Original Article

Understanding tumor anabolism and patient catabolism in cancer-associated cachexia

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Received March 8, 2017; Accepted April 3, 2017; Epub May 1, 2017; Published May 15, 2017

Abstract: Cachexia is a multifactorial paraneoplastic syndrome commonly associated with advanced stages of cancer. Cachexia is responsible for poor responses to antitumoral treatment and death in close to one-third of affected patients. There is still an incomplete understanding of the metabolic dysregulation induced by a tumor that leads to the appearance and persistence of cachexia. Furthermore, cachexia is irreversible, and there are currently no guidelines for its diagnosis or treatments for it. In this review, we aim to discuss the current knowledge about cancer-associated cachexia, starting with generalities about cancer as the generator of this syndrome, then analyzing the characteristics of cachexia at the biochemical and metabolic levels in both the tumor and the patient, and finally discussing current therapeutic approaches to treating cancer-associated cachexia.

Keywords: Cachexia, cancer, biochemistry, metabolism

Introduction

Although there have been important advances in cancer therapy aimed at different types of neoplasia, achievements have commonly been directed at treating the tumor instead of the concomitant syndromes that are present due to metabolic aberrations caused by the presence of the malignancy. One of the most relevant syndromes that increases as cancer progresses is cachexia, which compromises the life of the patient and irremediably causes weakness and death. Since there is increasing evidence demonstrating the implications of systemic biochemical pathways in the initiation and development of cancer-associated cachexia, in this review, we will focus our discussion on biochemical tumor aberrations and their impact on the maintenance of cachexia as well as on the host damage at different levels due to the chronic systemic inflammation induced by the presence of cancer. We will also discuss current therapies that attempt to obstruct the progression of cachexia in cancer.

Cancer as a metabolic entity

Cancer is commonly seen as a plethora of diseases that modify the cellular metabolism for the continuous preservation of proliferative signaling with an immortal replicative state of cells while they evade anti-growth signals, immune suppression and cell death [1]. For a healthy cell to transform into a malignant cell, it must develop genomic instability that permits mutability for the overexpression of oncogenes such as the transcription factor c-Myc, growth factor receptors such as epidermal growth factor receptor (EGFR), signal transduction proteins such as Ras and phosphatidylinositol-3' kinase (PI3K), and inhibitors of apoptosis such as Bcl2 [1]. Additionally, tumor suppressor genes, which include proteins that inhibit cell division and cell proliferation (such as p53 and p16INK4a) and those related to the stimulation of cell death (p53), become inhibited in cancer [1]. However, the upregulation of oncogenes is not sufficient for the tumorigenesis process, and rapidly dividing cells need to increase their ATP production for high energy demands, increase the biosynthesis of biomolecules, and regulate the reduction-oxidation state [2]. This is where tumor metabolism must intervene to secure the success of malignant cells.

Metabolism, which is composed of interrelated biochemical reactions that promote the prolif-
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1108 Am J Cancer Res 2017;7(5):1107-1135

Mechanical obstruction

Tumour mass

Chronic systemic inflammation

Muscle wasting

Adipose wasting

Anorexia and weight loss

Cachexia

Figure 1. Elements of cancer-associated cachexia. Neoplasia generates cachexia through the chronic presence of systemic inflammation, which is associated with muscle and adipose wasting as well as anorexia. Anorexia can also be promoted by the gastrointestinal obstruction caused by the physical presence of the tumor mass. Together, these aberrations lead to weight loss and, irremediably, to cachexia.

Generalities of cachexia

Etymologically, the word “cachexia” refers to a poor disease prognosis; this term originates from the Greek *kakos* and *hexia*, meaning “bad condition” [9, 10]. Cachexia is a multiorgan syndrome characterized by a progressive and involuntary loss of body weight [11, 12], particularly from skeletal muscle and adipose tissue [13, 14] due to alterations related to carbohydrate, lipid and protein metabolism [15]. Indeed, according to the literature, the primary tissues affected during the progression of cachexia are both skeletal muscle and white adipose tissue [16]. While the prominent clinic-
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The origin of cachexia is associated with reduced food intake along with abnormal metabolism induced by factors derived from both the tumor and the host, which irremediably lead to weight loss [20]. Cachexia involves an energy imbalance resulting from anorexia and an increase in energy expenditure derived from the hypermetabolic condition of the underlying disease [15]. Therefore, cachexia is considered a state of “autocannibalism” in which the tumor grows at the expense of the health of the subject [20] through the consumption of biomolecules necessary for the function of other organs. Typically, advanced cancer individuals develop cachexia [21], but it can also be present with localized neoplasia [22]. However, cachexia is not a pathognomonic syndrome in cancer. It can also occur in advanced stages of several diseases, including chronic obstructive pulmonary disease, malabsorption, chronic heart failure, acquired immunodeficiency syndrome, severe sepsis and trauma [11, 23]. When present in cancer, cachexia is the cause of death of close to one-third of patients [24-26], mostly when weight loss exceeds 30% [27]. Furthermore, the development of cachexia is related to an increase in chemotherapy toxicity and mortality [18, 28].

Up to 50-80% of advanced cancer patients will experience cachexia during the course of their disease [25, 29, 30], but this percentage is variable depending on the specific type of neoplasm. Cachexia is more common in tumors of upper gastrointestinal tract origin because these tumors may lead to obstruction and, consequently, to a reduction in food intake [20], as will be discussed in the next section.

Due to the complex clinical findings and lack of medical classifications for cachexia, a 2006 international consensus graded cachexia into cachexia and pre-cachexia. This group defined pre-cachexia as the medical condition of <5% body weight loss over a period of 6 months that is related to a primary chronic disease and characterized by metabolic alterations, inflammation and anorexia [17]. Cachexia, on the other hand, can be defined as a weight loss of >5% over the same period of time, also secondary to a chronic disease and with the same systemic alterations [31]. This new term, pre-cachexia, can be employed in epidemiological and intervention studies aimed at preventing or delaying changes in body composition associated with chronic diseases [32].

The diagnosis of cachexia should exclude other clinical conditions, such as primary depression, starvation, hyperthyroidism, malabsorption, and age-related muscle loss [18]. However, the growing prevalence of obesity and sarcopenic obesity may hinder the diagnosis of cachexia. In fact, in cancer patients with an elevated body mass index and unplanned weight loss of ≥5% could pass unnoticed, and clinical intervention would thus be delayed [18]. Therefore, it is recommended that the body composition of patients should be continuously assessed by computed tomography (CT) image analysis or dual-energy X-ray absorptiometry (DEXA) to analyze fat and skeletal muscle depots [18].

Tumoral origins of cachexia

The specific etiology of cancer-related cachexia is complex, and it may be incompletely understood in some patients. Additionally, the heterogeneity of the clinical presentation of cancer-related cachexia can lead to its underdiagnosis. In this section, we will describe in detail the pathophysiology of the alterations induced by the tumor that irremediably lead to cachexia, starting with the mass effect of the neoplasm, which retards food transit toward the gastrointestinal tract, continuing with the chronic systemic inflammation that is generated as a response to the presence of the tumor, and ending with the biochemical disruption inside the cancer cells that promotes cachexia.

Mechanical influence of the tumor in cachexia

One specific effect of the tumor on the patient is its mechanical impact on the digestive tract, which reduces food ingestion and in turn may promote anorexia, therefore leading to dimin-
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Figure 2. The effects of chronic systemic inflammation are strong promoters of cancer-induced cachexia. A permanent and uncontrolled inflammatory environment has multiple effects on the host at different levels in the pathogenesis of cachexia. LIF is a recognized inducer of myotube atrophy that damages myocytes. CNTF inhibits the gene expression of neuropeptide Y, a potent appetite stimulant in the arcuate nucleus of the brain. VEGF, PGE₂, MMP-9 and an acidic environment are associated with tumoral angiogenesis. Furthermore, a reduction in the pH of the tumor microenvironment stimulates the expulsion of acetate from malignant cells, which promotes histone acetylation aberrations within the tumor mass. Both IFN-γ and TNF-α block myosin heavy chain mRNA production to minimize the myogenesis process. Moreover, TNF-α-induced NF-κB functions as an alternative route to impede myogenesis via the blockade of myoD. Lipolysis is indirectly allowed through the NF-κB-mediated inhibition of perilipins. TNF-α also induces oxidative stress in muscle, which degrades muscle proteins. The upregulation of IL-6 is associated with inhibition of PGC-1α, which makes systemic cells susceptible to reactive oxygen species damage secondary to a reduction in mitochondrial biogenesis. IL-6 and CRP are promoters of weight loss. Abbreviations: LIF: leukemia inhibitory factor; CNTF: ciliary neurotrophic factor; pH: potential of hydrogen; VEGF: vascular endothelial growth factor; MMP-9: metalloproteinase 9; PGE₂: prostaglandin E₂; IFN-γ: interferon-γ; TNF-α: tumor necrosis factor α; NF-κB: nuclear factor kappa beta; myoD: myogenic differentiation I; PGC-1α: peroxisome proliferator-activated receptor gamma co-activator 1-α; IL6: interleukin 6; CRP: C-reactive protein; ROS: reactive oxygen species.

ished body weight. Indeed, close to 50% of cancer patients at diagnosis affirmed irregularities in their eating behavior, and this percentage grew to 65% in terminally ill cancer patients [33].

