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REVIEW ARTICLE

BPC157 as Potential Agent Rescuing from Cancer Cachexia

Eun A. Kang[1], Young-Min Han[1], Jeong Min An[1], Yong Jin Park[2], Predrag Sikiric[3], Deok Hwan Kim[4], Kwang An Kwon[5], Yoon Jae Kim[5], Donghwa Yang[5], Hann Tchah[5] and Ki Baik Hahm[1,4,*]

[1]*CHA University School of Medicine, CHA Cancer Prevention Research Center, CHA Bio Complex, Pangyo, Korea;* [2]*GI Medics, Ltd, Seoul, Korea;* [3]*Department of Pharmacology, University of Zagreb, Zagreb, Croatia;* [4]*Digestive Disease Center, CHA University Bundang Medical Center, Seongnam, Korea;* [5]*Department of Pediatrics and Gastroenterology, Gachon University Gil Hospital, Incheon, Korea*

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Abstract: Cancer cachexia, one of the metabolic syndromes caused by cancer, is a devastating and miserable condition encountered in more than 50% of terminal cancer patients presenting with significant weight loss associated with skeletal muscle atrophy and fat loss. Though cachexia may account for up to 20% of cancer deaths, no significant treatment is still lacking and is of urgent unmet medical need in cancer treatment. Therefore, understanding the underlying molecular mechanisms is essential for anticipating therapeutic approaches. Since the primary events driving cachexia are mediated via either the central nervous system related- or inflammation related-anorexia, hypoanabolism, and hypercatabolism, therapy usually targets nutritional support to compensate reduced food intake along with some anti-inflammatory agents to cover specific inflammation-related metabolic derangement, and encourages exercise to supplement reduced physical activity, but all proven to be not so effective so far. Therefore, combination therapies such as a standard multi-modal package including an anorexic agent, megestrol acetate, and anti-inflammatory agent coupled with the development of potential novel therapeutics promise a new era in rescuing patients from cancer cachexia. In this review, we propose the potential application of BPC157, one of the active cytoprotective agents isolated from gastric juices for cancer cachexia. Before clinical trial, we introduced the evidence showing BPC157 rescued from cancer cachexia supported with explored mode of actions,

Keywords: Cancer cachexia, BPC157, muscle atrophy, cytoprotection, hypercatabolism, hypoanabolism.

1. INTRODUCTION

Cancer-associated cachexia, simply cancer cachexia, is a devastating and miserable clinical condition presenting with the loss of body weight accompanied with specific losses of skeletal muscle as well as adipose tissue, driven by excess energy expenditure, excess catabolism, and cancer-basis inflammation accompanied with increased proinflammatory cytokines, enhanced proteasomic degradation of muscle, and considerable level of lipolysis. From the point of metabolism, cancer cachexia is described by an abrupt disruption in energy balance, drastic changes of metabolism, a significant decrease in fat mass, apparent skeletal muscle atrophy in either anatomy or physiology point under perturbating proinflammatory cytokines [1]. Since anorexia is usually coincides with cancer development, reduced food intake compounded by secondary nutrition derangement is inferred as core initiative to cancer cachexia, but concurrent hypermetabolism, hypercatabolism, and hypoanabolism provoked by systemic inflammation and catabolic factors, which are not reversed by conventional nutritional support led to progressive and irreversible impairment of cancer cachexia [1]. Our preliminary study obtained from cachexia animal model showed that the real amount of food consumption was not the initial finding, the decrease in food intake was the finding noted at a later stage of cachexia. That is, anorexia is accompanying condition, one of the general constitutional symptoms in cancer, not major phenomenon in initiating cancer cachexia. Recent advances in understanding cancer cachexia are completed in either animal model or clinical trials covering cell signaling and underlying molecular changes of cancer cachexia such that cancer cachexia involves diverse mediators derived from the cancer cells and cells within the tumor microenvironment, including inflammatory and immune cells, endocrine, metabolic and central nervous system perturbations, and catabolic changes in skeletal muscle and adipose tissue, respectively, focusing the changes in representational contributors of inflammation, proteolysis, autophagy, and lipolysis [2] (Fig. **1**). As a representative animal model of cancer cachexia, mice bearing colon-26 (C-26 colon adenocarcinoma cells) tumors are commonly used for cancer cachexia as seen in Fig. **2A** and Fig. **3A** because they showed significant reductions in body weights, apparent shrinkage of muscle and fat mass, and increased expression of inflammatory genes and ubiquitin ligases associated with protein degradation [3-7] as well as loss of muscle strength and muscle fatigue, quite similar features as seen in patients with cancer cachexia [8]. To date, unfortunately, practical guidelines to counteract cancer cachexia are lacking, mainly because of the multi-modal pathogenesis and lack of agent modulating multi-factorial targets. Since a single therapy may not be effective, only a multi-modal approach involving different treatment combinations or agents imposing multi-targeted amelioration is likely to be successful in either prevention or treatment of cancer cachexia [9]. Since the exact and principal mediators implicated in the cancer cachectic process have not been clearly revealed, therapies aimed at such signaling systems might be more effective in preventing tissue atrophy beyond the current mitigating approach such as nutrition, progesterone such as megestrol, and orexic agents such as ghrelin or thalidomide (Table **1**). New pharmacological therapies and conventional nutritional treatments will soon integrate the parallel pathway and potential biomarker for cachexia. In this review, we introduce the potential of BPC157 as a fundamental agent targeting

*Address correspondence to this author at the CHA Cancer Prevention Research Center, CHA Bio Complex and CHA University Bundang Medical Center, Seongnam, 463-812, Korea; Tel: +82 31 780 5220; Fax: +82 31 780 5219; E-mail: hahmkb@cha.ac.kr

Table 1. Potential future candidates for the treatment of cancer cachexia.

