Review

In Vitro Models for Cancer-Associated Cachexia: The Complex Modelling of a Multiorgan Syndrome

Isabel Meireles 1,2, Rui Medeiros 1,3,4 and Fátila Cerqueira 1,3,4,*

1 Molecular Oncology and Viral Pathology Group, Research Center of IPO Porto (CI-IPOP)/RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto)/Porto Comprehensive Cancer Center (Porto.CCC), Raquel Seruca, Rua António Bernardino de Almeida, 4200-072 Porto, Portugal; isabelmeireles@gmail.com (I.M.);
ruimedei@ipoporto.min-saude.pt (R.M.)
2 Faculty of Medicine of the University of Porto (FMUP), Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal
3 FP-I3ID, FP-BHS, GIT-LoSa, University Fernando Pessoa, Praça 9 de Abril, 349, 4249-004 Porto, Portugal
4 Faculty of Health Sciences, University Fernando Pessoa, Rua Carlos da Maia, 296, 4200-150 Porto, Portugal
* Correspondence: fatima@ufp.edu.pt

Abstract: Cancer-associated cachexia is a multifactorial syndrome characterised by systemic inflammation and hypermetabolism that affects different tissues and organs. Is characterised by progressive and irreversible weight loss, mainly due to skeletal muscle wasting and often accompanied by loss of fat mass. Due to its complexity, and lack of effective treatment, this syndrome is a sign of poor prognosis in cancer patients. Cellular models constitute a valuable and powerful tool offering insights into the molecular pathways and cellular responses associated with cancer cachexia. Currently, there are robust and widely used cell lines used to establish models to study the pathophysiology of muscle wasting and adipose tissue loss. Various methods can be used to induce the cachectic phenotype in the cells, utilising genetic engineering or different inducing agents such as hormones, inflammatory factors and chemotherapeutic drugs. The available experimental data on their metabolic properties and transcriptional and proteomic profiles allows the selection of the most suitable research model to replicate the relevant aspects of cachexia. In this review, we make an overview of the in vitro models used to study biological aspects of cancer-associated cachexia and analyse their strengths and limitations in replicating the complex physiological environment and pathological processes of the syndrome. Herein, we also briefly approach the difficulty of modelling the contribution of different organs and crosstalk between different tissues.

Keywords: cancer-associated cachexia; in vitro models; skeletal muscle wasting; cardiac cachexia; adipose tissue loss

1. Cancer-Associated Cachexia Overview

Cancer-associated cachexia (CAC) is a complex hypercatabolic syndrome characterised by an ongoing and involuntary loss of muscle mass and body weight, accompanied or not with the loss of adipose tissue, that cannot be reversed by nutritional supplementation, leading to progressive functional impairment [1]. Other physiological abnormalities including insulin resistance, loss of appetite or anorexia, asthenia, sarcopenia and anaemia are also included in the clinical traits of this comorbidity [2]. CAC is a multi-organ syndrome involving the brain [3,4], heart [5,6], liver [7,8], pancreas [9] and gut [10], which prompts systemic metabolic rewiring and systemic inflammation. However, the mechanisms of impairment occurring in these organs are understudied [11]. Overall, CAC is a burden affecting approximately 80% of cancer patients culminating in death in 20–30% of cases [12]. The prevalence of the comorbidity varies according to the cancer type and stage, reaching up to ~70% in pancreatic cancer, ~60% in gastroesophageal and head–neck cancers, 50–40%
in lung, colorectal and certain haematological cancers and ~15–25% in breast and prostate cancers [13]. Yet, regardless of the tumour location or stage, CAC is considered a negative prognostic factor [14]. Since chemotherapy dose is administered considering body surface area or lean body mass from the patient, cachexia reduces antitumor efficacy and tolerance to treatment, increases susceptibility to side effects and toxicity and therefore increases mortality in patients and reduces the disease’s overall survival rate [15,16].