The proportion of patients who experience cancer-associated cachexia depends on the specific type of cancer and its state of progression [8]. The reported frequency of weight loss was 30-40% in patients suffering from acute non-lymphocytic leukemia, non-Hodgkin’s lymphoma or breast cancer, while the frequency of weight loss was close to 60% in both colon and pulmonary cancers [9, 33, 34]. On the other hand, the highest incidence of weight loss can be found in tumors of upper gastrointestinal origins, such as esophageal and head and neck cancers (with an incidence over 70%) and particularly in pancreatic and in gastric cancers (with a frequency over 80%) [9, 33, 35, 36]. This effect can be associated with stenosis of the gastrointestinal tract, particularly in head and neck, esophageal and gastric malignancies, due to primary dysphagia, which mechanically limits food ingestion [37]. In the case of pancreatic cancer patients, tumor invasion can obstruct the pancreatic duct and the second part of the duodenum, which induces pain, gastroparesis, duodenal stenosis, and constipation, among other symptoms [38].

Another consequence of the presence of the tumor is early satiety, which at any stage of cancer is linked to a 30% increase in the risk of death [33]. Early satiety is related to malabsorption secondary to alterations at the mucosal level as well as to the obstruction of food passage through the gut [34]. Indeed, obstruction is common in bowel neoplasia and tumors of the abdominal area, with a frequency ranging from 4 to 24% in colorectal cancer and 5 to 42% in ovarian tumors [39]. Additionally, abdominal tumors can disturb motility and promote ileus, which may contribute to emetic symptoms, which minimize food ingestion [37].

Initiation of cachexia by tumor-induced chronic systemic inflammation

Inflammation is acknowledged as a driving force in several chronic diseases and functions as a strong outcome predictor in the patient. In this subsection, we will cover the general implications of systemic inflammation in cachexia. Subsequently, in each section of this review, we
will discuss the specific role of inflammation in every aspect of cancer-associated cachexia.

According to one proposed mechanism for the development of cancer cachexia, it is the result of a global physiological response driven by the increase in the chronic production and secretion of pro-inflammatory cytokines as the disease progresses [40] (Figure 2). Cytokines are proteins that act as paracrine intercellular mediators, and they can induce or inhibit the immune response. Chronic inflammation is the consequence of permanently elevated pro-inflammatory cytokine levels, in opposition to the acute inflammation represented by cytokine waves [41]. In fact, the notion of a continuous systemic inflammatory background helps to distinguish this syndrome from other conditions, such as anorexia [26].

It is recognized that the acute-phase response is produced by the presence of the tumor itself [26]. Innate immune effectors, such as macrophages, are some of the principal sources of immune mediators, such as tumor necrosis factor (TNF-α) [26]. Immunohistochemical analyses of subcutaneous fat tissue from gastrointestinal cancer cachexia patients have revealed abundant macrophage markers, including CD68 [42]. Currently, there are controversial results regarding the presence of high circulating TNF-α levels in cancer cachexia, which could be due to the short half-life of TNF-α and/or its possible localized paracrine secretion [43]. TNF-α, together with interleukin (IL)-1, promotes the activation and nuclear translocation of nuclear factor-kappa beta (NF-κB) to alter gene expression, which causes catabolic signals that induce protein loss in skeletal muscle cells through specific muscle ubiquitin ligases [43-45], as will be discussed later. TNF-α also stimulates lipolysis in human adipocytes through the activation of extracellular signal-regulated kinases (ERKs) and mitogen-activated protein kinase (MAPK), leading to the malfunction of perilipins [46], which regulate the integrity of lipid droplets (as will be discussed later).

Other Th1 response-related cytokines associated with cachexia are IL-6 and interferon (IFN)-γ [43]. IL-6, which is mostly produced as an acute-phase protein by the liver, is related to the development of cachexia [26], and supraphysiological concentrations of this cytokine led to a reduction in lean mass [47]. However, several tumors also secrete IL-6 [47]. One proposed mechanism of the IL-6 involvement in cachexia in this regard is based on the knowledge that IL-6, through JAK signaling and the activation of the transcription factor signal transducer and activator of transcription 3 (STAT3), modulates the gene expression of acute-phase proteins, leading to mitochondrial biogenesis disruption [26]. Additionally, monoclonal antibodies against Th1 cytokines prevent body mass loss in mouse models of melanoma and prostate cancer [43, 46]. IFN-γ is predominantly synthesized by T lymphocytes and NK cells [43]. Together with TNF-α, IFN-γ is a well-known inhibitor of myosin heavy chain IIb mRNA in skeletal muscle cells [48]. In the Lewis lung tumor mouse model, early immunological therapy with monoclonal antibodies directed against IFN-γ inhibited both neoplastic growth and tumor-associated wasting [49].

C-reactive protein (CRP), an acute-phase protein released by the liver, also contributes to inflammation [41]. An increase in CRP concentrations is ubiquitously employed in different clinical scenarios to measure systemic inflammation [19]. CRP concentrations, together with the consistent hypoalbuminemia observed in cachexia patients, are utilized in the Glasgow Prognostic Score (GPS) to predict outcomes of diverse tumor types [26, 41]. One British longitudinal study of more than 20,000 patients with diverse tumor types showed a correlation between elevated CRP concentrations and hypoalbuminemia among different cancers, which suggested that the GPS might work as a prognostic factor independent of tumor site [50]. Another longitudinal study aiming to analyze the relationship between cachexia and GPS in pancreatic adenocarcinoma cachectic patients under palliative care demonstrated that elevated CRP levels were related to decreased albumin concentrations and poorer survival [19]. Indeed, albumin levels below 3 g/dL have been related to worse outcomes in patients with stage 3 or 4 ovarian cancer [51]. Furthermore, there is an association between CRP and weight loss [52]; weight loss was favored in patients with gastrointestinal cancer cachexia with serum CRP concentrations higher than 5 µg/mL [42].
Figure 3. Biochemical and metabolic changes within the tumor. Tumor cells are organized as an anabolically active group that continuously interchanges molecules with the environment. A. Usually, as the tumor progresses, its cells secrete lactate as an anaerobic product of energy metabolism. Lactate, in turn, reduces the pH of the surroundings to promote a change in the phenotype of infiltrating macrophages from M1 to M2. M2 macrophages, together with myeloid-derived suppressor cells, are associated with tolerogenic functions that enable the tumor to evade the immune action. Lactate is also mobilized to the liver with the employment of the high vascularization of the microenvironment to produce glucose via the Cori cycle, which then can return to the tumor in an endless loop. At
the same time, malignant cells release acetate from lysines within histones as a protective mechanism against the acidic pH. At the cellular level, the neoplasm develops point mutations in the genome, such as mutations that induce the Ras-Akt-mTOR pathway, to increase glycolysis within the cell. The continuous activation of glycolysis, together with the Akt-induced ACLY enzyme, led to citrate generation within the mitochondria to fuel the Krebs cycle. Malate, through the malic enzyme, increases the pyruvate concentration. Both citrate and pyruvate are transformed into acetyl-coenzyme A, which can either be employed as an acetate group donor for the DNA acetylation process or can be extruded from the cell to the microenvironment to function as a regulator of intracellular pH. Enhanced glycolysis can also be promoted via the upregulation of hexokinase II and by the high glucose concentration secondary to the HIF-1α-mediated increase in glucose transporters. HIF-1α is also related to the transcription of glutamine transporters at the cellular membrane; along with adipophilin, it induces the formation of lipid droplets in close contact with the mitochondria. These lipid droplets contain a high amount of fatty acids secondary to the high expression of fatty acid synthase by SREBPs. Fatty acids are assembled into triacylglycerides, which are lysed by the hormone-sensitive lipase to provide energy to the tumor cell. The translocation of glutamine transporters into the cellular membrane promotes an increase in intracellular glutamine, which is transformed into glutamate via the up-regulation of glutaminase, which produces ammonia as a waste product. Ammonia, in turn, is a signal for autophagy that the cancer cell employs to secure a continuous pool of energy and biomolecules for its anabolic processes. Inside the mitochondria, isocitrate dehydrogenase is transformed into an aberrant form of α-ketoglutarate termed 2-hydroxyglutarate. This reaction can be promoted by the anaplerotic reaction of glutamate, which is introduced as α-ketoglutarate by either GDH or ALT enzymes, depending on the presence of low or high glucose metabolism within the cell, respectively. Glutamate, with the help of NADPH, is associated with the generation of glutathione, which is the most potent antioxidant that safeguards the malignant cell from reactive oxygen species-mediated death. Abbreviations: FASN: fatty acid synthase; HIF-1α: hypoxia-inducible factor-1α; GLS: glutaminase; NH4+: ammonia; SREBP: sterol regulatory element-binding protein; HSL: hormone-sensitive lipase; IDH: isocitrate dehydrogenase; 2-HG: 2-hydroxyglutarate; ATP: adenosine triphosphate; GDH: glutamate dehydrogenase; ALT: alanine transaminase; NADPH: nicotinamide adenine dinucleotide phosphate; ACLY: ATP-citrate lyase; acetyl-CoA: acetyl-coenzyme A; HK2: hexokinase II; ME: malic enzyme; α-KG: α-ketoglutarate.

