American Journal of Cancer Biology

American Journals of Cancer Biology
http://www.ivyunion.org/index.php/ajcb
Vol. 1, Article ID 201300117, 16 pages

Review Article

Role of NF-KB in Loss of Skeletal Muscle Mass in Cancer Cachexia and its Therapeutic Targets

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Abstract

Cancer cachexia is a type of cancer metabolic syndrome characterized by wasting of energy storage tissue of the body such as loss of skeletal muscle or fat body mass. It is one of the most common forms of cachexia. Researchers have shown that around 50%–80% cancer patients are cachectic and about 22% cancer related deaths are due to cachexia. Recent evidences confirm that skeletal muscle loss is induced by different mediator based mechanisms such as cytokines, tumor-derived factors, TNF-alpha receptors, androgen receptors, negative modulator of growth factors, and ubiquitin-proteasome pathways. These mediators interact with their unique receptors on skeletal muscles and activate nuclear factor-kappa-B (NF-κB) which is a transcription factor essential for atrophy based muscle protein degradation. Inhibition of NF-κB ameliorates cancer-induced muscle loss are suggestive of a new set of drug targets for clinical intervention in cancer cachexia. Here we summarize the recent advances concerning involvement of NF-κB in loss of skeletal muscle mass in cancer cachexia and its therapeutic targets. Future experimental efforts may focus on discovering of new drugs that act as potential therapeutic targets in cancer cachexia against the myriad of signaling pathways to precisely understand the mechanisms of loss of skeletal muscle mass and adipose tissue which could demonstrates significant improvement in treatment outcome, functional status and quality of life of the patients.

Keywords: Skeletal muscle atrophy; NF-kB; Cancer Cachexia; Therapeutic targets; Muscle cells

Peer Reviewers: Belamy Cheung, PhD, Children's Cancer Institute Australia, University of New South Wales, Australia.

Editor: Sihua Peng, PhD, Department of Pathology, Zhejiang University School of Medicine, China

Received: March 17, 2013; **Accepted**: June 5, 2013; **Published:** July 2, 2013

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Cachexia can occur under a variety of disease states such as AIDS, heart failure, sepsis, diabetes and cancer. Cancer cachexia is a multi-factorial syndrome defined by an ongoing wasting of skeletal muscle mass (with or without loss of fat moss) that cannot be completely reversed by nutritional treatments and leads to progressive functional impairment [1].Thus, the loss of skeletal muscle mass is the main characteristic feature of cancer cachexia where intracellular protein degradation and several extracellular alterations including the breakdown of muscle basement membrane and intramuscular connective tissues, have been attributed. Published literature indicates that out of total cancer patients, 50%–80% patients become cachectic and about 20-30% deaths are due to cancer cachexia [2]. Cancer patients suffering from cachexia have lower survival rate due to loss of skeletal muscles, which in turn affects the muscle strength, mobility, fatigue, and quality of life [1-5].

Accumulating evidences suggest that various stimuli trigger muscle wasting either by reducing protein synthesis, or enhancing the degradation of skeletal muscle protein or sometimes by both [3, 6-8]. Although the triggering mechanisms for each type of muscle wasting may be unique, there are common sets of transcriptional and biochemical changes augmenting the cell capacity for protein breakdown in skeletal muscle loss. Earlier studies have suggested different mediator based mechanisms that cause loss of skeletal muscle mass such as; cytokines, tumor-derived factors, TNF-alpha receptors, androgen receptors, ubiquitin-proteasome pathway, negative modulators of muscle growth, and intracellular signaling pathways [3,4,7,8,10-11]. Earlier published reports have established that increased expression of muscle specific ubiquitin-conjugating enzymes (E3 ligases i.e. MAFbx/ atrogin-1, MMP9, MuRF1, and E3IIα) play major role in degradation of a bulk of muscle proteins in various distinct conditions [3, 12-15]. Although etiopathogenesis of cachexia is poorly understood, several studies suggested that expression of various genes such as MyoD, cyclin [12, 16-19], ubiquitin-conjugating E2 enzyme UbcH2/E2$_{20k}$ [11,20], MuRF1 [6,21], MMP9 [22] and inflammatory cytokines [6,23] are tightly controlled by coordinated action of NF-kB signaling pathways. Moreover, some studies on cachexia indicate that increased proteolysis and apoptosis induction are mediated by NF-κB up-regulation [24-26].