The complexity and heterogenicity of this syndrome rely on the involvement of many mediators (such as systemic pro- and anti-inflammatory mediators, hormones, neuropeptides and tumour-derived factors) and signalling pathways, making the clinical management very challenging [17]. Despite representing a major burden in the cancer patients’ quality of life the scientific knowledge of the disease is still scarce. The underlying pathophysiological mechanisms of this disorder are not completely understood, therefore, there are no approved standard treatments for CAC. Even so, in clinical practice, a comprehensive approach involving pharmacological interventions, nutritional supplementation and the recommendation of physical exercise is usually prescribed [18].

Studying the intertwining of dysregulated mechanisms during CAC could provide new therapeutic targets for the disease [19].

The most deleterious characteristic of CAC is the loss of skeletal muscle. This organ comprises almost half of the body weight and has an essential role in maintaining homeostasis, in whole-body energy production and protein metabolism, serving as a reservoir for amino acids sustaining protein synthesis [20]. Skeletal muscle mass is preserved due to an elaborate balance between protein anabolism and catabolism [21]. During tumour progression followed by frequent occurrence of CAC, this equilibrium is disrupted, resulting in acceleration of protein breakdown and decline of protein synthesis [22].

The loss of skeletal muscle is induced by the secretion of various pro-inflammatory and factors promoters of catabolism by immune and cancer cells such as proteolysis inducing factor (PIF), interleukin-1 (IL-1), IL-6, IL-8, tumour necrosis factor-α (TNF-α), myostatin, angiotensin II and activin A potentiating catabolism [23].

In combination these stimuli activate different signalling pathways involved in muscle wasting: ubiquitin-proteasome system (UPS), autophagy and calcium-activated protease calpains [24]. It is also noteworthy that during cachexia, the capacity for regeneration of muscle cells is damaged [25].

Simultaneously, it also accounts for muscle wasting and the decrease in pro-anabolic signals including insulin, insulin-like growth factor-1 (IGF-1), growth hormone and cytokines hampering inflammation namely interleukin-4 (IL-4) and interleukin-10 (IL-10) [24].

Muscle depletion varies from 7 to 30%, with higher percentages directly related to the severity of the syndrome [19]. In cases of advanced disease, skeletal muscle wasting is established as an independent predictor of mortality [26–28]. Aggravation of skeletal muscle loss has a negative impact on progression-free survival during chemotherapy or surgery in patients with advanced-stage ovarian [29] and unresectable colorectal [30] cancers.

2. In Vitro Models to Study CAC

Substantial knowledge about the underlying mechanism of CAC comes from animal models. However, observations from the existing preclinical models could not be consistently replicated in studies using human samples of muscle biopsies or yield successful clinical trials or drug discovery [17]. Variations in human and animal model results could partly be explained by differences in physiology between species, tumour biology, tumour-host interactions, pre-existent co-morbidities, previous treatments for cancer, or lack of interaction between the tumour and the host immune system [17,31].

2.1. Skeletal Muscle Wasting

2.1.1. C2C12 Cell Line

The muscle precursor cell line C2C12, firstly isolated from mouse skeletal muscle myoblasts [32], is a common model to study muscle atrophy [33] but also cellular senes-
cence [34], dystrophy [35], sarcopenia [36], diabetes mellitus [37], obesity [38], and hepatic steatosis [39]. In culture, the cells are mononucleated and spindle-shaped. During differentiation, upon serum withdrawal from the culture medium and low concentration of horse serum cells can fuse forming multinucleated structures turning into elongated fibre-shaped myotubes [40]. As such, the cell line can be induced to undergo stepwise differentiation since a muscle precursor (satellite cells) to a myoblast, followed by a myocyte, and ultimately a multinucleated myotube [41]. These features enable the characterisation of the mechanisms that regulate each step of muscle development and functioning [41,42].

Therefore, the C2C12 cell line provides an invaluable tool to study the molecular biology and mechanisms of myogenic regulation [43,44], muscle differentiation [45], regeneration, and muscle atrophy [41].