Other pro-inflammatory molecules involved in inflammation-associated cachexia are leucemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF). LIF is a pleiotropic cytokine produced, among others, by embryonic stem cells [53]. LIF acts via STAT3 to induce myotube atrophy in vitro [54], and a murine model implanted with LIF-secreting neoplasms developed cachexia [43]. On the other hand, CNTF is mostly produced by glioma cells of the peripheral nervous system, and it induces anorexia and body weight loss through the repression of neuropeptide Y (NPY) gene expression in the hypothalamic arcuate nucleus (ARC) [55]. Accordingly, it was observed that mice engrafted with glioma cells experienced strong cachectic effects [43].

In inflammatory environments, local cells express molecules associated with leukocyte trafficking, including P-selectin glycoprotein ligand 1 [56]. In fact, myeloid suppressor cells are locally concentrated around the tumor and produce matrix metalloproteinase (MMP)-9, which promotes cancer angiogenesis [26]. Since healthy mammalian cells are located 100-200 µm away from blood vessels due to the diffusion limit of oxygen, when the cell mass grows beyond this limit, it is mandatory to recruit new blood vessels via angiogenesis or the intussusception of pre-existing capillaries or post-capillary venules [57, 58]. Particularly in cancer, a neoplasm can only continue to grow and metastasize with an efficient blood supply; angiogenic signals, such as the metabolic stress induced by local low pH and the pressure promoted by proliferating cells, are provided in the tumor microenvironment to induce the production of vascular endothelial growth factor (VEGF) and angiopoietin factors, which, in turn, induce angiogenesis [57]. Angiogenic promoters are also stimulated with the secretion of prostaglandin E2 (PGE2) by the immune cellular infiltrate enriched with the cyclooxygenase (COX)-2 enzyme, which in breast cancer cells has been shown to bind to G-protein receptors to promote both proliferation and tube formation in endothelial cells via the generation of pro-angiogenic factors, including VEGF [59]. PGE2 is also produced in prostate cancer cells under hypoxic conditions, where it promotes hypoxia-inducible factor (HIF)-1α nuclear accumulation [60]. HIF-1α, a transcription factor responsible for the major transcriptional responses of over 100 genes under hypoxic conditions, is stable under limited oxygen concentrations and dimerizes with its β subunit to regulate neovascularization, intracellular pH regulation, cell survival, tumor growth and energy metabolism, particularly by increasing the expression of transporters and enzymes associated with glycolysis [5], as will be discussed later.
The anabolic phenotype of the tumor

Cachexia is characterized by a negative protein and energy balance [56] due to the hypercatabolic activation of both protein and glucose, together with fat degradation, by systemic inflammation [15] (Figure 3). In this subsection, we will discuss the anabolic aberrations in glycolysis, glutaminolysis and fatty acid synthesis that are associated with the progression of tumor and the development of cachexia.

Increase in aerobic glycolysis and mitochondrial malfunction

To preserve homeostasis, all living entities on earth are dependent on cellular energy in the form of ATP, the universal currency of metabolic reactions. ATP is generated in eukaryotes from glucose via glycolysis, which produces pyruvate in the cytosol. Pyruvate is then oxidatively metabolized in the mitochondria to CO₂ and water through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS), respectively [61-63]. Although ATP is produced and consumed at almost the same rate under normal circumstances, energy wasting is one of the most prominent elements of cachexia that promotes the persistence of the underlying illness. In cancer, one possible explanation for this phenomenon is the existence of high glucose catabolism, which constantly provides energy for the tumor.

Most cells are commonly exposed to a constant supply of nutrients, but they are not able to accept them unless they are appropriately stimulated by growth factors [64]. In cancer, however, cells acquire genetic mutations (such as in the oncogenes hras or kras) [63] that may modify receptor signaling pathways for the continuous uptake of diverse nutrients [64]. Cells transformed with Ras increase the macropinocytosis process for the uptake of extracellular molecules, particularly glucose [7]. Otto Warburg demonstrated that after glucose is internalized by cancer cells, it is employed to produce ATP by glycolysis rather than by OXPHOS, even in the presence of oxygen; interestingly, oxygen is consumed at the same rate as in normal tissue cells [65, 66]. This process, known as the ‘Warburg effect’ or ‘aerobic glycolysis’, produces ATP less efficiently and requires increased glucose consumption (almost tenfold the level of many healthy cells in the same amount of time) [61, 65].

The introduction of glucose into the cell is dependent on its membrane translocation by glucose transporters (GLUTs), which is mediated by the recognition of insulin by insulin receptors [67] and can be perturbed by the K-Ras oncogene [68, 69] and the oncogenic transcription factor c-Myc [70]. While under healthy circumstances, c-Myc links the cell cycle with mitochondrial biogenesis, the upregulation of c-Myc is associated with an increase in the respiratory capacity of the cell by the elevated mitochondrial replication and metabolism required to sustain rapid proliferation [71]. However, under oncogenic transformation, glucose transport could occur independently of insulin [72], such as by HIF-1α [60], in tumors under inflammatory and thermogenic conditions, in both normoxia and hypoxia [73]. Additionally, HIF-1α is induced under oncogenic pressure by, among others, H-Ras, Her2, and FRAP, as well as by the downregulation of tumor suppressors such as VHL, PTEN and p53 [73]. HIF-1α is regulated by the Ras-Raf-MEK-ERK signaling pathway [73], and it binds to the glut1 promoter site to stimulate the expression of GLUT1 mRNA, which, in turn, internalizes glucose to the cell [74]. Once inside, the first step in glycolysis is the irreversible conversion of glucose to glucose 6-phosphate, catalyzed by hexokinases (HKs) I-IV [63, 75, 76]. Specifically, HK-II is the predominant isoform in insulin-sensitive tissues [76] and can be upregulated up to 100-fold in cancer [75]. Furthermore, HK-II competitively binds to the voltage-dependent anion channel in the outer mitochondrial membrane, which prevents the union with the pro-apoptotic molecule Bax and, in turn, inhibits cytochrome c release; therefore, the malignant cell evades apoptosis [1]. On the other hand, pyruvate kinase M2, the enzyme that controls the generation of pyruvate in the last step of glycolysis, suffers from decreased activity under oncological conditions, which indirectly promotes the redirection of glycolytic intermediates into the biosynthesis of other biomolecules to promote anabolism in the tumor [77].

In fermentation, the Warburg effect generates high quantities of lactate [78] by the HIF-1α-induced lactate dehydrogenase [5]. Lactate can be employed to synthesize glucose via the

Figure 3

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Cory cycle in the liver, an energetically inefficient pathway that requires 6 ATP molecules from the host for each glucose molecule produced for the tumor [12], and to recycle the reduced form of β-nicotinamide adenine dinucleotide (NADH) to its oxidized form (NAD⁺) for glycolysis [78]. Additionally, lactate induces acidosis in the tumor microenvironment [62]. Lactate can be removed from the tumor cell by monocarboxylic acid transporter (MAT)-4, a protein upregulated by HIF-1α, with the symport employment of hydrogen ions, which in turn lowers the extracellular pH [5]. The acidic environment is harmful to normal cells, but cancer cells seem to tolerate [66] and even take advantage of it, since the high abundance of hydrogen affects the uptake of weak base chemotherapeutic drugs, including doxorubicin [6]. The microenvironmental pH can lower the intracellular pH, which stimulates the extrusion of acetate as a mechanism to eliminate protons from the cell to maintain its homeostatic pH [79]. Additionally, with glycolysis, there is reduced production of reactive oxygen species (ROS), allowing the genome of neoplastic cells to elude the damage provoked by ROS and resulting in apoptosis resistance [66]. Indeed, the 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) technique has revealed a poor prognosis in patients with tumors showing increased glucose uptake, and in vitro, the non-invasive MCF-7 breast cancer cell line consumes less aerobic glucose than the highly invasive MDA-MB-231 breast cancer cell line [63].

Another target site of energy wasting in cachexia is mitochondria [80]. Healthy mitochondria combust substrates for the generation of ATP; in the process, some energy is released as heat secondary to an inefficient coupling of ATP synthesis to oxygen consumption [81]. Part of this energy is employed in proton leak reactions, characterized by the passage of the protons out of the mitochondrial matrix back into the mitochondria by the employment of proton conductance pathways that avoid the ATP synthase [82]. Proton leak has been linked to both phospholipids and proteins in the mitochondrial inner membrane, particularly polyunsaturated fatty acids, such as the mitochondria-specific cardiolipin, and uncoupling proteins (UCPs) -1, -2 and -3, which are involved in uncoupled respiration [83]. In this process in particular, respiration is not coupled to ATP production and therefore produces heat through the dissipation of the mitochondrial proton gradient [84]. The UCPs are mitochondrial anion carriers of the inner membrane that play a thermogenic role [85] and exert a “browning” effect on white adipose tissue (WAT) [10], as will be discussed later. UCP-1 is a protein mostly expressed in brown fat, while UCP-2 is observed in most tissues, and UCP-3 is found in thermogenic tissues including skeletal muscle [86]. Due to their function, the presence of UCPs is related to a decrease in oxidative capacity by the mitochondrial OXPHOS complex IV [87]. In fact, such proteins have been linked to a lean phenotype in transgenic mice [88]. While the mRNA level of UCP-1 has been observed to be increased in the brown adipose tissue (BAT) of cancer cachectic mice [16], the UCP2 gene was overexpressed in skeletal muscle from cachectic rats [87], and UCP-3 mRNA levels were more than five-fold higher in cancer cachectic patients compared with controls and with patients without weight loss [16]. Interestingly, a transgenic mouse model overexpressing the UCP-3 protein in skeletal muscle exhibited a lean phenotype and even displayed hyperphagic behavior, with a 50% increase in food ingestion compared with non-transgenic controls [89]. This, together with the similar plasma concentrations of both triglycerides and non-esterified fatty acids in controls and transgenic mouse models, suggests that fat combustion was higher in the latter [89].

