Am J Cancer Res 2017;7(5):1107-1135
www.ajcr.us /ISSN:2156-6976/ajcr0052446

Original Article

Understanding tumor anabolism and patient catabolism in cancer-associated cachexia

Alejandro Schcolnik-Cabrera[1], Alma Chávez-Blanco[1], Guadalupe Domínguez-Gómez[1], Alfonso Dueñas-González[2]

[1]Division of Basic Research, Instituto Nacional de Cancerología, México; [2]Unidad de Investigación Biomédica en Cáncer, Instituto de Investigaciones Biomédicas UNAM/Instituto Nacional de Cancerología, Mexico

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 (PI_3K), 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 $p16^{INK4a}$) 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-

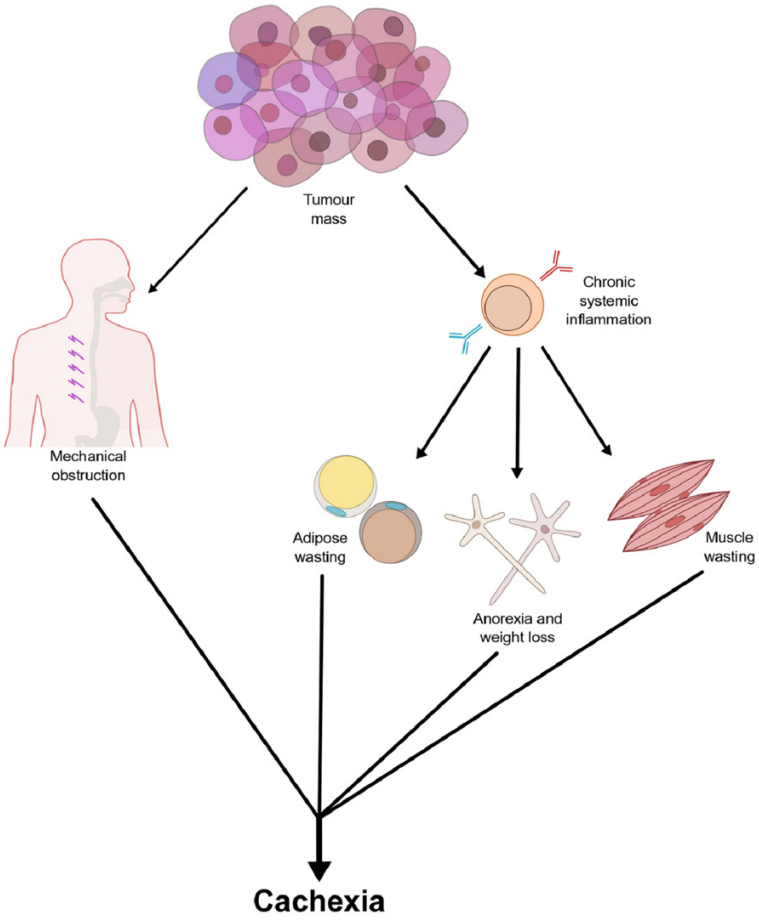

Figure 1. Elements of cancer-associated cachexia. Neoplasia generates cachexia through the chronic presence of systemic inflammation, which is associated with muscle and adipose wasting as well as anorexia. Anorexia can also be promoted by the gastrointestinal obstruction caused by the physical presence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

eration, survival and controlled growth of cells in the organism, can be separated into catabolic pathways, which generate energy in the form of adenosine triphosphate (ATP) by the rupture of molecules when nutrients are scare, and anabolic pathways, which consume energy to synthesize molecules under supplementation of abundant growth factors [1, 3]. Although under normal states, metabolism is highly regulated according to the cellular requirements [1], tumor cells are reprogrammed to enhance key metabolic pathways, such as aerobic glycolysis, glutaminolysis, and fatty acid synthesis [4]. Only cells that transform to adopt this malignant metabolic phenotype will be selected within the tumor microenvironment to survive and progress [5]. In fact, several anabolic alterations focused on cell growth and proliferation, and not on increasing ATP as with healthy cells [3], are commonly found in neoplasia. Regarding glucose and glutamine, most cancer cells develop high avidity for their consumption to generate energy and to build macromolecules for tumor progression [6], which, together with the constitutive activation of signaling pathways downstream of diverse growth factor receptors (even without circulating growth factors), doubles their total biomass to generate daughter cells [3, 7]. In particular, chemotherapy-resistant malignant cells commonly undergo aerobic glycolysis with high production of lactate [4, 6], unlike non-proliferating and differentiated cells, which depend on oxidative phosphorylation to produce the ATP required for their maintenance [3].

Together, a series of anabolic and catabolic dysregulation is involved in systemic inflammation, biochemical imbalance, tissue wasting, anorexia and weight loss due to tumor-associated metabolic stress, which induce a state known as cachexia in the patient [8].

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 clini-

cal element of cachexia in adults is weight loss, in children it is associated with growth failure [17]. Cachexia also involves systemic inflammation and anorexia (**Figure 1**), which together lead to physical disability, reduced quality of life, and diminished survival [11, 13, 14, 18]. The cachexia syndrome is multifactorial, cannot be fully reverted by nutritional support and leads to global functional impairment in patients [14, 19].

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 neoplasia. 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-

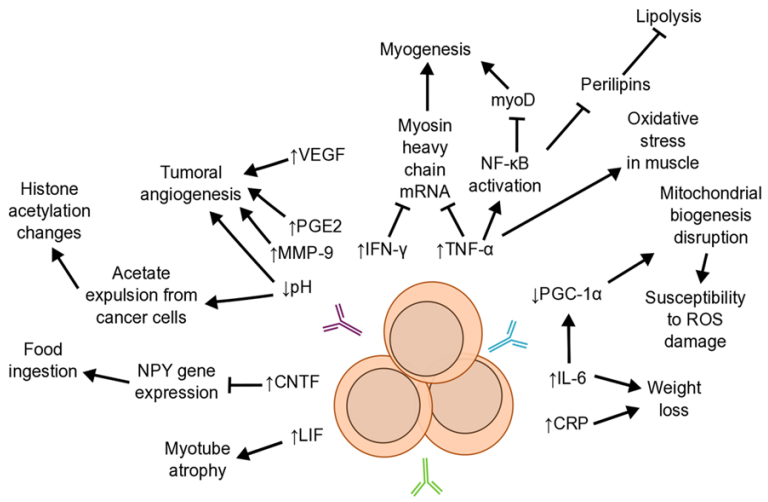

Figure 2. The effects of chronic systemic inflammation are strong promoters of cancer-induced cachexia. A permanent and uncontrolled inflammatory environment has multiple effects on the host at different levels in the pathogenesis of cachexia. LIF is a recognized inducer of myotube atrophy that damages myocytes. CNTF inhibits the gene expression of neuropeptide Y, a potent appetite stimulant in the arcuate nucleus of the brain. VEGF, PGE_2, 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; PGE_2: prostaglandin E_2; 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; IL-6: 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 mucosa 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].

