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Review

Biomarkers of cancer cachexia

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ABSTRACT

Cachexia is a complex multifactorial syndrome, characterized by loss of skeletal muscle and fat mass, which affects the majority of advanced cancer patients and is associated with poor prognosis. Interestingly, reversing muscle loss in animal models of cancer cachexia leads to prolong survival. Therefore, detecting cachexia and maintaining muscle mass represent a major goal in the care of cancer patients. However, early diagnosis of cancer cachexia is currently limited for several reasons. Indeed, cachexia development is variable according to tumor and host characteristics. In addition, safe, accessible and non-invasive tools to detect skeletal muscle atrophy are desperately lacking in clinical practice. Finally, the precise molecular mechanisms and the key players involved in cancer cachexia remain poorly characterized. The need for an early diagnosis of cancer cachexia supports therefore the quest for a biomarker that might reflect skeletal muscle atrophy process. Current research offers different promising ways to identify such a biomarker. Initially, the quest for a biomarker of cancer cachexia has mostly focused on mediators of muscle atrophy, produced by both tumor and host, in an attempt to define new therapeutic approaches. In another hand, molecules released by the muscle into the circulation during the atrophy process have been also considered as potential biomarkers. More recently, several "omics" studies are emerging to identify new muscular or circulating markers of cancer cachexia. Some genetic markers could also contribute to identify patients more susceptible to develop cachexia. This article reviews our current knowledge regarding potential biomarkers of cancer cachexia.

1. Introduction

1.1. Definition of cancer cachexia

Cachexia is a complex multifactorial syndrome associated with underlying illness and characterized by loss of skeletal muscle and fat mass. In contrast to malnutrition, cachexia cannot be reversed simply by conventional nutritional support [1]. Cachexia affects the majority of advanced cancer patients and is associated with poor prognosis regardless the tumor nature [2]. More precisely, the loss of skeletal muscle mass is recognized as an independent predictor of mortality and is associated with functional impairment, altered quality of life and reduced tolerance and response to anticancer therapies [1,3]. Interestingly, it has been shown that reversal of muscle loss leads to prolong survival in animal models of cancer cachexia [4]. These observations support that maintaining muscle mass is *per se* helpful in improving survival in cachectic conditions. Therefore, detecting cachexia and maintaining muscle mass represent a major goal in the care of cancer patients.

1.2. Molecular mechanisms of cancer cachexia

The precise molecular mechanisms of cancer cachexia remain poorly characterized. It is thought to result from abnormal metabolism and anorexia. Inflammation, induced by both tumor- and host-derived factors seems to be central in the wasting process [5]. The development of muscle atrophy results from an imbalance between muscle protein synthesis and degradation inducing a decrease in myofibrillar and sarcoplasmic proteins illustrated by muscle fiber shrinkage. However, the nature of the key players responsible for muscle atrophy in cancer cachexia is still elusive. Therefore, early diagnosis and clinical management of cancer cachexia are currently limited.

1.3. Challenges of the diagnosis

Cachexia development is variable according to the tumor nature, stage and site, but also to interindividual variations (genetic predisposition, initial BMI and body composition, physical activity, food intake, comorbidities and gut microbiota) [2,5,6]. Therefore, due to the heterogeneity of presentation and to the lack of well-established definition, the diagnosis of cachexia is often not done in a timely fashion. In

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2011, Fearon et al. marked an evolution in the field, bringing a consensus definition of cancer cachexia, based on body weight loss in association with low muscularity [1]. However, measuring and monitoring muscle mass remain challenging for several reasons. Foremost, in contrast to body weight loss, skeletal muscle mass measurement is a static parameter, which does not allow to properly appreciate muscle mass changes over time. In addition, muscle mass is extremely variable across the general population. Hence, a normal muscularity does not exclude a previous loss of muscle mass. Finally, the tools used to measure skeletal muscle mass, such as CT-scan, DEXA or bioimpedance are limited by their accessibility, repeatability, cost and irradiation.

1.4. The ideal biomarker of cancer cachexia

The need for an early diagnosis of cancer cachexia supports the quest for a biomarker that might reflect skeletal muscle atrophy process. The clinical perspectives of such a marker are multiple. Since the efficiency of care depends of the early start of therapy, such biomarker could allow an early diagnosis and an effective therapy as well as the monitoring of this therapy. The best marker of cachexia should be a marker of the muscle wasting process more than a marker of skeletal muscle mass, given the extremely variability of muscle mass across the general population. It should therefore allow to detect precociously the muscle loss process, even in patients with initial high muscle mass and before reaching a low muscularity level. Furthermore, it should be highly specific to skeletal muscle wasting and only weakly influenced by other intercurrent processes, such as infections, immobilization and anti-cancer therapies. Finally, the ideal biomarker should be easy to quantify in circulation, without requiring invasive muscle biopsy.