After differentiation, C2C12 myotubes remain stable until 10 days in culture [46]. However, the differentiation potential of satellite cells is limited [47]. Late passages of C2C12 myoblasts present significant impairment of myogenic differentiation potential, caused by the reduced expression of myogenic regulatory factors, being the lifespan of the cell line restricted to 50 passages before senescence (for cell differentiation even lower passages are required) [48].

Muscle contraction and insulin can stimulate peripheral glucose uptake. Despite the cell line being insulin-responsive and therefore perfectly capable of serving as a model of study insulin- or contraction-stimulated glucose uptake, C2C12 cells are not as generally used because the expression levels of the glucose transporter 4 (GLUT-4) protein are lower when compared to other cell models, as L6 rat cell line [40,46,49]. Overall, this insulin-stimulated response is perfectly capable of serving a as model of study insulin resistance and other metabolic conditions [40].

This cell model’s particular features and the resemblance in myosin and glycogen content with the human muscle depict C2C12 as a remarkable model to study exercise and stress [40]. A unique characteristic of this cell line is its capability to develop a contractile apparatus of sarcomere units [49]. C2C12 cells contract when subjected to electrical stimulation, in fact, following an extended period of time after differentiation, cells can spontaneously contract [43]. The cells express high levels of myosin heavy chain genes MYH1 and MYH4, which are associated with glycolytic fibres (type II), indicating contractility properties [40]. The expression level of MHC and sarcomeric organisation are responsible for the capacity of force generation in C2C12 cells, which in part mirrors the most important attribute of skeletal muscles [50]. As a result, murine cells have been often used in pharmaceutical science and biomedical research proving to be useful to successfully identify drug candidates with high specificity resourcing to screening methods based on their phenotypic traits, such as myotube formation [51,52].

In opposition to the traditional 2-dimensional (2D) monolayer cultures, 3-dimensional (3D) has been gaining popularity. The most recent method to manufacture complex 3D architecture and alignment of muscle fibres uses bioprinting, which allows the combination of biomimetic materials to resemble the native tissue and generate more faithful models [53–56]. The microenvironment created consists of bioink (the most usual biomaterial is hydrogel) and biological material (cells) [56]. This technique significantly improves cell differentiation and response to stimuli [53,56].

2.1.2. L6 Cell Line

The rat L6 cell line was established from rat skeletal muscle myoblasts isolated by Yaffe. These cells are a common precursor model that has been used in muscular research, particularly constituting the main in vitro model for metabolic studies [46]. The complete and successful myotube differentiation process of L6 cells takes 5–6 days [48]. After that time, the cells become less stable lasting only 6 more days in culture, showing a reduction in myogenic differentiation 1 (MYOD1) and simultaneously creatine kinase M type (CKM) [46].
L6 cells express lower levels of GLUT-1 and GLUT-3, the transporters responsible for baseline glucose uptake, in an expression pattern analogous to fully differentiated mammalian muscle. However, the model has higher expression levels of GLUT-4, the only insulin-dependent glucose transporter existing in skeletal muscle [46]. This suggests that L6 cells have a better response to insulin-stimulated glucose uptake even though the baseline glucose uptake is inferior [57]. Therefore, this suggests that L6 cells are the most promising candidate as an in vitro model system to investigate glucose uptake mechanisms, insulin resistance mechanisms and their interplay with other metabolic diseases [40,46].

Additionally, L6 myotubes express high levels of glucocorticoid receptors [58]. L6 cells do not develop organised sarcomere, instead, cells possess many contractile units forming myofibrils that easily differentiate in myotubes, giving the differentiated cells culture the appearance of a disorganised network [46,48]. The myotubes formed by L6 do not contain α-actin or muscle myosin II, neither exhibit any obvious contractile activity upon stimulation [46,48]. Collectively, these constraints make them less indicated to study stimuli–muscle contraction. On the other hand, L6 cells present a force–Ca\(^{2+}\) curve resembling the muscle in vivo [48,49].