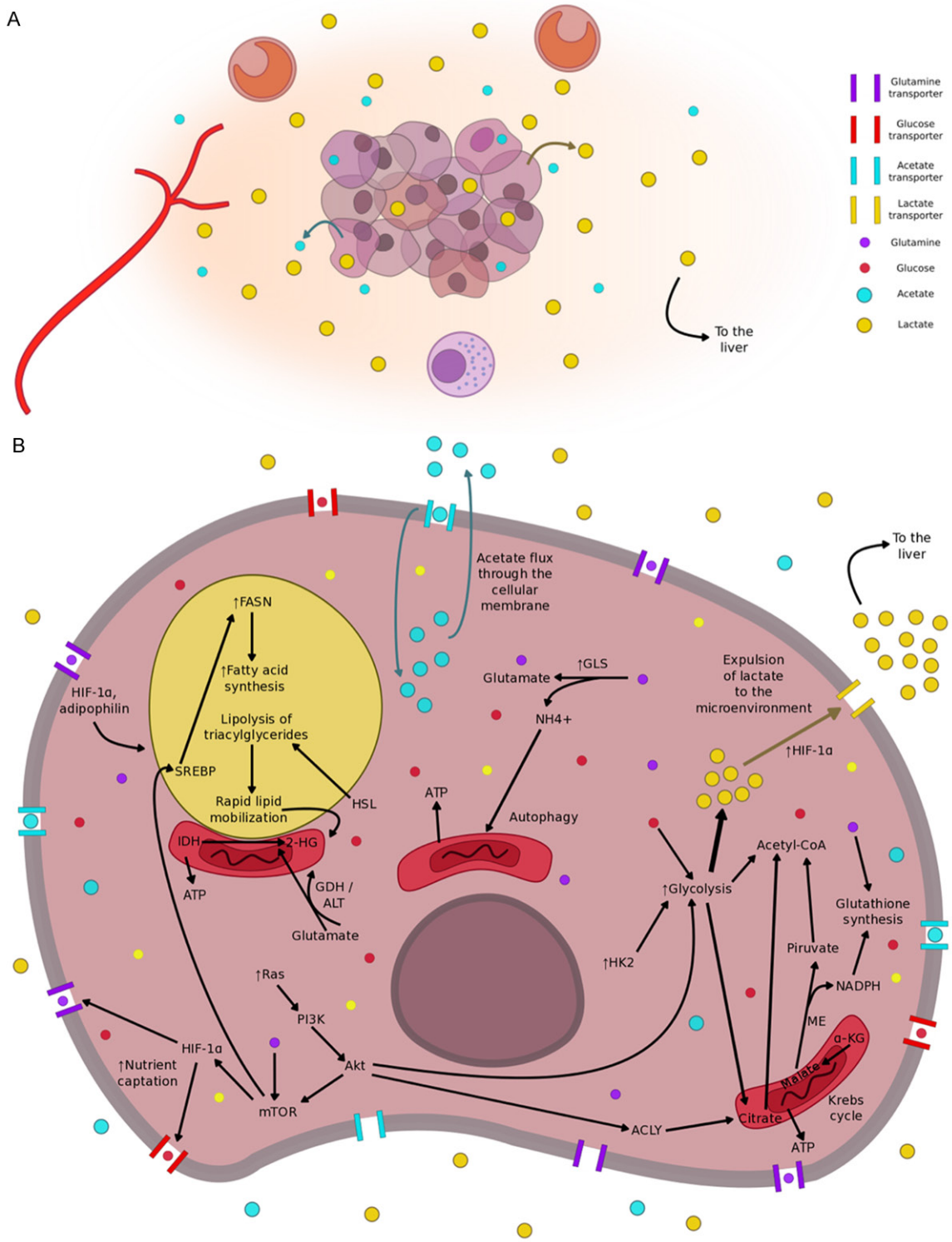

Figure 3. Biochemical and metabolic changes within the tumor. Tumor cells are organized as an anabolically active group that continuously interchanges molecules with the environment. A. Usually, as the tumor progresses, its cells secrete lactate as an anaerobic product of energy metabolism. Lactate, 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. B. 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 upregulation 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 leukemia 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 E_2 (PGE_2) 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]. PGE_2 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 CO_2 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

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 ^{18}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 cristae 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 PI_3K-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 $FADH_2$ 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 multienzyme complex [114].

FASN is 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 PI_3K-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 prostatic 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, PI_3K-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 is 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 palmitoyltransferase 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 PI_3K for the translocation of GLUT4 and the secondary introduction of glucose into the cell [128]. Phosphoinositide phos-

phatidylinositol 3,4,5-triphosphate (PIP_3), 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 I (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

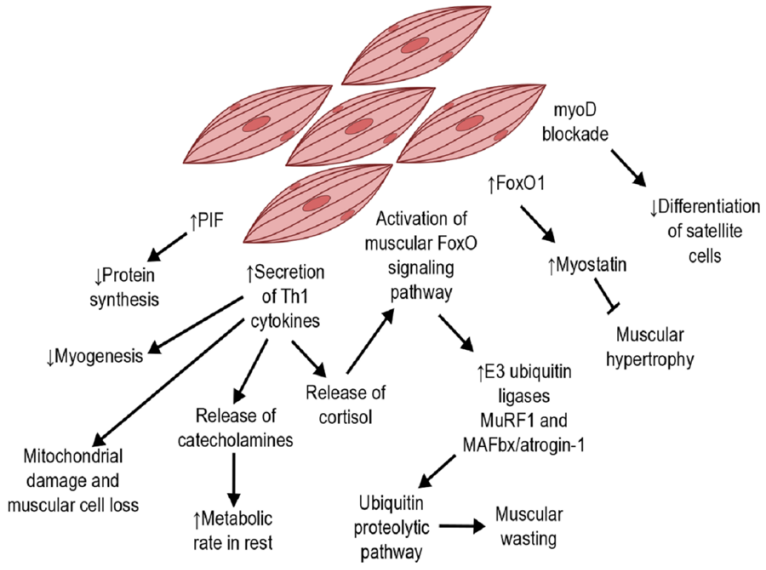

Figure 4. Muscle cells are direct targets in cachexia. At the muscle level, an increase in proteolysis-inducing factor is related to a reduction in protein synthesis. This effect is potentiated by the secretion of Th1 cytokines, such as TNF-α, which minimize myogenesis, promote mitochondrial damage with concomitant muscle cell wasting, induce the release of diverse catecholamines that increase the metabolic rate at rest, and provoke direct muscle wasting by the release of cortisol, which activates the muscle-specific ubiquitin ligases MuRF1 and MAFbx/atrogin-1 mediated by the transcription factor FoxO. In particular, the upregulation of FoxO1 increases myostatin, which blocks muscle hypertrophy in the subject. Furthermore, the inhibition of the myoD transcription factor is accompanied by a reduction in the differentiation of satellite cells, which are precursors of new myocytes under healthy conditions. Abbreviations: PIF: proteolysis-inducing factor; FoxO: Forkhead box O proteins; MuRF1: muscle RING finger 1 ubiquitin ligase; MAFbx: muscle atrophy F-box ubiquitin ligase; myoD: myogenic differentiation I.

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 (AVVVs) 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 LC3AI 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/atrogin-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-PI_3K-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 atrogin-1 and MuRF1 promoter reporter, which led to an increase in atrogin-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 overexpressing 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-