Drug	Composition	Possible Mechanism
Megestrol acetate	Active progesterone derivative	Neuropeptide Y release, possible
BPC157	Pentadecapeptides isolated from gastric juices	TNF-α and IL-6 inhibition Proteasome ubiquination inhibition Anti-inflammatory action
Ghrelin	Gastric peptide hormone	Growth hormone receptor secretagogue
Delta-9-tetrahydrocannabinol	Cannabinoid	Endorphin receptor activation
Melanocortin antagonists	Adrenocorticotropic hormone antagonist	Melanocortin-4 receptor antagonism
Thalidomide	Immunomodulatorycompound	Decrease TNF-α, pro-inflammatory cytokines, nuclear factor kappa B, and angiogenesis
Etanercept	Biologics	Decrease TNF effect
Omega-3-fatty acids	Lipids	Decrease pro-inflammatory cytokines
Non-steroidal anti-inflammatory drugs (NSAIDs)	COX enzyme inhibitor	Anti-inflammatory action

the signaling process implicated in cancer cachexia. Though described in detail below, BPC157, a pentadecapeptide first described in 1991, also called BPC (Body Protection Compound) 15, PL-10, PLD-116 or PL14736, of which the first studies were focused on its prominent beneficial effects on gastric and intestinal injuries induced by several kinds of irritants and ulcerogenic. Through later ensuing publications, it's beneficial effects on other organs such as the liver, pancreas, intestine, brain, and heart became also evident., among which the basic and common mode of action of BPC157 was claimed to be "cytoprotective", "angiogenic", "regenerative", and "anti-inflammatory" in the gastrointestinal mucosa, after which, collectively it is an agent supporting "epithelial integrity" [10]. Based on our preliminary study that BPC157 was very effective antagonizing TNF-α and IL-6 pivotally implicated in cachexia, we set hypothesis that BPC157 can be against cancer cachexia because these actions were pivotal for mitigating cancer cachexia.

2. GENERAL CONCEPT OF CANCER CACHEXIA

Cachexia is a term originating from the Greek, *kakos* and *hexis*, meaning 'bad condition', by which cancer cachexia means that the combination condition presenting with weight loss mainly from loss of skeletal muscle and body fat and cancer-related inflammation [11]. In some, Quality of Life (QoL) and even morbidity of cancer patients involves asthenia, weakness, anorexia, anemia, and fatigue, totally very poor QoL also correlated with poor prognosis. It is generally accepted that beyond poor QoL, cachexia is indirectly responsible for the death in at least 20% of all cancer patients as well as non-cancer patients such as chronic obstructive pulmonary disease, diabetes mellitus, congestive heart failure, and chronic renal failure. Just like the obscurity of cancer origin, cancer cachexia also seems to be defined unanimously, but from the hints from tumor microenvironment, chronic inflammation and relevant signaling seems to be a hallmark of cancer cachexia [12]. In detail, proinflammatory cytokines such as IL-6, TNF-α, and TGF-β, have been widely examined for their regulation of cancer cachexia. In another aspect, cancer cachexia can be defined as a complicated metabolic syndrome associated with wasting symptoms seen in mitochondrial disorder, thereafter, summarized cancer cachexia as a dysfunctional regulation of mitochondrial dynamics, mitophagy, and biogenesis involving cachectic muscle and a disrupted capacity for oxidative metabolism in skeletal muscle [13]. Regarding inflammatory signaling relevant to cancer cachexia, activation of either MAPK-NF-κB, IL-6-JAK-STAT3 [14, 15], or TGF-β-Smad3 signaling (Fig. **1**) had been associated with cancer-induced muscle mitochondria dysfunction in C26 colon adenocarcinoma- or Lewis lung carcinoma (LLC)-bearing mice [12-15]. Prominent appearance of wasting in cancer cachexia is associated with skeletal muscle atrophy. As learned from our investigation, anorexia and decreased appetite are also frequently encountered findings in cancer cachexia, our model in the current review on C26 adenocarcinoma-cancer cachexia [47]; these phenomena were observed at rather a later stage of cachexia before mortality. For instance, to identify the mechanism by which activin signaling leads to increased muscle protein catabolism and thereby to cachexia, Mathew SJ [16] compared ubiquitin-proteasome pathway in muscles from cachectic animals with and without treatment with ActRIIB. As a result, they found that the muscle-specific E3 ubiquitin ligases, *Atrogin*-1 and *MuRF*-1, as well as ubiquitin, were significantly elevated in atrophic muscles. Also they found that the level of phospho-SMAD2, a downstream transcription factor in the activin signaling cascade, was increased in cachectic muscles and myostatin-activin-SMAD cascade has been shown to activate *Fox*O transcription factors. In summary, if the maintenance of mitochondrial content and capacity for ATP production with the final restoration of proteasomic degradation triggered with oncogenic inflammatory stimuli, they can be preserved in cachectic muscle, that can be the choice of treatment option for cancer cachexia [17]. While our preliminary studies have revealed that BPC157 can compensate mitochondrial content loss with wasting to maintain mitochondrial function, we set hypothesis BPC157 can preserve mitochondrial function, maintain the capacity for ATP production through oxidative phosphorylation and beta-oxidation as well as mitigation of cachexia-driven muscle atrophy.

2.1. Specific Concept of Cancer Cachexia Development

2.1.1. Muscle Atrophy

Many metabolic changes are responsible for the loss of muscle mass as seen in cancer cachexia. That is, abnormalities in protein including deranged synthesis, excess degradation, and abnormal amino acid metabolism are seen in the cachectic muscle. Central to these protein degradations, there is an autophagy event (Fig. **1A** and **1B**). Since many intracellular signals are involved in protein turnover and, therefore, in the wasting process (Fig. **2B**),

(A)

Causes of anorexia leading to cancer cachexia

1) Substances released from tumors, proinflammatory cytokines, lactate
2) Tumors altering gut function
3) Tumors altering nutrients, zinc deficiency
4) Tumors causing hypoxia
5) Increased central serotonin
6) Alterations of peripheral hormones that alter feeding

Leading mechanisms associated cancer cachexia

1) Protein degradation pathways
   - Ubiquitin proteasome pathway
   - Autophagy/Lysosomal pathway
2) MAPK-NF-κB pathway
3) IL-6-JAK-STAT3 pathway
4) TGF-β-smad-activin pathway

(B)