The number and morphology of mitochondria within a given cell vary with cell type, and mitochondrial dysfunctional has been linked to cancer [90]. Since mitochondria are required for the production of key metabolites for bioenergetic processes such as NAD⁺, ATP, α-ketoglutarate (α-KG) and acetyl-coenzyme A (acetyl-coA), mutations in the mitochondrial genome are associated with altered gene expression [91]. The mitochondrial enzyme isocitrate dehydrogenase (IDH), which catalyzes the formation of α-KG in the Krebs cycle, has mutant forms in cancer that produce 2-hydroxyglutarate (2-HG) instead of α-KG [92]. In turn, 2-HG is associated with the induction of the transcription factor nuclear factor kappa beta (NF-κB) via ROS-dependent extracellular signal-regulated kinase (ERK) activation to promote the proliferation of malignant cells [93]. Furthermore, mitochon-
drial morphological changes, including the presence of electron-lucent areas and swelling, which are indicative of crista loss and ATP depletion, respectively [94], as well as the loss of the normal homogeneous matrix, have been reported in the mitochondria of the gastrocnemius muscles in the colon-26 carcinoma mouse model of cachexia [95]. Furthermore, mitochondria can be found with different morphologies, including punctate, intermediate or filamentous, based on computational 3-D modeling algorithms [90]. Interestingly, punctate mitochondria are correlated with increased glycolysis levels and decreased oxygen consumption [90]. All the aforementioned events are suggested to be related to defective OXPHOS and therefore to a reduction in the production of ATP [95].

The role of “glutamine addiction” in glutaminolysis

Both glucose and glutamine are highly metabolized by several neoplastic cells for the production of amino acids, ribonucleotides, lactate, glutathione and ammonium ions through glutaminolysis [78, 96, 97]. If the cell enters a highly proliferative state that is no longer sustainable with the employment of glucose derivatives alone, then glutamine becomes a major energy source [98].

Glutamine is a five-carbon non-essential amino acid found at a concentration of 0.6-0.9 mmol/L in plasma [99] and is the main amino acid that transfers carbons and nitrogens from proteolysis to central tissues for further processing [78]. Indeed, glutamine is the most abundant amino acid in plasma; almost one of every five circulating amino acids is glutamine [100]. The cell transporters of the SLC6 family, including SLC6A19 and SLC6A14, use Na+ transmembrane gradients for the uptake of neutral amino acids into the cell, including glutamine [98]. Other cell transporters for glutamine belong to the SLC38 family, particularly SLC38A1, SLC38A3, SLC38A5, and SLC38A7, which are specific for glutamine [98, 101]. For the purpose of energy generation, glutamine also needs to enter mitochondria; it has been hypothesized that the SLC25 family is responsible for this process [98]. The diversity of glutamine transporters reveals the pleiotropic distribution of this amino acid in the body. In the brain in particular, it is employed for the glutamine/glutamate cycle due to the high loss of the excitatory neurotransmitter glutamate [98, 102]. However, if the cell harbors low levels of ATP, the glutamine/glutamate cycle is disrupted, and the equilibrium is shifted to the creation of glutamate [97].

Glutamine is an essential source of anabolic metabolism in highly proliferating cells, including tumors [98, 99]. In elevated energy demand states, including cancer, endogenous glutamine is insufficient to fulfill survival requirements, and it must be taken up from other corporal sites [98]. Commonly, glutamine is released from skeletal muscle, and to a less extent from lungs, by proteolysis in periods of metabolic stress to be internalized by the tumor [97, 100]. Once inside the neoplastic cell, glutamine is deamidated via glutaminase (GLS) 1 and 2 into glutamate and ammonia in the mitochondrial matrix [98, 100, 103]. A high ammonia concentration, together with PI3K-Akt-mTOR signaling [2], is a signal that activates autophagy via mitochondrial dehydrogenase and caspases 3 and 7 [104], which is useful for malignant cells to recycle cellular molecules into metabolic precursors and therefore to extend cell survival [97]. Furthermore, the GLS product glutamate, together with the γ-glutamylcysteine synthetase, stimulates the generation of the major cellular antioxidant glutathione to give tumor cells the advantage of higher resistance to chemo- and radiotherapy approaches [97, 100, 105]. In fact, one metabolomics study conducted in 138 clear-cell renal cell carcinoma samples revealed that higher levels of both glutathione and glutamine were found as the tumor progressed and generated metastasis [106].

Glutamine supplies the TCA cycle by replenishing α-ketoglutarate in a two-step reaction of deamination with the help of glutamate dehydrogenase (GDH) and/or aminotransferases in a process called anaplerosis [107-109]. These reactions may occur in either the cytosol or the mitochondria, according to the glucose concentration in the cell: when glucose metabolism predominates, the transamination pathway with the alanine transaminase (ALT) enzyme is preferred; otherwise, GDH is employed [100]. One molecule of glutamine can theoretically produce 8 NADH, 3 FADH2, and 3 GTP molecules.
after its complete oxidation [98]. Actually, some cancer cells depend on the oxidation of glutamine for the synthesis of more than 50% of all their ATP [110], which has led to the term “glutamine addiction” being applied to those cells [100, 109, 111].

Even without entering the TCA cycle, as in glioblastoma, glutamine is synthesized from glutamine synthetase to support nucleotide biosynthesis for tumor growth [99]. However, glutamine can also be produced by the transformation of the amino acid proline via proline dehydrogenase to pyrroline-5-carboxylate, which then is converted to glutamate and finally to glutamine [77]. The promotion of cellular proliferation in cancer is also enhanced by an excess of glutamine inside the cell, which may be exported to exchange for essential amino acids; this facilitates the activation of the serine/threonine kinase mTOR, which positively regulates cell growth [100]. The process of tumor growth requires growth factors as well, which are glycosylated by hexosamines that use the nitrogen skeleton provided by glutamine [100].

The relevance of glutamine in cancer survival and progression is correlated with the mutations expressed by the specific cell line, as has been demonstrated by several studies. If a Myc-overexpressing cell experiences glutamine deprivation, it will undergo apoptosis; if a cell overexpresses K-Ras and is not allowed to metabolize glutamine, it will arrest in the mitotic S- and G2/M-phases [109]. However, normal cells are also dependent on glutamine, as its deprivation establishes a blockade in the G1 phase of the cell cycle [112]. In line with this notion, GLS activity has been shown to be correlated with tumor growth and malignancy, and its suppression inhibits tumor growth [100]. Furthermore, glutaminolysis is associated with cisplatin resistance in gastric cancer [4].

Promotion of de novo fatty acid synthesis

In healthy humans, the de novo synthesis of fatty acids (FAs) only occurs in adipose tissue, liver, kidney and lactating breast tissue [113]. This process is a sequential enzymatic reaction that relies on fatty acid synthase (FASN), a multifunctional polypeptide encoded in the 17q25 region of the human genome [115]. This complex possesses seven catalytic domains and acts as an acyl-carrier enzyme that catalyzes the repeated condensation of two-carbon groups derived from malonyl-coenzyme A (malonyl-CoA) to an acetyl-CoA primer from citrate by the ATP-citrate lyase (ACLY) [59] to generate the saturated 16-carbon FA palmitate [113, 116, 117]. Palmitate then can be elongated and desaturated to form multiple lipid classes [113] with the help of the acyl carrier protein (ACP), which mobilizes the FA cargo between enzymes to generate the final lipid [118]. After activation through coupling to CoA, FAs are incorporated into triacylglycerides for energy storage or into sterols, glycerophospholipids and sphingolipids for membrane generation and signaling functions [113, 114]. In particular, such molecules are employed at the cellular membrane to construct microdomains known as lipid rafts, which are sites of co-localization of proteins that form signaling complexes for transduction networks [119].

In healthy subjects, most lipids are acquired from dietary fat, and cells prefer to employ circulating lipids rather than de novo lipids [116]. In that sense, FASN is expressed at low levels under normal conditions. The regulation of this enzymatic complex relies on growth factors, insulin, carbohydrate and fat ingestion, glucocorticoids, exercise and thyroid hormone [117, 119, 120]. Additionally, FASN is under the transcriptional control of sterol regulatory element-binding proteins (SREBPs) [113], which are induced through the PI3-K-Akt-mTOR pathway [117] and are negatively regulated by the tumor suppressors p53 and retinoblastoma (Rb) [1].