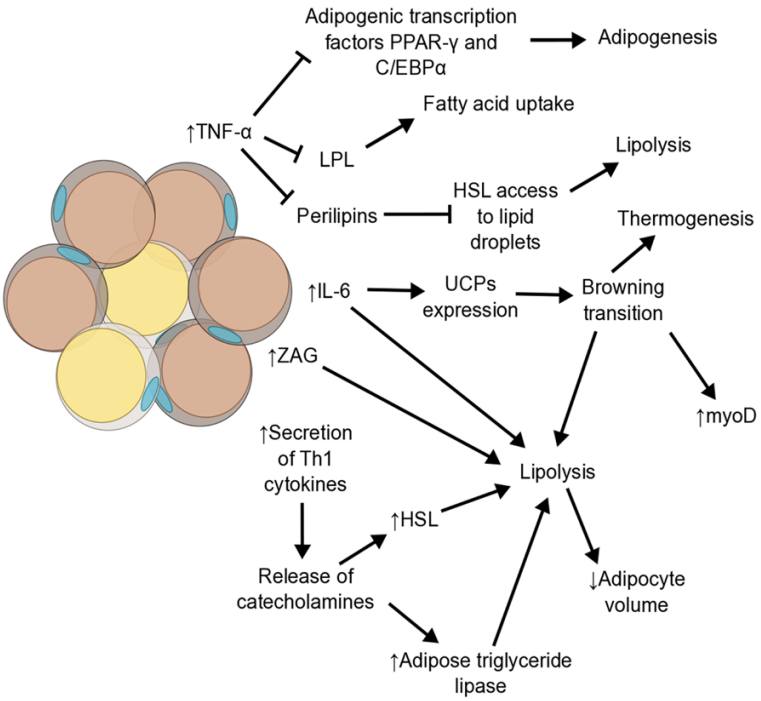

Figure 5. Adipose tissue undergoes browning transition and lipolysis during the progression of cachexia. Systemic inflammation causes an increase in pro-inflammatory cytokines that have an impact on adipose tissue. TNF-α impedes three pathways associated with adipose metabolism. First, by the inhibition of the adipogenic transcription factors PPAR-γ and C/EBPα, the adipogenesis process is stopped. Second, lipoprotein lipase fails to take up fatty acids to construct complex lipids within the adipocyte. Finally, perilipins are unable to prevent hormone-sensitive lipase from inducing lipolysis in the adipose tissue. On the other hand, the high abundance of IL-6 stimulates the expression of uncoupling proteins, which in turn are related to the browning transition in adipocytes and, thus, a permanent thermogenic state. The browning transition is also associated with an increase in the skeletal muscle transcription factor myoD. Furthermore, lipolysis can be stimulated through different routes. IL-6, together with the browning transition and the ZAG protein, directly induces lipolysis. Th1 cytokines are related to the secretion of catecholamines into circulation, which upregulates both hormone-sensitive lipase and adipose triglyceride lipase. Both enzymes generate lipolysis. Together, these processes stimulate adipose wasting via a reduction in adipocyte volume. Abbreviations: TNF-α: tumor necrosis factor α; PPAR-γ: peroxisome proliferator-activated receptor-gamma; C/EBP: CCAAT/enhancer-binding protein; LPL: lipoprotein lipase; HSL: hormone-sensitive lipase; IL-6: interleukin 6; UCPs: uncoupling proteins; ZAG: zinc-α2-glycoprotein; myoD: myogenic differentiation I.

chondria content [149]. 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

[16]. It is also recognized that catecholamines promote an increase in the expression of the triglyceride-lysis 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 downregulation 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-

tures, 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 a low level of subcutaneous fat related to advanced tumor stages was an independent predictor of early death [51]. However, the obese cachectic patient is a challenge in the clinical scenario, since a high body mass index can complicate the analysis of the contribution of fat mass loss to the progression of cachexia [36].

Body fat composition is evaluated in cancer patients using diverse imaging approaches, including DEXA, magnetic resonance imaging (MRI), and CT scan analysis [138]. With the aforementioned techniques, now it is clear that the fat loss associated with cachectic cancer states in patients follows a pattern according to the specific cancer type. Indeed, one study of cachectic gastrointestinal cancer patients 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-

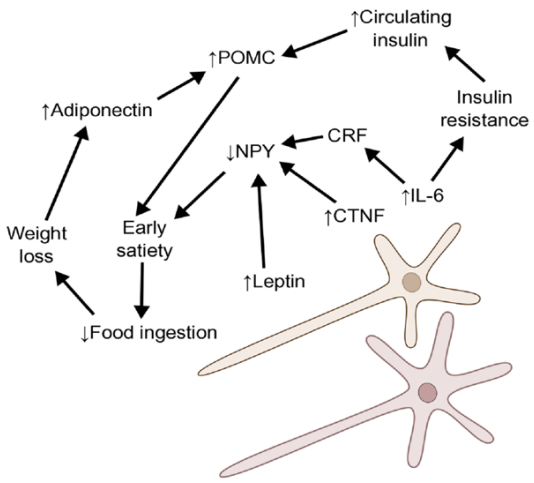

Figure 6. Cancer-associated cachexia stimulates anorexia and weight loss. A pro-inflammatory systemic environment is a direct promoter of insulin resistance, and high circulating levels of insulin cause the POMC neurons at the arcuate nucleus of the hypothalamus to become activated. IL-6 also induces 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.

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].

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 docosahexanoic 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 IIB 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 progesterone, 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 β_2-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.

Address correspondence to: Alfonso Dueñas-González, Unidad de Investigación Biomédica en Cáncer, Instituto de Investigaciones Biomédicas UNAM/Instituto Nacional de Cancerología, Mexico. E-mail: alfonso_duenasg@yahoo.com

References

[1] Tarrado-Castellarnau M, Atauri P, Cascante M. Oncogenic regulation of tumor metabolic reprogramming. Oncotarget 2016; 7: 62726-62753.

[2] Trotta AP, Chipuk JE. Mitochondrial dynamics as regulators of cancer biology. Cell Mol Life Sci 2017; 74: 1999-2017.

[3] Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. Cancer Cell 2012; 21: 297-308.

[4] Zhao Y, Butler EB, Tan M. Targeting cellular metabolism to improve cancer therapeutics. Cell Death Dis 2013; 4: e532.

[5] Tennant DA, Duran RV, Boulahbel H, Gottlieb E. Metabolic transformation in cancer. Carcinogenesis 2009; 30: 1269-1280.

[6] Morandi A, Indraccolo S. Linking metabolic reprogramming to therapy resistance in cancer. Biochim Biophys Acta 2017; 1868: 1-6.

[7] Anastasiou D. Tumour microenvironment factors shaping the cancer metabolism landscape. Br J Cancer 2017; 116: 277-286.

[8] Oliveira AG, Gomes-Marcondes MC. Metformin treatment modulates the tumour-induced wasting effects in muscle protein metabolism minimising the cachexia in tumour-bearing rats. BMC Cancer 2016; 16: 418.

[9] Donohoe CL, Ryan AM, Reynolds JV. Cancer cachexia: mechanisms and clinical implications. Gastroenterol Res Pract 2011; 2011: 601434.

[10] Argiles JM, Busquets S, Stemmler B, Lopez-Soriano FJ. Cancer cachexia: understanding the molecular basis. Nat Rev Cancer 2014; 14: 754-762.

[11] de Matos-Neto EM, Lima JD, de Pereira WO, Figueredo RG, Riccardi DM, Radloff K, das Neves RX, Camargo RG, Maximiano LF, Tokeshi F, Otoch JP, Goldszmid R, Camara NO, Trinchieri G, de Alcantara PS, Seelaender M. Systemic inflammation in cachexia-is tumor cytokine expression profile the culprit? Front Immunol 2015; 6: 629.

[12] Tisdale MJ. Mechanisms of cancer cachexia. Physiol Rev 2009; 89: 381-410.

[13] Tisdale MJ. Cancer anorexia and cachexia. Nutrition 2001; 17: 438-442.

[14] Naing A, Dalal S, Abdelrahim M, Wheler J, Hess K, Fu S, Hong DS, Janku F, Falchook GS, Ilustre A, Ouyang F, Kurzrock R. Olanzapine for cachexia in patients with advanced cancer: an exploratory study of effects on weight and metabolic cytokines. Support Care Cancer 2015; 23: 2649-2654.

[15] Argiles JM, Busquets S, Stemmler B, Lopez-Soriano FJ. Cachexia and sarcopenia: mechanisms and potential targets for intervention. Curr Opin Pharmacol 2015; 22: 100-106.

[16] Bing C, Trayhurn P. Regulation of adipose tissue metabolism in cancer cachexia. Curr Opin Clin Nutr Metab Care 2008; 11: 201-207.

[17] Argiles JM, Lopez-Soriano FJ, Toledo M, Betancourt A, Serpe R, Busquets S. The cachexia score (CASCO): a new tool for staging cachectic cancer patients. J Cachexia Sarcopenia Muscle 2011; 2: 87-93.

[18] Dodson S, Baracos VE, Jatoi A, Evans WJ, Cella D, Dalton JT, Steiner MS. Muscle wasting in cancer cachexia: clinical implications, diagnosis, and emerging treatment strategies. Annu Rev Med 2011; 62: 265-279.