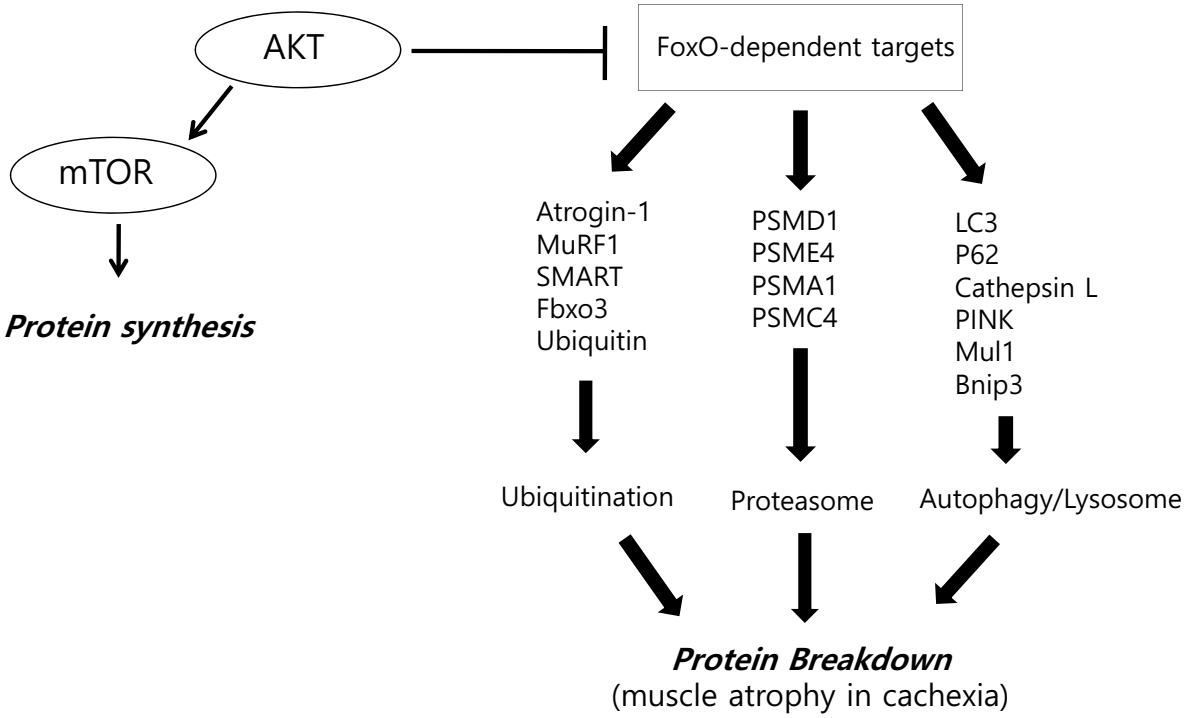

Fig. (1). Anorexia and muscle atrophy noted in cancer cachexia (**A**) Anorexia is commonly present in cancer cachexia. Though multiple causes of anorexia exist in cancer such as substances released from or by the tumor, tumors causing dysphagia or altering gut function, tumors altering nutrients, tumors causing hypoxia, increased peripheral tryptophan leading to increased central serotonin, and alterations of release of peripheral hormones that alter feeding led to depression and pain and decreased desire to eat, according to our model of C26-induced cancer cachexia, these derangement of anorexia and loss of appetite developed lately, whereas as described in lower box, significant molecular mechanisms are intervened in cancer cachexia. (**B**) Protein breakdown in cancer cachexia. The *Fox*O targets related to ubiquitin-proteasome and autophagy-lysosome system. In detailed changes of these signaling disturbance in cancer cachexia are presented in following Fig. **2** – Fig. **4**.

