase. However, the prostaglandins and leukotrienes deriving from the degradation of EPA exert less proinflammatory activities when compared to the prostaglandins and leukotrienes deriving from the degradation of omega-6 fatty acids. Therefore, reduced production of omega-6 fatty acid-derived mediators of inflammation through supplementation of pharmacological doses of omega-3 fatty acids is now considered to play a contributory role in reducing inflammation and promoting preservation of nutritional status in cancer patients [16].

2. Interaction between Neuroinflammation and Neurotransmission

During cancer, the physiological functioning of the brain areas controlling energy homeostasis is disrupted. Consistent evidence indicates that increased hypothalamic expression and release of mediators of inflammation play a large role in this event (Figure 1). Proinflammatory cytokines such as IL-1 and TNFα have been recognized for many years as principal actors in the pathogenesis of anorexia and cachexia [17]. Hypothalamic IL-1 mRNA expression and IL-1 levels are significantly increased in the cerebrospinal fluid of anorexic tumor-bearing rats and inversely correlate with energy intake [7, 18]. The causative role of brain IL-1 in cancer anorexia and cachexia is supported by data showing that anorexia ameliorates after intrahypothalamic injection of the IL-1 receptor antagonist [19]. Intraperitoneal injection of recombinant human soluble TNFα receptor in experimental models improves anorexia thus confirming the role of TNFα in the negative modulation of appetite [12]. Finally, megestrol acetate, a potent orexigenic drug largely used in cancer patients, improves food intake by reducing the expression of IL-1 by mononuclear cells and by increasing hypothalamic concentrations of the prophagic mediator neuropeptide Y (NPY), which confirms the significant role of IL-1 in mediating cancer-associated anorexia in humans [20, 21].

Proinflammatory cytokines appear to exert their effects through their influence on the physiological hypothalamic pathway promoting catabolism, that is, the melanocortin

system. Intracerebroventricular injection of IL-1 increases the frequency of signaling of melanocortin neurons in the arcuate nucleus of hypothalamus which express the type 1 IL-1 receptor. In addition, IL-1 stimulates the release of α-MSH [22]. Also, the classical neurotransmitter serotonin appears to be involved (Figure 1).

Serotonin contributes to energy balance by triggering satiety through its effects in the hypothalamus [23, 24]. Increased hypothalamic serotonin levels have been associated with the onset of cancer anorexia in experimental *in vivo* models and increased expression of serotonin receptors (5-HTRs). The link between serotonergic neurotransmission and disease-related anorexia is confirmed by the restoration of energy intake after tumor resection and normalization of hypothalamic serotonin concentrations and receptor expression [25, 26]. Intrahypothalamic injection of the serotonin antagonist mianserin ameliorates energy intake in experimental models of anorexia [13]. The synthesis of the hormone melatonin is determined by its precursor serotonin. Melatonin modulates the activity of the hypothalamic suprachiasmatic nucleus and alters biological rhythms. Disrupted melatonin synthesis and secretion in patients with cachexia and in wasted animals may contribute to serotonin accumulation in the hypothalamus [27, 28]. Serotonin plays a role in disease-associated anorexia, as confirmed by increased plasma and cerebrospinal fluid levels of the amino acid tryptophan, the precursor of serotonin, in anorexic and cachectic cancer patients [29]. Catabolic effects may be the consequence of the brain accumulation of tryptophan during the disease [30]. Brain tryptophan is also crucial in determining the release of kynurenine and its derivatives, molecules able to modulate immune functions [30]. Kynurenine represents the most important pathway, because tryptophan is mostly degraded via this pathway, producing 3-hydroxykynurenine and 3-hydroxyanthranilic acid, which represent acid free radical generators. The rate of tryptophan degradation through the kynurenine pathway is mediated directly by inflammation. In this light, the accumulation of brain tryptophan coupled with increased release of proinflammatory cytokines may maintain tryptophan metabolism toward increased free radicals production, determining enhanced oxidative stress. In experimental models of cancer-associated anorexia, increased concentrations of markers of oxidative stress have been measured in hypothalamic regions involved in the control of energy homeostasis [31].

As previously mentioned, melatonin biosynthetic pathway might be involved in the pathogenesis of anorexia. Melatonin exerts antioxidant function, and since the brain is largely composed of unsaturated fatty acids, preferential targets of reactive oxygen species, it could be speculated that melatonin supplementation may limit brain oxidation-induced inflammation and thus ameliorate anorexia and cachexia. However, Del Fabbro et al. have recently reported that oral melatonin 20 mg at night did not improve appetite, weight, or quality of life compared with placebo [32]. However, since the trial involved, among others, patients with gastrointestinal cancer, a role for the mechanical impact of tumor burden on the lack of clinical effects cannot be excluded.