To optimise the organisation of skeletal muscle tissue, L6 cells have been cultured in 3D micro-grooved scaffolds of collagen capable of orientating the alignment of myoblasts forming multi-layered cell sheets according to specific patterns. Such application has great potential in investigating cell–cell interactions, while maintaining the muscle development process and integrity [59].

2.1.3. Human Skeletal Muscle Cells (HSMC)

Human skeletal muscle cells (HSMC) are a relevant model, mainly used due to their closeness to human physiology, even though comparative research with other widely used models is rare [46]. Similarly to C2C12 cells, HSMC cells remain stable until 10 days in culture. Using this model is scarcer due to the invasiveness of the sample collection in patients [48].

2.1.4. Comparative Analysis of In Vitro Models Using Skeletal Cell Lines

A study using available databases of the transcriptomic data of C2C12, L6 and HSMC cell lines analysed the similarities between them and concluded that L6 cells were more enriched in genes associated with metabolic and proliferative pathways, with few genes related to muscle physiology. While C2C12 cells presented augmented genes related to muscle pathways. Similarly, HSMC indicated a higher level of genes associated with muscle development [46]. Overall, the study demonstrated that differences between C2C12, L6 and HSMC cell lines can be credited to species and specific intrinsic singularities of each model, which translates into different cellular responses. Still, there is a lack of studies comparing the different cell models in muscle biogenesis, metabolism, proliferation, cell biology and their suitability for different research purposes [46].

2.2. Cancer-Associated Cardiac Cachexia

Preceding the cachexia diagnosis, cancer patients often report symptoms of fatigue and muscle weakness. However, those symptoms are aggravated by the decline in muscle mass all of which can impair the patient’s quality of life [19,60]. Cardiac abnormalities are often unaddressed pathological symptoms of CAC, they manifest as shortness of breath, pulmonary incapacity, and diminished exercise capacities, which are indicative of chronic heart failure [5]. These abnormalities are associated with enlarged oxygen consumption, thus prolonging of this occurrence increases energy expenditure. Consequently, this might result in energy inefficiency leading to a harmful energy balance and wasting, hence the designation of cardiac cachexia [11]. This occurrence is an independent predictor of increased morbidity and mortality in cancer patients [5,27]. Cardiac wasting is mainly caused by loss of diaphragm and cardiac proteins, in part, as a result of patients’ cancer
treatment with cardiotoxic drugs to treat cancer or by tumour secretions that increase atrophy of cardiomyocytes and affect cardiac contractility [5,11].

Overall, cardiac cachexia is promoted by the same mechanisms causing muscle wasting: cytokines and other pro-cachectic mediators secreted by the primary tumour, metastases and activated immune cells. In cardiac cachexia UPS and ALP (autophagy lysosomal pathway) are hyperactivated, but autophagy has a more preponderant role than in muscle wasting. This might justify the greater metabolic rate and protein turnover in the heart compared to skeletal muscle [5].

H9C2 Cell Line

Derived from embryonic BD1X rat ventricular tissue, the H9C2 cardiomyoblasts cells have been extensively used as an in vitro model for skeletal and cardiac muscle [61]. The cell line can differentiate into cardiac myocytes when cultivated in a differentiation medium for 7 days supplemented with all-trans-retinoic acid (RA) increasing the expression of genes codifying proteins [62]. Their morphological features mirror immature embryonic cardiomyocytes’ properties in electrophysiology, morphology (cell membrane), signalling (expression of g-signalling protein), and hormonal response [63,64]. When stimulated with a hypertrophic agent these cells present nearly identical responses as those obtained in primary cardiomyocytes [65]. This discovery supports the relevance of H9C2 as a useful and reliable model to study cardiac hypertrophy in a molecular context and the development of the heart and diseases affecting the organ [65]. Still, the suitability of the murine cardiomyocytes model has been under scrutiny because the cell line is able to proliferate contrarily to primary cardiomyocytes. Additionally, H9C2 cells are unable to beat, unlike primary cardiac myocytes, for that limited function this line is not appropriate for contraction-based studies [65,66]. The cells are rather easy to maintain in culture, however, since they are not immortalised, their lifespan is very limited and can only be cultured for a restricted number of passages [64]. The rise in passage number also increases the sensitivity to toxicity, and oxidative stress and the morphology of untreated cardiomyocytes also suffers alterations, meaning that cell parameters also vary [64]. It was found that the H9C2 cell line is reliable only in the first five passages. So, it is necessary to keep in mind that further passages of cardiomyocytes may not ensure the repeatability and reliability of the results because of the ageing culture [64]. The cell line is remarkably interesting in cytotoxic studies to analyse the cardiotoxicity safety of anticancer drugs namely, Doxorubicin [67], Mitoxantrone [68] and Pixantrone [69], focusing also on the mechanisms of myocyte damage and effect on cell apoptosis and necrosis.