Since the loss of muscle mass is crucial to cancer cachexia and mostly determine the survival prognosis, most of the putative biomarkers described so far have focused on the muscle wasting process. However, the contribution of inflammation and anorexia to the development of cancer cachexia pinpoints new avenues also susceptible to lead to biomarkers identification. Although the quest for a biomarker of cancer cachexia has mostly focused on mediators of muscle atrophy, other molecules, some of them being released by the muscle or eventually adipose tissue into the circulation, might reflect this process and be therefore considered as potential biomarkers. On the other hand, the non-hypothesis driven “omic” technic approach should lead to highlight new muscular or circulating markers of muscle wasting process. Finally, some genetic markers could contribute to identify patients more susceptible to develop cachexia. This article reviews our current knowledge regarding the potential biomarkers of cancer cachexia.

2. Biomarkers derived from mediators of cancer cachexia

2.1. Pro-inflammatory cytokines

Inflammation, induced by both tumor- and host-derived factors is a key player in the development of cancer cachexia. Several early studies have therefore focused on the role of pro-inflammatory cytokines, such as Tumor Necrosis Factor α (TNFα) and Interleukin-6 (IL-6) as biomarkers of this muscle wasting process. Indeed, TNFα and IL-6 are upregulated in different cancer cachexia animal models [4,7–9] and are released by some tumor cell lines [7]. Moreover, administration of anti-TNFα or anti-IL-6 antibodies abolishes the increase of muscle proteolysis makers observed during cachexia, albeit not sufficient to reverse completely the muscle atrophy [10,11]. Finally, an elevation of systemic concentrations of TNFα and IL-6 to supraphysiological levels leads to cachexia and early death in animals [12,13].

In humans, however, the increase of circulating TNFα in cancer cachexia is controversial [14,15]. Moreover, circulating TNFα levels are not associated with survival in cancer patients [16] and anti-TNFα therapies, such as infliximab, have failed to prevent the muscle loss in cancer patients [17]. These clinical data do not therefore support TNFα as neither a critical driver nor a biomarker of muscle wasting during cancer cachexia. In contrast to TNFα, the clinical relevance of IL-6 has been better delineated, mainly concerning its role as a predictive factor of survival in advanced cancer patients [15,16,18]. However, the link between an elevation of circulating IL-6 levels and body weight loss in cancer patients is variable across studies and the association between IL-6 and the low muscularity is poorly investigated [14,19–24]. Furthermore, in contrast to animal models, treatment with an IL-6 specific monoclonal antibody has beneficial effects on anemia and anorexia, but no clear effects on lean mass in cancer patients [25]. These data suggest that IL-6 might be involved in cachexia process through the development of anorexia and fat mass loss rather than *via* a direct muscle atrophic effect. Thenceforth, IL-6 could be a candidate as biomarker of inflammation associated with cancer cachexia and a good predictive factor of survival.

On the other hand, Penafuerte et al., have identified in plasma of cachectic and pre-cachectic cancer patients compared to those without cachexia an increase in mRNA expression of neutrophil-derived proteases, angiotensin II, TGFβ1 and CRP levels [24], supporting the role of inflammation in cancer cachexia development.

2.2. Transforming Growth Factor (TGF)-β superfamily members

Since Zhou et al. showed in 2010, that treating tumor-bearing mice with an antagonist of Activin A and Myostatin, reverses cancer cachexia and dramatically extends survival, despite high circulating levels of classical pro-inflammatory cytokines [4], the interest for these members of the TGF-β superfamily, in the cancer cachexia field, has rapidly grown.

2.2.1. Activin A

Activin (Act) A, the most known member of the Activin family, is expressed in a wide range of tissues and exerts many biological activities by binding to the Activin type IIB receptor (ActRIIB) [26]. ActA is present in the circulation either in bioactive free form or bound to Follistatin, the main regulator of its biological activity. ActA concentrations are increased in various pathologies, such as cancer, acute respiratory distress syndrome, chronic obstructive pulmonary disease, and in many conditions associated with inflammation [27].