[19] Bye A, Wesseltoft-Rao N, Iversen PO, Skjegstad G, Holven KB, Ulven S, Hjermstad MJ. Alterations in inflammatory biomarkers and energy intake in cancer cachexia: a prospective

study in patients with inoperable pancreatic cancer. Med Oncol 2016; 33: 54.
[20] Fearon KC, Glass DJ, Guttridge DC. Cancer cachexia: mediators, signaling, and metabolic pathways. Cell Metab 2012; 16: 153-166.
[21] Petruzzelli M, Schweiger M, Schreiber R, Campos-Olivas R, Tsoli M, Allen J, Swarbrick M, Rose-John S, Rincon M, Robertson G, Zechner R, Wagner EF. A switch from white to brown fat increases energy expenditure in cancer-associated cachexia. Cell Metab 2014; 20: 433-447.
[22] Fox KM, Brooks JM, Gandra SR, Markus R, Chiou CF. Estimation of cachexia among cancer patients based on four definitions. J Oncol 2009; 2009: 693458.
[23] Tisdale MJ. Biology of cachexia. J Natl Cancer Inst 1997; 89: 1763-1773.
[24] DeBerardinis RJ, Cheng T. Q's next: the diverse functions of glutamine in metabolism, cell biology and cancer. Oncogene 2010; 29: 313-324.
[25] Solheim TS, Blum D, Fayers PM, Hjermstad MJ, Stene GB, Strasser F, Kaasa S. Weight loss, appetite loss and food intake in cancer patients with cancer cachexia: three peas in a pod? - analysis from a multicenter cross sectional study. Acta Oncol 2014; 53: 539-546.
[26] Onesti JK, Guttridge DC. Inflammation based regulation of cancer cachexia. Biomed Res Int 2014; 2014: 168407.
[27] von Haehling S, Anker SD. Cachexia as major underestimated unmet medical need: facts and numbers. Int J Cardiol 2012; 161: 121-123.
[28] Inui A. Cancer anorexia-cachexia syndrome: current issues in research and management. CA Cancer J Clin 2002; 52: 72-91.
[29] Wang X, Pickrell AM, Zimmers TA, Moraes CT. Increase in muscle mitochondrial biogenesis does not prevent muscle loss but increased tumor size in a mouse model of acute cancer-induced cachexia. PLoS One 2012; 7: e33426.
[30] Iwata Y, Suzuki N, Ohtake H, Kamauchi S, Hashimoto N, Kiyono T, Wakabayashi S. Cancer cachexia causes skeletal muscle damage via transient receptor potential vanilloid 2-independent mechanisms, unlike muscular dystrophy. J Cachexia Sarcopenia Muscle 2016; 7: 366-376.
[31] Meriggi F. Cancer cachexia: one step ahead. Rev Recent Clin Trials 2015; 10: 246-250.
[32] Muscaritoli M, Anker SD, Argiles J, Aversa Z, Bauer JM, Biolo G, Boirie Y, Bosaeus I, Cederholm T, Costelli P, Fearon KC, Laviano A, Maggio M, Rossi Fanelli F, Schneider SM, Schols A, Sieber CC. Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics". Clin Nutr 2010; 29: 154-159.
[33] Laviano A, Meguid MM, Inui A, Muscaritoli M, Rossi-Fanelli F. Therapy insight: Cancer anorexia-cachexia syndrome--when all you can eat is yourself. Nat Clin Pract Oncol 2005; 2: 158-165.
[34] Dhanapal R, Saraswathi T, Govind RN. Cancer cachexia. J Oral Maxillofac Pathol 2011; 15: 257-260.
[35] Parry-Billings M, Leighton B, Dimitriadis GD, Curi R, Bond J, Bevan S, Colquhoun A, Newsholme EA. The effect of tumour bearing on skeletal muscle glutamine metabolism. Int J Biochem 1991; 23: 933-937.
[36] Tsoli M, Robertson G. Cancer cachexia: malignant inflammation, tumorkines, and metabolic mayhem. Trends Endocrinol Metab 2013; 24: 174-183.
[37] Ockenga J, Valentini L. Review article: anorexia and cachexia in gastrointestinal cancer. Aliment Pharmacol Ther 2005; 22: 583-594.
[38] Tan CR, Yaffee PM, Jamil LH, Lo SK, Nissen N, Pandol SJ, Tuli R, Hendifar AE. Pancreatic cancer cachexia: a review of mechanisms and therapeutics. Front Physiol 2014; 5: 88.
[39] Baracos VE. Cancer-associated cachexia and underlying biological mechanisms. Annu Rev Nutr 2006; 26: 435-461.
[40] Aoyagi T, Terracina KP, Raza A, Matsubara H, Takabe K. Cancer cachexia, mechanism and treatment. World J Gastrointest Oncol 2015; 7: 17-29.
[41] Seelaender M, Batista M Jr, Lira F, Silverio R, Rossi-Fanelli F. Inflammation in cancer cachexia: to resolve or not to resolve (is that the question?). Clin Nutr 2012; 31: 562-566.
[42] Batista ML Jr, Henriques FS, Neves RX, Olivan MR, Matos-Neto EM, Alcantara PS, Maximiano LF, Otoch JP, Alves MJ, Seelaender M. Cachexia-associated adipose tissue morphological rearrangement in gastrointestinal cancer patients. J Cachexia Sarcopenia Muscle 2016; 7: 37-47.
[43] Argiles JM, Busquets S, Lopez-Soriano FJ. Anti-inflammatory therapies in cancer cachexia. Eur J Pharmacol 2011; 668 Suppl 1: S81-86.
[44] Guttridge DC, Mayo MW, Madrid LV, Wang CY, Baldwin AS Jr. NF-kappaB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. Science 2000; 289: 2363-2366.
[45] Miyamoto Y, Hanna DL, Zhang W, Baba H, Lenz HJ. Molecular pathways: cachexia signaling-A targeted approach to cancer treatment. Clin Cancer Res 2016; 22: 3999-4004.
[46] Bing C. Lipid mobilization in cachexia: mechanisms and mediators. Curr Opin Support Palliat Care 2011; 5: 356-360.