2.3. Adipose Tissue Loss

During CAC, the loss of skeletal muscle is, to a lesser extent, accompanied by extensive remodelling and loss of adipose tissue. In fact, it was discovered that lipolysis precedes muscle wasting in some cases, therefore loss of adipose tissue can be considered an overall survival predictor in cachectic patients [70,71]. Adipose atrophy results in increased lipolysis and fat oxidation, reduced lipogenesis, disrupted lipid accumulation and adipogenesis, as well as browning [2,23]. In the initial stages of CAC, the white adipose tissue (WAT) representing a major energy reserve and therefore being responsible for lipid storage undergoes a process of transformation in brown adipose tissue (BAT), an active participant in thermogenesis, in a process denominated browning. Both BAT and the skeletal muscle are enriched in mitochondria expressing high levels of uncoupling proteins (UCPs) responsible for shifting the ATP production by the mitochondria to an increase in heat production and consequent lipolysis and energy expenditure [72,73].

3T3-L1 Cell Line

The preadipocyte cell line 3T3-L1 is originally from the fibroblast cell line 3T3, established from disaggregated 17- to 19-day-old mouse embryos [72]. Over the last few years, the well-characterised cell line has become a suitable proxy model for primary human
preadipocytes commonly used to study the WAT, to identify molecular markers, transcription factors and molecular pathways involved in the differentiation of preadipocyte cells (also known as adipogenesis), lipid metabolism, the activity of hormones and assess the potentiality of nutrients and other compounds, particularly those used in the treatment of obesity [74,75]. More recently, a characterisation of the lineage of 3T3-L1 adipocytes showed that even though the basal bioenergetic expression profile corresponds to white adipocytes, the cells present attributes from both white and brown adipocytes [76]. Under appropriate culture conditions, the fibroblast-like preadipocytes can differentiate and acquire a mature adipocyte-like phenotype [77]. The current and most refined process for differentiation consists of a cocktail of pro-differentiation agents: insulin, dexamethasone and 3-isobutyl-1-methyl-xanthine (IBMX). 3T3-L1 cell differentiation results in a process of sequential expression of specific adipogenic transcription factors, each of them regulated by pro-differentiation agents. The initiation of the adipogenic gene expression program is promoted by dexamethasone which stimulates the expression of CCAAT enhancer binding protein (C/EBP)δ and IBMX which stimulates the expression of C/EBPβ. Both transcription factors heterodimerise activating C/EBPα and peroxisome proliferator-activated receptor (PPAR)y eventually starting adipogenesis [74,75,77]. 3T3-L1 preadipocyte cells present a fibroblast-like morphology, throughout their conversion into adipocytes, cells lose their primitive mesenchymal character and accumulate triglycerides into lipid droplets intracellularly bound to the membrane [77–79]. Differentiation takes up to 10 to 12 days and the culture lasts until cell culture passage 10. However, with an increasing number of cell passages, the capacity of cells to differentiate decreases [77]. For the study of adipogenesis 3D technique has been applied using a system of single cells suspended in agarose capable of promoting cell differentiation without using a cocktail of agents for induction [80,81]. Spheroids can also be made using a method of hanging droplets capable of better reproducing the adipocyte-like properties [81].