Several evidences have pinpointed the possible role of ActA as a critical driver of muscle atrophy in cancer cachexia. Firstly, inhibin-deficient mice, characterized by high concentrations of circulating ActA, exhibit a loss of skeletal muscle leading to death [4]. Secondly, even in the absence of underlying disease or tumor, an increase of local or circulating concentrations of ActA induces skeletal muscle atrophy [4,9,28,29]. Furthermore, in contrast to IL-6, the body weight loss observed in rodents in response to an elevation of circulating ActA levels is mainly caused by a loss of lean mass [9]. Indeed, ActA exerts a direct atrophic effect on skeletal muscle by binding to its muscle ActIIB receptor, leading to an activation of an atrophy gene program [28]. Finally, circulating levels of ActA have been reported to be elevated in animal models of cancer cachexia and ActA expression is increased in various malignant tumor tissues [9,22,30,31].

Our team has recently shown in a population of cancer patients that, cachexia, characterized by a decreased of muscle mass and function, is associated with high circulating concentrations of ActA [32]. In the same line, Lerner et al., have found an elevation of ActA in cancer patients with body weight loss and a negative correlation between ActA levels and lean mass [21,22,33]. Furthermore, high level of ActA is a prognostic factor of poor survival in different types of cancer [34–37]. Taken together, these observations indicate that ActA might appear as a circulating marker of muscle wasting during cancer cachexia. Albeit future studies will be necessary to validate the value of ActA as a marker of cachexia in cancer patients, this hypothesis is particularly attractive, since inhibitors of ActA are currently under clinical investigation.

2.2.2. Myostatin

Myostatin (Mstn), also called Growth Differentiation Factor-8 (GDF-8), is produced predominantly by skeletal muscle and acts mainly as an autocrine/paracrine factor on muscle itself [38]. Mstn exerts an inhibitory effect on muscle growth, by binding to the ActIIB receptor, which is shared with ActA [39].

Although Mstn is doubtless an inhibitor of skeletal muscle mass development, its role in muscle atrophy remains unclear. Indeed, *in vitro*, Mstn reduces diameter of myotubes [39]. Similarly, in rodents, muscle overexpression [40] or systemic administration [41] of Mstn lead to skeletal muscle atrophy. In addition, inhibition of Mstn increases dramatically muscle mass [38]. However, although muscle Mstn expression or protein levels are often increased in muscle atrophy models such as cancer cachexia [4], denervation [42], and sarcopenia [43], circulating Mstn level is not increased in most situations of muscle atrophy and particularly in cancer cachexia models [44,45].

In humans, the role of circulating Mstn in cancer cachexia is uncertain. Early observations reported elevated levels of Mstn in conditions characterized by muscle wasting, such as ageing [46], HIV infection [47] and heart failure [48]. At the opposite, recent studies showed a decrease of circulating Mstn in conditions characterized by reduced skeletal muscle mass such as ageing, without correlation between Mstn levels and muscle mass [49–52]. In cancer patients with weight loss, Breitbart et al., observed a decrease of circulating Mstn propeptide levels, reflecting a decrease in Mstn production [53]. Consistent with this observation, we showed that patients, exhibiting cancer cachexia, had low circulating Mstn levels in comparison to those without cachexia, and Mstn levels were correlated positively with skeletal muscle mass [32]. The lack of consistency between these studies might be explained by the use of various immunoassays detecting different forms of Mstn (free or binding with its propeptide) and a possible cross-reactivity with other TGF-β members. But, most likely, this data suggest that circulating Mstn does not play a major role in the cancer cachexia development. On the other hand, a decrease in circulating Mstn in cancer cachexia could be interpreted as a protective down-regulatory mechanism in response to muscle wasting. In conclusion, circulating Mstn does not seem to reflect the muscle wasting process during cancer cachexia.

2.2.3. GDF-11

The Growth Differentiation Factor 11 (GDF-11) is the TGF-β family member closest to Mstn. GDF-11 acts also by binding to a shared ActIIB receptor, suggesting that it reproduces the biological actions of Mstn on skeletal muscle. However, in contrast to Mstn, GDF-11 is widely expressed in several tissues and a deletion of GDF-11 gene leads mainly to skeletal defects, implying a distinct role from Mstn [54]. Hammers et al., have recently delineated the effects of GDF-11 on skeletal muscle [55]. They showed that, like Mstn, GDF-11 causes an atrophy of C2C12 myotubes. Moreover, an elevation in circulating GDF-11 leads to skeletal muscle atrophy in mice. In humans, data concerning circulating GDF-11 and ageing or sarcopenia are controversial due to difficulties related to its measurement, owing to its great similarity with Mstn and its low concentration in circulation. With a more specific assay (mass spectrometry), Schafer et al., highlighted a link between high GDF-11 and frailty [52]. Definitely, these observations support the need of additional studies to assess the association between an elevation of GDF-11 and the muscle wasting process.