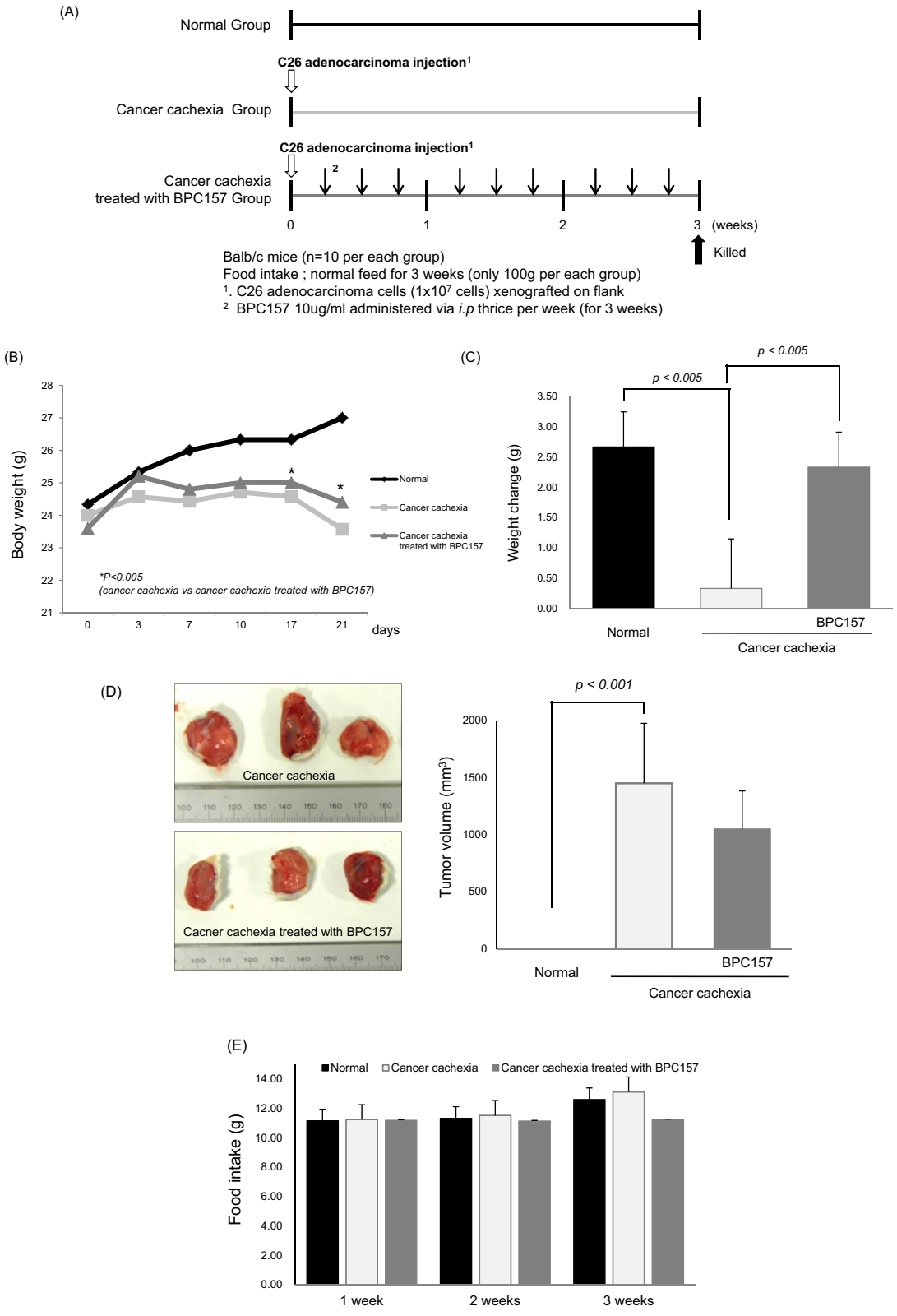

Fig. (2). Cachexia relieving effects of BPC157 in C26-induced cancer cachexia (**A**) Scheme of study We generated C26 colon adenocarcinoma-induced cancer cachexia model and in order to document the efficacy of 10μg/ml BPC157 in cancer cachexia model, BPC157 was administered three times per week intraperitoneally in 1x10^7 C26 cells-induced cancer cachexia model. (**B-C**) Statistically significant amelioration of cachexia-associated weight loss was noted in BPC157 administered group (*P<0.005* vs. cancer cachexia control). Significantly difference in body weight was noted between cancer cachexia and animal treated with BPC 157 (*P<0.005*). Due to the possible anti-cancer results with BPC157, xenografted tumors were all resected. There was no statistical difference in either (**D**) tumor volumes or (**E**) food intake, leaving the possibility that BPC157 affected muscle and fat rather than cancer-associated system factors influencing body weight.

inflammatory cytokines that are secreted by either immune cells or tumors directly induce signaling pathways that can up-regulate enzymes influencing skeletal muscle protein turnover. For instance, pro-inflammatory and pro-cachectic cytokines such as TNF-α or IL-6 are majorly involved in two established pathways, one is the nuclear factor-κB (NF-κB) pathway and the other is p38- or ERK-MAPK pathway [18]. These mediators induce an up-regulation of the expression of the key E3ubiquitin ligases such as muscle RING finger-containing protein 1 (*MuRF*1) and muscle atrophy F-box protein (*Atrogin* 1), which mediated structural muscle protein breakdown and inhibition of protein synthesis. In addition to intense proteolysis associated with cancer cachexia, an impaired regenerative capacity of myogenic cells may also be involved in muscle wasting. For instance, *paired box* 7 (PAX7), a transcription factor implicated in muscle regeneration, was significantly increased in muscle of cancer cachexia reflecting the deranged and decreased regenerative capacity in response to NF-κB. In contrast to these pathways of muscle atrophy, an anabolic condition reflected in skeletal muscle is insulin-like growth factor-1 (IGF-1) signaling (Fig. **1**), for which, IGF1 activated insulin receptor substrate 1 (IRS1)–PI3K–AKT signaling and AKT induce protein synthesis by blocking the repression of mTOR (Fig. **1** & Fig. **5**). AKT also phosphorylates the fork headbox (*Fox*O) family of transcription factors. These have a key role in inducing transcriptional up-regulation of MURF1. These transcription factors seem to be essential, as IGF-1–AKT-mediated *Fox*O phosphorylation is sufficient to block the up-regulation of the ubiquitin E3 ligases that participate in muscle proteolysis. As a consequence to cytokine changes, protein degradation is increased and protein synthesis is decreased by the transforming growth factor-β (TGF-β)-family ligand myostatin through pathways involving the activation of the SMAD complex and by p38 and JAK.

2.1.2. Fat Loss

Lipolysis with fat loss is a key feature of cancer cachexia and has been attributed to increased adipocyte lipolysis. Though the exact mechanism behind this alteration is unknown, Agustsson T [19] studied mature subcutaneous fat cells and differentiated preadipocytes from cancer patients with and without cachexia. In reality, body fat was reduced by 40% and *in vivo* lipolytic activity was 2-fold increased in patients with cancer cachexia. In mature adipocytes, the lipolytic effects of catecholamines were twice or thrice increased in cachexia, leading to the conclusion that adipocyte lipolysis is definitely increased in cancer cachexia not due to non-epigenetic factors, but due to enhanced expression and function of adipocyte lipase such as HSL. Though white adipose tissue is responsible for energy storage and found to have endocrine and inflammation-modulatory activities, brown adipose phenotype, in others, termed as "beige" or "brite" adipose tissue in a process referred to as “browning” [20], concluded that all three types of adipose tissue, white, beige, and brown contributed to the development and progression of cancer cachexia.

2.2. Current and Future Treatment Options for Cancer Cachexia (Table 1)

2.2.1 Multi-Modal Approach for Cancer Cachexia

In clinic, altering all major clinical outcomes is not possible in terms of cancer cachexia due to limited elucidation of exact pathogenesis, but on the other hand, unmet medical needs developing rational, practical and effective cachexia management are very urgent. Therefore, successful randomized clinical trials of novel agents or efficient combination treatments are prerequisites. Though earlier intervention during active adjuvant or palliative cancer therapy or during follow surveillance is a better setting, but not feasible even in the era of precision medicine, it is more due to insufficient pathogenic mechanisms of cancer cachexia [21]. As stated already, since cancer cachexia is a multi-modal syndrome, there are several specialized multi-disciplinary trials that are either adding treatment elements or establishing regimens for multi-modal intervention such as cancer chemotherapy, nutritional intervention, and adding specific empirical combination such as orexin, ghrelin or its mimetics, exercise, and others biologics targeting inflammation mediators, for instance, etanercept or adalimumab to block TNF-α, finally the correction of frail, anorectic, elderly, and co-morbid condition. With intense nutritional intervention combined with appetite promoting agent, there was a beneficial effect only on some aspects of quality of life [22].

2.2.2 Conventional Drugs

In brief, until now no widely approved drug for the treatment of cancer cachexia is available. However, steroid hormones such as megestrol acetate (Megace®) have been shown to be effective in stimulating appetite, for instance, corticosteroids and progestins being more effective than androgens; however, some limitations and disadvantages are that corticosteroids are associated with additional unwanted effects with short-lasting efficacy and weight gain in patients receiving progestins, harboring the risk of thromboembolism and more likely lean body tissue those receiving androgens [23]. In some patients, targeting the inflammatory response with cyclooxygenase inhibitors (NSAIDs) such as indomethacin, ibuprofen, and celecoxib, in order either to reduce the level of acute-phase reactants such as CRP and ESR or to reduce resting energy expenditure, but of limited efficacy until now burdened with fatal side effects. In others, ω-3 fatty acids are known to be competitive antagonists of inflammatory arachidonic acid, but large-scale Cochrane meta-analysis concluded that evidence from a very few randomized controlled studies was insufficient to give a recommendation [24-29]. Thereafter, thalidomide inhibited the production of the inflammation-related cytokine TNF-α by human macrophages and was also tested in patients with cachexic esophageal cancer, but in spite of the teratogenic risk of thalidomide with severe adverse effects such as high costs, peripheral neuropathy, constipation, thromboembolism, pulmonary edema, no expected outcomes were noted.