3. Generation of a Cachexia-Induced Phenotype

To establish a cachexia-induced phenotype several methods and agents have been employed to induce skeletal muscle wasting, adipocyte tissue loss and myocardial atrophy (Figure 1). From direct agents such as cell medium from tumour cells [82], inflammatory factors [23], hormones [83], chemotherapeutic drugs [84] and gene regulation (upregulation and downregulation) using miRNAs [85]. The methods used to establish CAC cell models and cell mechanisms are summarised in (Table 1).

Table 1. A summary table of differently induced cancer-associated cachexia in vitro models and their mechanisms of action.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Induction Method</th>
<th>Cell Effect</th>
<th>Mechanism of Action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2C12</td>
<td>Conditioned medium from patient-derived pancreatic cancer cells</td>
<td>Upregulation of UPS proteins, and increase in autophagy</td>
<td>Activation of p38 MAPK and expression of transcription factor C/EBPβ</td>
<td>[86,87]</td>
</tr>
<tr>
<td></td>
<td>Conditioned medium from PC3 and DU145 cell lines</td>
<td>Inhibition of the formation of microtubes; decreasing of differentiation</td>
<td>Expression of transcription factor C/EBPβ</td>
<td>[87]</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone (10 mM for 48 h)</td>
<td>Reduction in myotubes number and width; increased expression of Atrogin-1 and Murf1</td>
<td>Protein O-GlcNAcylation</td>
<td>[88]</td>
</tr>
<tr>
<td></td>
<td>Cisplatin (5 mM for 24 h)</td>
<td>Increased expression of ubiquitin ligases; decrease in myogenic differentiation</td>
<td>Autophagy pathway, myostatin pathway and UPS system</td>
<td>[89]</td>
</tr>
</tbody>
</table>
### Table 1. Cont.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Induction Method</th>
<th>Cell Effect</th>
<th>Mechanism of Action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2C12</td>
<td>Microvesicles from A549, PANC-1 and A549 cell lines</td>
<td>Increased apoptosis</td>
<td>Expression of miR-21, signalling through the Toll-like 7 receptor (TLR7)</td>
<td>[85,90]</td>
</tr>
<tr>
<td></td>
<td>miR-195a-5p and miR-125b-1-3p mimics</td>
<td>Increased apoptosis</td>
<td>Bcl-2-mediated apoptosis</td>
<td>[85]</td>
</tr>
<tr>
<td>L6</td>
<td>Conditioned medium from MCF7 and BT474</td>
<td>Reduction of insulin responsiveness</td>
<td>Downregulation of PI3-kinase/PKB pathway</td>
<td>[91]</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone (1 µM for 6 h)</td>
<td>Protein degradation; increased expression of ubiquitin and proteosome proteins</td>
<td>Downregulation of NF-κB signalling pathway</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin (0 to 1 µMol for 48)</td>
<td>Decrease in glucose uptake</td>
<td>Reduction of AMPk activity</td>
<td>[93]</td>
</tr>
<tr>
<td></td>
<td>Conditioned medium from SW480 and H1299 cell lines</td>
<td>Reduction in myotubes number and diameter</td>
<td>Cannabinoid receptors (CB1R and CB2R)</td>
<td>[94]</td>
</tr>
<tr>
<td>H9C2</td>
<td>Methylprednisolone (0.5 µg/mL for 24 h)</td>
<td>Increased expression of Atrogin-1 and Murf1</td>
<td>NF-κB-inducing kinase (NIK)</td>
<td>[95]</td>
</tr>
<tr>
<td></td>
<td>Ascites from CT26-induced cachexia mice model</td>
<td>Reduction of cell proliferation; inhibition of mitochondrial respiration and ATP production; increase in oxidative stress</td>
<td>Expression of TNF-α and high mobility group box (HMGB1)</td>
<td>[96]</td>
</tr>
<tr>
<td></td>
<td>TNF-α, IFN-γ, IL-6, IL-8, and IL-1β (50 ng/mL for 48h)</td>
<td>Increase in mitochondrial membrane potential</td>
<td>Upregulation of dynamin-related protein 1 (DRP1) and the mitochondrial outer membrane protein (hFIS1)</td>
<td>[97]</td>
</tr>
<tr>
<td></td>
<td>LLC cell-derived extracellular vesicles</td>
<td>Adipocyte lipolysis and WAT browning</td>
<td>Parathyroid hormone-related protein (PTHrP) mediated protein kinase A (PKA) pathway</td>
<td>[98]</td>
</tr>
<tr>
<td>3T3-L1</td>
<td>Extracellular vesicles from C26 cells (3.12–100 µM for 48 h) and IL-6</td>
<td>Adipocyte lipolysis</td>
<td>Upregulation of STAT3/PKM2/SNAP23 pathway</td>
<td>[99]</td>
</tr>
<tr>
<td></td>
<td>Breast cancer derived extracellular vesicles (miR-204-5p)</td>
<td>Increase in adipocyte lipolysis and WAT browning</td>
<td>Hypoxia-inducible factor 1A (HIF1A) induced by leptin signalling pathway</td>
<td>[100]</td>
</tr>
</tbody>
</table>