2.2.4. GDF-15

Growth differentiation factor-15 (GDF-15), also known as Macrophage Inhibiting Factor-1, is produced by several tissues and its circulating levels rise in pathological situations, such as inflammation, injury, cancer and cardiovascular diseases [56]. One of its best known functions is to regulate appetite. In fact, in mice, an elevation in circulating GDF-15 induces an important decrease of body weight, due to a strong reduction in food intake, which are both completely reversible with the return of GDF-15 levels in normal ranges [57]. The body weight loss, in this case, is characterized by a loss of both lean and fat mass. The anorectic effect of GDF-15 results from a direct action of the circulating cytokine on feeding centers in the brainstem [57]. In contrast, the data concerning a direct effect of GDF-15 on skeletal muscle are scare. Nevertheless, an increase in expression of proteolysis markers, such as Atrogin-1 and MuRF-1, is observed in C2C12 myotubes exposed to GDF-15 and, more importantly, overexpression of GDF-15 in skeletal muscle causes muscle atrophy *in vivo* [58]. Taken together, these data suggest than in addition to its central anorectic effect, GDF-15 might exert a direct atrophic effect towards skeletal muscle.

The substantial elevation of circulating GDF-15 observed in cancer patients [57,59] and its role in appetite regulation have therefore led to postulate its implication in cancer cachexia development. Indeed, GDF-15 is expressed and secreted in various malignant tumors. In addition, Lerner et al., observed that treating mice, bearing different human tumor xenografts, with an anti-GDF-15 antibody, prevented body weight loss, particularly skeletal muscle mass and prolonged survival, without affecting tumor growth [22].

In cancer patients, a high level of circulating GDF-15 appears to be associated with body weight loss and negatively correlated with lean mass [33,57]. Unexpectedly, no association between GDF-15 and appetite scores was observed, suggesting that other factors could influence appetite in cancer patients or that GDF-15 could be involved in body weight loss through other mechanisms. Finally, as demonstrated for ActA, high level of GDF-15 was associated with shorter survival in cancer patients [21,22,33,60]. GDF-15 appears therefore as a potential biomarker of cancer cachexia, which deserves further investigations.

2.3. PTHrP

Parathyroid Hormone release Peptide (PTHrP) is known to induce paraneoplastic hypercalcemia by stimulating bone resorption and calcium renal reabsorption. But, a recent work has pinpointed its role in the development of cancer cachexia in mice through fat browning and increased energy expenditure [61]. Indeed, Kir et al., showed that circulating PTHrP is increased in tumor-bearing mice. More interestingly, treating these animals with an antibody against PTHrP reverses partially skeletal muscle and fat loss without changing tumor size. According to this study, PTHrP might act by increasing expression of thermogenic genes, such as UCP1, Dio2 and PGC1α in adipose tissue, inducing hypermetabolism, elevated heat production and then increased energy expenditure. However, PTHrP does not seem to have a direct atrophic effect on skeletal muscle, as demonstrated by the lack of effects of PTHrP on muscle gene atrophy expression. Moreover, treating primary myotubes with PTHrP did not lead to atrophy. But, the improvement of skeletal muscle mass and function observed in cancer cachexia models treated with anti-PTHrP antibody suggests that PTHrP play a role in the skeletal muscle wasting process likely in collaboration with other factors or by a crosstalk between adipose and skeletal muscle tissues [62].

In the line of these preclinical observations, circulating PTHrP has been shown to be predictive of body weight loss in a cohort of cancer patients [63]. Furthermore, cancer patients with detectable circulating PTHrP exhibit a lower lean mass and a higher resting energy expenditure, compared to those with undetectable PTHrP [61]. These recent data suggest that circulating PTHrP could be a new candidate to consider as biomarker of cancer cachexia. However, future clinical studies are needed to characterize deeply the role of PTHrP in cachexia development and its capacity to reflect the skeletal muscle wasting process.

These potential mediators of cancer cachexia could be produced by the tumor itself, since an increase in their expression is found in several tumor cell lines or tissues. However, the production and the secretion of this kind of peptide vary probably widely from one type of tumor to another. For this reason, it is unlikely that cancer cachexia results from

the action of a unique mediator, regardless of the nature of the tumor. Given this issue, large clinical studies which investigate several potential biomarkers of cachexia in different types of cancer are required to delineate the best biomarker or the best combination of biomarkers of cancer cachexia. In this context, Lerner et al., published recently several interesting studies assessing the link between several cytokines levels, weight loss and lean mass in patients with different types of cancer, mainly lung, gastro-intestinal and pancreatic cancers [21,22,33]. In their analysis, circulating GDF-15 and ActA appeared to be the two best biomarkers of weight loss: they were negatively correlated with lean mass and also prognostic factors of survival. However, other molecules, such as PTHrP, were not investigated in these studies.

