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Abstract

Purpose of review: Cancer Cachexia (CC) is a frequent syndrome that affects patient quality of life, anti-cancer treatment effectiveness, and overall survival. The lack of anti-CC therapies likely relies on the complexity of the syndrome, that renders difficult to design appropriate clinical trials and, on the other side, on the insufficient knowledge of the underlying pathogenetic mechanisms. Aim of this review is to collect the most relevant latest information regarding CC with a special focus on the experimental systems adopted for modeling the disease in translational studies.

Recent findings: the scenario of preclinical models for the study of CC is not static and is rapidly evolving in parallel with new prospective treatment options. The well-established syngeneic models using rodent cancer cells injected ectopically are now used alongside with new ones featuring orthotopic injection, human cancer cell or patient derived xenograft, or spontaneous tumors in genetically engineered mice.

Summary: the use of more complex animal models better resembling CC, ideally including also the administration of chemotherapy, will expand the understanding of the underlying mechanisms and will allow a more reliable evaluation of prospective drugs for translational purposes.

Keywords: cancer cachexia, muscle wasting, animal models

Cancer cachexia (CC) is a complex syndrome mainly characterized by body weight loss, muscle wasting and metabolic abnormalities that affects 50 to 80% of cancer patients and accounts for about 20% of cancer deaths [1]. Body weight loss mainly depends on wasting of skeletal muscle mass which is considered the main hallmark of CC. Also systemic inflammation participates to the onset of CC. The major symptoms of CC include anorexia, anemia, asthenia and fatigue, further impairing patient quality of life. In addition, cachexia is associated with decreased anti-cancer treatment tolerance and effectiveness, resulting in reduced survival.

Promising strategies aimed to counteract cachexia basically target anorexia and metabolic disturbances. Several drugs are currently tested in clinical trials, but none of these treatments proved effective enough to be applied routinely in the clinical practice[2]. The design of effective treatments is limited by both the difficulty to recruit patients for clinical trials and by the insufficient knowledge of the pathogenesis of CC [3]. Most of the information regarding the mechanisms of CC and the possible therapeutic options are firstly developed in preclinical models. These systems are useful for modeling the human condition, circumvent the restrictions and handling problems of clinical studies and explore different therapeutic strategies for translational purposes. However, it is imperative that animal models for preclinical studies closely mimic the human condition in order to maximize the translation of findings. Given the heterogeneity of human cancer and the differences interspecies, each animal model does not totally represent the complexity of human CC. Therefore, it is fundamental that researchers use models that are appropriate for their endpoints. Pre-clinical models should be compared with CC in patients mainly in terms of tumor burden, effects on food intake, body weight and composition, inflammatory status and the tendency to metastasize. In this regard, several well-established animal models of CC are available (Table 1 and next chapter) and new ones are emerging in the attempt to circumvent the limitations of the syngeneic heterotopic models currently used

Novel mechanisms of CC in well-established animal models

Syngeneic models, consisting of rodent cancer cell lines injected subcutaneously (s.c.), intramuscularly (i.m.) or intraperitoneally (i.p.) in recipient mice or rats, are characterized by reproducible, synchronized and rapid tumor growth in immune-competent hosts. The most frequently used models and their main features are reported in Table1 and were extensively described in previous reviews [3][14*].

Starting from the C26 coloncarcinoma, the degree of wasting may vary likely depending on cell passage number, storage conditions and injection site [15, 4]. For example, anorexia doesn't develops when C26 cells are injected s.c. or i.m. [5], whereas it is present when the cells are injected i.p [4]. A major drawback of this model is the relatively short period between the beginning of CC symptoms and the animal death (few days)[3], reducing the therapeutic window to study interventional approaches. Conversely, a positive feature is that the wasted phenotype occurs when the tumor weight comprises only about 2% of carcass weight [3], such condition being closer to human cancers. Also systemic inflammation drives CC in C26 model and is mainly due to the high serum levels of IL-6 [16]. However, Seto and colleagues [17] underlined the role of Leukemia Inhibitor Factor (LIF) in the onset of cachexia using this model, showing increased LIF levels and its effect on muscle atrophy via activating the JAK2-STAT3 pathway [17]. Cytokines can modulate CC not only in the skeletal muscle, indeed, the TWEAK receptor Fn14 was shown to trigger muscle atrophy activating a specific cachectic signal in the tumor, independently from the activation of the host receptor [18**].