Recently significant progress has been made in understanding the signaling mechanisms linked to cachexia such as smads [27], AP-1[28], IFNγ [29], Foxo1 [30-31], and NF-κB [6, 31]. Among them, the activation of the nuclear factor-kappa B (NF-kB) signaling pathway has been reported to be essential for degradation of muscle protein in humans and rodents [6-9, 21]. In fact, several genetic and pharmacological studies in experimental animal models have shown that inhibition of NF-kB can prevent skeletal muscle loss in a wide variety of stimuli suggesting that NF-kB can serve as an important molecular target in future therapies [4,6,7,8,21-23]. Although the underlying mechanisms in skeletal muscle loss have been elucidated, but there is no comprehensive review discussing the NF-κB mediated therapeutic targets in skeletal muscle loss in cancer cachexia. In present review, we have summarized the recent advances in NF-κB mediated skeletal muscle loss due to

cancer cachexia along with its therapeutic targets.

NF-κB signaling pathway

NF-kB is a REL family of transcription factors, which regulates >150 genes that are involved in a variety of cellular processes depending on the cell types and upstream triggers such as: cytokines, immune cell receptors, antigen-presenting receptors, regulators of redox status, acute-phase response, apoptosis, disuse atrophy, host defense and cancer cachexia [4,8,10, 23].

NF-κB consists of a family of 5 members: p105/p50, p100/p52, p65 (RelA), RelB, and c-Rel, all sharing the Rel homology domain which allows their dimerization and translocation to the nucleus in mammalian cells. Evidences from human and rodent studies suggest that p50-p65 heterodimer plays a major role in activation of NF-κB transcription factor in skeletal muscle cells [3,6,7,9]. Before activation, NF-κB dimers are retained in the cytoplasm by binding to specific inhibitors of NF-κB known as inhibitory kappa-B (IκB) proteins. Seven different IκB are found in mammalian cells including; IκBα, IκBβ, IκBε, IκBγ, BCL-3 and precursor proteins of IκB- p100 and p105. They contain five to seven C-terminal ankyrin repeats which bind to of NF-κB dimerization domain and prevent nuclear translocation and maintain NF-κB in an inactive state. NF-κB activation is mediated by phosphorylation of IκB through the IκB kinases that allow Rel heterodimers to translocate into the nucleus to activate NF-kB dependent genes [32].

There are two pathways for NF-κB activation: 1) classical or canonical, and 2) alternative or non-canonical. The classical signaling pathway is primarily involved in the activation of NF-κB transcription factor in response to inflammatory mediator stimuli. This is followed by phosphorylation of IKB kinase-β (IKK β) to IkBα, leading to its ubiquitination and degradation, and thus allowing NF-kB to translocate into the nucleus.

In alternative signaling pathway specific activation of p52: RelB heterodimers results into NF-kB activation under the control of NF-kB-inducing kinase (NIK) in response to various stimuli. NIK has been shown to activate IKK-1, leading to inducible processing of p100 with preferential nuclear translocation of p52-RelB dimmers [33-34].

The IκB kinase (IKK) complex is mainly involved in regulation of cell stimulation. It consists of two catalytic domains (IKK-α and IKK- β) along with a regulatory subunit (IKK-γ/NEMO) [35]. After activation, NF-κB-bound IκB proteins are phosphorylated, by the activated IκB kinase, and are then targeted for poly-ubiquitination and rapid degradation through the 26S proteasome pathways by creating a binding site for the SCF β TrCP ubiquitin ligase complex [36]. Activated NF-κB allows generation of p50 and p52 by the partial processing of p100 and p105, and eventually results in the formation of dimers which are translocated into the nucleus. In nucleus they are involved in transcriptional activation of several target genes such as MuRF1 [7, 21], MMP9 [22, 37], MyoD cyclin [16,17,19], and several inflammatory cytokines (Figure 1) [6,8,23]. Besides, NF-κB transcription factor is involved in the modulation of transactivation of several genes by the posttranslational modifications of p65 (by phosphorylation, acetylation, and ubiquitination). This suggests that NF-κB serves as a crucial transcription factor with multiple levels of regulation, mediating a battery of biological functions.