3. The Melanocortin System and Its Role during Inflammation

Melanocortin system mediates the anorectic effects of serotonin, as demonstrated by the activation of the central melanocortin pathway after the administration of fenfluramine, a serotonin reuptake inhibitor [33]. Studies have focused on 2 subtypes of serotonin receptors, the 5-HT2cR and the 5-HT1bR which are located within the arcuate nucleus of the hypothalamus. Anorexigenic neurons express 5-HT2cRs, whereas orexigenic NPY neurons express 5-HT1bRs. Agonists activate these receptors thus hyperpolarizing the NPY neurons while dramatically reducing the inhibitory postsynaptic potentials in melanocortin neurons [34]. An improvement in glucose tolerance and a decrease in plasma insulin levels were consequent to the administration of doses of 5-HT2cR agonists in experimental models of obesity via melanocortin-4 receptor signaling pathways [35]. Serotonin, IL-1, and TNFα are able to influence the activity of the central melanocortin system. In fact, peripheral infusion of IL-1 causes anorexia by increasing brain tryptophan levels and serotonin synthesis [36]. TNFα and IL-1 are able to regulate neuronal serotonin transporter [37]. Experimental data suggest that catabolic states are associated with increased hypothalamic expression of IL-1 together with enhanced release of serotonin. The function of the melanocortin system is conditioned by the interaction between serotonin and IL-1 within the arcuate nucleus. The consequences are the inhibition of NPY neuronal activity and the stopping of the inhibition of melanocortin neurons. These effects alter the melanocortin system by enhancing the release of α-MSH, an endogenous melanocortin receptor type 4 (MC4R) agonist, and suppressing the release of agouti-related peptide (AgRP), an endogenous MC4R antagonist. Interestingly, binding of α-MSH on MC1R reduces TNFα secretion by macrophages, therefore determining anti-inflammatory effects [38, 39].

The activation of the melanocortin system during peripheral acute stress is likely related to the direct sensing by hypothalamic cells of humoral or nervous triggers. However, during chronic stress, the role of neuroinflammation, and particularly of brain microglia, is key (Figure 1). The most important immune effector cells of the brain are microglia, the tissue macrophages of the brain, and they are involved in the onset, maintenance, relapse, and progression of brain inflammation. Under healthy conditions, microglia is characterized by a ramified morphology, which is used to continuously scan the environment. Upon any homeostatic disturbance, microglia rapidly change their phenotype and contribute to processes including inflammation, tissue remodeling, and neurogenesis. During activation, microglia releases neurotrophic factors, as well as neurotoxic factors and proinflammatory cytokines. Host defense is dependent on microglial activation, although detrimental effects have been also reported. However, robust and consistent evidence shows that microglia stimulates myelin repair, removal of toxic proteins, and prevention of neurodegeneration [40]. Recent data show that functional phenotypes of microglia differ according to the diverse brain regions and to the different

types of stress (i.e., neuroinflammation, neurogenesis, brain tumour homeostasis, and aging) [41].

4. From Neuroinflammation to Systemic Inflammation

Consistent evidence supports the concept that inflammation drives a multifactorial central and peripheral network of signaling pathways involved not only in the pathogenesis of cancer cachexia, but in tumor development and progression as well. In addition, inflammatory response is associated with increased circulating levels of specific cytokines, such as IL-1, IL-6, IFNγ, TNFα [42], and acute-phase proteins that lead to hypermetabolism and weight loss in patients with anorexia and cachexia [43]. Also, in advanced stages of cancer, IL-1β is strongly associated with loss of appetite, weight loss, sarcopenia, and general weakness [44]. Despite this robust evidence, it should be also acknowledged that Kayacan et al. did find increased concentrations of TNFα and IL-6 in patients with lung cancer, but they could not observe any significant difference between cachectic and noncachectic patients [45]. This highlights the importance of considering the circadian rhythm of cytokine production and release when measuring their circulating levels.

The mechanistic interaction between neuroinflammation, systemic inflammation, and tumor development has not yet been completely clarified. Evidences for a causal relationship between neuroinflammation and systemic inflammation and features of cachexia are increasing [46]. In models of anorexia and cachexia, administration of proinflammatory cytokines induced acute-phase protein response, anorexia, weight loss, protein and adipose tissue catabolism, and higher concentration of cortisol and glucagon, as well as decreased insulin resistance and a positive modulation of energy homeostasis [47]. In addition, high IL-6 levels correlated with cachexia phenotype, while treatment with monoclonal antibody to IL-6 reversed this picture [48]. When the specific role of neuroinflammation in the development and progression of cancer is considered (Figure 1), results obtained show that the sympathetic nervous system modulates the antitumor immune defense response. In fact, chemically sympathectomized tumor-bearing rats had significantly increased neutrophil-to-lymphocyte ratio, an indicator of disease progression, although no significant changes in tumor growth and survival were observed [49]. Also, Magnon et al. found that the formation of autonomic nerve fibers in the prostate gland regulates prostate cancer development and dissemination in mouse models. Moreover, a retrospective blinded analysis of prostate adenocarcinoma specimens from 43 patients revealed that the densities of sympathetic and parasympathetic nerve fibers in tumor and surrounding normal tissue, respectively, were associated with poor clinical outcomes [50]. Whether increased tumor innervation by autonomic nervous system could be regulated by increased brain inflammatory response remains to be ascertained. However, microglial activation has been demonstrated to contribute to the endocrine dysregulation and the elevated sympathetic nerve activity reported in streptozotocin-treated rats [51].