3. Markers of muscle protein degradation

In addition to the mediators of cachexia, several other molecules, present in muscle tissue or released by the muscle into the circulation, might reflect the atrophy process and therefore be considered as potential biomarkers of muscle wasting.

3.1. In the circulation

Many studies have been interested to investigate as biomarkers of cancer cachexia the products of muscle proteins degradation, released in circulation during atrophy process, also called neoepitopes and reflecting directly the muscle tissue destruction [64]. These molecules are produced by modifications of an existing molecule, by proteolytic cleavage or addition of a chemical group. The main hurtle with this kind of approach is to find a protein specific of skeletal muscle atrophy in order to avoid contamination by products of non-muscular proteins degradation.

3.1.1. 3-Methylhistidine

For several decades, 3-methylhistidine (3MH) has been known as a marker of muscle protein degradation and therefore as a biomarker of muscle wasting process. Myofibrillar proteins, actin and myosin, undergo a post-translational methylation of specific histidine residues [65]. During proteolysis of these proteins, these residues are released in bloodstream and excreted in urine, without being recycled for protein synthesis. Measuring 3MH in plasma or urine could therefore reflect muscle protein breakdown. The main hindrance to use this measurement in clinical practice is that it must be performed on 24-h urine collection after 3 days without eating meat. An alternative is to measure an index of 3MH production in urine or plasma after oral administration of a stable isotope tracer of 3MH [66]. However, during the last years, the validity of this technique has been questioned. Firstly, it measures also 3MH residues resulting from proteolysis of non-skeletal muscle proteins bound to 3MH (smooth muscle from intestine) [67]. Secondly, it does not seem to respond to interventions which increase muscle protein degradation [68]. For these reasons, 3MH has not been further investigated as biomarker of cancer cachexia.

3.1.2. Titin fragments

Titin is a large and abundant protein present in striated muscle (skeletal and cardiac) [69]. Its main function is the maintenance of myofibril assembly and the stabilization of sarcomere. After degradation by protease, titin fragments are released in circulation and excreted in urine. In murine (dexamethasone treatment) and human (bedrest) models of muscle atrophy, changes (decrease and then increase) in serum titin fragments concentrations are observed, reflecting wasting process [69]. Moreover, titin fragments are increased in urine of patients with Duchene muscular dystrophy compared to control subjects [70]. However, further studies are request to delineate if serum or urine concentrations of titin fragments are correlated with loss of muscle mass or function, in particular in cancer cachexia.

3.1.3. Collagen fragments

Fragments of collagen III and VI, which are both components of extracellular matrix of skeletal muscle, are released in circulation during increased muscle turnover and might reflect muscle atrophy process. Interestingly, different fragments of collagen can be identified due to the action of different proteases depending on the pathophysiological situation (matrix-metalloproteinase [MMP]-generated collagen VI fragment [C6M], collagen type VI peptides containing IC6 epitope [IC6], N-terminal peptide of procollagen type III [P3NP] or [ProC3], C-terminal agrin fragment [CAF]) [71]. In humans, circulating collagen VI fragments are increased in cancer patients compared to healthy controls and are correlated with lean body mass but only in healthy subjects [71]. Furthermore, changes in circulating collagen III and VI fragments are also observed respectively after resistance exercise training [72] or immobilization [73]. Further clinical investigations are required to characterize which type of collagen fragments could reflect muscle wasting during cancer cachexia.

3.1.4. Carnosine dipeptidase 1

Based on a serum proteomic analysis, Arner et al., have identified a decrease of circulating carnosine dipeptidase 1 (CNDP1) in cachectic patients in two different cohorts of cancer patients (gastro-intestinal and pancreatic cancer) [74]. Furthermore, a correlation between CNDP1, weight loss, more advanced disease states and reduced survival has been demonstrated. More studies are clearly necessary before considering CNDP1 as a marker of cancer cachexia.

3.2. Into the skeletal muscle

Some markers of cancer cachexia have been identified in the skeletal muscle itself, but their interest is limited by the need of an invasive muscle biopsy.