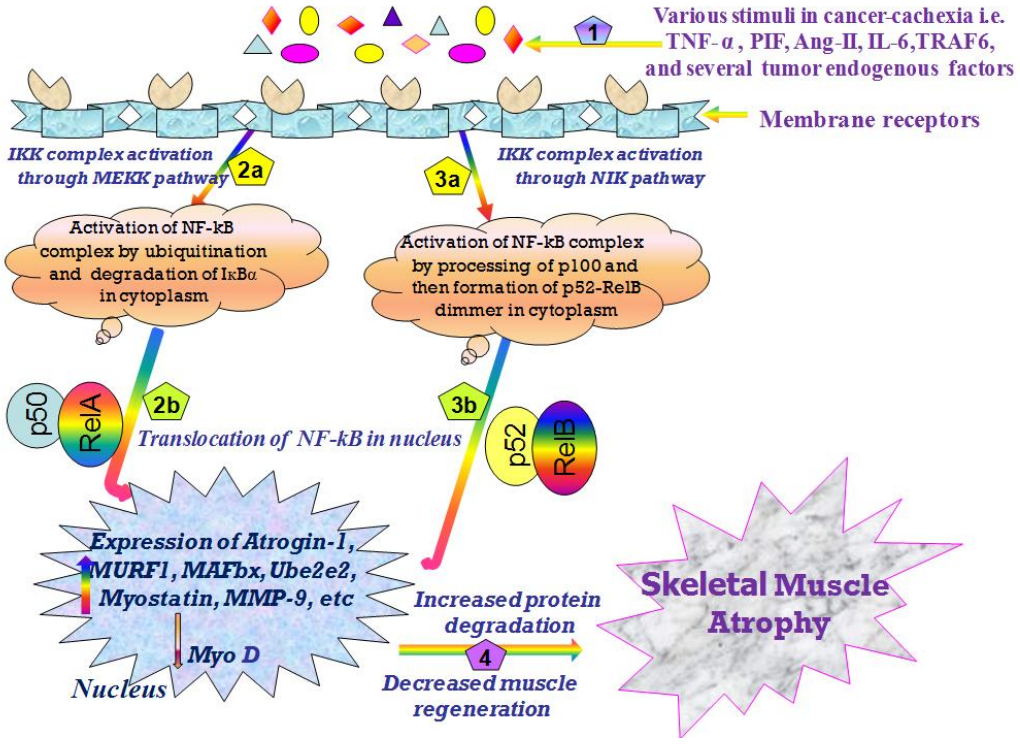

Figure 1 Role of NF-kB in loss of skeletal muscle mass in cancer cathexia. NF-kB signaling pathways in skeletal muscle atrophy due to cancer cachexia. Step 1: Cancer cachexia is mediated by various stimuli i.e. TNF- α , PIF, Ang-II, IL-6,TRAF6, as well as several tumor derived endogenous factors and their interactions with skeletal muscle cells. These interactions at the membrane side of the muscle receptors activate IKK complex through MEKK pathway (step 2a)/or by NIK pathway (step 3a). MEKK pathway mediated activation of IKK complex results in the activation of NF-kB complex by ubiquitination and degradation of IκBα in cytoplasm (step 2b). In NIK pathway, activation of NF-kB complex takes place by the processing of p100, which results in the formation of p52-RelB dimmer in cytoplasm (step 3b). Consequently, activated dimmer of NF-kB (p50-RelA or p52-RelB) is translocated into nucleus where it regulates muscle wasting through the increased expression of Atrogin-1, MURF1, MAFbx, Ube2e2, Myostatin, MMP-9 or decreased expression of MyoD protein causing lowered muscle regeneration and increase in degradation of muscle protein through the ubiquitin proteasome dependent mechanism as shown in step 4 and finally cancer cachexia.

Role of NF-κB in muscle loss due to cancer cachexia

Muscle atrophy is a characteristic feature of cancer cachexia, and the degree of muscle loss has been correlated with the reduced survival rate of cancer patients. Recent literature has demonstrated that skeletal muscle loss is caused by different mediator based mechanisms [4, 6, 8, 10, 23, 25, 38].