2.2.3. Investigational Developing Drugs

In accordance with the rapidly expanding knowledge of the basic and translational basis of cancer cachexia, newly initiated trials are assessing the potential clinical benefit of new agents, but still they fail in showing an anticipating improvement. However, recent advances in medicinal chemistry can provide a hope in the near future due to high levels of unmet medical needs in cancer cachexia. For instance, compared with saline infusion, infusion of the gastric-amino-acid hunger-stimulating peptide ghrelin in seven anorexic patients with cancer increased food intake significantly by 31%. The orally active ghrelin receptor agonist, anamorelin hydrochloride, showed similar activity in patients with cancer cachexia. A phase III, randomized, placebo-controlled, clinical trial assessing anamorelin hydrochloride in patients with lung cancer-associated cachexia is currently recruiting patients. Selective androgen receptor modulators have been developed for the treatment of muscle wasting and osteoporosis. Enobosarm was shown to significantly increase lean body mass and muscle function in a double-blind- placebo controlled-trial in 120 candidates. Since IL-6 is a putative mediator of muscle wasting, humanized anti-IL-6 antibody has been shown to be effective in the treatment of cancer cachexia. Another finding resonates with the results of phase II clinical trial that assessed selumetinib, an inhibitor of MAPK1 and of IL-6 secretion, in patients with bile duct cancer. Growth Differentiation Factor-8 (GDF 8), a member of the TGF-β family, the action of which is to limit muscle mass, resulted in significant increases in muscle mass. Similarly, another effort to develop agents capable of modulating myostatin signaling, LY2495655, an anti-myostatin monoclonal antibody is under RCT in patients with metastatic pancreatic cancer presenting with cancer cachexia. BYM338, another anti-myostatin antibody, is also under phase II evaluation in patients with advanced cachexic lung or pancreatic

cancer. Conclusively, all of these trials still face further promising results in the treatment of cancer cachexia.

2.2.4. Hype and Hope

Since cancer cachexia evolves through different stages of clinical relevance, that is, pre-cachexia, cachexia, and refractory cachexia, the strategy should be included to either prevent or delay the progression of cancer cachexia or to ameliorate life-threatening cancer cachexia [21]. Therefore, earlier and organized multi-modal interventions in conjunction with nutritional supplementation, optimal physical exercise, and third efficient pharmacological interventions are mandatory. Recently, as exemplified by Petruzzelli M publication [24] that the inhibition of white adipose tissue browning can represent a promising approach to ameliorate cachexia in cancer patients and by the data provided by authors as listed below, soon agents can be available after RCT for cancer cachexia.

3. BPC157 AS POTENTIAL CANDIDATE FOR CANCER CACHEXIA, WHAT, WHY AND HOW?

3.1. Developmental History of BPC157

The pentadecapeptide body protective compound (BPC)-157, *Mr*1419, with the sequence *Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val,* a 15-amino acid fragment existing in gastric juice, is thought to be essential for BPC. BPC 157 has a strong anti-inflammatory activity, spurting healing, and restored marked angiogenic effect [25]. Besides anti-inflammatory actions as shown in inflammatory bowel disease model [26], muco-protective actions as shown in ethanol-induced gastritis [27], and anti-oxidative actions as shown in cysteamine-induced duodenal ulcer [28], significantly facilitate the healing of bone fracture in rats through an osteogenic effect significantly improving the healing of segmental bone defect. Furthermore, BPC157 accelerated the healing of transected rat Achilles tendon and transected rat quadriceps muscle [10]. Thereafter, BPC157 has been fully characterized and investigated in quite diverse diseased organs such as the liver, lung, colon, and gastric lesions besides anti-anxiety and anti-depressant effects, and Parkinson's disease. Recently, common and basic to all of these beneficial models with BPC157, accelerated healings after BPC157 were published. BPC157 exerted its enhancement effects on proliferation, migration, and tube formation of endothelial cells, for which phosphorylated levels of ERK1/2 were pivotal in this strong healing acceleration.

3.2. Documented Peculiar Actions of BPC157

Using duodeno-cutaneous fistulas model in rats, the healing of troublesome fistula along with the documentation of mechanism has been documented [30]. As a result, they found that BPC157 afforded significant closure of fistulas and skin healing with interaction with NO system [26, 31-33], and as a novel mediator explaining Dr. Andrea Robert's cytoprotection and adaptive cytoprotection [34], it could reverse toxicity caused by non-steroidal anti-inflammatory drugs (NSAIDs) [34]. BPC 157 significantly antagonized lesions after repeated doses of diclofenac, primarily rescued from NSAID-induced gastrointestinal lesion [35], and also improved other systemic lesions of NSAID-induced liver and brain toxicity. To elaborate the advantage of the stable gastric pentadecapeptide, BPC 157 could have a particular relevance in colo-cutaneous fistula healing. Given in the same dose range, it ameliorated the skin and visceral wound healing recently improved ileo-ileal anastomosis healing. The most important finding is that BPC157 is very stable in human gastric juice [26] and recent experiments demonstrated that recombinant human platelet-derived growth factor homo-dimer of B-chains and BPC157 had similar selectivity for stimulation of granulation tissue in both sponge granuloma and in healing wounds. However, BPC157 was more active in stimulating early collagen organization. It also stimulated the expression of the early growth response 1 (*egr*-1) gene.