5. Brain and Muscle-Adipose Tissue Axis

Robust data indicate that the control of energy intake and expenditure is largely mediated by the hypothalamus, and centrally produced proinflammatory cytokines participate in activating the molecular modifications inducing the development of cancer-associated anorexia and cachexia [46]. Moreover, experimental models of wasting showed that muscle catabolism during disease is activated by hypothalamic stimuli and cytokines may enhance the activity of the hypothalamic melanocortin system promoting muscle and adipose wasting [46].

The interaction between inflammatory mediators and the central nervous system may occur at the peripheral levels and may play a relevant role in triggering the host inflammatory response. This inflammatory response, when constantly present, may lead to the development of cachexia. At peripheral levels, tumour growth could be sensed by the vagus nerve, possibly by sensing the paracrine release of proinflammatory cytokines [52]. This information is conveyed to brainstem regions and finally to the hypothalamus, activating the melanocortin system through specific neural intermediates and receptors [53]. The melanocortin system, when activated, enhances the release of cytokines to reduce food intake and promote muscle catabolism. Consequently, inhibition of the brain inflammatory response that is induced by cytokines may result in better clinical outcome than systemic immune suppression. In this light, exploration of the possible pathogenic and clinical roles of fatty acid-derived modulators of inflammation may yield relevant results.

As previously mentioned, EPA supplementation contributes to anticachexia therapy by reducing inflammatory response. Docosahexaenoic acid (DHA) is the major brain omega-3 fatty acid and has been shown to be involved in the biosynthesis of potent anti-inflammatory and proresolving mediators by macrophages, maresins [54]. Although their biological function has been investigated in experimental models of acute inflammation, a possible role during clinical conditions characterized by mild to moderate, yet chronic, inflammatory response, including cancer, cannot be excluded. Greater relevance for the pathogenic link between neuroinflammation and cachexia appears to be exerted by neuroprotectins.

Similarly to maresins, DHA is the precursor of neuroprotectins as well [55]. Consistent evidence showed that neuroprotectins attenuate brain damage following ischemia and restore nerve integrity and function after experimental surgery. Also, neuroprotectin D1 has been shown to induce homeostatic regulation following proteotoxic stress induced by misfolding proteins [56]. Such type of stress appears more similar to that induced by a growing tumour and therefore suggests that neuroprotectins could be a relevant therapeutic target to specifically inhibit the brain contributory role to cachexia of cancer.

6. Conclusion

During the last few years, our knowledge of the mechanisms regulating neural inflammation has been largely improved. However, the impact on clinical practice of these advancements in the pathophysiology of neuroinflammation and its link with systemic inflammation is still lacking. This may be determined by the heterogeneity of the symptoms characterizing anorexia and cachexia in human conditions. It is extremely likely that the different clinical conditions induced by inflammation are determined by the polymorphisms of different genetic profile [57], which in turn regulates the neurochemical/metabolic response to similar challenges. In this light, it appears mandatory to focus our research on the identification of polymorphisms of key genes, regulating the expression of inflammatory markers and possibly serotonin. This approach will allow the use of preventative or early anticatabolic therapies.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

A Survey of Attitudes towards the Clinical Application of Systemic Inflammation Based Prognostic Scores in Cancer

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Introduction. The systemic inflammatory response (SIR) plays a key role in determining nutritional status and survival of patients with cancer. A number of objective scoring systems have been shown to have prognostic value; however, their application in routine clinical practice is not clear. The aim of the present survey was to examine the range of opinions internationally on the routine use of these scoring systems. *Methods.* An online survey was distributed to a target group consisting of individuals worldwide who have reported an interest in systemic inflammation in patients with cancer. *Results.* Of those invited by the survey ($n = 238$), 65% routinely measured the SIR, mainly for research and prognostication purposes and clinically for allocation of adjuvant therapy or palliative chemotherapy. 40% reported that they currently used the Glasgow Prognostic Score/modified Glasgow Prognostic Score (GPS/mGPS) and 81% reported that a measure of systemic inflammation should be incorporated into clinical guidelines, such as the definition of cachexia. *Conclusions.* The majority of respondents routinely measured the SIR in patients with cancer, mainly using the GPS/mGPS for research and prognostication purposes. The majority reported that a measure of the SIR should be adopted into clinical guidelines.

1. Introduction

Cancer remains a major problem worldwide with 12.7 million new cases diagnosed in 2008. In the UK alone, 331,000 people were diagnosed with cancer in 2011 [1]. Despite major advances in detection and treatment of cancer as well as the introduction of several cancer screening programmes, outcomes following cancer remain poor with only half of people diagnosed with cancer surviving at 5 years [1].

