Invited Review: Michelangelo, the Sistine Chapel and the “secret” of cancer cachexia

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Abstract

The clinical journey of chronic diseases, including cancer, renal failure and chronic obstructive pulmonary disease, is frequently characterised by the progressive deterioration of nutritional status, leading to increased morbidity and mortality, and impinges upon quality of life. Disease-associated malnutrition is characterised by anorexia and reduced food intake, but nutritional depletion cannot be accounted for by insufficient energy intake only. Indeed, wasting of muscles and adipose tissue also occurs, which is not suppressed by the provision of nutrients. Disease-associated malnutrition is defined as the anorexia-cachexia syndrome, to differentiate this clinical condition from malnutrition resulting from simple starvation, which responds to nutritional support. The pathogenesis of the anorexia-cachexia syndrome is multifactorial, but moderate yet persistent inflammation plays a prominent role in mediating the observed changes of eating behavior and of the metabolism of peripheral tissues. Peripheral tissue wasting and disease-associated anorexia have been classically considered as involving different molecular pathways, the former being mediated by increased muscle proteolysis and adipose tissue lipolysis, the latter being induced by neurochemical alterations. However, recent data seem to support the concept that disease-associated anorexia and wasting represent the clinical phenotypes of common pathogenic mechanisms, involving brain areas controlling energy homeostasis. In this regard, the different clinical pictures of disease-associated malnutrition, characterised by a variety of combinations of anorexia and wasting, reflect the different interactions occurring between the genotypes of the host and the underlying disease. Therefore, it has been proposed that the anorexia-cachexia syndrome is better defined as cachexia, which now encompasses the countless clinical expressions of the host’s response to a chronic insult. Surprisingly, such a unifying concept was already left by Michelangelo as a hidden message on the ceiling of the Sistine Chapel 500 years ago.

Introduction

When visiting Rome, the Vatican and its museums are a “must see”. Thousands of tourists visit the Sistine Chapel and admire the famous frescos by Michelangelo decorating the ceiling. Many are overwhelmed by the beauties of the scenes depicted. Others are more concerned on how Michelangelo could have painted these masterpieces lying on his back. But very few, when looking at the “Creation of Adam”, realise that Michelangelo was hiding a message in that scene, which is now becoming scientific evidence after 500 years.

In cancer patients, a progressive and apparently unstoppable deterioration of nutritional status frequently occurs, leading to worsened clinical outcome. Accumulating evidence indicates that this multifaceted syndrome is the clinical manifestation of a persistent, yet moderate inflammatory response, induced by the growing tumour. Although a number of symptoms contribute to the onset of severe malnutrition in cancer patients, anorexia (i.e. reduced appetite and energy intake) and peripheral tissue wasting, which is traditionally referred to as cachexia, are the leading causative factors. Therefore, the term anorexia-cachexia syndrome has been largely used to define the cancer-associated nutritional deterioration. More recently, however, the better knowledge of the molecular mechanisms of anorexia and tissue wasting revealed that a number of pathogenic pathways are shared by these two clinical conditions. In particular, the activity of specialised hypothalamic areas modulating energy homeostasis appears to be involved in mediating the behavioural and nutritional complications associated with cancer. Consequently, the term “cancer cachexia” is now becoming more frequently used in the clinical setting to define the progressive deterioration of nutritional status of cancer patients, irrespective of the relative contribution of anorexia and metabolic changes.

Cancer anorexia

The pathological and persistent loss of appetite, i.e. anorexia, is a clinically relevant symptom since it is highly prevalent and is a robust predictor of increased risk of death in hospitalised patients, particularly in cancer patients. Also, anorexia impinges on the quality of life, and is a key determinant of cancer patients’ quality of life.
Uncertainties still exist in clearly defining and diagnosing anorexia. In order to provide a diagnostic tool allowing a qualitative and quantitative assessment of cancer anorexia, the European Society for Clinical Nutrition and Metabolism (ESPEN) has recently developed an Anorexia Questionnaire, adapted from the AC/S-12 of Functional Assessment of Anorexia/Cachexia Therapy (FAACT) questionnaire. This diagnostic tool includes 12 questions related to appetite and eating behaviour, whose multiple answers are anchored to a score. A total score ≤ 24 has been proposed as being sufficient to make diagnosis of anorexia.