Alterations in muscle protein synthesis and degradation arise from the presence of tumor-derived factors, such as proteolysis-inducing factor (PIF), IL-6, or increased production of several endogenous factors, such as cytokines or Ang II [4, 5, 23]. PIF is a molecule which has a role in tumor and mediates cancer cachexia. Earlier data in cancer cachexia showed that decrease in muscle mass is regulated by PIF and tumor produced sulphated glycoproteins which are responsible for increased protein degradation

and protein synthesis inhibition [39]. Furthermore, same investigators have confirmed that protein degradation in muscle mass is due to enhanced expression of components involved in ubiquitin-proteasome pathway. Another observation in murine myotubes shows that induction of proteosome by PIF results in increased DNA binding activity of NF-κB, and decreased IkBα concentration in cytoplasm [40]. Similarly, a study by Smith et al. (2004) on skeletal muscle cells showed that NF-κB transcriptional factor is involved in the intracellular action of PIF for protein degradation by the induction of proteasome dependent pathway [41]. Some other investigators have also demonstrated that activation of PKC is an important signaling event in the induction of proteasome expression by both PIF and Ang II through the phosphorylation and degradation of IkBα and subsequent activation of NF-κB [40-42]. A relationship between the down regulation of protein synthesis and the increased degradation of the muscle protein in skeletal muscle cells has been established through PIF and Ang II mediated induction of the ubiquitin-proteasome pathway via activation of NF-κB [43]. Same group further established that PIF and Ang II are responsible for degradation of IkBα and nuclear accumulation of NF-κB in myotubes of muscle cells transfected with pcDNA3 (-) and wild-type PKR but not in the mutant PKRαΔ6. The results suggested that PKR phosphorylation is responsible for enhancement of protein degradation by increased phosphorylation and degradation of IkBα through the activation of upstream kinase IKK [44]. Increased phosphorylation of PKR and eIF 2 in gastrocnemius muscle of weight loosing mice, bearing the MAC16 tumor (cachectic mice bearing murine adenocarcinoma cells), suggested that similar processes may occur in cancer cachexia [44]. Lewis lung carcinoma (LLC) cells are used to create a model for cancer cachexia-induced muscle wasting in mice. Cai et al. (2004) study showed that subcutaneous injection of LLC cells leads to the growth of large tumors at the injection sites and diffuse tumors in the lungs. Same group also observed enhanced activity of NF-kB (6-fold) in the muscles of wt mice (sham and denervated mice) with tumors however no effect on MISR (denervated) mice with similar tumor burdens [7]. Increased NF-kB activity was presumably due to the effects of circulating "cachexia" factors. However, when cancer cachexia bearing mice were treated with selective NF-kB inhibitor, decreased muscle wasting and prolonged survival rate were observed. Same group also confirmed that activation of NF-kB in MIKK mice results in higher expression of the E3 ubiquitin ligase, and MuRF1 [7]. Another study by Kawamura et al. (1999) demonstrated that when double stranded oligodeoxynucleotides were injected into the tumors of C-26 in mice, an attenuation of the loss of body weight, gastrocnemius muscle mass, and epididymal fat mass were observed [45].

The pro-inflammatory cytokines such as TNF-α, interleukin-6 and IL-1 play significant role in the pathogenesis of muscle wasting in cachexia [23]. Adler et al. (1999) have demonstrated that increased production of IL-6 is primarily regulated by NF-kB mediated transcription factor in cachexia of prostate cancer [46]. Some studies have suggested that NF-kB inhibition may result in amelioration of cancer cachexia in mouse tumor model [47] as well as in cultured human leukemia Jurkat cells in mice [48]. Recently, a significant association between IL-6 and cachexia in patients with prostate cancer as well as in JCA-1 tumor bearing mice has been established [49]. Furthermore, these authors demonstrated that by inhibiting NF-κB activation in the JCA-1 tumor bearing mice, prostate cancer induced development of