Allocation of patients to the correct form of treatment, be that surgical, oncological, or palliative, remains a difficult decision. However, if patients were allocated to the most appropriate treatment, then outcomes for all patients would improve, irrespective of new, more effective treatments. Traditionally, in those with early stage operable disease the treatment decision has been made largely based on staging of the cancer itself for example the Tumour, Node, Metastasis (TNM) staging system whereas in advanced stage inoperable disease the treatment decision has been made largely based on the general health and fitness and whether the patient had lost weight (cachexia).

In the last decade or so it has become apparent that a host inflammatory response, in particular the systemic inflammatory response, plays a key role in determining cachexia and the survival of patients with cancer [2, 3]. With this new knowledge, a number of prognostic scoring systems that provide an objective measurement of the systemic inflammatory response have been developed and have been shown to have prognostic value in patients with cancer. These include the Glasgow Prognostic Score/modified Glasgow Prognostic Score (GPS/mGPS), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), white cell-lymphocyte ratio (WLR), and others [4, 5]. The mGPS (combination of the values of preoperative serum albumin and C-reactive protein) and the NLR (ratio of neutrophil and lymphocyte counts) are the most widely reported prognostic scores worldwide and

both have been shown to have prognostic value in a variety of common solid tumours [6–8]. For example, by the end of 2012, the GPS/mGPS had been shown to have independent prognostic value in cancer patients in 51 studies involving 28,500 patients [6]. Furthermore, the NLR has been shown to have independent prognostic value in 100 studies involving greater than 40 000 patients, with greater than 50% of these studies published since the start of 2012 [9].

Despite the plethora of reported studies for these prognostic scores, their value in routine clinical practice either as tools to stratify patients in terms of outcomes or for consideration for therapies such as adjuvant chemotherapy or in clinical trials is not clear. With this in mind, the aim of the present survey, in an international cohort, was to examine the range of opinions on the routine use of systemic inflammation based prognostic scoring systems and their potential incorporation into clinical guidelines.

2. Methods

A worldwide survey designed to establish opinions on the use of systemic inflammation based prognostic scoring systems was created. This was a web-based survey that included 10 questions on "systemic inflammation based prognostic scores in cancer" as follows.

Survey Questions

(1) What is your discipline (surgeon/oncologist/pathologist, etc.) and in which country are you based?

(2) Do you or your colleagues routinely assess the systemic inflammatory response as part of the clinical assessment of patients with cancer?

Since 2008, could you estimate how many patients have been assessed (a) in total and (b) per year?

(3) If you answered yes to question (2), for what purpose?

Audit
Prognostication
Treatment Allocation
Research

(4) If you answered yes to question (2), what measure of the systemic inflammatory response do you use?

GPS
NLR
Other

(5) Would you use a measure of the systemic inflammatory response to stratify patients entering into clinical trials?

(6) If you answered yes to question (5), which would you prefer to use?

GPS
NLR
Other

(7) In which clinical scenario do you think a measure of the systemic inflammatory response offers most benefit to patients?

In making decisions about allocation of surgical treatment for primary operable disease
In making decisions on allocation of neoadjuvant treatment
In making decisions on allocation of adjuvant treatment
In making decisions on palliative chemotherapy

(8) Do you think that a measure of the systemic inflammatory response should be adopted into clinical guidelines?

(9) If yes, which would you prefer to use?

GPS
NLR
Other

(10) If you do not think that a measure of the systemic inflammatory response is useful in the routine clinical assessment of cancer patients, please comment.

The survey was generated through the SurveyMonkey website (http://www.surveymonkey.com/, SurveyMonkey, Paulo Alto, USA) and the access link emailed to the target group. The target group was selected primarily from two recent reviews [6, 7] and by performing a more recent literature search for articles using the keywords cancer, inflammation, recurrence, survival, mGPS, and NLR. This literature search was performed at the end of January 2014. Once a comprehensive list of articles was obtained, the email addresses of corresponding authors from each article formed the basis of a mailing list for distribution. The email sent out clearly stated that the aim of the survey was to establish whether there was a role for the application of systemic inflammation based prognostic scores in routine clinical practice and research and that participation was voluntary. Software on the website ensured duplication of responses from the same individual was not recorded. No incentives were used to promote or encourage participation.

The survey was first sent out on 26th February 2014 with a reminder sent out one week later. The survey remained open for 4 weeks and was closed on the 26th March 2014. Data was analysed and graphs of results were compiled using Microsoft Excel 2007 (Redmond, WA, USA).