Pathogenesis of anorexia and reduced energy intake

Regulation of food intake. Under physiological conditions, the homeostasis of food intake is controlled by complex and redundant mechanisms. Neural, metabolic, and humoral signals from peripheral tissues inform the brain whether energy stores are being repleted or depleted. The hypothalamus receives and integrates peripheral signals. Within the hypothalamus, the infundibular nucleus in humans (i.e. the arcuate nucleus in rodents), is considered to act as an important sensor of alterations in energy stores to control appetite and body weight. Involved in this role are two distinct subsets of neurons.

The first population of neurons express pro-opiomelanocortin (POMC). POMC is an inert polypeptide precursor which is cleaved into the biologically active melanocortins, i.e. α-MSH, β-endorphin, γ-melanocyte-stimulating hormone (MSH). The biological effects of melanocortins are mediated through a family of five melanocortin receptors, termed MC1R to MC5R. Among them, MC4R is a crucial molecular component of the homeostatic circuit that regulates energy balance by mediating anorectic and catabolic responses.

The second subset of neurons expresses the potent orexigenic peptides neuropeptide Y (NPY) and agouti-related protein (AgRP). Interestingly, AgRP is the endogenous antagonist of MC4Rs, thereby antagonising the anorexigenic effects of melanocortins. This evidence underlines the reciprocal functional relationship between the two subsets of hypothalamic neurons. The POMC and NPY/AgRP neurons project to related hypothalamic nuclei, and these downstream second-order neurons expressing melanocortin receptors are included in the hypothalamic melanocortin system.

The melanocortin system plays a crucial role in the homeostasis of energy metabolism. In the presence of excess energy, POMC neurons are activated and trigger the release of melanocortins, which activate MC4R thereby leading to suppressed food intake and increased energy expenditure. Simultaneously, the activity of acutely AgRP/NPY system is suppressed, which would otherwise antagonise the effects of melanocortins on MC4R. In contrast, in times of energy depletions, the activity of anorexigenic POMC neurons is decreased but the activity of orexigenic NPY/AgRP neurons is increased.

Peripheral factors and cancer anorexia. Under physiological conditions, the hypothalamus integrates a number of peripheral inputs and modulates eating behaviour accordingly. These signals arise from peripheral tissues, mainly from the gastrointestinal tract, and are conveyed to the hypothalamus. The biological functions of these signals are different and time-specific. Consequently, peripheral signals are usually classified as short-term (i.e. visual-oronasal stimuli, ghrelin, cholecystokinin), medium-term (i.e. rising concentrations in plasma of specific nutrients, polypeptide YY) and long-term (i.e. leptin, insulin) signals.

Considering the role of peripheral signals in mediating the onset of appetite and satiety, it was tempting to speculate that changes in peripheral signals could mediate the development of cancer anorexia. However, consistent experimental data suggest that cancer anorexia mainly results from the resistance of hypothalamic neurons to peripheral signals.

Brain neurochemistry and cancer anorexia. The hypothalamic inappropriate response to these peripheral signals appears to be mediated by the persistent activation of POMC neurons. Consistent evidence indicates that MC4Rs are key factor in mediating anorexia and body weight loss during cancer. Furthermore, experimental data suggest that decreased activity of NPY/AgRP neurons should parallel the hyperactivation of POMC neurons in the presence of cancer. Immuncytochemical studies in tumour-bearing rats with anorexia-cachexia show decreased NPY innervation of hypothalamic nuclei, which is reversed by tumour resection. Direct measurement of NPY concentrations in the hypothalamus of tumour-bearing rats with anorexia-cachexia reveals a significant decrease of this orexigenic peptide. In humans, data on hypothalamic NPY levels and activity during cancer are lacking. However, significantly lower plasma levels of NPY have been measured in anorectic cancer patients when compared to controls. Furthermore, animal studies show that megestrol acetate, an orexigenic drug used in the treatment of human cachexia, increases hypothalamic NPY levels.