3.2.1. 14-kDa actin fragment

In catabolic situations, muscle actomyosin complexes and myofibrils are degraded in a first step by caspase-3, producing a characteristic 14-kDa actin fragment [75]. In these situations, the muscle level of 14-kDa actin fragment is closely associated with the proteolysis rate [76]. Moreover, the muscle level of 14-kDa actin fragment is higher in hemodialyzed and severely burned patients and decreases after endurance training compared to control subjects. However, the muscle level of 14-kDa actin fragment remains unchanged in young subjects with muscle atrophy due to immobilization. So, the validity of quantifying muscle 14-kDa actin fragment as marker of atrophy process has yet to be investigated in different pathological muscle wasting situations.

3.2.2. β-Dystroglycan

In an attempt to identify a biomarker of cancer cachexia, Stephens et al., have assessed the levels of different candidate proteins in the muscles of cancer patients with cachexia [77]. This research showed that β-dystroglycan content is increased in muscle of cachectic cancer patients. β-dystroglycan is a muscle transmembrane protein, acting as a linkage between the extracellular matrix and the cytoskeleton and therefore contributing to structural integrity of muscle. Furthermore, they observed that low levels of two structural muscle proteins, myosin heavy chain and dystrophin, are associated with short survival. These data suggest therefore the existence of structural alterations in muscle of cachectic patients. Earlier, some works highlighted the possible role of alterations in dystrophin glycoprotein complex in muscle atrophy development during cancer cachexia. Indeed, in cachectic mice, a reduced muscle dystrophin content and an increase in aberrant glycosylations of β-dystroglycan and β-sarcoglycan, without change in their levels, leading to muscle membrane abnormalities, have been demonstrated [78]. Given that, more studies are clearly necessary before considering quantitative or qualitative alterations of β-dystroglycan as reflect of muscle damage during cancer cachexia.

3.2.3. Transcriptomic analyses

In preclinical models of cancer cachexia, the muscle expression of genes involved in atrophy or atrogenes such as MuRF1 or Atrogin-1 is generally increased, involving the role of ubiquitin/proteasome pathway in cachexia development [79]. However, in humans, different studies failed to show similar changes in the expression of these atrogenes, suggesting the role of alternative pathways (autophagy) [80,81]. Based on a microarray analysis, the muscle expression of calcium/calmodulin-dependent protein kinase (CaMK)IIβ and endothelial receptor tyrosine kinase (TIE1) have been demonstrated to be correlated with weight loss in two cohorts of cancer patients [80]. Muscle CaMKIIβ levels are known to be increased after physical activity [82] and Ca (2 +)-CaM-eEF2K signaling is implicated in inhibition of muscle protein synthesis induced by exercise [83]. TIE1 is involved in vascular network development [84] and its muscle expression increases during exercise training [85]. However, the role of these two proteins in muscle atrophy and their potential interest as biomarkers of this process have not been yet investigated.

3.2.4. Proteomic analyses

Muscle proteomic studies are currently emerging in order to highlight new potential biomarkers of cachexia.

In animal models of cancer cachexia (colon-C26), Barreto et al., have identified 269 proteins differentially expressed in muscle of tumor bearing mice [86]. Particularly, proteins involved in mitochondrial function and biogenesis are downregulated, such as OPA-1 (optic atrophy protein-1), mitofusin-2, DRP-1 (GTPase dynamin related protein-1), cytochrome C and PGC-1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha) as well as proteins related to TCA cycle, fatty acid metabolism and calcium signaling.

Changes in muscle structure and composition occur also during cancer cachexia. This is supported by a recent proteomic analysis, performed by Ebhardt et al., in a population of cancer patients [87]. In this study, they observed a decrease of level of Tropomyosin (TPM1,2) and an increase in myosin heavy chain (1, 4, 8, 3-MYHs) content in the muscle of cancer cachectic patients. In addition, dysregulation of proteins, involved in mitochondrial electron transport chain and focal adhesion has been observed, bringing new potential candidates to be assessed.

Definitely, these works open ways to further investigations to identify a protein signature of cancer cachexia and to understand underlying mechanisms.

4. MicroRNAs

MicroRNAs (miRNAs) are a class of small non-coding RNAs regulator of gene expression. Several miRNAs have been identified as being involved in muscle development or atrophy [88,89].