3. Results

In February 2014, the survey was emailed to 238 individuals worldwide who had published articles related to systemic inflammation in patients with cancer. 43% were from Asia, 42% from Europe, 12% from America, and 3% from Australia. The response to survey question (1) is shown in Figures 1(a) and 1(b). In total, 60 people completed the survey (25%). 26 respondents (43%) were surgeons, 15 (25%) oncologists, and 19 (32%) from other medical specialties. The proportion of

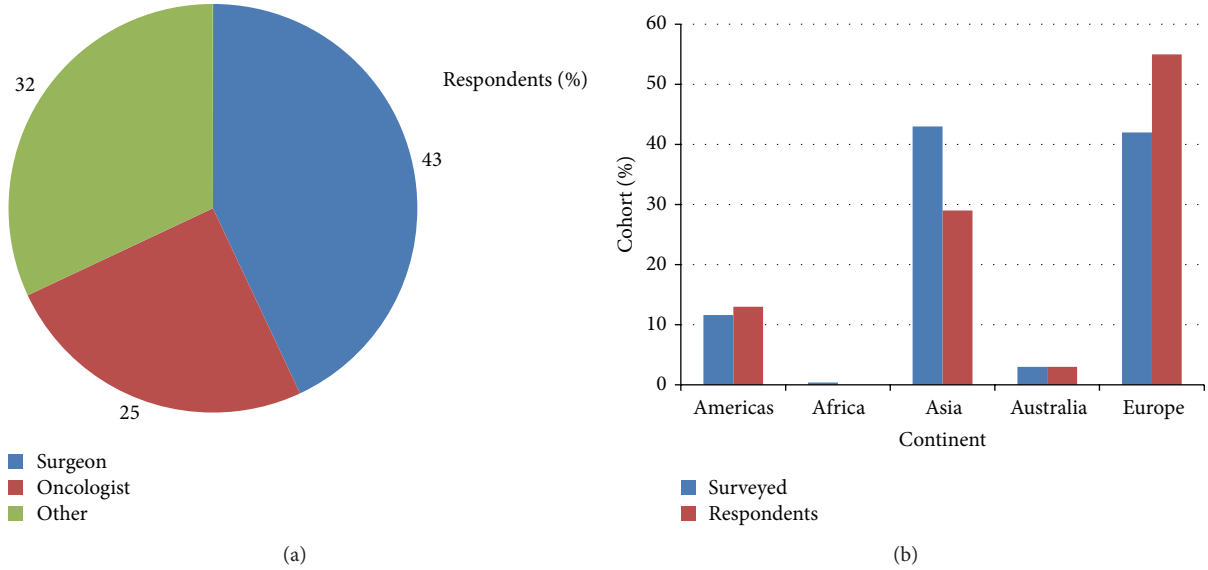

Figure 1: (a) What is your discipline? (Respondents = 60) and (b) in which country are you based? (Respondents = 31).

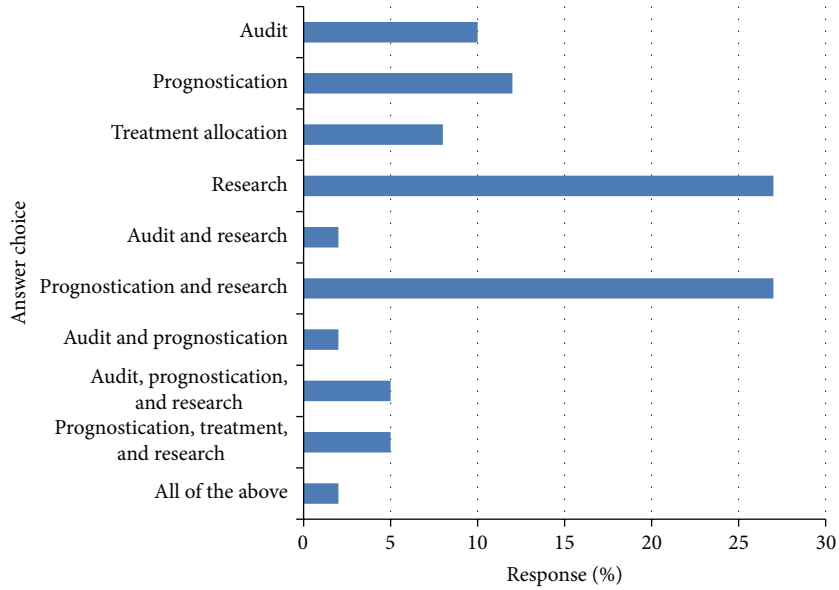

Figure 2: For what purpose do you measure the systemic inflammatory response? Respondents (n = 41).

respondents is shown in Figure 1(b) with 55% of respondents being from Europe, 29% from Asia, 13% from Americas, and 3% from Australia.

In response to question (2), 39 (65%) of the respondents answered yes that they routinely measured the systemic inflammatory response in patients with cancer. The median number of patients each participant assessed per year was 100 and the median number of patients each participant assessed in total was 330.

The response to question (3) is shown in Figure 2. Of the respondents, 11 (27%) reported its use for the purpose of prognostication and research, 11 (27%) reported its use for research purposes alone, 5 (12%) reported its use for the purpose of prognostication alone, 4 (10%) reported its use for audit purposes, and 3 (8%) reported its use for the purpose of treatment allocation.

The response to question (4) is shown in Figure 3. Of those who responded, 16 (40%) answered that the measure of the systemic inflammatory response they used was the GPS, 8 (20%) the GPS/NLR, and 6 (15%) the NLR alone.