The mechanisms responsible for the dysfunction of the melanocortin system have been investigated in experimental studies, and results suggest the involvement of pro-inflammatory cytokines and the neurotransmitter serotonin. The role of pro-inflammatory cytokines, and particularly IL-1, IL-6 and TNF-α, in the pathogenesis of cancer anorexia has been recognised for many years. In tumour-bearing rats with anorexia, hypothalamic IL-1 mRNA expression is significantly increased. In humans, IL-1 appears to play a significant role in mediating anorexia since megestrol acetate has been shown to improve appetite and food intake via reduced expression of IL-1 by mononuclear cells, which may explain its effects on hypothalamic NPY concentrations. Interestingly, POMC neurons express the type 1 IL-1 receptor, and intracerebroventricular injection of IL-1 increases the frequency of action potentials of POMC neurons and stimulates the release of α-MSH. These data strongly suggest that IL-1 is involved in mediating the dysfunction of the melanocortin system by increasing the activity of POMC neurons in the hypothalamus.

Serotonin is a neurotransmitter that contributes to the regulation of energy balance, by mediating satiety through its effects in the hypothalamus. In experimental tumour models, the onset of anorexia is associated with increased hypothalamic serotonin levels, as assessed by in vivo microdialysis, and increased expression of serotonin receptors (5-HT3). Also, tumour resection restores energy intake, which is associated to normalised hypothalamic serotonin...
concentrations and receptor expression. In cancer patients, the role of serotonin in cancer anorexia has been inferred by measuring increased plasma and cerebrospinal fluid levels of the precursor of serotonin, the amino acid tryptophan, in anorectic cancer patients. Intriguingly, the anorectic effects of serotonin appear to be mediated by the melanocortin system.

These data indicate that during cancer, increased hypothalamic expression of IL-1 occurs in conjunction with increased release of serotonin. Serotonin and IL-1 interact within the arcuate nucleus (infundibular nucleus in humans) to influence the activity of the melanocortin system, yielding and maintaining the inhibition of NPY/AgRP neuronal activity. These biochemical events facilitate the release of the endogenous MC4R agonist, α-MSH, while suppressing the release of the endogenous MC4R antagonist, AgRP, thus resulting in dysfunction of the melanocortin system.

**Hypothalamic energy metabolism and cancer anorexia.** The hypothalamic regulation of food intake involves different pathways. Indeed, fatty acid metabolism within hypothalamic neurons controls food intake and energy metabolism in a leptin-independent way. In particular, inhibition of fatty acid synthase (FAS) blocks fasting-induced upregulation of orexigenic neuropeptides and downregulation of anorexigenic neuropeptides. Hypothalamic malonyl-coenzyme A (CoA), a substrate of FAS, is an indicator of global energy status. Its concentration is low in fasted mice and rapidly increases on refeeding. Therefore, high intrahypothalamic malonyl-CoA induces anorexia by inhibiting fatty acid oxidation, whereas low levels have the converse effect and elicit food intake.

The FAS/malonyl-CoA pathway could be involved in the pathogenesis of cancer anorexia, because in-vitro studies show that pro-inflammatory cytokines, particularly TNF-α and IL-1, inhibit fatty acid oxidation. If this effect also applies to the in-vivo situation, then pro-inflammatory cytokines, and particularly IL-1, may cause an inappropriate switch in hypothalamic neurons from fatty acid oxidation to fatty acid synthesis, increase hypothalamic malonyl-CoA concentrations and suppress food intake.

**Cancer cachexia**

Changes in intermediary metabolism largely contribute to wasting and mediate a number of clinically relevant symptoms and signs in cancer patients. Robust experimental and clinical data indicate that chronic inflammation, as already discussed for the pathogenesis of cancer anorexia, represents the main trigger of the changes in intermediary metabolism.