4. EXPERIMENTAL EVIDENCE :BPC157 IS RESCUED FROM CANCER CACHEXIA

4.1. Cachexia Relieving Effects of BPC157 in C26-Induced Cancer Cachexia Model

In order to document the efficacy of 10ug/ml BPC157 in cancer cachexia model, we have injected BPC157 three times per week intraperitoneally in $1x10^7$ C26 adenocarcinoma cell-induced cancer cachexia model (Fig. **2A**) [8, 36]. C26 adenocarcinoma cells, 1x107, administered into Balb/c mice significantly led to cancer cachexia around 3-4 weeks [47]. As seen in Fig. **2B**, a statistically significant improvement in halting weight loss was noted in BPC157 administered group (*P<0.005* vs Cancer cachexia control, Figs. **2B** & **2C**). Ethical committee prohibits extended observation of cancer cachexia, by which we compared the body weights up to 3 weeks of C26-induced cancer cachexia, and maintenance of more than 4-5 weeks definitely showed a significant difference in mortality (data not shown). Significantly, big difference was noted between cancer cachexia and animal treated with BPC 157 (*P<0.005*, Fig. **2C**). If BPC157 afforded anticancer effect, this might affect cancer cachexia. In order to prove that there were no anti-cancer results achieved with BPC157, xenograft tumors were all resected. As seen in Fig. **2D** & **2E**, there was no statistical difference in either tumor volumes or food intake, leaving the possibility that BPC157 affected muscle and fat rather than cancer-associated system factors influencing body weight.

4.2. BPC157 as Smoothening Effects on Cachexia-Associated Muscle Atrophy

Whole thigh and leg muscles were observed in Group 3A. Whole muscle mass was also evaluated amongst the members of the group. As expected, leg muscle mass in cancer cachexia group was significantly decreased (*P<0.05*, Fig. **3A**), whereas muscle mass was significantly preserved in the group treated with BPC157 (arrow), signifying that BPC157 significantly retarded C26-induced muscle atrophy. In *In vivo* observation of mice activities, the activities of Group 3 were quite similar as seen in Group 2, but slow and retarded in Group 2. With real observation of muscle bundle and muscle fiber, the characteristic features of muscle change in cancer cachexia group were noted, being thin in bundle, inflammatory cell infiltrations and considerable levels of muscle atrophy accompanied with muscle degeneration (Fig. **3B**). Looking at the changes of Atrogin-1 and MuRF-1, muscle related E3 ubiquitin ligase, both Atrogin-1 and MuRF-1 expressions were significantly decreased in Group 2(*P<0.05*, Fig. **3C**). However, the expression of MuRF-1 significantly decreased in Group 3 (*P<0.001*, Fig. **3C**). Then, the changes in Mfn-2 were also significant. Mfn-2 mRNA and their protein expressions significantly decreased in Group 2, while these expressions were significantly preserved in cancer cachexia group treated with BPC157 (*P<0.001*, Fig. **3D**). All of these changes in skeletal muscle transcription were reflected by the changes in PAX-7, muscle-specific transcription factor. As seen in Fig. **3E**, significant changes in either PAX-7 or PGC-1α were observed (*P<0.005*), all findings consistently signified that BPC157 retards cancer cachexia-induced muscle atrophy through encouraging muscle regeneration.

4.3. BPC157 Corrected Cancer-Induced Deranged Inflammation and Metabolism

Basic to cancer cachexia, oncologic inflammations prevail. As seen in Fig. **4A**, the expressions of TNF-α and IL-6 mRNA were significantly increased in group 2 (*P<0.001*) [37]. However, no changes in the expressions of TNF-α and IL-6 were noted in cancer cachexia group treated with BPC157. Also looking at muscle metabolism relevant to cancer cachexia, BPC157 significantly corrected deranged muscle proliferation as well as myogenesis, bringing changes in the expression of FoxO3a, p-AKT, p-mTOR, and P-GSK-3β (*P<0.001*, Fig. **4B**). Taken all together, BPC157