cachexia was significantly attenuated through the down regulation of IL-6 production. Similarly, a recent study by Nai et al. (2007) on cachexia in colon 26 tumor-bearing mice (Cachexia was induced by subcutaneous grafting of colon carcinoma C26 cells in the dorsal region of 7-week-old mice) had suggested that NF-κB inhibitor attenuated the development of cachexia through the inhibition of IL-6 expression, and by inhibition of activation of the NF-κB transcription factor in tumor tissues [50]. An earlier study showed that interleukin-6 (IL-6) is involved in muscle wasting through the lysosomal (cathepsin) and non-lysosomal (proteasome) mediated pathways in tumor bearing animals [51]. However, recently a report on mouse bearing colon 26 adenocarcinoma in experimental cancer cachexia, demonstrated enhanced concentration of TNF-alpha and IL-6 in spleen of tumor-bearing animals in comparison to control, which is mediated by the activation of NF-κB transcription factors. Furthermore authors suggest that low dose of indomethacin alleviates the cachexia and prevents body weight loss in muscle atrophy by decreasing the activation of NF-κB transcription factor as well as lowering the serum levels of TNF-alpha and IL-6 [52]. Some studies have demonstrated that TNF-alpha has catabolic effect on energy storage body such as skeletal muscle and adipose tissue [53]. TNF-alpha produces reactive oxygen species that cause increased expression and activation of the NF-kB transcription factor, which in turn results into skeletal muscle atrophy by decreased protein synthesis, and increased protein degradation through ubiquitin–proteasome mediated pathway [54-55].

Earlier published report by Ladner et al. (2003) indicates that TNF-alpha induces NF-κB activation in a biphasic manner. First, transient phase is terminated within 1 h of cytokine addition. Second phase persists for an additional 24-36 h. The transient phase is mediated by the degradation and consequent re-synthesis of IkB alpha. Second phase activity correlated with down-regulation of both IkBα and IkBβ proteins. Authors further demonstrated that cytokines involved in activation of NF-κB transcription factor and protein degradation are mediated by IKK/26 S proteasome pathway dependent mechanism in skeletal muscles associated with cachexia [56]. Another in-vitro study confirmed that TNF-induced activation of NF-κB is responsible for inhibition of skeletal muscle differentiation by the decreased expression of MyoD mRNA. However in mouse muscle, both TNF alpha and IFNγ signaling were required for the NF-κB-dependent down-regulation of MyoD and dysfunction of skeletal myofibers [17]. Tumor necrosis factor alpha and gamma interferon also induce muscle degeneration by activating the transcription factor NF-κB by the nitric oxide (NO) production which stimulates MyoD mRNA loss. Furthermore, cytokine treatment of iNOS(-/-) mice, activated NF-κB and did not trigger MyoD mRNA degeneration, demonstrating that NF-κB-mediated muscle wasting requires an active iNOS-NO pathway [19]. This suggests that iNOS may play an important role in cytokine-induced cachexia, and may act as potential therapeutic target for cancer cachexia. A recent study by Bera and Ray (2009) demonstrated that TNF-alpha involved in degradation of MyoD and myogenin mRNA, which may lead to severe muscle wasting and cachexia in carcinogen-induced sarcoma of mouse muscle. These authors further suggested that the activation of TNF-alpha mediated nitric oxide production pathway is involved in degradation of MyoD and myogenin mRNA by activation of NFkappaB transcription factor [57]. Matrix metalloproteinases (MMPs) are a family of proteinases that can degrade proteins involved

in intracellular and extracellular remodeling. Recent in-vivo and in-vitro study by us [22] and another group [58] has suggested that TWEAK (Tumor necrosis factor-like weak inducer of apoptosis) and TNF-α play a major role in skeletal muscle loss by production of metalloproteinase-9 (MMP-9). We further demonstrated that when dominant-negative inhibitor of NF-κB (IκBαΔN) was transected in muscle cells, expression of MMP9 was completely blocked in myotubes; thus confirming that NF-κB plays a major role in the production of MMP-9 in skeletal muscle cells.

A recent study on Lewis lung carcinoma (LLC) model of induced cancer cachexia showed that TRAF6 (TNF receptor associated factor) plays a major role in cancer cachexia along with simultaneous loss of muscle mass [59]. Authors further showed that when TRAF6 mko and TRAF6 f/f were injected in LLC cell in left flank of mice, significant reduction in fiber cross-sectional area was observed in skeletal muscle of TRAF6 f/f mice. However, in LLC- bearing TRAF6mko mice, fiber cross-sectional area was completely preserved. LLC-bearing TRAF6mko mice showed significant inhibition of activity of NF-κB in skeletal muscle as compared to TRAF6f/f mice. Furthermore, authors confirmed that LLC injected TRAF6mko mice had complete inhibition of expression of MuRF1, LC3B, and Beclin1 in comparison to TRAF6f/f mice and suggested that inhibitor of TRAF6 may play major role in prevention of tumor-induced activation of ubiquitin–proteasome systems in skeletal muscle cells [59].