However, under neoplastic conditions such as breast, ovarian, renal, prostate and colorectal cancers, FASN is overexpressed independently of the levels of circulating lipids [113, 120], and an increase in its expression has been correlated with the stage progression of the malignant disease [119, 121]. Furthermore, the upregulation of FASN is related to gemcitabine and radiation resistance in pancreatic cancer and to docetaxel/trastuzumab/adriamycin resistance in breast cancer [4]. The increase in FASN expression is more evident in steroid-responsive tumors, which commonly store lipids, such as breast and prostate cancers [60]. Particularly in the prostate cancer cell lines PC-3 and LNCaP, there is a notable increase in
the fasn gene copy number; however, it is more common to find upregulation of the transcriptional expression of fasn [115, 117]. One study of prostate cancer cell lines showed that the expression of the lipogenic transcription factor SREBP-1 was associated with the induction of FASN expression for the generation and accumulation of lipid droplets in prostate cancer cells [122]. Furthermore, the same study demonstrated that in a clinical prostate cancer cohort, SREBP-1 expression was increased with higher Gleason scores, which correlated with the progression of the malignancy [122].

Regardless of the source of FASN activity, the de novo synthesis of esterified FA has been previously associated with more than 90% of all esterified FA in tumor models [116]. The same signaling pathway involved in FASN activity, namely, PI3K-Akt-mTOR, is a common signature of aggressive tumors since it is involved in glucose uptake, protein synthesis, and cell growth and survival [119]. However, the high dependence on FASN of neoplastic cells has a severe consequence: the inhibition of FASN, such as by the anticancer drug orlistat, leads to apoptosis in tumors, while normal cells remain almost unaffected [117, 120]. This response might be encouraged by the reduced fat absorption in the gut of murine cancer cachexia models [123].

Activation of de novo FA synthesis would render the tumor less dependent on the local blood supply and would promote cell growth and survival in insufficiently vascularized cancer cells [113]. Under conditions of lipid excess, tumors store lipids as droplets via HIF-1α and adipophilin for protection against ROS [60]. Moreover, lipid droplets tend to be present in direct contact with mitochondria, and it has been suggested that this conformation allows cells to rapidly mobilize lipids in stressful situations [124]. Indeed, there is a progressive increase in the number of lipid droplets in muscle cells with the advance of cachexia in cancer patients [124], since a predisposition for the generation of such droplets is related to protection from death by starvation because autophagy allows cells to sustain their energy supply under stress [59]. Furthermore, the presence of lipid droplets in tumor cells is associated with cancer progression [125]. In fact, in cancer patients with cachexia, there is an upregulation of hormone-sensitive lipase (HSL) mRNA, which regulates the lipolysis of triacylglycerol molecules in lipid droplets [46].

FASN is crucial for de novo lipid synthesis, but the precursors of FAs are relevant as well. The acetyl-CoA molecule is primarily generated from glucose, such as in human mammary epithelial cells, where the oncogene Ras induces the serine/threonine kinase Akt, which activates glycolytic metabolism [119]. Moreover, acetyl-CoA can be obtained from glutamine or FAs through anaplerotic reactions to produce the TCA cycle molecule citrate [126, 127]. Irrespective of its origin, citrate is a well-known precursor of acetyl-CoA, which requires ACLY, an enzyme upregulated under oncological conditions [97, 109] and activated by Akt [59]. Interestingly, since Akt enhances the nuclear translocation of SREBPs to promote FA synthesis while directly phosphorylating ACLY to stimulate FA synthesis, Akt links increased glycolysis with amplified lipogenesis in malignant cells [1]. In such cells, mainly under hypoxic conditions and as an alternative to glucose, there is an increase in the capture of acetate, which can donate carbons to sustain the acetyl-CoA pool [126]. Currently, when glutamine is the major energy source in the cell, mitochondria export the malate generated by the TCA cycle enzyme fumarase into the cytosol, and this malate is then transformed to pyruvate by malic enzyme (ME) [98]. This reaction produces a secondary product, nicotinamide adenine dinucleotide phosphate (NADPH), for FA and glutathione synthetase (GSH) production [98]. Furthermore, under hypoxic conditions, glutamine provides a carbon skeleton for lipogenesis through α-ketoglutarate via the IDH1-reductive pathway [60, 98].

In additional to de novo lipogenesis, lipid catabolism is altered under oncological states. The overexpression in tumors of carnitine palmityltransferase 1 isoforms A and C, enzymes that are involved in the fat oxidation process, is induced by AMP-activated protein kinase (AMPK) and p53, and it allows cells to survive under hypoglycemic and hypoxic conditions [60]. Indeed, lipid excess is related to the phosphorylation of insulin receptor substrate 1 (IRS-1), which activates PI3K for the translocation of GLUT4 and the secondary introduction of glucose into the cell [128]. Phosphoinositide phos-
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Phosphatidylinositol 3,4,5-triphosphate (PIP₃), which is produced in response to growth factors and functions as a second messenger in the cell, acts both as a substrate for the oncogene phosphatase and tensin homolog (PTEN) and as a mediator of the recruitment and activation of Akt [59]. The aggregation of lipid rafts with diverse death receptors, including Fas and TRAIL, forms clusters of apoptotic signaling molecule-enriched rafts (CASMERs), which act as regulators of apoptosis signals in cancer cells [125]. In contrast, sphingolipid ceramide, which promotes growth-inhibitory pathways and apoptosis in malignant cells, is deregulated in cancer [59].

Increase in host catabolism in cancer-induced cachexia

In this section, we will shift our focus from the tumor to the patient. We will list the systemic changes that the tumor generates in the host, beginning with wasting at the muscle and adipose tissue levels and then moving on to anorexia and weight loss. In every subsection, we will analyze these modifications from the metabolic and biochemical points of view. However, it must be noted that not every patient may develop all of the discussed alterations.

Muscle mass wasting

Regardless of the definition of cachexia, there is general agreement about the necessity of loss of skeletal muscle mass, with or without reduction in body fat reservoirs [18]. Skeletal muscle atrophy and loss could be induced by disuse, muscle denervation, decreased food intake, cachexia and sarcopenia, among other causes [129]. Muscle wasting in cachexia, which should not be confused with sarcopenia since the latter is related to the biological process of aging [130] and does not involve either muscle protein degradation or inflammation [18], is directly related to weakness in patients [16] and has been recognized as a predictor of obscure treatment outcomes and increases in chemotherapy toxicity and mortality [18]. In fact, unlike other conditions that are conducive to loss of muscle mass, aggressive caloric supplementation is unable to reverse muscle wasting under cachectic conditions [18].

Skeletal muscle stores almost half of the whole-body protein mass in young adults [131, 132]. In healthy individuals, there is a normal balance between catabolic and anabolic processes in skeletal muscle, which requires a constant renewal of muscle protein to maintain the muscle mass [18]. Under physiological conditions and concomitantly with an increase in age, the loss of muscle mass is accompanied by gains in fat mass, with the lower-limb muscle groups being the most notable area of this transition [132]. This series of events is accompanied by a nearly 40% reduction in basal protein synthesis rates, loss of functionality and diminished skeletal muscle oxidative capacity [132]. In cachexia, however, there is solely an accelerated process of skeletal muscle mass loss secondary to the underlying clinical condition [133]; this loss can be as high as 75% in patients with cancer cachexia and advanced metastatic disease compared with controls [18]. Actually, muscle wasting may be present early in cachexia, and much of the weight loss in such patients can be attributed to the loss of skeletal muscle mass, while the protein content in visceral organs is relatively preserved [18]. Several molecules have been suggested as possible targets in muscle undergoing cachexia, including actin, actomyosin, and myosin [133].

Nevertheless, the primary catabolic activators triggering muscle cachexia may be those involved in the immune response (Figure 4). It is recognized that an elevated neutrophil:lymphocyte ratio and CRP are related to low skeletal muscle mass [134], and the latter has been employed to predict outcomes in patients with biliary, colorectal and prostate cancers [26]. Other immune mediators related to the Th1 response, which include the cytokines IL-1β, IL-6, IFN-γ, and TNF-α, are also associated with muscle wasting in cachexia [18]. The cytokines that are involved in the Th1 response, such as IL-1β, IFN-γ and TNF-α, activate NF-κB, which in turn reduces muscle protein synthesis [135] and promotes an increase in muscle catabolism [136]. Such cytokines are also related to the downregulation of the expression of the master regulator of myogenesis, the myogenic differentiation 1 (myoD) transcription factor, which normally binds to the myosin heavy chain IIb promoter region to stimulate myosin expression [135]. Indeed, the expression of myoD1 was reduced in the quadriceps of cancer cachectic model mice, which secrete many...
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Inflammatory factors, such as IL-1β, IL-6 and TNF-α [137]. TNF-α, which is also known as cachectin, is associated with increased oxidative stress in skeletal muscle during cancer [15] and cooperates with IFN-γ to inhibit myosin heavy chain mRNA [135]. Initially, TNF-α was thought to play a direct role in cachexia since it is known to function as an inhibitor of lipoprotein lipase (LPL), which mediates FA uptake in adipose tissue by the hydrolysis of very-low-density lipoproteins and chylomicrons [138]. However, demonstrating a direct correlation between TNF-α and the degree of cachexia has proven complicated [26]. Conversely, levels of IL-6 have been shown to be correlated with the development of cachexia in rodent models [26], and IL-6 is recognized as a sensitive predictor of weight loss in patients with advanced small-cell lung and colon cancers [139]. In fact, one group working with recombinant adeno-associated viral vectors (AVVs) carrying IL-6 transgenes in Balb/c mice implanted with cachexia-inducing colon-26 (C26) adenocarcinoma cells showed that activin A initiated muscle wasting after 7 days due to the upregulation of atrogin-1 and MuRF1, and it promoted an increase in both the expression of the autophagy indicator LC3A1 and in its transformation into the phosphatidylethanolamine-conjugated form, which was correlated with the number of autophagosomes [47]. The same group demonstrated that the effects of activin on skeletal muscle cells were potentiated by IL-6, although both cytokines also worked together to promote reductions in WAT mass and adipocyte size through the activation of the FA catabolism pathway and browning of WAT [47]. With the blockage of IL-6 activity, it is possible to revert skeletal muscle wasting in vivo [140]. Additionally, cytokines promote the secretion of catecholamines and cortisol from the adrenal gland, which in turn increase the metabolic rate at rest and activate the ubiquitin-related proteolytic pathway in skeletal muscle cells, respectively [135]. Nonetheless, it has been suggested that even chemotherapies based on cisplatin, adriamycin, etoposide or CPT-11 alone may directly promote muscle wasting via activation of the NF-κB pathway, which leads to degradation via the ubiquitin-proteasome pathway [141].