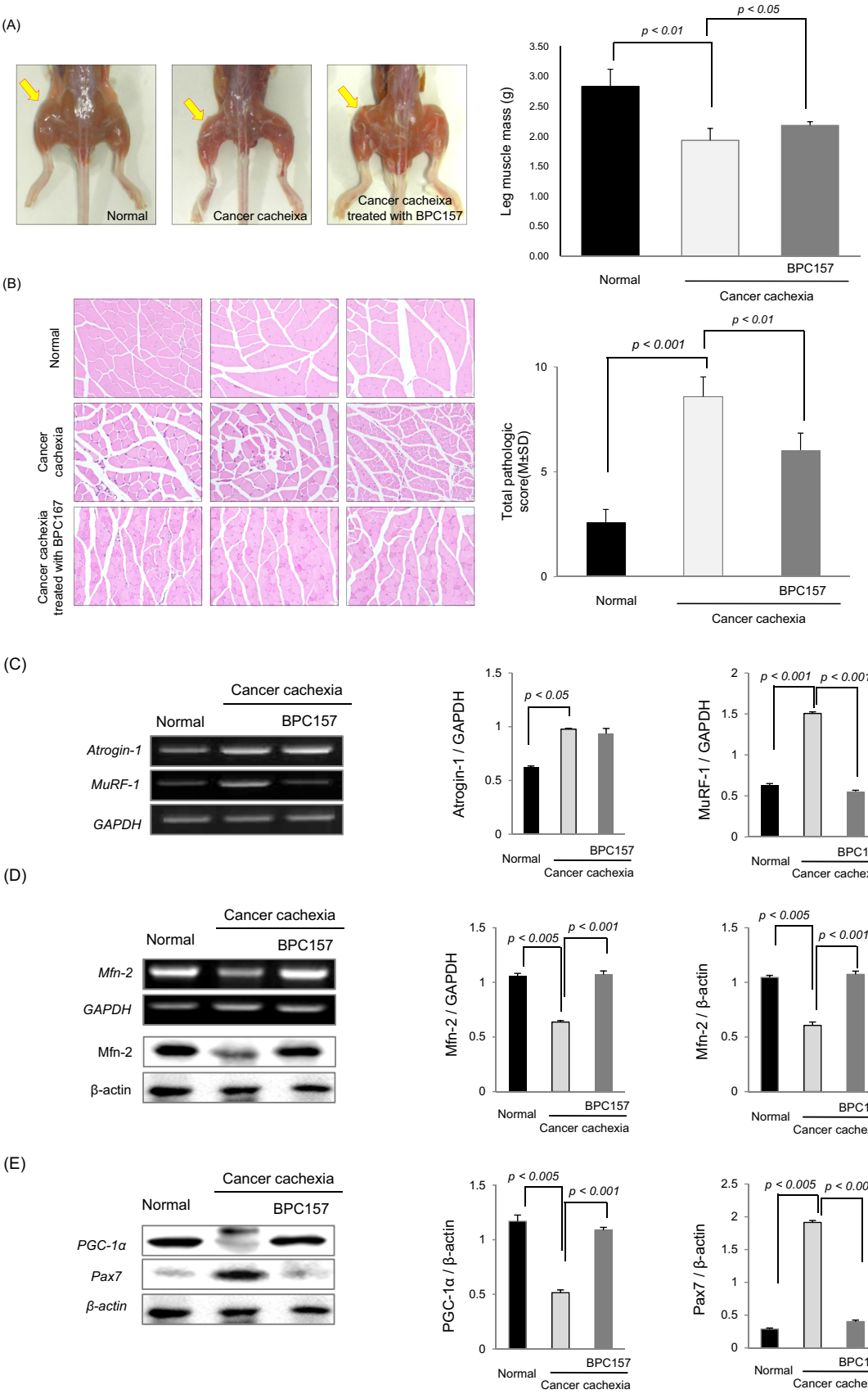

Fig. (3). BPC157 as smoothening effects on cachexia-associated muscle atrophy (**A**) Whole thigh and leg muscle was observed according to group. Whole muscle mass was evaluated among group. As expected, leg muscle mass in cancer cachexia group was significantly decreased (*P<0.05*), whereas muscle mass was significantly preserved in group treated with BPC157. (**B**) Pathology of muscle bundle and muscle fiber according to group.The characteristic features of

muscle change in cancer cachexia group were noted, thin in bundle, inflammatory cell infiltrations and considerable levels of muscle atrophy accompanied with muscle degeneration. (**C**) RT-PCR of *Atrogin*-1 and *MuRF*-1mRNAM muscle related E3 ubquitin ligase, both *Atrogin*-1 and *MuRF*-1mRNA expressions were significantly decreased in Group 2(*P<0.05*). However, the expression of MuRF-1 were significantly decreased in Group 3 (*P<0.001*). (**D**) *Mfn* mRNA and its expression according to group The changes of Mtn-2 were significant. *Mfn-2* mRNA and their protein expressions were significantly decreased in Group 2, while these expressions were significantly preserved in cancer cachexia group treated with BPC157 (*P<0.001*). (**E**) All of these changes in skeletal muscle transcription were reflected by the changes of PAX-7. Muscle specific transcription factor. (Significant changes in either PAX-7 or PGC-1α were observed (*P<0.005*).

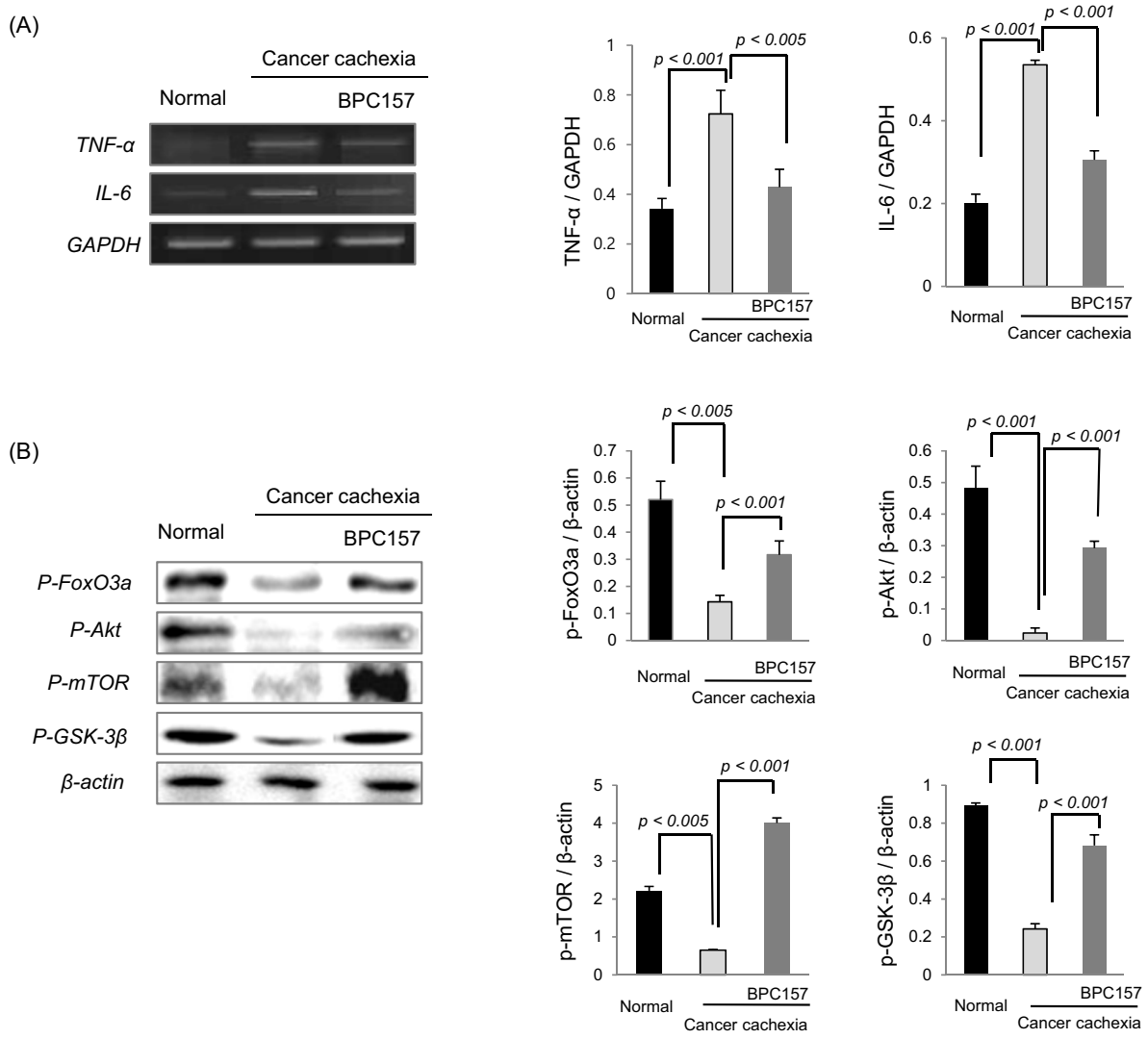

Fig. (4). BPC157 corrected cancer-induced deranged inflammation and metabolism (**A**) RT-PCR for *TNF-α*and *IL-6* mRNA. The expressions of TNF-α and IL-6 mRNA were significantly increased in group 2 (*P<0.001*). However, no changes in the expressions of TNF-α and IL-6 were noted in cancer cachexia group treated with BPC157. (**B**) Western blot for FoxO, AKT, mTOR, and GSK-3β. BPC157 significantly corrected deranged muscle proliferation as well as myogenesis, the changes in the expression of FoxO3a, p-AKT, p-mTOR, and P-GSK-3β (*P<0.001*).

afforded significant mitigating action against cancer cachexia-induced muscle degeneration, inflammation, and catabolism (Fig. **5**).