Rhoads et al. (2010) investigated the expression of nuclear levels of p50, p65, Bcl-3, phospho-p65 (Ser536), and IκBα in human skeletal muscle of 14 gastric cancer patients (similar to animal models of cancer cachexia) and 10 healthy controls [9]. The study showed that there was no change in nuclear levels of p50, Bcl-3, and p65 in controls and patients; however, a significant (25%) increased expression in phospho-p65, and 25% decreased expression of IκBα were observed in skeletal muscle of patients as compared to controls. Moreover, no correlation between the stage of cancer and extent of decrease in IkB alpha was observed, suggesting that activation of NF-kB is an early and sustained event in humans with gastric cancer [9].

NF-kB mediated therapeutic targets to inhibit muscle atrophy in cancer cachexia

Cachexia therapy for last one decade is mainly based on skeletal muscle target molecules which are able to stimulate the anabolic pathways and have ability to reduce catabolic processes. Recent evidence demonstrates the relationship between the depression of protein synthesis in skeletal muscle by different mediators, and the enhanced degradation of the myofibrillar protein myosin through activation of NF-kB, resulting in an increased expression and activity of the ubiquitin-proteasome proteolytic pathway in cancer cachexia [3,4,6,10]. These studies imply that the molecules which interfere with activation of NF-kB may be effective in the treatment of skeletal muscle atrophy in cancer cachexia. Hence, we summarize the effect of important molecules which possibly play significant role in inhibition of NF-κB transcription factor and can be useful in treatment of cancer cachexia.

Eicosapentaenoic acid (EPA): EPA is a polyunsaturated fatty acid with antitumor and anticachectic effects. Recent studies have shown that in cachexia, EPA may attenuate protein degradation, by preventing NF-kB accumulation in the nucleus [24, 26]. A study

using murine MAC16 cachexia model has shown that it preserves muscle mass by down regulation of increased expression of the ubiquitin–proteasome pathway [60], and prevented PIF-induced nuclear migration of NF-kB by stabilizing the IkB/NF-kB complex through inhibition of upstream signaling pathways [40]. Similar opinions have been advanced to substantiate that EPA may block the action of PIF-induced nuclear migration of NF-kB and attenuates the development of cachexia in pancreatic cancer patients [61-62].

Resveratrol: It is a natural phytoalexin commonly found in red wine. It is an inhibitor of NF-kB activation and acts through inhibition of IKK dependent NF-kB activation [63]. It has ability to inhibit PIF-induced proteasome expression and acts as anticachectic agent in mice bearing the MAC16 tumour, which induces profound cachexia involving wasting of skeletal muscle [64]. Wyke et al (2004) demonstrated that resveratrol (1 mg/ kg body weight) significantly attenuated the PIF-induced expression of the ubiquitin-proteasome proteolytic pathway, and decreased muscle protein degradation and loss of body weight by the lowering of NF-kappaB DNA-binding activity in mice bearing the MAC16 tumor [65]. A previous study has shown that resveratrol inhibits loss of skeletal muscle mass, induced by C26 adenocarcinoma tumors through its inhibition of NF-κB (p65) activity in skeletal muscle and suggest that use of oral resveratrol therapy may protect from cancer-induced atrophy through the inhibition of NF-κB and increases the gain of skeletal muscle mass [66].

Epigallocatechin-3-gallate (EGCG): EGCG is a polyphenolic component in green tea. It acts as a potent preventive agent against cancer induced cachexia. EGCG counteracts cachexia-provoked muscle wasting by regulating the expressions of NF-κB as well as downstream mediators, MuRF1 and MAFbx. Wang et al. (2011) have suggested that EGCG may be a potential molecule in therapeutics of the tumor-induced muscle atrophy [67].