The response to question (5) is shown in Figure 4(a). Of the respondents, 31 (56%) answered yes they would use a measure of the systemic inflammatory response to stratify patients entering clinical trials.

The response to question (6) is shown in Figure 4(b). Of the respondents, 20 (57%) answered that they would use the GPS, 4 (11%) the NLR, and 4 (11%) the GPS/NLR for stratifying patients entering clinical trials.

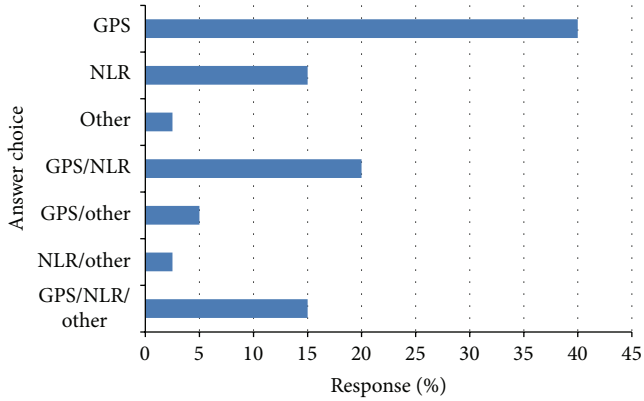

Figure 3: What measure of the systemic inflammatory response do you use? Respondents (n = 40).

The response to question (7) is shown in Figure 5. Of the respondents, 12 (25%) reported that the clinical scenarios where a measure of the systemic inflammatory response offers most benefit were making decisions on palliative chemotherapy, 10 (21%) making decisions on allocation of adjuvant therapy, 6 (12%) making decisions about either adjuvant therapy or palliative chemotherapy, and 5 (10%) all 4 categories. Only 2 (4%) reported on making decisions on allocation of surgical treatment.

The response to question (8) is shown in Figure 6(a). Of the respondents, 46 (81%) answered yes to whether a measure of the systemic inflammatory response should be adopted into clinical guidelines.

The response to question (9) is shown in Figure 6(b). Of those who responded, 30 (60%) answered that the measure of the systemic inflammatory response they would prefer to use in clinical guidelines was the GPS, 7 (14%) GPS/NLR, and 5 (10%) NLR.

4. Discussion

The results of the present study showed that the majority of respondents routinely measured the systemic inflammatory response and used the GPS/mGPS, mainly for research and prognostication purposes and that the majority of respondents reported that a measure of the systemic inflammatory response should be adopted into clinical guidelines.

A small number of people responded to our survey (25%) although this rate falls within the average response rate of between 20 and 30% [10]. Factors that are known to improve the survey response rate include incentives, reduced survey length, reduced complexity of questions, and reminder emails [10]. In the present study the questions were intentionally simple and limited to 10 in total and a reminder email was sent to encourage respondents but did not employ any incentive for completing the survey.

The survey was sent to potential participants worldwide with the majority to Asia and Europe. The majority of respondents of this survey were surgeons (43%) with oncologists making up a quarter of respondents. The location of the respondents did not closely match the locations of the potential survey participants. Those invited to participate were mainly from Asia and Europe; however, only 29% of respondents were from Asia while 55% were from Europe. Perhaps this lack of response from Asia is due to cultural differences which were not present in those from Europe or due to greater language barriers. Whatever the reason, the poor response rate from Asia was disappointing given that the majority of work using these prognostic scores has been carried out in Europe and Asia. In the present study, respondents were asked to estimate how many patients with cancer they had assessed using these systemic inflammation based scores in each year. The response was approximately 100 per year. With this volume of work it could be considered that those who responded were specialists and had an interest in systemic inflammation based scores.

It has been widely reported that markers of the systemic inflammatory response are good prognostic markers in patients with cancer. The majority of survey respondents reported that they routinely assessed the systemic inflammatory response in patients with cancer and the majority used this assessment for research or prognostication purposes. This is not unexpected since the majority of studies examining these scoring systems were performed for research purposes or were performed retrospectively to aid prognostication of patients into high and low risk groups. Whilst CRP has been shown to have prognostic value in a number of tumours, the mGPS, which utilises a combination of CRP and albumin at standard thresholds, has been shown to have superior prognostic value and obviates the problem of different CRP threshold values being used within and across different tumour types. In the present study, the majority of respondents reported that they would use GPS/mGPS as their method of assessing the systemic inflammatory response. This would appear to be consistent with the literature and whilst the participants of this survey have an interest in this field, it was not clear, prior to this survey, what views they had on the clinical application of systemic inflammation based prognostic scores, in particular which, if any, score that they would prefer to use clinically.