**Pathogenesis of wasting**

**Protein metabolism in cancer patients.** Under physiological conditions, protein degradation in muscle is offset by compensatory protein synthesis to preserve muscle mass. During cancer, muscle catabolic pathways are hyperactivated, while a compensatory increase of muscle anabolism does not occur. The net result is the progressive loss of muscle mass and muscle function. There are three main proteolytic pathways that are responsible for protein catabolism in skeletal muscle. The first pathway is the lysosomal system, which is involved in proteolysis of extracellular proteins and cell-surface receptors. The second pathway is the cytosolic calcium-regulated calpains, which are mainly involved in tissue catabolism in skeletal muscle. The third pathway, and probably the most significant proteolytic contributor to muscle wasting in cancer cachexia is the ATP ubiquitin proteasome proteolytic pathway, whose preferred substrate is myosin heavy chain. Interestingly, the ubiquitin system is hyperactivated also in weight stable cancer patients. This evidence highlights the need to start the nutritional intervention as early as possible, since absence of clinical signs may not imply absence of metabolic derangements.

A number of mediators of muscle wasting have been identified. The pro-inflammatory cytokines TNF-α and IL-1 have been shown to activate the ubiquitin-proteasome system. Similar effects have been suggested to be mediated by the tumour-derived 24-kDa sulfated glycoprotein proteolysis-inducing factor (Pif), whose role in cancer cachexia has been recently questioned. In addition, there has been significant progress in identifying new signalling pathways that contribute to muscle atrophy that are potentially pertinent in cancer. Among others, these include the down-regulated insulin and insulin-like growth factor pathways that lead to Akt inactivation and reversal of muscle hypertrophy; the angiotensin system that operates through the activation of caspases; the transforming growth factor-β family member myostatin; and the mediator of terminal muscle differentiation MyoD. Interestingly, most, if not all, of these emerging pathways seem to mediate their effects through the activation of the ubiquitin-proteasome system.

**Carbohydrate metabolism in cancer patients.** In the 1920s, Warburg demonstrated that tumours differ in glucose metabolism from normal tissues by exhibiting an enhanced glycolytic pathway. Under normal oxygen-rich conditions, tissues metabolise glucose to pyruvate, which is then completely oxidised to CO2 and H2O via the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. Conversely, in states of low oxygen tension, as may occur in some solid tumours, cells can rely solely on anaerobic glycolysis, in which glucose is converted to pyruvate and then lactic acid in the cytosol to meet energy needs; this is known as the Pasteur effect. Many invasive cancer cells selectively metabolise glucose to lactic acid in the presence of oxygen, a phenomenon known as the Warburg effect. There are several defects in cell biochemistry that may facilitate the anaerobic glycolytic phenotype in cancer cells over the more efficient oxidative phosphorylation. The excess of lactate surrounding tumour cells is transported to the liver, where the carbon skeleton undergoes an energy-consuming gluconeogenic process to re-form glucose. It is important to underline that the creation of a futile Cori cycle can account for as much as a 300 kcal/day energy loss.

Tumour-induced alterations in glucose metabolism include also insulin resistance, and 37% of all patients with cancer show glucose intolerance and abnormal insulin responses. Consistent evidence implicates the action of cytokines on glucose metabolism directly or by stimulation of glucoregulatory hormones.

**Lipid metabolism in cancer patients.** In cancer patients, an extensive depletion of adipose tissue is frequently observed. The loss of fat
stores cannot be explained by reduced appetite alone. Increased lipolysis appears to be a key factor underlying the loss of adipose tissue in cancer cachexia. Enhanced expression and activity of hormone-sensitive lipase (HSL), a rate-limiting enzyme of the lipolytic pathway, is thought to underlie the increase in lipolysis. In addition to increased lipolysis, there is a fall in lipoprotein lipase (LPL) activity.

Various factors produced by tumours, or by the host’s immune cells responding to the tumour, can alter lipid metabolism. Pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 have been implicated in adipose atrophy in cachexia. Certain tumours produce circulating factors such as the 41 kDa zinc-α2-glycoprotein (ZAG). The biological functions of ZAG were largely unknown until a lipid-mobilising factor (LMF), purified from the urine of cancer patients with cachexia, was shown to be identical to ZAG.