A recent finding demonstrates that C26-bearing mice have increased matrix metalloproteinase levels in both cardiac and skeletal muscle compared with control mice, which may contribute to altered heart and skeletal muscle function associated with CC [19].

During the last years exercise training has been proposed to be useful to revert CC. Contrasting data were obtained using the C26 model, where aerobic exercise proved either able to protect from skeletal muscle wasting [20] or not [6], the prevention of muscle loss being associated with the removal of the myogenic differentiation block [21]. It is noteworthy that the same exercise protocol ineffective in the C26 proved effective in the LLC model [6], stressing the concept that, similarly to the human condition, CC is a

heterogeneous syndrome. Moving to mechanism-based prospective therapy to counteract CC using the C26 model, the administration of two histone deacetylase inhibitor (valproic acid [22] and AR-42[23*]) proved able to attenuate skeletal muscle loss. Similarly, an angiotensin (AT) 1-receptor antagonist prevented muscle wasting and myocardial dysfunction [24], although decreasing tumor mass, thus rendering difficult to understand if the drug is directly affecting CC.

In the past few years, growing evidences show that supplementation with different types and doses of antioxidants may induce muscle atrophy trough downregulation of endogenous antioxidant defense [25*]. In this regard, another study showed that a combination of antioxidants enhanced rather than preventing CC in C26-bearing mice [26].

The widely used Walker-256 carcinosarcoma has a major drawback in the excessive tumor burden [10]. Nevertheless, it mimics the majority of CC features, such as decrease of body weight, food intake, skeletal muscle and fat mass and the occurrence of systemic inflammation [27]. A study by Beluzi *et al*. [10] demonstrates that pioglitazione (PGZ), a peroxisome proliferator activated receptor gamma (PPARγ) activator, increases survival of Walker 256-bearing rats and preserves body mass, although reducing tumor growth. Recent studies underline the effects of supplementation with natural compounds on CC and tumor growth. In this regard, oral administration of Aloe vera and honey is able to attenuate CC in Walker 256 model, whereas it promotes oxidative stress and damage in the tumor [28]. In another study, the supplementation with fish oil and Oro Inca oil (the latter rich in alpha-linolenic fatty acid) reduced tumor mass, increased blood glucose and decreased circulating triacylglycerol, TNF-α and IL-6 [11]. Moreover, Deminice *et al*. [29] provides initial evidence that creatine supplementation can mitigate tumor growth and body weight loss, while improving hepatic homocysteine metabolism and oxidative stress in Walker 256-bearing rats.

The growth of the Yoshida AH-130 tumor causes severe anorexia and body wasting in the rat host. Similarly to the C26 model, cachexia in the AH-130 hosts occurs when the tumor burden (cellularity) is very low [30]. Using this model, our group showed that proteasome inhibition by bortezomib does not attenuate

skeletal muscle wasting [12]. Supporting the concept that human CC modeling should include the use of chemotherapy, and that a prospective anti-CC strategy should be multimodal, the administration of megestrol acetate (MA; an appetite stimulant) and formoterol (β2-agonist) proved effective in counteracting cachexia in AH-130-bearing rats receiving sorafenib (a multi-kinase inhibitor used against liver malignancies) [13*] .MA effectiveness in this experimental model was confirmed by another study, showing that the drug protects from heart atrophy and dysfunction [31].

The use of the Lewis Lung Carcinoma (LLC) cells to model CC is common across several laboratories. This tumor induces rapid and progressive body and tissue wasting, while anorexia occurs only at late stages [30], despite the development of large tumors. Distinctly from other models, LLC cells frequently metastasize [3]. While this peculiarity better resembles human cancer, it could be a confounding factor when an anti-CC treatment is adopted (see below). LLC-induced muscle wasting has been described to be also associated with Edoplasmatic Reticulum (ER) stress and Unfolded Protein Response (UPR). In this study, the inhibition of ER stress enhanced muscle atrophy and decreased muscle function, suggesting that ER stress in required for muscle homeostasis [32*]. Moving to anti-CC treatments, the combination of formoterol with soluble ActRIIb, an inhibitor of myostatin, completely reversed muscle wasting even prolonging survival in parallel to a decreased metastatic spreading [33**]. Another orexigenic compound currently under investigation for its property to increase appetite, fat and muscle mass is ghrelin, that exerts these effects downregulating pro-inflammatory cytokines and activating myogenin, myoD and Akt in the skeletal muscle in both C26- and LLC-bearing mice [7*].