In cancer cachexia, skeletal muscle protein degradation commonly occurs to maintain the supply of amino acids for the tumor. However, in the early stages of muscle degradation, free amino acids may be employed by the organism to be transformed in the liver and other tissues into substrates for gluconeogenesis and acute-phase protein synthesis [142]. Alanine, aspartic acid and glutamic acid are amino acid ana-
logs of α-keto acids, all of which can be found in muscle fibers [78]. Among the diverse proteolytic events that occur in cachexia, it seems that the triphosphate-dependent ubiquitin-proteasome proteolytic pathway is the most important for the degradation of proteins [15, 18, 142]. This system is induced through the upregulation and activation of the muscle-specific E3 ubiquitin ligases muscle RING finger 1 (MuRF1) and muscle atrophy F-box (MAFbx/atrogen-1), which selectively ubiquitinate specific substrates in skeletal muscle proteins to mark them for degradation by the proteasome [18, 129, 142, 143]. The transcriptional activation of both MuRF1 and MAFbx is increased up to seven- to ten-fold in animal models of muscle atrophy [18]. These ligases are induced by the three members of the Forkhead box O (FoxO) signaling pathway: FoxO3, FoxO4, and especially FoxO1 [144]. The FoxO factors are induced during fasting and treatment based on glucocorticoids; when dephosphorylated, they enter the nucleus to promote growth suppression or apoptosis [144]. The muscle-specific overexpression of both FoxO1 and FoxO3a has been observed in the soleus and tibialis anterior muscles of tumor-bearing cachectic mouse models, which was associated with muscle wasting [145]. Indeed, the activation of FoxO1 has been shown to be related to the activation of the muscle-specific hormone myostatin, a TGF-β ligand that blocks the skeletal muscle hypertrophy induced by the IGF1-PI3-K-Akt anabolic pathway [94], by both blocking protein degradation and increasing protein biosynthesis [146] through the phosphorylation of SMAD2 and SMAD3, which translocate together with SMAD4 to the nucleus to ultimately lead to muscle wasting [45]. In fact, TGF-β family proteins, which include myostatin, activins, and growth/differentiation factor (GDF)-15, play a widely recognized role in muscle wasting in cachexia [47]. On the other hand, overexpression of FoxO3a was sufficient to activate an atrogen-1 and MuRF1 promoter reporter, which led to an increase in atrogen-1 mRNA in skeletal muscle [145]. Furthermore, the knockdown of FoxO transcription activity has been related to myotube hypertrophy (via increased diameter) in vitro [146].

Other molecules are also related to muscle loss in cachexia. Proteolysis-inducing factor (PIF) is a glycoprotein discovered in the circulation of mice bearing cachexia-inducing tumors, but not in mice with non-cachexia-inducing tumors [147]. PIF is produced by both murine and human cancers, and it induces the loss of skeletal muscle by decreasing protein synthesis and promoting protein degradation [148]. In humans, PIF is detectable mostly in advanced tumors of gastrointestinal origin and in the urine of such patients, demonstrating a strong correlation between a degree of weight loss and the presence of PIF in both tumors and patient urine [147]. It was demonstrated that being isolated from a human melanoma cell line, PIF could be administered to non-tumor-bearing mice to actively generate a decrease in body weight without reducing food intake [148]. On the other hand, myoblast proliferation is inhibited by myostatin, and mice with a transgenic myostatin gene develop a cachexia-like syndrome, which manifests as severe muscle wasting [135].

The accelerated muscle wasting process also relies on both increased myocyte apoptosis due to a lack of differentiation of satellite cells and mitochondrial abnormalities in skeletal muscle cells [15]. The skeletal muscle of cachectic patients characteristically develops mitochondrial dysfunction and disrupted mitochondrial dynamics [29]. Particularly, there is a reduction in the content of transcriptional peroxisome proliferator-activated receptor gamma co-activator 1-α (PGC-1α) protein. PGC-1α is a positive regulator of mitochondrial biogenesis; it increases the expression of nuclear respiratory factors that control the expression of diverse mitochondrial genes and induces several ROS-detoxifying enzymes [84]. While it is recognized that the ectopic expression of PGC-1α in WAT generates a drastic increase in mitochondrial biogenesis and the induction of UCP-1 protein, such as in BAT [84], previous studies of transgenic mouse models over-expressing PGC-1α revealed that increases in muscle mitochondrial biogenesis and activity did not seem to prevent muscle loss [29]. This response differs from that found in mitochondrial myopathies and sarcopenia, in which elevated expression of PGC-1α in skeletal muscle cells protects against the progression of those diseases [29]. Indeed, in cachexia mouse models with severe weight loss, there was a reduction in the amount of muscle PGC-1α protein with a concomitant decrease in muscle mito-
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Additionally, the remaining mitochondria exhibited variable sizes with a tendency to be smaller, and the aforementioned events could be attenuated by IL-6 inhibition via the employment of an IL-6 receptor antibody [149].

Adipose tissue loss

Adipose tissue is a major endocrine organ that secretes hormones and adipokines to modulate appetite and nutrient metabolism. This tissue is mostly composed of stored lipid droplets and is associated with systemic energy homeostasis [124]. Lipids such as triacylglycerides constitute approximately 90% of normal adult fuel reserves, and WAT releases them during energy deprivation [16]. Additionally, WAT secretes adipokines such as leptin, adiponectin, TNF-α, IL-6, plasminogen activator inhibitor-1 and visfatin, which (among other adipokines) can regulate appetite, energy expenditure, insulin sensitivity, and the inflammatory response [16].

The extensive loss of adipose tissue is a hallmark of cancer cachexia, in which it contributes to the negative energy balance [16, 36]. Various elements contribute to cachexia-related adipose wasting (Figure 5), and this effect cannot be solely explained by diminished appetite since experimental models have revealed that it is more severe than food restriction [16]. One explanation for the reduction in adipose tissue depots is the evident increase in lipid mobilization due to enhanced adipocyte triglyceride lipolysis, reduced lipogenesis and FA esterification secondary to decreased LPL activity and impaired adipocyte turnover (pre-adipocytes/mature adipocytes) [42, 46, 138, 150]. Furthermore, adipose wasting in cancer has been correlated with alterations in the circulating levels of the adipose tissue-protective hormone insulin and in catecholamines, which are pro-lipolytic [16, 138]. Indeed, there was an over two-fold increase in the lipolytic effects of catecholamines in mature adipocytes isolated from subcutaneous fat of gastrointestinal adenocarcinoma cachectic patients compared with controls...
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[16]. It is also recognized that catecholamines promote an increase in the expression of the triglyceride-lysing enzymes adipose triglyceride lipase (ATGL) and HSL. In particular, HSL has been shown to induce lipolysis at the surface of lipid droplets [138, 150, 151], and elevated HSL mRNA levels in the adipose tissue of colorectal, pancreatic, ovarian, esophageal, and stomach cancer patients were associated with high free fatty acids (FFAs) in serum [152, 153].

Inflammation of adipose tissue is common in cachectic patients and is most evident as the disease progresses [42]. Indeed, cancer cachexia murine models have revealed the active expression and secretion by WAT [154] and visceral adipose tissue (VAT) [138] of pro-inflammatory molecules, such as TNF-α and IL-6, which promote fat depletion [16, 36, 136, 151]. TNF-α has been associated with the induction of cachexia in chronic illnesses such as cancer by the suppression of adipocyte differentiation via blocking adipogenic transcription factors, such as peroxisome proliferator-activated receptor-gamma (PPAR-γ) and CCAAT/enhancer-binding protein-α (C/EBPα), which increases the Wnt/β-catenin transcriptional activity [155]. TNF-α also promotes both the blockade of LPL function and the expression of perilipins, phosphoproteins that are located at the lipid droplet surface and normally prevent access to lipases, such as HSL [16, 156]. In addition, TNF-α inhibits the expression of GLUT4 and insulin receptor, thus altering glucose transport in adipose cells [157]. Insulin-resistant adipocytes in VAT, in particular, have been reported to be more sensitive to catecholamine-induced lipolysis than adipocytes in the subcutaneous adipose tissue [138]. Lipid breakdown in VAT leads to the direct delivery of FFAs to the liver for the rapid production of both hepatic triglycerides and low-density lipoproteins, which exacerbates the dysregulated metabolic state [138]. Furthermore, gastrointestinal cancer cachectic patients displayed high circulating levels of IL-6 [151] and elevated IL-6 mRNA expression in subcutaneous fat compared with controls [154]. There is also evidence that IL-6 promotes lipolysis in human adipose tissue; high circulating levels of this cytokine have been associated with the progression of cancer cachexia [16].