[47] Chen JL, Walton KL, Qian H, Colgan TD, Hagg A, Watt MJ, Harrison CA, Gregorevic P. Differential effects of IL6 and Activin A in the development of cancer-associated cachexia. Cancer Res 2016; 76: 5372-5382.
[48] Acharyya S, Ladner KJ, Nelsen LL, Damrauer J, Reiser PJ, Swoap S, Guttridge DC. Cancer cachexia is regulated by selective targeting of skeletal muscle gene products. J Clin Invest 2004; 114: 370-378.
[49] Matthys P, Heremans H, Opdenakker G, Billiau A. Anti-interferon-gamma antibody treatment, growth of Lewis lung tumours in mice and tumour-associated cachexia. Eur J Cancer 1991; 27: 182-187.
[50] Proctor MJ, Morrison DS, Talwar D, Balmer SM, O'Reilly DS, Foulis AK, Horgan PG, McMillan DC. An inflammation-based prognostic score (mGPS) predicts cancer survival independent of tumour site: a Glasgow Inflammation Outcome Study. Br J Cancer 2011; 104: 726-734.
[51] Torres ML, Hartmann LC, Cliby WA, Kalli KR, Young PM, Weaver AL, Langstraat CL, Jatoi A, Kumar S, Mariani A. Nutritional status, CT body composition measures and survival in ovarian cancer. Gynecol Oncol 2013; 129: 548-553.
[52] Mahmoud FA, Rivera NI. The role of C-reactive protein as a prognostic indicator in advanced cancer. Curr Oncol Rep 2002; 4:250-255.
[53] Nicola NA, Babon JJ. Leukemia inhibitory factor (LIF). Cytokine Growth Factor Rev 2015; 26: 533-544.
[54] Seto DN, Kandarian SC, Jackman RW. A key role for leukemia inhibitory factor in C26 cancer cachexia. J Biol Chem 2015; 290: 19976-19986.
[55] Inui A. Cancer anorexia-cachexia syndrome: are neuropeptides the key? Cancer Res 1999; 59: 4493-4501.
[56] Fearon K, Arends J, Baracos V. Understanding the mechanisms and treatment options in cancer cachexia. Nat Rev Clin Oncol 2013; 10: 90-99.
[57] Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature 2000; 407: 249-257.
[58] Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer 2003; 3: 401-410.
[59] Baenke F, Peck B, Miess H, Schulze A. Hooked on fat: the role of lipid synthesis in cancer metabolism and tumour development. Dis Model Mech 2013; 6: 1353-1363.
[60] Deep G, Schlaepfer IR. Aberrant lipid metabolism promotes prostate cancer: role in cell survival under hypoxia and extracellular vesicles biogenesis. Int J Mol Sci 2016; 17.
[61] Alam MM, Lal S, FitzGerald KE, Zhang L. A holistic view of cancer bioenergetics: mitochondrial function and respiration play fundamental roles in the development and progression of diverse tumors. Clin Transl Med 2016; 5: 3.
[62] Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol 2011; 27: 441-464.
[63] Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004; 4: 891-899.
[64] Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009; 324: 1029-1033.
[65] Xu XD, Shao SX, Jiang HP, Cao YW, Wang YH, Yang XC, Wang YL, Wang XS, Niu HT. Warburg effect or reverse Warburg effect? A review of cancer metabolism. Oncol Res Treat 2015; 38: 117-122.
[66] Wu W, Zhao S. Metabolic changes in cancer: beyond the Warburg effect. Acta Biochim Biophys Sin (Shanghai) 2013; 45: 18-26.
[67] Djiogue S, Nwabo Kamdje AH, Vecchio L, Kipanyula MJ, Farahna M, Aldebasi Y, Seke Etet PF. Insulin resistance and cancer: the role of insulin and IGFs. Endocr Relat Cancer 2013; 20: R1-R17.
[68] Yun J, Rago C, Cheong I, Pagliarini R, Angenendt P, Rajagopalan H, Schmidt K, Willson JK, Markowitz S, Zhou S, Diaz LA Jr, Velculescu VE, Lengauer C, Kinzler KW, Vogelstein B, Papadopoulos N. Glucose deprivation contributes to the development of KRAS pathway mutations in tumor cells. Science 2009; 325: 1555-1559.
[69] Sasaki H, Shitara M, Yokota K, Hikosaka Y, Moriyama S, Yano M, Fujii Y. Overexpression of GLUT1 correlates with Kras mutations in lung carcinomas. Mol Med Rep 2012; 5: 599-602.
[70] Dang CV. Glutaminolysis: supplying carbon or nitrogen or both for cancer cells? Cell Cycle 2010; 9: 3884-3886.
[71] Vyas S, Zaganjor E, Haigis MC. Mitochondria and Cancer. Cell 2016; 166: 555-566.
[72] Bollig-Fischer A, Dewey TG, Ethier SP. Oncogene activation induces metabolic transformation resulting in insulin-independence in human breast cancer cells. PLoS One 2011; 6: e17959.
[73] Lim JH, Lee ES, You HJ, Lee JW, Park JW, Chun YS. Ras-dependent induction of HIF-1alpha785 via the Raf/MEK/ERK pathway: a novel mechanism of Ras-mediated tumor promotion. Oncogene 2004; 23: 9427-9431.
[74] Chen C, Pore N, Behrooz A, Ismail-Beigi F, Maity A. Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. J Biol Chem 2001; 276: 9519-9525.

[75] Mathupala SP, Ko YH, Pedersen PL. Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. Oncogene 2006; 25: 4777-4786.
[76] Roberts DJ, Miyamoto S. Hexokinase II integrates energy metabolism and cellular protection: akting on mitochondria and TORCing to autophagy. Cell Death Differ 2015; 22: 364.
[77] Johnson C, Warmoes MO, Shen X, Locasale JW. Epigenetics and cancer metabolism. Cancer Lett 2015; 356: 309-314.
[78] Phang JM, Liu W, Hancock C. Bridging epigenetics and metabolism: role of non-essential amino acids. Epigenetics 2013; 8: 231-236.
[79] Carrer A, Wellen KE. Metabolism and epigenetics: a link cancer cells exploit. Curr Opin Biotechnol 2015; 34: 23-29.
[80] Antunes D, Padrao AI, Maciel E, Santinha D, Oliveira P, Vitorino R, Moreira-Goncalves D, Colaco B, Pires MJ, Nunes C, Santos LL, Amado F, Duarte JA, Domingues MR, Ferreira R. Molecular insights into mitochondrial dysfunction in cancer-related muscle wasting. Biochim Biophys Acta 2014; 1841: 896-905.
[81] Porter RK. Mitochondrial proton leak: a role for uncoupling proteins 2 and 3? Biochim Biophys Acta 2001; 1504: 120-127.
[82] Stuart JA, Cadenas S, Jekabsons MB, Roussel D, Brand MD. Mitochondrial proton leak and the uncoupling protein 1 homologues. Biochim Biophys Acta 2001; 1504: 144-158.
[83] Stefanyk LE, Coverdale N, Roy BD, Peters SJ, LeBlanc PJ. Skeletal muscle type comparison of subsarcolemmal mitochondrial membrane phospholipid fatty acid composition in rat. J Membr Biol 2010; 234: 207-215.
[84] Austin S, St-Pierre J. PGC1alpha and mitochondrial metabolism--emerging concepts and relevance in ageing and neurodegenerative disorders. J Cell Sci 2012; 125: 4963-4971.
[85] Rousset S, Alves-Guerra MC, Mozo J, Miroux B, Cassard-Doulcier AM, Bouillaud F, Ricquier D. The biology of mitochondrial uncoupling proteins. Diabetes 2004; 53 Suppl 1: S130-135.
[86] Bing C, Brown M, King P, Collins P, Tisdale MJ, Williams G. Increased gene expression of brown fat uncoupling protein (UCP)1 and skeletal muscle UCP2 and UCP3 in MAC16-induced cancer cachexia. Cancer Res 2000; 60: 2405-2410.
[87] Julienne CM, Dumas JF, Goupille C, Pinault M, Berri C, Collin A, Tesseraud S, Couet C, Servais S. Cancer cachexia is associated with a decrease in skeletal muscle mitochondrial oxidative capacities without alteration of ATP production efficiency. J Cachexia Sarcopenia Muscle 2012; 3: 265-275.
[88] Busiello RA, Savarese S, Lombardi A. Mitochondrial uncoupling proteins and energy metabolism. Front Physiol 2015; 6: 36.
[89] Clapham JC, Arch JR, Chapman H, Haynes A, Lister C, Moore GB, Piercy V, Carter SA, Lehner I, Smith SA, Beeley LJ, Godden RJ, Herrity N, Skehel M, Changani KK, Hockings PD, Reid DG, Squires SM, Hatcher J, Trail B, Latcham J, Rastan S, Harper AJ, Cadenas S, Buckingham JA, Brand MD, Abuin A. Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. Nature 2000; 406: 415-418.
[90] Giedt RJ, Fumene Feruglio P, Pathania D, Yang KS, Kilcoyne A, Vinegoni C, Mitchison TJ, Weissleder R. Computational imaging reveals mitochondrial morphology as a biomarker of cancer phenotype and drug response. Sci Rep 2016; 6: 32985.
[91] Shaughnessy DT, McAllister K, Worth L, Haugen AC, Meyer JN, Domann FE, Van Houten B, Mostoslavsky R, Bultman SJ, Baccarelli AA, Begley TJ, Sobol RW, Hirschey MD, Ideker T, Santos JH, Copeland WC, Tice RR, Balshaw DM, Tyson FL. Mitochondria, energetics, epigenetics, and cellular responses to stress. Environ Health Perspect 2014; 122: 1271-1278.
[92] Schulze A, Harris AL. How cancer metabolism is tuned for proliferation and vulnerable to disruption. Nature 2012; 491: 364-373.
[93] Chen JY, Lai YS, Tsai HJ, Kuo CC, Yen BL, Yeh SP, Sun HS, Hung WC. The oncometabolite R-2-hydroxyglutarate activates NF-kappaB-dependent tumor-promoting stromal niche for acute myeloid leukemia cells. Sci Rep 2016; 6: 32428.
[94] Padrao AI, Oliveira P, Vitorino R, Colaco B, Pires MJ, Marquez M, Castellanos E, Neuparth MJ, Teixeira C, Costa C, Moreira-Gonçalves D, Cabral S, Duarte JA, Santos LL, Amado F, Ferreira R. Bladder cancer-induced skeletal muscle wasting: disclosing the role of mitochondria plasticity. Int J Biochem Cell Biol 2013; 45: 1399-1409.
[95] Shum AM, Mahendradatta T, Taylor RJ, Painter AB, Moore MM, Tsoli M, Tan TC, Clarke SJ, Robertson GR, Polly P. Disruption of MEF2C signaling and loss of sarcomeric and mitochondrial integrity in cancer-induced skeletal muscle wasting. Aging (Albany NY) 2012; 4: 133-143.
[96] Li XB, Gu JD, Zhou QH. Review of aerobic glycolysis and its key enzymes-new targets for lung cancer therapy. Thorac Cancer 2015; 6: 17-24.
[97] Michalak KP, Mackowska-Kedziora A, Sobolewski B, Wozniak P. Key roles of glutamine pathways in reprogramming the cancer metabolism. Oxid Med Cell Longev 2015; 2015: 964321.