Emerging animal models

Beyond the well-established models reported above, a relevant piece of information from the last year papers comes from less used or newly characterized animal systems, either syngeneic or 'closer' to human CC. As for syngeneic mouse models, a quite new one was used to assess the importance of gut microbiota in the onset of cachexia, using mice injected with murine pro-B leukemic cells (Ba/F3), where a synbiotic approach partially prevented muscle wasting and prolonged survival [34**].

Orthotopic tumors

During the last years, most of the preclinical studies in the cancer field moved from ectopically to orthotopically growing tumors, in order to provide cancer cells its original stroma and microenviroment, both elements being fundamental for cancer cell behavior and for recapitulating human tumors. Such procedure requires considerable technical skills and most of the cell lines adopted for orthotopic injection were not previously used or well characterized in vivo for modeling cachexia. Indeed, new models were set up very recently and it is likely that many more will appear in the future. Starting from the simplest ones, intravenous injection of human leukemic cells easily reproduced hematologic malignancies and specific infusion of human primary juvenile myelomonocytic leukemia cells in irradiated mice induced cachexia [35]. The study by Chen and coworkers aimed to demonstrate the effectiveness of the Chinese herb magnolol in preventing cachexia in athymic mice injected with T24 human bladder cancer cells [36*]. In this model, atrophy is mediated by suppression of the IGF-1 dependent signaling and by FoxO3 activation. In another report, human pancreatic cancer cells (S2-013) injected orthotopically in athymic mice [37] were used to mimic pancreatic ductal adenocarcinoma (PDAC), a tumor type with very high cachexia incidence in humans. On the whole, three new models are now available for the study of cachexia induced by those specific tumors in their own physiologic anatomic site, however all of them require the use of immune-compromised mice, thus potentially limiting the recapitulation of the human disease.

Humanized models and patient derived xenografts (PDX) in mice

Similarly to the above-mentioned models based on the injection of human cells into mice, other human cell lines (ectopically injected) were reported to be able to induce cachexia in mice. A very elegant reporter system for muscle atrophy was obtained expressing the tdTomato fluorescent protein under the control of human MuRF1 promoter in SCID mice injected ectopically (subcutaneously in the flank) with pancreatic cancer Panc1 or Pa04C cells [38*]. Another report focused on the role of hypermetabolism in the onset of cachexia, demonstrating that inflammatory mediators trigger excessive fatty acid oxidation in the skeletal muscle, eventually driving cachexia, in NOD-SCID mice injected in the flank with RXF393 cells (a

human renal cell carcinoma cell line; [39**]), whereas 498, 786-O, SKRC39 or SN12C cells proved unable to trigger cachexia. These models on one side offer the possibility to compare the effect of human cancer cells to those of animal cells, although keeping on the limitations of ectopic tumor growth and the lack of an intact immune system.

In order to closely reproduce human tumors, patient-derived bioptic fragments have been implanted into mice either ectopically (most of the PDXs actually commercially available) or orthotopically [40] and biobanks have been created for this scope. The main aim of the researchers in setting up these models is to test anti-cancer drugs, even in a personalized mode, in order to predict the most effective drug for a given patient. So far, no data are available regarding the use of PDXs for modeling CC; in this regard, the need to use immune-deficient recipient mice might limit their use. In the future, the engineering of a human-like immune system and tumor microenvironment in mice will increase the predictive value of these preclinical models [41].