Another circulating factor related to adipose tissue loss is zinc-α2-glycoprotein (ZAG). ZAG is a protein that belongs to the class I major histocompatibility complex and has been observed to be overexpressed in prostate and breast cancer patients [158]. It has been demonstrated that lipid mobilization factor (LMF), which is secreted by tumors under cachectic conditions to stimulate triglyceride hydrolysis and increase the expression of UCPs to promote FFA oxidation [159], shares high amino acid sequence homology with ZAG [158]. ZAG stimulated lipolysis in isolated murine and human adipocytes, and experimental treatment with ZAG in healthy mice and the obese murine model ob/ob induced adipocyte atrophy [160]. Furthermore, cancer cachexia is associated with the down-regulation of genes related to adipogenesis, including the key adipogenic factors C/EBP-α and -β [16, 42]. In the WAT of the MAC16 colon adenocarcinoma mouse model, the mRNA levels of both C/EBP-α and C/EBP-β were significantly diminished, with a 100-fold reduction in the C/EBP-α isoform [161].

Whole-body lipolytic activity is measured in patients with elevated fasting circulating levels of glycerol and FFAs, which result from the cleavage of triglycerides [16, 138]. This excess of FFAs produces energy through mitochondrial oxidation due to the upregulation of genes involved in fat oxidation, including PGC-1α and UCP-2 [138]. The aforementioned events promote a “browning transition” of WAT, which is accompanied by changes in the usual functions of this tissue. WAT abandons its role as an energy depot and instead gains a thermogenic function, which diminishes mitochondrial electronic transport and results in permanent energy loss [10]. The fat cells undergoing this browning transition are called “beige adipocytes” to distinguish them from the native brown adipocytes in healthy organisms. WAT browning, which contributes to fat loss in cancer, occurred before skeletal muscle wasting in mouse models of cancer cachexia [21]. However, while brown adipocytes express UCP-1 under normal conditions, beige adipocytes only express this protein secondary to the recognition of activators such as PGC-1α agonists, IL-6, and tumor-derived parathyroid hormone-related protein (PTHrP) [88, 138].

Fat loss can also be reflected in morphological changes in adipose tissue, such as a reduction in adipocyte size secondary to the downregulation of pathways linked to cell and tissue struc-
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Tissues, including the cytoskeleton and cell adhesion [46]. Gastrointestinal cancer patients experiencing weight loss exhibited a reduction in adipocyte volume but not in the total fat cell number [46, 138]. In fact, adipocytes in the subcutaneous adipose tissue of such patients have shown decreases of 32.9% in cell size and 18.5% in cell perimeter compared with controls [42].

Experimental models of cancer cachexia have revealed that the loss of adipose tissue occurs before protein mass is lost and food intake is diminished [46, 150]. Moreover, advanced pancreatic cancer patients exhibit elevated total fat loss, both from VAT and subcutaneous fat mass, which is higher than muscle tissue wasting [162]. Regardless of the source, fat loss in cachexia is associated with shorter survival time [138]. In fact, a retrospective study of ovarian cancer evaluated with CT demonstrated that fat was preferentially lost from VAT compared with control subjects [16]. DEXA, which quantifies regional lean body mass [138], was applied in a cohort study of gastrointestinal cancer patients under palliative care and revealed preferential fat tissue wasting from the trunk, followed by leg and arm adipose compartments [163]. Moreover, that study demonstrated that total body fat wasting in progressive cancer cachexia was more pronounced compared with lean tissue mass [163].

Anorexia

Reduced food intake in patients with cancer is caused by anorexia, and it is usually referred to as the cachexia-anorexia syndrome (CAS) [164]. However, anorexia alone cannot explain the reduced body weight in cancer subjects, and cachexia-associated wasting cannot be completely reversed by increasing the nutritional intake alone [138]. For CAS to occur, the patient not only must experience weight loss and increased catabolism but also must diminish his food ingestion [165]. In fact, cachectic patients commonly exhibit significant loss of appetite and early satiety [18] (Figure 6). The mechanical effect of the tumor mass due to the spatial area of growth of the neoplasm can cause the loss of appetite, primarily in upper gastrointestinal cancers, but emotional distress, taste and odor perception variations, and the side effects of chemo- and radiotherapies may also be responsible for this effect [16, 164].

Regulation of food intake involves the integration of peripheral and neural signals, primarily in the hypothalamus [16]. Two principal neuronal populations promote and reduce food ingestion: orexigenic and anorexigenic neurons, respectively. In the hypothalamic ARC, the most potent appetite stimulant, NPY, promotes food intake and activates the parasympathetic output to diminish the resting energy expenditure (REE), while proopiomelanocortin (POMC) induces satiety and stimulates sympathetic activity to increase the REE [34, 166]. Indeed, NPY/agouti-related protein (AGRP) neurons and POMC/cocaine and amphetamine-related transcript (CART) neurons, both located at the ARC, exert opposite functions to control food consumption and are stimulated by different activators. Leptin, an adipose cytokine whose circulating concentration is proportional to body adipose tissue and decreases with diet, inhibits NPY/AGRP neurons and concomitantly stimulates POMC/CART neurons to reduce food intake and increase energy expenditure [167-169]. An analogous effect to depolarize leptin receptors in POMC neurons can be achieved by adiponectin [168], a cytokine that is also secreted by fat depots and whose serum concentrations are inversely related to body weight [170]. Insulin, a hormone produced by the pancreas that regulates glycemia, is also recognized as an anorexigenic molecule that acts on POMC to reduce food ingestion [167, 169]. On the other hand, ghrelin, which is mainly secreted by the stomach and duodenum under fasting states, activates NPY/AGRP neurons th-
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rough its high affinity for the growth hormone secretagogue receptor (GHSR) and induces food intake [165]. Plasmatic ghrelin levels increase before meals and are commonly low under obesity states [170]. Furthermore, NPY/AGRP neurons by themselves inhibit POMC/CART neurons through the secretion of γ-aminobutyric acid (GABA), which is released upon the binding of ghrelin to GHSR [167]. Between 15% and 40% of cancer patients develop anorexia, but this proportion may be as high as 80% in advanced stages [165]. The presence of CAS is associated with poor patient prognosis, as these patients concomitantly demonstrate poor responsiveness to anti-neoplastic treatments [165]. Dysregulation of the NPY pathway leads to reduced energy intake, and NPY-immunoreactive hypothalamic neurons are diminished in cancer anorexia models [16]. One possible explanation for this pattern in cancer cachexia is the observed correlation between high circulating leptin concentrations and the inhibition of NPY release [16, 135]. Additionally, the recognized role of leptin is to stimulate the activity of sympathetic nerves to BAT to increase UCP-1 expression, thus promoting thermogenesis and adipose wasting [169]. Th1 cytokines, including TNF-α and IL-6, are associated with the secretion of corticotrophin-releasing factor (CRF) in the brain, which promotes hypophagia by the blockade of NPY-producing neurons [16, 135]. On the other hand, the transcription of POMC in the hypothalamus is downregulated under food deprivation [171]. Indeed, mutations in chromosome 2p21, where the POMC gene is found, are related to variations in the serum concentration of leptin and, therefore, in food intake [171].

There are two forms of ghrelin: unacylated (UnAG) and acylated (AG). The acylated form is generated by the action of ghrelin-O-acyltransferase (GOAT) and binds to GHSR-1a to release GH for both the promotion of food intake and skeletal myocyte differentiation, which ameliorates cachexia in patients [129].

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Figure 6. Cancer-associated cachexia stimulates anorexia and weight loss. A pro-inflammatory systemic environment is a direct promoter of insulin resistance, and high circulating levels of insulin cause the POMC neurons at the arcuate nucleus of the hypothalamus to become activated. IL-6 also induces corticotrophin-releasing factor, which, together with ciliary neurotrophic factor and leptin, decreases neuropeptide Y levels. The upregulation of POMC and the downregulation of neuropeptide Y are linked to early satiety in the patient and therefore to reduced food ingestion, which leads to weight loss. The latter is related to an increase in adiponectin levels, which concomitantly augments POMC neuronal activity. This response creates a loop of early satiety and weight loss, and it is potentiated by chronic systemic inflammation. Abbreviations: IL-6: interleukin 6; CRF: corticotrophin-releasing factor; POMC: proopiomelanocortin; NPY: neuropeptide Y; CNTF: ciliary neurotrophic factor.