Energy metabolism in cancer patients. Coupling of energy intake with energy production is a mechanism to conserve energy when energy intake is low while disposing of excess ingested energy when energy intake is excessive. However, in cancer patients there is an uncoupling of the balance between energy production and energy intake in favour of increased energy production. One possible explanation for this increased energy expenditure is that the tumour-bearing host metabolism is energetically inefficient due to increased futile cycle activity. The recycling of lactate between the tumour and the host is certainly energetically inefficient. Pro-inflammatory cytokines IL-6 and TNF-α have been implicated in stimulating gluconeogenesis and activating a futile cycle of glucose use and lactate formation in hepatocytes and myocytes, respectively.

Nonshivering thermogenesis takes place in brown adipose tissue (BAT) whereby energy uncoupled from oxidative phosphorylation is released as heat energy instead of fuelling ATP production. During cachexia, there is an increase in BAT thermogenesis. Brown adipocytes contain mitochondria that are characterised by the presence of uncoupling proteins 1,2,3 (UCP1, UCP2, UCP3). The UCP proteins are a family of mitochondrial membrane proteins that decrease the coupling of oxidative phosphorylation to ATP formation, culminating in the production of heat rather than ATP. Increased UCP-mediated thermogenesis in BAT increases energy expenditure and may exacerbate cancer cachexia.

Cancer cachexia: a unifying hypothesis of its pathogenesis

For decades, the accepted model of the pathogenesis of cancer cachexia was based on the assumption that behavioural changes (i.e. anorexia) and metabolic alterations (i.e. muscle wasting, increased energy expenditure) were mediated by the derangement of different molecular pathways. Accumulating evidence now challenges this approach by suggesting that anorexia and wasting represent in part different phenotypes of common neurochemical/metabolic alterations. Hypothalamic signalling and changes in energy expenditure. As previously described in detail, the inhibitory effects of pro-inflammatory cytokines on fatty acid oxidation contribute to the dysregulation of the melanocortin system, thus leading to reduced energy intake. However, inhibition of fatty acid oxidation within hypothalamic neurons influences metabolism in peripheral tissues. The “malonyl-CoA signal” is rapidly transmitted to peripheral tissues by the sympathetic nervous system, increasing mitochondrial biogenesis, fatty acid oxidation and uncoupling protein-3 expression, and thus energy expenditure. Also, since mitochondria are among the main sources of reactive oxygen species production, it is postulated that this “brain–muscle axis” may contribute to increased oxidative stress, which in turn increases muscle protein degradation.

Hypothalamic signalling and muscle mass regulation. Experimental studies aimed at suppressing the activity of the central melanocortin system during cancer resulted in increased food intake, which was paralleled by amelioration of energy expenditure and improved lean body mass. The mechanisms by which MC4R antagonism in cancer models exerts its metabolic effects are still a matter of investigation. Recent data suggest that the improvement of basal metabolic rate is partly mediated by the normalisation of the expression of uncoupling proteins. Much less is known about the mechanisms leading to preserved body weight and lean body mass. In particular, it is yet to be determined whether central melanocortin antagonism influences the activity of the peripheral ATP-dependent ubiquitin-proteasome system. Nevertheless, the existence of a “brain–muscle axis” is likely, in which the brain not only regulates energy intake, but influences metabolic rate and the balance in muscles between anabolism and catabolism. The exact mechanisms of the interplay between central and peripheral pathways await better detailing, however it appears to involve the balance between inhibitory and stimulatory factors of the regulation of muscle mass, as recently demonstrated in an animal model of uraemic cachexia. In particular, the expression of myostatin, an important inhibitory factor of muscle mass accretion, is increased in the muscles of uraemic rats. In contrast, the expression of insulin-like growth factor-I, a factor promoting muscle accretion, is reduced. Interestingly, the injection of AgRP in the third ventricles of uraemic rats partially corrected these uraemia-induced changes, resulting in a gain of body mass.

Does clinical cancer cachexia match accumulating molecular evidence?