### 3.1. Cancer Cells’ Secreted Factors

It is consensual and well-established that tumour cells’ secretion factors could from a distant site dysregulate protein metabolism and initiate or maintain CAC [101,102]. Research on CAC focused on the identification of different factors contributing to the cachexia mechanisms of wasting consisting of incubating the cell model (muscle, adipocytes or cardiomyocytes) with conditioned mediums from tumour cell lines [102]. This is the most common method to induce an in vitro model of cancer cachexia. So far, several secreted factors have been shown to be involved in CAC, such as the leukaemia inhibitory factor (LIF) was found in elevated concentrations in a conditioned medium from mouse colon carcinoma 26 model (C26) [103]. The signal transducer and activator of transcription 3 (STAT3) were found to be an inducer of myotube atrophy in addition to adipocyte lipolysis [104]. Heat shock protein (HSP) levels, such as Hsp70 and Hsp90, were found to be elevated in mice bearing cachectic tumours and in the serum of cancer patients with higher pathological grades and in a more advanced clinical stage [105,106]. C2C12
myotubes treated with CM from pancreatic cancer cells release high levels of Hsp70- and Hsp90-induced muscle wasting mediated by p38β MAPK [86].
Dexamethasone is a synthetic glucocorticoid widely applied for the nutritional treatment of cancer cachexia to improve patient appetite. However, in high doses or long-term use, the hormone promotes side effects including muscle atrophy by decreasing protein synthesis and breakdown of muscle proteins [118]. Dexamethasone activates the UPS system promoting the expression of Atrogin-1 and MuRF1 disrupting the autophagy flux and increasing cell apoptosis, inducing in vitro and in vivo muscle atrophy [118,119]. Dexamethasone induces skeletal muscle atrophy without disturbing cell viability in a serum-free culture medium [120].

Angiotensin II has been associated with cardiac and cancer, which is involved in the pathogenesis of associated cachexia. Clinical studies indicate elevated levels of plasma in these patients [121]. C2C12 myotubes treated with angiotensin II presented a reduced size in myotube diameter by increasing Atrogin-1 and Murf-1 expression [122]. Moreover, Angiotensin II is induced by i PPARγ decrease, which is an upstream regulator of miR-29b involved in muscle atrophy [123]. Also, in primary rat cardiomyocytes and H9C2 cells, it was demonstrated that angiotensin II and endothelin-1 promote a hypertrophic response [65].

Myostatin (GDF-8) and Activin A belong to the TGF-β family and are negative regulators of skeletal muscle growth, by one hand inhibiting protein synthesis and the other potentiating protein degradation dependent on p38β MAPK-activated signalling [124,125]. Muscle cells treated with myostatin presented a lower expression of myoD and pax3, two genes involved in myogenesis, and increased expression of UPS-related genes (atrogin-1, MuRF-1, and E214) [126]. 3T3-L1 cells treated with myostatin demonstrated a significant reduction in the intracellular lipid content compared with non-treated cells [127].