Weight loss

Differentiation from other weight loss syndromes is mandatory for the early recognition and correct management of cachexia [135]. Weight loss can result from starvation, sarcopenia and dehydration; however, unlike cachexia, weight loss in those conditions can be reversed [135]. In cachexia, primary anorexia causes a reduction in food intake, and together with both hypercatabolism and hypoanabolism, it generates relevant weight loss [56]. Particularly in cancer, patients with pancreatic or gastric tumors have the highest frequency of weight loss, and subjects with non-Hodgkin lymphoma, breast cancer, acute non-lymphocytic leukemia, or sarcomas have the lowest frequency [12]. Furthermore, weight loss is present in 15-40% of all cancer patients, and it indicates an obscure prognosis [22, 135]: the greater the weight loss, the shorter the survival time [12]. Indeed, weight loss is a relevant prognostic factor in cancer [172]; when it surpasses 6% compared with basal weight, it is linked to a shorter survival time in patients with breast, colon and prostate cancers, among oth-
ers [173]. In addition, weight loss has been reported to be responsible for 25-30% of all cancer-related deaths [94]. One observational European multicenter study evaluating cachexia, appetite and food intake in subjects with different types of cancer revealed that weight loss was higher in patients not treated with chemotherapy [29]. However, special attention should be placed on certain conditions, such as ovarian cancer, in which weight loss may not be completely evident due to the concomitant presence of ascites or even the weight of both the tumor and its metastases [174].

Weight loss in cancer is also associated with higher basal REE in these patients and with the treatment per se. In oncological subjects, the REE has been observed to increase; this effect may be attributable to UCP upregulation and to the Cory cycle recycling tumor-derived lactate to the liver [16]. With respect to treatment-induced weight loss, the administration of the FOLFOX treatment, specifically the FOLFIRI chemotherapy scheme, in a colorectal cancer mouse model caused adipose tissue and skeletal muscle weight loss, with nearly 10% corporal weight loss by the end of a 5-week treatment regimen [141]. It should be mentioned that changes in diet and/or treatment with appetite stimulants, such as corticosteroids and progestational agents, may transitionally increase weight in these patients; however, this effect is related to water retention and gains in fat mass, rather than muscle [18].

**Current therapeutic approaches for cancer-associated cachexia**

To date, there are no therapies for the successful management of the cachectic patient. In fact, current care measures for cachectic individuals are focused on nutritional supplementation, physical therapy, and prescription of both appetite stimulants and anti-inflammatory drugs, rather than a curative approach [175].

Furthermore, it has been demonstrated that not only the tumor but also chemotherapy drugs are able to induce and sustain the presence of cachexia. In particular, cisplatin promotes NF-κB activity, which, together with its ability to upregulate the expression of myostatin, induces anabolic activity for muscle wasting [176]. Another therapy that has shown pro-cachectic activity is the FOLFIRI approach for colorectal cancer; in a healthy CD2F1 murine model, this approach induced body weight loss at the expense of adipose and muscle tissue wasting due to the hyperphosphorylation and activation of both the ERK1/2 and p38 MAPK pathways, with concomitant reductions in the mitochondrial protein PGC-1α and the number and size of skeletal muscle mitochondria [176]. Therefore, it is mandatory to modify standard treatments in cancer to reduce the number of cachectic subjects.

Since cachexia is a complex syndrome with systemic involvement, proposed treatments that are in development must address all of its individual components. Starting with the detonator of cachexia, named chronic systemic inflammation, several current experimental assays and clinical trials aim to reduce or eradicate the persistent inflammatory environment in the patient. Omega-3 polyunsaturated FAs, named eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been evaluated due to their anti-inflammatory properties via the suppression of pro-inflammatory cytokines and arachidonic acid-derived mediators in cancer cachectic patients; the results showed increases in body weight and lean body mass [33]. TNF-α inhibitors, such as etanercept, a recombinant fusion protein of TNF-α type II receptor that blocks TNF-α activity, and the recombinant anti-TNF-α antibody infliximab, have contradictory effects according to the literature. Studies have revealed that these drugs neither show a significant benefit versus placebo in diminishing muscle wasting nor restore lean body mass in cancer cachectic patients [45]. However, other reports have demonstrated that infliximab, at least, is capable of reverting muscle wasting under chronic inflammatory states [177]. On the other hand, it has been demonstrated that CTNO-328, a monoclonal antibody directed against IL-6, was able to reverse cancer-induced cachexia in nude mice [16]. Another promising antibody against IL-6 is tocilizumab, which has been employed in chemotherapy-resistant metastatic lung cancer patients to diminish cachexia symptoms [178].

Mitochondrial biogenesis is disrupted early in the development of cachexia, but this could be rescued by the administration of an IL-6 receptor antibody and by exercise [26]. Indeed, exercise increases survival in cancer patients [179],
since it potentially increases muscle blood flow by 20-fold with the concomitant transport of nutrients and immune cells to the affected tissues [180]. Moreover, although one report showed that 2 weeks of IL-6 over-expression in a cachexia murine model reduced gastrocnemius muscle mass by 12% compared with controls, this effect was prevented when the mice underwent exercise training during the same period of IL-6 over-expression [149].

Type IIIB activin receptor (ActRIIB) ligands, such as myostatin, activins and GDF-11, are elevated under muscle wasting states. When activated, they phosphorylate SMAD2/3 to repress protein synthesis through the inhibition of the Akt/mTOR signaling pathway while translocating together with SMAD4 to the nucleus to increase protein degradation [175]. ActRIIB ligands are increased in cachexia, and one study aimed to target their effects to striated muscle in a C57BL/6 murine model bearing C26 tumor cells that employed AVVVs that upregulate SMAD7 [175]. This SMAD produces negative feedback that prevents SMAD2/3 phosphorylation and promotes ActRIIB complex degradation. The abovementioned study demonstrated the promotion of skeletal muscle hypertrophy throughout the body and the prevention of muscle wasting by inhibiting the transcription of the E3 ubiquitin ligases MuRF1 and MAFbx [175]. Another study of a Balb/c mouse model transplanted with CT26 colon adenocarcinoma cells demonstrated that the MEK1 inhibitor selumetinib ameliorated cancer-induced cachexia through the prevention of skeletal muscle and adipose tissue wasting, which was associated with reduced body weight loss compared with controls [181]. The same study revealed that selumetinib promoted the inhibition of the expression of the E3 ubiquitin ligases MuRF1 and Fbx32 through the activation of the mTOR/Akt pathway concomitantly with the inhibition of FoxO3a and the MEK/ERK pathway of muscle ubiquitination [181]. Interestingly, a study conducted using male Wistar rats implanted with the breast carcinoma cell line Walker 256 demonstrated that even the antidiabetic drug metformin, a biguanide that is commonly prescribed for type 2 diabetes mellitus, reduced gastrocnemius protein mass loss up to 30% compared with non-tumor-bearing controls. This effect was due to a reduction in proteasome expression [8].

Anabolic steroids have been used to treat muscle wasting because they increase muscle mass and strength, but their administration is associated with adverse effects on the prostate, skin and hair [182]. Enobosarm, a nonsteroidal selective androgen receptor modulator that possesses anabolic properties without the risks of anabolic steroids, is currently being assessed in phase 3 POWER clinical trials. In these trials, DEXA is being used to assess the lean body mass of patients with non-small cell lung cancer to evaluate enobosarm for the prevention and treatment of muscle wasting [183]. Results from the POWER clinical trials will be released soon.

The synthetic compound megestrol acetate, a steroidal progestin and a derivative of progestrone, is an appetite stimulant that has been used in clinical trials as an approach to both CAS and muscle wasting. Indeed, one study with 102 CAS patients, mostly with lung or gastrointestinal malignancies, revealed that the therapeutic combination of megestrol acetate with the antiemetic/anti-inflammatory drug thalidomide for 8 weeks increased both appetite and body weight while reducing pro-inflammatory cytokines, such as TNF-α and IL-6, compared with the control [184]. In another clinical research study involving 13 patients with diverse advanced malignancies that employed megestrol acetate and the β2-agonist formoterol fumarate, which is suggested to arrest muscle atrophy and increase muscle mass, patients showed improvements in muscle strength, size and function [185].

Since ghrelin induces increases in body weight, body fat mass, and lean tissue mass, both ghrelin and ghrelin agonists, such as anamorelin, have been used to stimulate food intake and appetite [182]. It has been shown that the administration of ghrelin protects against cisplatin-induced cachexia by promoting muscle anabolism in experimental models; therefore, it helps prevent weight loss due to its affinity for GHSR [141]. Additionally, anamorelin, which has a longer half-life than ghrelin, produced an elevation in body weight gain compared with placebo in a clinical trial of 226 patients with stage 3 or 4 non-small cell lung cancer [186]. On the other hand, because the hypothalamus contains receptors for both TNF-α and IL-1β, a therapy based on ibuprofen, an inhibitor of
cyclooxygenase, has been demonstrated to block anorexia in a rat model [187]. Additionally, blocking POMC neurons with AGRP in a tumor cachexia mouse model was able to restore reduced food ingestion and therefore promoted an increase in body weight [16].

Conclusions

Cachexia continues to be a health problem that presents, to different degrees, in patients with chronic diseases, such as cancer. There are currently no effective therapeutic schemes to adequately treat cachexia, and our knowledge about the integrative pathogenesis of cachexia, starting from the genetic and biochemical levels, is still insufficient. It is crucial to continue research that systemically evaluates the causes of cachexia, including biochemical and metabolic aberrations as well as new potential treatment targets to reduce the high mortality associated with this syndrome.

Acknowledgements

Alejandro Schcolnik-Cabrera is a student belonging to the Plan de Estudios Combinados en Medicina (PECEM), UNAM.

Disclosure of conflict of interest

None.

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