[98] Scalise M, Pochini L, Galluccio M, Indiveri C. Glutamine transport. From energy supply to sensing and beyond. Biochim Biophys Acta 2016; 1857: 1147-57.
[99] De Vitto H, Perez-Valencia J, Radosevich JA. Glutamine at focus: versatile roles in cancer. Tumour Biol 2016; 37: 1541-1558.
[100] Hensley CT, Wasti AT, DeBerardinis RJ. Glutamine and cancer: cell biology, physiology, and clinical opportunities. J Clin Invest 2013; 123: 3678-3684.
[101] Pochini L, Scalise M, Galluccio M, Indiveri C. Membrane transporters for the special amino acid glutamine: structure/function relationships and relevance to human health. Front Chem 2014; 2: 61.
[102] Shen J, Petersen KF, Behar KL, Brown P, Nixon TW, Mason GF, Petroff OA, Shulman GI, Shulman RG, Rothman DL. Determination of the rate of the glutamate/glutamine cycle in the human brain by *in vivo* 13C NMR. Proc Natl Acad Sci U S A 1999; 96: 8235-8240.
[103] Xiao D, Ren P, Su H, Yue M, Xiu R, Hu Y, Liu H, Qing G. Myc promotes glutaminolysis in human neuroblastoma through direct activation of glutaminase 2. Oncotarget 2015; 6: 40655-40666.
[104] Svoboda N, Zierler S, Kerschbaum HH. cAMP mediates ammonia-induced programmed cell death in the microglial cell line BV-2. Eur J Neurosci 2007; 25: 2285-2295.
[105] Shanware NP, Mullen AR, DeBerardinis RJ, Abraham RT. Glutamine: pleiotropic roles in tumor growth and stress resistance. J Mol Med (Berl) 2011; 89: 229-236.
[106] Hakimi AA, Reznik E, Lee CH, Creighton CJ, Brannon AR, Luna A, Aksoy BA, Liu EM, Shen R, Lee W, Chen Y, Stirdivant SM, Russo P, Chen YB, Tickoo SK, Reuter VE, Cheng EH, Sander C, Hsieh JJ. An integrated metabolic Atlas of clear cell renal cell carcinoma. Cancer Cell 2016; 29: 104-116.
[107] Owen OE, Kalhan SC, Hanson RW. The key role of anaplerosis and cataplerosis for citric acid cycle function. J Biol Chem 2002; 277: 30409-30412.
[108] Brunengraber H, Roe CR. Anaplerotic molecules: current and future. J Inherit Metab Dis 2006; 29: 327-331.
[109] Saqcena M, Mukhopadhyay S, Hosny C, Alhamed A, Chatterjee A, Foster DA. Blocking anaplerotic entry of glutamine into the TCA cycle sensitizes K-Ras mutant cancer cells to cytotoxic drugs. Oncogene 2015; 34: 2672-2680.
[110] Rajagopalan KN, DeBerardinis RJ. Role of glutamine in cancer: therapeutic and imaging implications. J Nucl Med 2011; 52: 1005-1008.
[111] Wise DR, Thompson CB. Glutamine addiction: a new therapeutic target in cancer. Trends Biochem Sci 2010; 35: 427-433.
[112] Pardee AB. A restriction point for control of normal animal cell proliferation. Proc Natl Acad Sci U S A 1974; 71: 1286-1290.
[113] Peck B, Schulze A. Lipid desaturation - the next step in targeting lipogenesis in cancer? FEBS J 2016; 283: 2767-2778.
[114] Riezman H. The long and short of fatty acid synthesis. Cell 2007; 130: 587-588.
[115] Shah US, Dhir R, Gollin SM, Chandran UR, Lewis D, Acquafondata M, Pflug BR. Fatty acid synthase gene overexpression and copy number gain in prostate adenocarcinoma. Hum Pathol 2006; 37: 401-409.
[116] Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer 2007; 7: 763-777.
[117] Liu H, Liu JY, Wu X, Zhang JT. Biochemistry, molecular biology, and pharmacology of fatty acid synthase, an emerging therapeutic target and diagnosis/prognosis marker. Int J Biochem Mol Biol 2010; 1: 69-89.
[118] Beld J, Lee DJ, Burkart MD. Fatty acid biosynthesis revisited: structure elucidation and metabolic engineering. Mol Biosyst 2015; 11: 38-59.
[119] Ventura R, Mordec K, Waszczuk J, Wang Z, Lai J, Fridlib M, Buckley D, Kemble G, Heuer TS. Inhibition of *de novo* palmitate synthesis by fatty acid synthase induces apoptosis in tumor cells by remodeling cell membranes, inhibiting signaling pathways, and reprogramming gene expression. EBioMedicine 2015; 2: 808-824.
[120] Ogino S, Kawasaki T, Ogawa A, Kirkner GJ, Loda M, Fuchs CS. Fatty acid synthase overexpression in colorectal cancer is associated with microsatellite instability, independent of CpG island methylator phenotype. Hum Pathol 2007; 38: 842-849.
[121] van der Mijn JC, Panka DJ, Geissler AK, Verheul HM, Mier JW. Novel drugs that target the metabolic reprogramming in renal cell cancer. Cancer Metab 2016; 4: 14.
[122] Huang WC, Li X, Liu J, Lin J, Chung LW. Activation of androgen receptor, lipogenesis, and oxidative stress converged by SREBP-1 is responsible for regulating growth and progression of prostate cancer cells. Mol Cancer Res 2012; 10: 133-142.
[123] Mulligan HD, Beck SA, Tisdale MJ. Lipid metabolism in cancer cachexia. Br J Cancer 1992; 66: 57-61.
[124] Stephens NA, Skipworth RJ, Macdonald AJ, Greig CA, Ross JA, Fearon KC. Intramyocellular lipid droplets increase with progression of cachexia in cancer patients. J Cachexia Sarcopenia Muscle 2011; 2: 111-117.