Recent studies have identified some miRNAs differentially regulated in muscle of cachectic tumor-bearing mice [90] and cancer patients [91] and showed their biological targets could be implicated in wasting process. In particular, Narasimhan et al., have identified 8 miRNAs differentially upregulated in muscle of cancer cachectic patients and their putative target mRNAs [91]. Some of them are notably involved in muscle mass regulation through Insulin-like Growth Factor I, TGF-β, bone morphogenic protein and Wnt/β-catenin signaling, in body weight regulation, appetite and energy homeostasis and in lipid biosynthesis. Albeit this new type of candidate biomarkers of cachexia seems promising, it should be validated in larger populations.

In order to have a more accessible biomarker, it would be suitable to identify some circulating miRNAs reflecting cachexia in cancer patients. In this regard, some candidates have been highlighted previously. Firstly, a decrease of circulating miR-486 has been observed in a population of breast cancer patients compared to healthy individuals [92]. In addition, studies showed that a reduction of miR-486 in circulation may result from tumor-induced factor such as TNFα and may impact muscle protein synthesis pathways, as miR-486 is a positive regulator of the IGF-I/Akt pathway [93]. Secondly, miR-21, whose muscle expression is upregulated in pathological situations leading to muscle atrophy, such as denervation and diabetes [94] may be also produced by tumor and may induce apoptosis of murine myoblasts [95]. However, miR-21 has not yet been evaluated in humans.

These data indicate that molecules different than proteins may also serve as marker of cancer cachexia. However, additional investigations are needed to establish more accurately the connection between changes in miRNA levels in muscle or in circulation and cancer cachexia or survival in cancer patients.

5. Markers of fat loss

Likely less harmful than muscle wasting, fat mass loss is nevertheless a key feature of cancer cachexia. Some circulating lipolytic factors, produced by adipose or tumor tissue, could therefore be also considered as biomarker of cancer cachexia [96]. Additionally, adipose tissue in weight-losing cancer patients seems more sensitive to hormones which stimulate lipolysis, such as catecholamines and natriuretic peptide.

5.1. In the circulation

5.1.1. Leptin

Because circulating leptin, which is produced mainly by adipose tissue, has been shown to reflect accurately the fat mass in different populations, including cancer patients, it could be considered as a circulating marker of decreased fat mass during cancer cachexia [97]. Indeed, some clinical studies observed relative hypoleptinemia in cancer patients [98–100]. However, leptin is likely a delayed marker of fat mass loss. Moreover, leptin is also influenced by age, sex, BMI, underlying diseases, making difficult the interpretation of its circulating concentration.

5.1.2. Free fatty acids and glycerol

During lipolysis in adipose tissue, free fatty acids (FFAs) and glycerol are produced from the degradation of stored triglycerides and released in circulation [96]. An increase of their circulating levels could therefore reflect fat loss. Indeed, high levels of FFAs and glycerol have been observed in cachectic cancer patients [96,101,102]. Moreover, both FFAs and glycerol levels were correlated positively with weight loss and negatively with the amount of visceral fat tissue [101]. Albeit high levels of FFAs and glycerol have been found robustly in different small groups of patients with cancer cachexia, further larger studies are requested to delineate the link between their plasma levels, cachexia and survival in cancer patients.

5.2. In adipose tissue

5.2.1. Protein zinc-α_2-glycoprotein

The protein zinc-α_2-glycoprotein (ZAG) is a lipolytic factor which may represent a candidate biomarker of cancer cachexia. Indeed, ZAG is expressed and secreted in several healthy and tumor tissues and stimulates lipolysis in rodent and human adipocytes [103]. In addition, ZAG expression and secretion from adipose tissue increase proportionally to weight loss in cancer patients and in obese patients subjected to caloric restriction and are well correlated with nutritional status [103,104]. However, in these populations, no correlation between circulating levels of ZAG and nutritional parameters (nutritional score, BMI and weight loss) has been observed. These data suggest that ZAG level in fat tissue but not in circulation is a marker of nutritional status and weight loss, bringing few evidences to use it as a marker of cachexia.