To strengthen the hypothesis that anorexia and wasting share similar pathogenic pathways, it would be important to verify whether they are clinically equivalent in the clinical practice. To this end, it is important to review the available definitions of cachexia and the clinical definition of cancer cachexia. Indeed, if the pathogenesis of cancer anorexia and tumour-associated tissue wasting is, at least in part, common, then it may be more appropriate to refer to the constellation of symptoms and signs leading to weight loss using a single term, i.e. cachexia, rather than anorexia-cachexia. Unfortunately, a widely accepted consensus on the definition of cancer cachexia does not exist yet. To promote the use of a common language among scientists and clinicians, ESPEN has recently endorsed the Washington definition which identifies cachexia as a complex metabolic syndrome associated to an underlying illness, and
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characterised by loss of weight and muscle mass with or without the loss of fat mass. Although this definition identifies cancer patients at risk of poorer outcome, on the other hand, patients meeting the criteria of cancer cachexia are likely to be in an advanced stage of nutritional depletion, which may limit the benefit of nutritional intervention. Therefore, the identification of symptoms related to the early phases of cachexia, or pre-cachexia, may help in identifying the higher risk patients and may favor the prompt start of nutrition therapy. ESPEN has recently proposed that pre-cachexia could be diagnosed in the simultaneous presence of underlying chronic disease, unintentional weight loss < 5% of usual body weight during the last six months, chronic or recurrent systemic inflammatory response, and anorexia or anorexia-related symptoms.

Although other symptoms are considered as complementary criteria for cachexia (i.e. anorexia, anaemia), it appears evident that the available definitions of cancer cachexia identify weight loss as the hallmark of this syndrome. Therefore, it could be assumed that in the absence of weight loss, patients cannot be defined as cachectic. But is this an approach that is clinically relevant? A critical answer to this questions is provided by the recent paper by Lasheen & Walsh. The authors studied the symptoms reported by patients with advanced cancer, in particular lung, colorectal, breast, pelvic and prostate cancer. Twenty-six percent of the patients did not complain of weight loss or anorexia. However, approximately 1/3 of patients complained of anorexia, 10% reported significant and involuntary weight loss and 31% reported anorexia and weight loss. Therefore, according to the Washington definition, 41% of the cancer population studied met the definition of cancer cachexia. In clinical medicine, any definition and the attendant diagnostic criteria are valuable since they identify patients at higher risk of negative clinical outcome. Therefore, it could be assumed that the outcome of the cachectic patients (i.e. those with weight loss or weight loss and anorexia) should be different from that of the cancer patients with anorexia but no weight loss. In contrast, the survival of cancer patients with weight loss only, anorexia only, and weight loss and anorexia did not differ. These results suggest that cancer cachexia is a multifaceted syndrome in which anorexia and weight loss due to wasting are clinically relevant symptoms which are not mutually exclusive.

Therefore, it seems that molecular and clinical evidence indicates that cancer cachexia should be better defined as a complex clinical syndrome, which is clinically characterised by a varying combination of anorexia and tissue wasting.

Conclusion

Accumulating evidence consistently reveals that the brain is key in mediating, influencing and determining not only human behaviour but a number of metabolic pathways in peripheral tissues as well. These results provide the molecular basis to evidence showing that transcendental meditation improves blood pressure and insulin resistance, and that willingness to take part in a clinical trial relates to a better prognosis. The fascinating unifying hypothesis of the pathogenesis of cancer cachexia, when corroborated by further evidence, will support the view that the brain and the central nervous system are key in controlling many, if not all, psychological and biological aspects of human life.

Not surprisingly for a genius, Michelangelo had already realised the key role of the brain in human life, revealing this concept in the “Creation of Adam” in the Sistine Chapel. Although a quick look at the fresco on the ceiling may not suggest the presence of any hidden message, a more accurate look will reveal that the angels and the robe around the body of God clearly form the shape of a sectioned brain. And God is in the centre of the brain. Michelangelo was a neo-aristotelic and in the painting he wanted to state that the presence of God is testified by the “divinity” of our brain. After 500 years, we may now use the painting of Michelangelo to state that the more we will know about our brain, the more we will learn about the biology of life and the best strategy to prevent and treat diseases.

References