[125] Beloribi-Djefaflia S, Vasseur S, Guillaumond F. Lipid metabolic reprogramming in cancer cells. Oncogenesis 2016; 5: e189.
[126] Gao X, Lin SH, Ren F, Li JT, Chen JJ, Yao CB, Yang HB, Jiang SX, Yan GQ, Wang D, Wang Y, Liu Y, Cai Z, Xu YY, Chen J, Yu W, Yang PY, Lei QY. Acetate functions as an epigenetic metabolite to promote lipid synthesis under hypoxia. Nat Commun 2016; 7: 11960.
[127] Mashima T, Seimiya H, Tsuruo T. De novo fatty-acid synthesis and related pathways as molecular targets for cancer therapy. Br J Cancer 2009; 100: 1369-1372.
[128] Hashmi S, Wang Y, Suman DS, Parhar RS, Collison K, Conca W, Al-Mohanna F, Gaugler R. Human cancer: is it linked to dysfunctional lipid metabolism? Biochim Biophys Acta 2015; 1850: 352-364.
[129] Porporato PE, Filigheddu N, Reano S, Ferrara M, Angelino E, Gnocchi VF, Prodam F, Ronchi G, Fagoonee S, Fornaro M, Chianale F, Baldanzi G, Surico N, Sinigagalia F, Perroteau I, Smith RG, Sun Y, Geuna S, Graziani A. Acylated and unacylated ghrelin impair skeletal muscle atrophy in mice. J Clin Invest 2013; 123: 611-622.
[130] Marcell TJ. Sarcopenia: causes, consequences, and preventions. J Gerontol A Biol Sci Med Sci 2003; 58: M911-916.
[131] Argiles JM, Busquets S, Felipe A, Lopez-Soriano FJ. Molecular mechanisms involved in muscle wasting in cancer and ageing: cachexia versus sarcopenia. Int J Biochem Cell Biol 2005; 37: 1084-1104.
[132] Koopman R, van Loon LJ. Aging, exercise, and muscle protein metabolism. J Appl Physiol (1985) 2009; 106: 2040-2048.
[133] Evans WJ. Skeletal muscle loss: cachexia, sarcopenia, and inactivity. Am J Clin Nutr 2010; 91: 1123S-1127S.
[134] Schaap LA, Pluijm SM, Deeg DJ, Visser M. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. Am J Med 2006; 119: 526, e529-517.
[135] Morley JE, Thomas DR, Wilson MM. Cachexia: pathophysiology and clinical relevance. Am J Clin Nutr 2006; 83: 735-743.
[136] Camargo RG, Quintas Teixeira Ribeiro H, Geraldo MV, Matos-Neto E, Neves RX, Carnevali LC Jr, Donatto FF, Alcantara PS, Ottoch JP, Seelaender M. Cancer cachexia and MicroRNAs. Mediators Inflamm 2015; 2015: 367561.
[137] Fukawa T, Yan-Jiang BC, Min-Wen JC, Jun-Hao ET, Huang D, Qian CN, Ong P, Li Z, Chen , Mak SY, Lim WJ, Kanayama HO, Mohan RE, Wang RR, Lai JH, Chua C, Ong HS, Tan KK, Ho YS, Tan IB, Teh BT, Shyh-Chang N. Excessive fatty acid oxidation induces muscle atrophy in cancer cachexia. Nat Med 2016; 22: 666-671.
[138] Ebadi M, Mazurak VC. Evidence and mechanisms of fat depletion in cancer. Nutrients 2014; 6: 5280-5297.
[139] Bonetto A, Aydogdu T, Jin X, Zhang Z, Zhan R, Puzis L, Koniaris LG, Zimmers TA. JAK/STAT3 pathway inhibition blocks skeletal muscle wasting downstream of IL-6 and in experimental cancer cachexia. Am J Physiol Endocrinol Metab 2012; 303: E410-421.
[140] Zaki MH, Nemeth JA, Trikha M. CNTO 328, a monoclonal antibody to IL-6, inhibits human tumor-induced cachexia in nude mice. Int J Cancer 2004; 111: 592-595.
[141] Barreto R, Waning DL, Gao H, Liu Y, Zimmers TA, Bonetto A. Chemotherapy-related cachexia is associated with mitochondrial depletion and the activation of ERK1/2 and p38 MAPKs. Oncotarget 2016; 7: 43442-43460.
[142] Hasselgren PO, Fischer JE. Muscle cachexia: current concepts of intracellular mechanisms and molecular regulation. Ann Surg 2001; 233: 9-17.
[143] Bodine SC, Baehr LM. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. Am J Physiol Endocrinol Metab 2014; 307: E469-484.
[144] Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A, Walsh K, Schiaffino S, Lecker SH, Goldberg AL. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. Cell 2004; 117: 399-412.
[145] Reed SA, Sandesara PB, Senf SM, Judge AR. Inhibition of FoxO transcriptional activity prevents muscle fiber atrophy during cachexia and induces hypertrophy. FASEB J 2012; 26: 987-1000.
[146] Shi J, Luo L, Eash J, Ibebunjo C, Glass DJ. The SCF-Fbxo40 complex induces IRS1 ubiquitination in skeletal muscle, limiting IGF1 signaling. Dev Cell 2011; 21:835-847.
[147] Cabal-Manzano R, Bhargava P, Torres-Duarte A, Marshall J, Bhargava P, Wainer IW. Proteolysis-inducing factor is expressed in tumours of patients with gastrointestinal cancers and correlates with weight loss. Br J Cancer 2001; 84: 1599-1601.
[148] Todorov PT, Field WN, Tisdale MJ. Role of a proteolysis-inducing factor (PIF) in cachexia induced by a human melanoma (G361). Br J Cancer 1999; 80: 1734-1737.
[149] White JP, Puppa MJ, Sato S, Gao S, Price RL, Baynes JW, Kostek MC, Matesic LE, Carson JA. IL-6 regulation on skeletal muscle mitochondrial remodeling during cancer cachexia in the ApcMin/+ mouse. Skelet Muscle 2012; 2: 14.
[150] Arner P, Langin D. Lipolysis in lipid turnover, cancer cachexia, and obesity-induced insulin