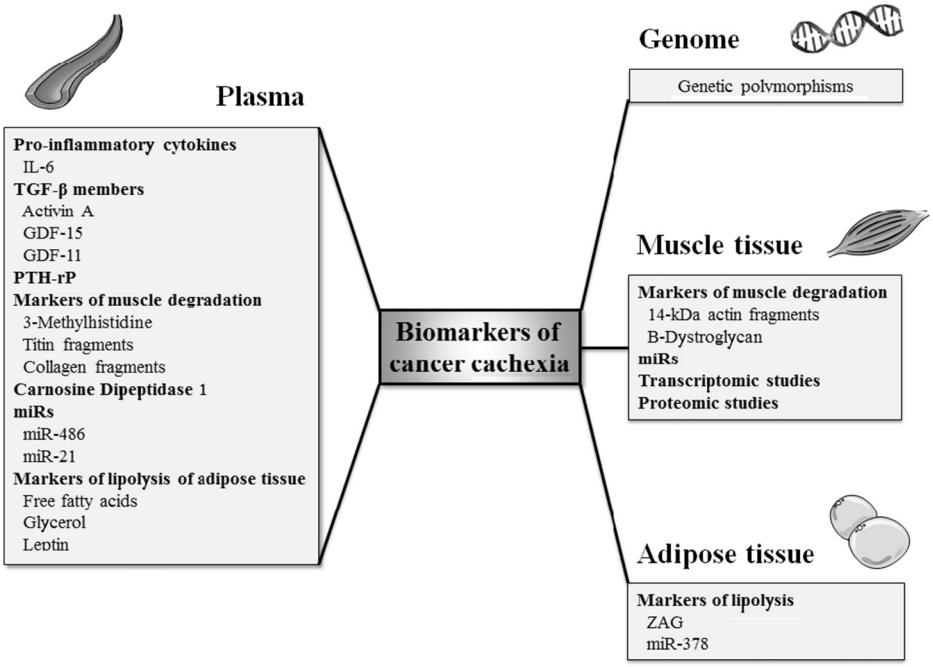

Fig. 1. Potential biomarkers of cancer cachexia.

5.2.2. miR-378

An upregulation of miR-378 in adipose tissue of cancer patients with cachexia has been shown [105]. Interestingly, overexpression of miR-378 enhances catecholamine-stimulated lipolysis in human adipocytes and conversely, inhibition of its expression decreases stimulated lipolysis and reduces expression of genes encoding for regulators of lipolysis. Further larger clinical studies are needed to demonstrate the involvement of this miR in fat loss during cancer cachexia but its interest in currently limited by the need of an adipose tissue sample.

6. Genetic biomarkers

The heterogeneity of cachexia presentation and speed of development is ascribed to individual variations, such as genetic predispositions. Highlighting such genetic markers could allow to identify patients susceptible to develop cachexia independently of the nature and stage of the underlying cancer. Different target genes are candidates for predisposition to cachexia. Indeed, polymorphism in genes encoding for pro-inflammatory cytokines or molecules involved in muscle or adipose tissues regulation, wasting process, immune response and appetite have been pinpointed and their clinical relevance in terms of muscle wasting and survival investigated [106].

Polymorphisms in IL-6 and IL-10 genes have been reported to be linked with systemic inflammation, prevalence of cachexia and survival in cancer patients [107,108]. On the other hand, an association between the C-allele of the rs6136 polymorphism in SELP gene which encodes P-selectin and a reduced risk of developing cancer cachexia has been demonstrated and confirmed in several studies [109–111]. More recently, Johns et al., have assessed polymorphisms of several candidate genes associated with cachexia and cachexia phenotype in a large cohort of cancer patients [111]. Interestingly, in patients with weight loss and low muscularity, variants of four genes encoding molecules involved in muscle metabolism (activin receptor type 2B [ACVR2B] and angiotensin-converting enzyme [ACE]), in fat metabolism (leptin receptor [LEPR]) and in cytokine production (tumor necrosis factor [TNF]) have been found. In patients, who exhibited only weight loss, polymorphism in genes encoding for P-selectin (SELP) and FOXO3 were observed. According with these results, skeletal muscle expression of some of them, such as ACVR2B, FOXO3 or LEPR was significantly associated with muscle mass and weight loss.

Polymorphisms in different genes encoding for molecules potentially involved in cancer cachexia process, have recently emerged with promising clinical implications, since they could allow an early identification of the predisposed individuals to develop cancer cachexia.

7. Conclusion

The etiology of cancer cachexia is multifactorial and therefore the many molecules involved in the wasting process represent as many candidate biomarkers of cachexia (Fig. 1). Several “omics” studies are emerging to identify muscular or circulating markers of wasting process during cancer cachexia. Not only proteins, but also miRNAs and metabolites, alone or in combination might serve as biomarkers of cancer cachexia. In this regard, direct characterization of the muscle secretome associated with muscle atrophy should identify specific muscle proteins, miRNAs and metabolites released by the muscle itself during atrophy. Before considering these molecules as biomarkers of cachexia, large clinical prospective studies will be requested to delineate which one among these attractive candidate biomarkers is the strongest and the most reproducible to carry a good prognostic value. There are no doubts that in the coming years, -omic techniques will allow us to characterize new biomarkers of cancer cachexia.

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