Interestingly, only a small number of respondents reported that they used assessment of the systemic inflammatory response to determine treatment allocation and this is an area where proponents of these scoring systems would hope to expand their use in order to better stratify patients to appropriate treatment modalities [11]. Of the survey respondents, 56% reported that they would use a measure of the systemic inflammatory response to stratify patients entering into clinical trials and 57% said they would choose mGPS/GPS for this. Moreover, of the survey respondents, 25% reported that these scores were used in making decisions about palliative chemotherapy, 21% in making decisions about allocation of adjuvant therapy, and 12% in making decisions either about adjuvant therapy or palliative chemotherapy. Only 4% reported that a measure of the systemic inflammatory response would be of benefit in making decisions about allocation of surgical treatment. This is of interest as the majority of respondents were surgeons, with the majority of research in these scoring systems having been undertaken by surgeons, yet the consensus was that it would not be of benefit

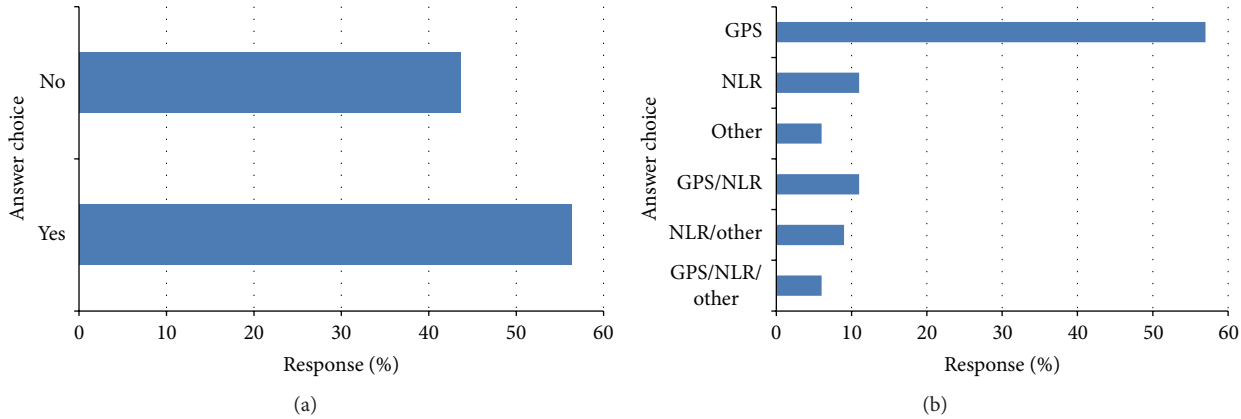

Figure 4: (a) Would you use a measure of the systemic inflammatory response to stratify patients entering into clinical trials? Respondents (n = 55) and (b) which measure of the systemic inflammatory response would you use to stratify patients entering into clinical trials? Respondents (n = 35).

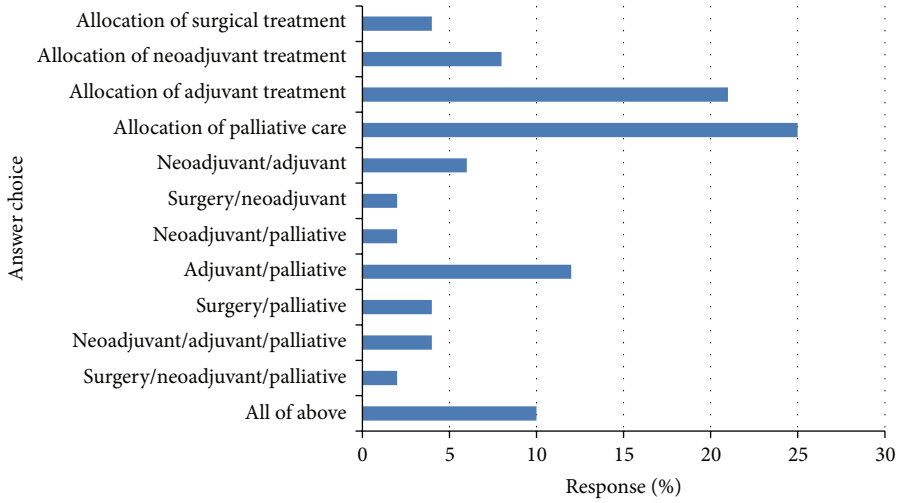

Figure 5: In which clinical scenario do you think a measure of the systemic inflammatory response offers most benefit to patients? Respondents (n = 49).