3.4. Anticancer Chemotherapeutic Agents

Cancer chemotherapy is a common primary treatment option for many types of cancer and considerable evidence indicates a major contribution to the development and sustainment of CAC, specifically driving skeletal muscle atrophy and simultaneously reducing body mass [128,129]. Cisplatin appears to mediate muscle wasting through activation of the NF-κB signalling pathway and independently forms the frequently implicated UPS [130]. Doxorubicin is an anthracycline chemotherapy drug whose main effect is cardiotoxicity leading to the primary cause of death in cancer survivors, that is cardiovascular disease [131]. In the cardiomyocyte cell line, H9C2 doxorubicin induces the production of reactive oxygen species, mitochondrial dysfunction and apoptosis [132]. However, a different outcome was reported regarding adipocytes, in which cell survival and chemoresistance were improved in breast cancer cells [133]. Similarly, melanoma cells exposed to a conditioned medium from adipocytes also showed increased cell proliferation and multidrug resistance to chemotherapeutics [134].

3.5. Gene Regulation

MicroRNAs (miRNAs) constitute a family of small molecules, noncoding RNA, ~18–22 nucleotides in length, evolutionarily conserved and tissue-specific that exert gene regulation [135]. They are typically found in biological body fluids or transported in extracellular vesicles such as exosomes [136]. In cachectic conditions, there have been identified several miRNAs involved in the deficit of lean and fat mass. Exosomes transporting miR-26a inhibited the transcription factor forkhead box O1 resulting in a blockage of muscle atrophy [137]. Overexpression of miR-21 in microvesicles from lung and pancreatic cancer cell lines can induce myoblast apoptosis in CAC activating Toll-like receptor 7 (TLR7) signalling, in a JNK-dependent manner [90]. Both miR-195a-5p and miR-125b-1-3p, identified in colon cancer exosomes, can induce Bcl-2-mediated apoptosis of C2C12 myoblasts [85]. In several in vivo and in vitro models of muscle atrophy, miR-29b is reportedly upregulated, and the impairment is mediated by two of its target genes insulin-like growth factor 1 (Igf-1) and phosphoinositide 3-kinases (Pi3k) [138]. Secreted by human breast cancer cells mir-155-5p
was proven to boost WAT browning, and promote BAT thermogenesis but had no effect in increasing lean mass loss [139].

4. Conclusions

The gross research in CAC has been focused on skeletal muscle and to a lesser extent adipose tissue loss, the two pivotal causes associated with poor quality of life and increased morbidity and mortality.

The in vitro models mentioned in this review constitute the bulk of research in CAC. However, to properly comprehend the complexity of cachexia as a multiorgan syndrome, the contribution of other organs such as the heart, brain, gut, liver and pancreas must be taken into consideration to develop effective novel therapeutic approaches. To our knowledge, there are no papers relying on in vitro models to elucidate the mechanisms of CAC in these organs. Therefore, there is no way to determine the effect of interplay between organs using in vitro models. While the in vivo models represent a step forward in biological complexity, the use of established cell lines serves as a beneficial platform for drug screening experiments, as they offer a repeatable and reproducible screening method of easy and fast manipulation, cost-effective, enabling the reduction of animals in further studies. Moreover, in vitro models have provided an inestimable contribution in the past to the unravelling of mechanisms and pathways. Despite research on in vitro 2D cultures that cannot accurately recreate in vivo conditions and the complex interactions between host tissues and tumours, the generation of 3D-engineered models is an evolving strategy to circumvent these limitations. Therefore, the development of in vitro models should be considered in further developments in this field to increase the knowledge of the specific mechanisms in other organs affected by CAC uncover potential biomarkers and assess therapeutic strategies to spur innovation in this field.

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Conflicts of Interest: The authors declare no conflicts of interest.

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