resistance. Trends Endocrinol Metab 2014; 25: 255-262.
[151] Ryden M, Agustsson T, Laurencikiene J, Britton T, Sjolin E, Isaksson B, Permert J, Arner P. Lipolysis--not inflammation, cell death, or lipogenesis--is involved in adipose tissue loss in cancer cachexia. Cancer 2008; 113: 1695-1704.
[152] Thompson MP, Cooper ST, Parry BR, Tuckey JA. Increased expression of the mRNA for hormone-sensitive lipase in adipose tissue of cancer patients. Biochim Biophys Acta 1993; 1180: 236-242.
[153] Balaban S, Lee LS, Schreuder M, Hoy AJ. Obesity and cancer progression: is there a role of fatty acid metabolism? Biomed Res Int 2015; 2015: 274585.
[154] Batista ML Jr, Olivan M, Alcantara PS, Sandoval R, Peres SB, Neves RX, Silverio R, Maximiano LF, Otoch JP, Seelaender M. Adipose tissue-derived factors as potential biomarkers in cachectic cancer patients. Cytokine 2013; 61: 532-539.
[155] Cawthorn WP, Heyd F, Hegyi K, Sethi JK. Tumour necrosis factor-alpha inhibits adipogenesis via a beta-catenin/TCF4(TCF7L2)-dependent pathway. Cell Death Differ 2007; 14: 1361-1373.
[156] Cawthorn WP, Sethi JK. TNF-alpha and adipocyte biology. FEBS Lett 2008; 582: 117-131.
[157] Ruan H, Hacohen N, Golub TR, Van Parijs L, Lodish HF. Tumor necrosis factor-alpha suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes: nuclear factor-kappaB activation by TNF-alpha is obligatory. Diabetes 2002; 51: 1319-1336.
[158] Kong B, Michalski CW, Hong X, Valkovskaya N, Rieder S, Abiatari I, Streit S, Erkan M, Esposito I, Friess H, Kleeff J. AZGP1 is a tumor suppressor in pancreatic cancer inducing mesenchymal-to-epithelial transdifferentiation by inhibiting TGF-beta-mediated ERK signaling. Oncogene 2010; 29: 5146-5158.
[159] Sanders PM, Tisdale MJ. Role of lipid-mobilising factor (LMF) in protecting tumour cells from oxidative damage. Br J Cancer 2004; 90: 1274-1278.
[160] Russell ST, Tisdale MJ. Studies on the antiobesity effect of zinc-alpha2-glycoprotein in the ob/ob mouse. Int J Obes (Lond) 2011; 35:345-354.
[161] Bing C, Russell S, Becket E, Pope M, Tisdale MJ, Trayhurn P, Jenkins JR. Adipose atrophy in cancer cachexia: morphologic and molecular analysis of adipose tissue in tumour-bearing mice. Br J Cancer 2006; 95: 1028-1037.
[162] Tan BH, Birdsell LA, Martin L, Baracos VE, Fearon KC. Sarcopenia in an overweight or obese patient is an adverse prognostic factor in pancreatic cancer. Clin Cancer Res 2009; 15: 6973-6979.
[163] Fouladiun M, Korner U, Bosaeus I, Daneryd P, Hyltander A, Lundholm KG. Body composition and time course changes in regional distribution of fat and lean tissue in unselected cancer patients on palliative care--correlations with food intake, metabolism, exercise capacity, and hormones. Cancer 2005; 103: 2189-2198.
[164] Esper DH, Harb WA. The cancer cachexia syndrome: a review of metabolic and clinical manifestations. Nutr Clin Pract 2005; 20: 369-376.
[165] Borner T, Loi L, Pietra C, Giuliano C, Lutz TA, Riediger T. The ghrelin receptor agonist HM01 mimics the neuronal effects of ghrelin in the arcuate nucleus and attenuates anorexia-cachexia syndrome in tumor-bearing rats. Am J Physiol Regul Integr Comp Physiol 2016; 311: R89-96.
[166] Millington GW. The role of proopiomelanocortin (POMC) neurones in feeding behaviour. Nutr Metab (Lond) 2007; 4: 18.
[167] Bell CG, Walley AJ, Froguel P. The genetics of human obesity. Nat Rev Genet 2005; 6: 221-234.
[168] Sun J, Gao Y, Yao T, Huang Y, He Z, Kong X, Yu KJ, Wang RT, Guo H, Yan J, Chang Y, Chen H, Scherer PE, Liu T, Williams KW. Adiponectin potentiates the acute effects of leptin in arcuate Pomc neurons. Mol Metab 2016; 5: 882-891.
[169] Dodd GT, Decherf S, Loh K, Simonds SE, Wiede F, Balland E, Merry TL, Munzberg H, Zhang ZY, Kahn BB, Neel BG, Bence KK, Andrews ZB, Cowley MA, Tiganis T. Leptin and insulin act on POMC neurons to promote the browning of white fat. Cell 2015; 160: 88-104.
[170] Wolf I, Sadetzki S, Kanety H, Kundel Y, Pariente C, Epstein N, Oberman B, Catane R, Kaufman B, Shimon I. Adiponectin, ghrelin, and leptin in cancer cachexia in breast and colon cancer patients. Cancer 2006; 106: 966-973.
[171] Pritchard LE, Turnbull AV, White A. Pro-opiomelanocortin processing in the hypothalamus: impact on melanocortin signalling and obesity. J Endocrinol 2002; 172: 411-421.
[172] Tisdale MJ. Mechanisms of cancer cachexia. Physiol Rev 2009; 89: 381-410.
[173] Chen SZ, Qiu ZG. Combined treatment with GH, insulin, and indomethacin alleviates cancer cachexia in a mouse model. J Endocrinol 2011; 208: 131-136.
[174] Rutten IJ, van Dijk DP, Kruitwagen RF, Beets-Tan RG, Olde Damink SW, van Gorp T. Loss of skeletal muscle during neoadjuvant chemotherapy is related to decreased survival in ovarian cancer patients. J Cachexia Sarcopenia Muscle 2016; 7: 458-466.

[175] Winbanks CE, Murphy KT, Bernardo BC, Qian H, Liu Y, Sepulveda PV, Beyer C, Hagg A, Thomson RE, Chen JL, Walton KL, Loveland KL, McMullen JR, Rodgers BD, Harrison CA, Lynch GS, Gregorevic P. Smad7 gene delivery prevents muscle wasting associated with cancer cachexia in mice. Sci Transl Med 2016; 8: 348ra398.

[176] Barreto R, Waning DL, Gao H, Liu Y, Zimmers TA, Bonetto A. Chemotherapy-related cachexia is associated with mitochondrial depletion and the activation of ERK1/2 and p38 MAPKs. Oncotarget 2016; 7: 43442-43460.

[177] Subramaniam K, Fallon K, Ruut T, Lane D, McKay R, Shadbolt B, Ang S, Cook M, Platten J, Pavli P, Taupin D. Infliximab reverses inflammatory muscle wasting (sarcopenia) in Crohn's disease. Aliment Pharmacol Ther 2015; 41: 419-428.

[178] Molfino A, Amabile MI, Rossi Fanelli F, Muscaritoli M. Novel therapeutic options for cachexia and sarcopenia. Expert Opin Biol Ther 2016; 16: 1239-1244.

[179] Pigna E, Berardi E, Aulino P, Rizzuto E, Zampieri S, Carraro U, Kern H, Merigliano S, Gruppo M, Mericskay M, Li Z, Rocchi M, Barone R, Macaluso F, Di Felice V, Adamo S, Coletti D, Moresi V. Aerobic exercise and pharmacological treatments counteract cachexia by modulating autophagy in colon cancer. Sci Rep 2016; 6: 26991.

[180] Liu Z, Long W, Fryburg DA, Barrett EJ. The regulation of body and skeletal muscle protein metabolism by hormones and amino acids. J Nutr 2006; 136: 212S-217S.

[181] Quan-Jun Y, Yan H, Yong-Long H, Li-Li W, Jie L, Jin-Lu H, Jin L, Peng-Guo C, Run G, Cheng G. Selumetinib attenuate skeletal muscle wasting in murine cachexia model through ERK inhibition and AKT activation. Mol Cancer Ther 2017; 16: 334-343.

[182] von Haehling S, Anker SD. Treatment of cachexia: an overview of recent developments. J Am Med Dir Assoc 2014; 15: 866-872.

[183] Crawford J, Prado CM, Johnston MA, Gralla RJ, Taylor RP, Hancock ML, Dalton JT. Study design and rationale for the phase 3 clinical development program of enobosarm, a selective androgen receptor modulator, for the prevention and treatment of muscle wasting in cancer patients (POWER Trials). Curr Oncol Rep 2016; 18: 37.

[184] Wen HS, Li X, Cao YZ, Zhang CC, Yang F, Shi YM, Peng LM. Clinical studies on the treatment of cancer cachexia with megestrol acetate plus thalidomide. Chemotherapy 2012; 58: 461-467.

[185] Greig CA, Johns N, Gray C, MacDonald A, Stephens NA, Skipworth RJ, Fallon M, Wall L, Fox GM, Fearon KC. Phase I/II trial of formoterol fumarate combined with megestrol acetate in cachectic patients with advanced malignancy. Support Care Cancer 2014; 22: 1269-1275.

[186] Bai Y, Hu Y, Zhao Y, Yu X, Xu J, Hua Z, Zhao Z. Anamorelin for cancer anorexia-cachexia syndrome: a systematic review and meta-analysis. Support Care Cancer 2017; 25: 1651-1659.

[187] Hellerstein MK, Meydani SN, Meydani M, Wu K, Dinarello CA. Interleukin-1-induced anorexia in the rat. Influence of prostaglandins. J Clin Invest 1989; 84: 228-235.