CONCLUSION

The pathophysiology of cancer cachexia is rather complex and unclear due to obscurity of cancer and includes symptoms that impact wasting and poor caloric intake as well as chronic inflammation, hypermetabolism, and hormonal alterations, after which cancer cachexia is very distressing to cancer patients as well as caregivers and families and also miserably and not reversible in spite of increasing caloric intake [38]. Its indefinite pathophysiology, complex molecular pathways leading to cancer cachexia, is also complex and troublesome resulted in the lack of specific treatment modality with no guidelines for the treatment of cancer cachexia [39, 40]. Though currently, megestrol acetate and glucocorticoids in order to stimulate appetite are actively prescribed in clinic, they do not warrant appetite and weight gain. Unfortunately, single pharmaceutical interventions alone for cachexia do not result in meaningful functional or real outcomes. Though serious interventions would include nutritional encouragement, assessing helps that has an impact on caloric intake, with a rational combination of some pharmacologic approaches combined with anti-inflammatory agent [41]; agents to meet unmet medical needs

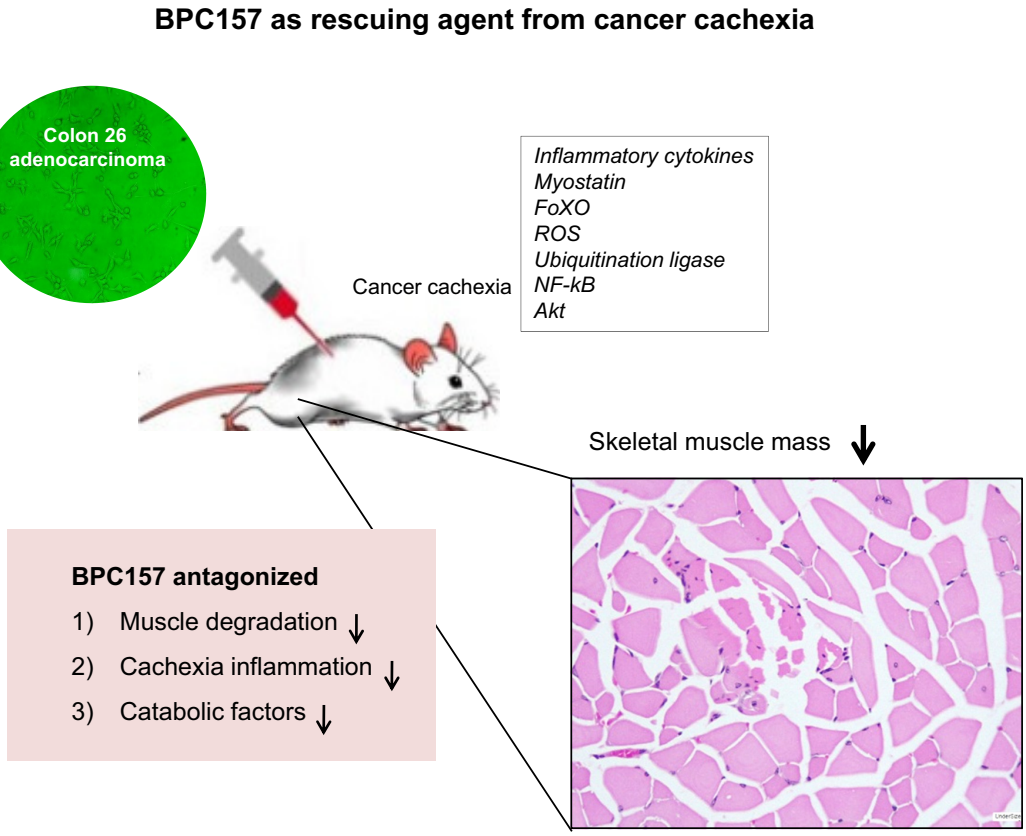

Fig. (5). Schematic figures explaining molecular mechanisms how BPC157 prevented cancer cachexia BPC157 afforded significant mitigating action against cancer cachexia-induced muscle degeneration, inflammation, and catabolism.

are mandatory. As candidates to meet unmet medical needs in cancer cachexia, agents to correct cancer cachexia-associated disruptions in muscle oxidative metabolism are under investigation. In detail, these are to match mitochondrial biogenesis, dynamics, autophagy, apoptosis, and function. In the cachectic environment, there is a remarkable decrease in mitochondrial biogenesis quantified by decreased peroxisome proliferator-activated receptor γ coactivator-1alpha (PGC-1α), *sirtuin*1 (Sirt1), nuclear respiratory factor 1 (NRF1), and mitochondria transcription factor A (TFAM). Others are mitochondrial fission proteins, fission 1 (Fis1) and dynamin-1-like protein (DRP1) while decreasing mitochondrial fusion proteins, mitofusion1 (Mfn1) and Mfn2 are implicated in mitochondria dynamics. Third is a significant reduction in present mitochondrial content due to increased mitochondrial autophagy and apoptosis reflected by increases in all isoforms of light chain 3 (LC3), parkin, PTEN-putative kinase 1 (PINK1), autophagy 5 (Atg5), voltage-dependent anion channel (VDAC), bcl-2-associated X protein (Bax), beclin, and BCL2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3). Last points to be implicated in cancer cachexia are impairments in muscle oxidative metabolism as shown with changes in adenosine monophosphate (AMP), 5′ adenosine monophosphate-activated protein kinase (AMPK), apoptosis-inducing Factor (AIF), cytochrome C (Cyt *c*) and *Fox*O. Luckily, as summarized in Fig. **5**, BPC157 can be a potential candidate to cover all of these derangements of cancer cachexia. However, further detailed clinical trials should be followed for either rescuing patients or improving QoL. Before BPC157, the royal approach in current is that since anorexia and metabolic alterations are the main components of the cachectic syndrome, ghrelin agonists, selective androgen receptor agonists, β-blockers and anti-myostatin peptides are actively under investigation to enhance food intake as well as convincing nutrient intake [42-46].

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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