Genetic engineered mouse models (GEMMs)

The use of human cancer cells or tumor xenografts implanted into mice for better recapitulating both tumor structure and behavior in humans may not be the best choice for reproducing human cachexia. Conversely, genetically manipulated mice that develop spontaneous tumors have been successfully used at this purpose. The main characteristics of these models were previously discussed [3], the major strengths being orthotopic tumor site, low tumor growth rate, and acceptable reproduction of multi-step carcinogenesis. The Apc Min/+ mouse develops colon cancer due to a mutation in the APC gene, and is the most widely used GEMM presenting with cachexia. As for CC mechanisms described using GEMMs, data obtained in Apc Min/+ mice exposed to eccentric exercise [42*] confirm that exercise training is an effective tool for preventing cachexia (see above; [6]). A less frequently adopted GEMM is the inhibin α-subunit knockout mouse (α-KO), featuring gonadal cancers, where activin-βC, a novel activin-A antagonist partially interferes with tumor growth and cachexia [43]. A new report adopting the same model, demonstrated that activin-βC modulates cachexia by repressing both the ubiquitin-proteasome and the

autophagic degradation pathways [44*]. Finally, data obtained using mice developing spontaneous pancreatic cancer upon the expression of Pdx1-cre, LSL-KrasG12D and INK4a/arffl/fl show that JAK2-STAT3 and NF-κB pathways are strongly activated and that the inhibition of STAT3 partially prevents body weight loss [45], confirming previous data obtained in syngeneic models [46].

Alternative models

A little implemented alternative option that has been proposed for modeling CC is the use of domestic animals (cats and dogs) [47]. A study has already been performed for cardiac cachexia, testing the effectiveness of anti-myostatin drugs [48]. Such system would closely reproduce human clinical trials in terms of cancer heterogeneity, individual's housing conditions and animal size. The other way round, the main potential limitation for CC studies is represented by the difficulty to find appropriate non-tumor-bearing controls.

Concluding remarks

In the last year, the results reported in animal models of CC have further increased the range of molecular mechanisms accounting for the clinical manifestations of this syndrome. The rapid evolution of the field will likely move towards two opposite directions. On one side, the dissection of single mechanisms for the definition of cause-effect relations will lead to the adoption of models as simple as possible. Recent examples are the overexpression of inflammatory mediators such as IL-6 or activin A as a strategy to dissect the single contribution of the specific mediator to the pathogenesis of CC [49*]. The results obtained suggested that IL-6 mainly drives fat loss while activin A promotes the wasting of lean mass. Another study reported about the relevance of chemotherapy to cachexia, showing that drugs used to treat colon cancer trigger muscle atrophy mainly impinging on MAP kinases and mitochondrial alterations [50*]. Conversely, more complex models (orthotopic, humanized models including chemotherapy) will be required to test the effectiveness of prospective anti-cachexia compounds leading to a rapid translation into clinical trials, despite an unavoidable raise in the costs.

Finally, similarly to clinical trials [51], the adoption of a specific CC models tailored to the specific aim of the research would require the definition of a consensus/guidelines on several aspects, including: choice of appropriate cancer cells, study design, endpoints and statistics.

Key points:

- The research of animal models of cancer cachexia rapidly evolves in front of the emerging need to define the underlying mechanisms of the disease and to find effective therapies.

- Besides the well-characterized and widely used syngeneic models of cancer cachexia, new models closer to the human disease have been established in order to maximize the translational value of the results.

- Given the heterogeneity of animal models, it is fundamental to adopt standard procedures and/or to provide guidelines for helping investigators to compare their results.

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Conflict of interest

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Table 1. Well-established models of CC.

The most commonly used syngeneic animal models of CC are listed, along with the main operative and CC-related characteristics.

Model	Host	Tumor injection site	Tumor mass (% of BW)	Experimental period (days)	Skeletal muscle and fat wasting	Anorexia	I
C26 carcinoma	Balb/c CD2F1	s.c. i.m. i.p.	2-6%	14-21	Yes	No or Yes	Yes (r
Lewis Lung Carcinoma (LLC)	C57B1/6	i.m. s.c.	20-30%	15-28	Yes	Yes	Yes (T
MAC 16 adenocarcinoma	NMRI mice	s.c.	<1%	20-30	Yes	No	No c
B16 melanoma	C57B1/6	s.c.	3,5-12%	14-16	Yes	Yes	Ye
Walker 256 carcinosarcoma	Sprague-Dawnely and Wistar rats	s.c.	10-20%	14-21	Yes	Yes	Yes Wal
Yoshida ascites hepatoma 130 (AH130)	Wistar rats	i.p.	<3%	14-21	Yes	Yes	Yes