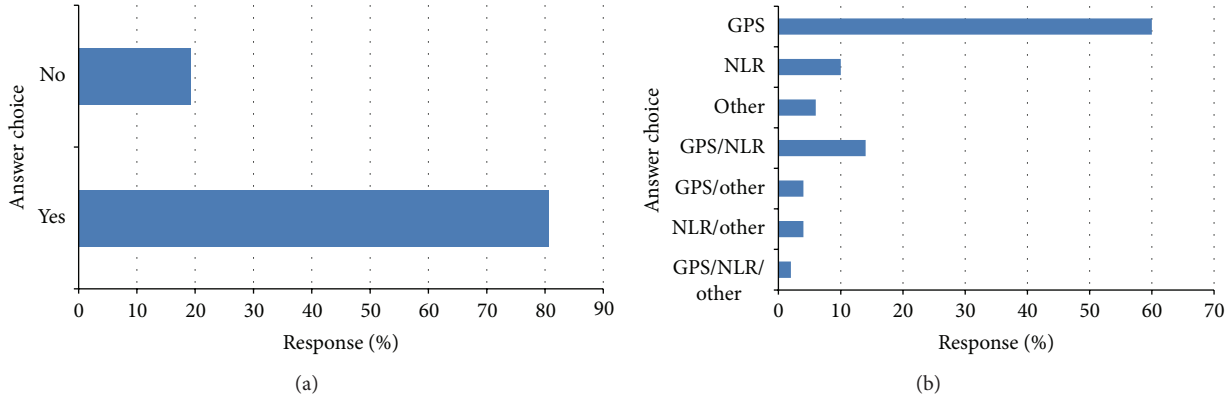

Figure 6: (a) Do you think that a measure of the systemic inflammatory response should be adopted into clinical guidelines? Respondents (n = 57) and (b) which measure of the systemic inflammatory response respondants think should be included in clinical guidelines? Respondents (n = 50).

to allocate surgical treatment based on these scoring systems. The basis of this approach is not clear. However it may be that surgeons wish to operate on all patients with potentially curable disease. It remains to be seen whether this approach will be maintained in the long term, particularly in aggressive cancers such as pancreatic cancer where neoadjuvant therapy is increasingly used as first line therapy.

Furthermore, recent work has suggested that markers of the systemic inflammatory response may be useful as a therapeutic target. The recent addition of an antiangiogenic monoclonal antibody to VEGF therapy, such as Bevacizumab to standard chemotherapy regimens, has resulted in improved efficacy of these regimens. However, recent studies have reported that patients with a raised neutrophil count, high NLR or mGPS 1 or 2, received no significant survival benefit from these regimens [12–14]. In addition, Botta and colleagues reported in their study that preoperative systemic inflammatory status was a marker of resistance to bevacizumab therapy [13]. Also, recent work has suggested that the mGPS may be useful in stratifying oncological treatment. Hurwitz et al. recently reported that Ruxolitinib (a Janus Kinase 1 (JAK1)/Janus Kinase 2 (JAK2) inhibitor) along with capecitabine improved overall survival and progression free survival in patients with metastatic pancreatic cancer with inflammation characterised by mGPS 1 or 2 [15].

Of the survey respondents, 80% reported that they felt that a measure of the systemic inflammatory response should be adopted into clinical guidelines and 60% reported that GPS/mGPS would be their preference. For example, cancer cachexia affects greater than 50% of patients with advanced disease and its clinical definition and symptoms have been intensively discussed in recent years [16, 17]. Recently, the European School of Oncology Task Force conducted a review of the literature on cancer cachexia. They concluded that cachexia is a complex process but that, along with anorexia, the presence of a systemic inflammatory response results in the features of the disease [16]. Furthermore, Douglas and McMillan (2014) recently proposed that the mGPS be used as the basis for formation of an objective and clinically relevant definition of cachexia [17]. The findings of the present study would appear to confirm that the mGPS is the most commonly used systemic inflammation based score and therefore appropriate for forming the basis of an objective definition of cancer cachexia.

The present study has a number of possible limitations. Firstly, respondents did not have to enter their location in order to complete the questionnaire, meaning the location for all the respondents was not obtained. In all surveys there is a tension between making the sample size as large as possible in order to eliminate bias and asking questions appropriate to those surveyed. In the present survey, we targeted those with a known interest in systemic inflammation based prognostic scores (those who had already published in this field) in order to maximise the number of appropriate and meaningful responses. The mGPS and NLR are the most popular scores as they have the largest evidence base. Although other systemic inflammation based prognostic scores such as the derived NLR (dNLR), lymphocyte monocyte ratio (LMR), and platelet-lymphocyte ratio (PLR) have been reported, they have not established a sufficient body of evidence in the literature. Moreover, where they have been directly compared, the mGPS had the greatest prognostic value in patients with cancer, independent of age, sex, deprivation, and tumour stage [4, 18]. Therefore, it is likely that the results of this survey reflect the reality of attitudes towards the application of these scores in those individuals with an interest in the field. It was of interest that 43% of the respondents were surgeons. This may reflect the activity of surgeons in this field. Indeed, it is recognised that surgeons are key members of the multidisciplinary team (MDT) that decides treatment allocation. Irrespectively, this would confirm that the survey was directed at clinicians in routine clinical practice.

In summary, the present study has shown that, in those who responded, the majority routinely measured the systemic inflammatory response in patients with cancer, with the majority using the GPS/mGPS, mainly for research and prognostication purposes. The majority reported that these scoring systems were of most clinical benefit in making decisions on adjuvant therapy and palliative chemotherapy and that the systemic inflammatory response, as evidenced by the GPS/mGPS, should be adopted into clinical guidelines, such as a new, objective and clinically relevant definition of cancer cachexia.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

David G. Watt and Campbell S. Roxburgh contributed equally to this study.

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