Beta-hydroxy-beta-methylbutyrate (HMB): HMB is an anticatabolic agent. Earlier reports illustrated that HMB attenuates PIF-induced protein degradation in muscle cells, by decreased expression of the ubiquitin-proteasome pathway enzymes [25, 68]. Furthermore, HMB is involved in prevention of NF-kB activation through decreased expression of PKC, resulting in IkB/NF-kB complex stabilization in murine model of cachexia [69]. Recently it has been found that beta-hydroxy-beta-methylbutyrate supplementation in tumor-bearing rats induced a lower tumor weight and tumor cell proliferation; and more than 100% reduction in tumor cell proliferation was accompanied by the increased expression of IkB-alpha [70]. Further, this study observed 17% suppression of NF-kappaB p65 subunit content by the HMB supplementation. This suggests that HMB supplementation may increase skeletal muscle mass in tumor-bearing rats by inhibiting the NF-kappaB activation. A study in placebo-controlled clinical trial of HMB in patients with cancer cachexia showed that using L-glutamine, L-arginine together with HMB in advanced cancer patients, results in increased body weight and an increase in lean body mass; however, no changes were observed in fat mass [71].

Pyrrolidine dithiocarbamate (PDTC): PDTC is an inhibitor of NFkappaB. A recent study on cachexia in colon 26 tumor-bearing mice suggested that it attenuates the development of cachexia in colon 26 tumor-bearing mice through the inhibition of IL-6 expression [72]. Furthermore, this compound also inhibited the wasting of gastrocnemius muscle, carcass weight and epididymal fat mass of tumor tissues by the diminishing of the NF-kB activation.

Dehydroxymethylepoxyquinomicin

(DHMEQ): DHMEQ shows anti–NF-kB activity in cultured human leukemia Jurkat cells [48]. A previous study by Kuroda et al. (2005) demonstrated a significant association between IL-6 and cachexia in patients with prostate cancer as well as in JCA-1 tumor bearing mice [49]. Furthermore, same group had established that NF-kB inhibitor DHMEQ has ability to prevent cachexia induced by prostate cancer through the inhibition of IL-6 production in a JCA-1 tumor bearing mice.

Indomethacin (IND): Zhou et al. (2003) investigated the role of IND by categorizing their experiment into five groups: (a) control, (b) tumor-bearing plus saline, (c) tumor-bearing plus IND (0.25 mg x kg(-1)), (d) tumor-bearing plus IND (0.5 mg x kg(-1)), and (e) tumor-bearing plus IND (2 mg x kg(-1). Then colon 26 adenocarcinoma cells of murine were inoculated subcutaneously to induce cachexia. Saline and IND were given intraperitoneally from the onset of cachexia upto seven days and thereafter serum levels of TNF-alpha, IL-6, and activity of NF-kappaB in the spleen of male BABL/c mice were observed [52]. The study demonstrated enhanced concentration of TNF-alpha and IL-6 in spleen of tumor-bearing animals as compared to control, which is mediated by the activation of NF-kB transcription factors. Furthermore, authors suggested that low dose of indomethacin (0.5 mg ŀkg-1) alleviated the cachexia, and prevented body weight loss in muscle atrophy by decreased activation of NF-kB and lowering the levels of TNF-alpha plus IL-6 expression [52].

Ghrelin: Earlier animal studies in mice, inoculated with human melanoma cells of cancer-cachexia demonstrated that Ghrelin treatment might attenuate cachexia by stimulation of food intake and gain in body weight loss [72-74]. Another study confirmed that tumor implanted rats treated with either Ghrelin or the Ghrelin analog BIM-28131 demonstrated improvement in food intake and gain of body weight [75]. Recent literature is suggestive of increased Ghrelin secretion from tumor tissues might be counteracting skeletal muscle mass wasting by stimulation of food intake and activation of anabolic pathways by the inhibition of NF-kB activation [76].

Curcumin: Curcumin is a natural product from turmeric which is believed to prevent activation of NF-kB by blocking phosphorylation and subsequent degradation of IkB-alpha. It has been shown to increase the rate and extent of muscle regeneration after trauma [77]. Wyke et al.(2004) study in murine myotube of mice bearing the MAC16 tumor suggested that PIF-induced expression of ubiquitin-proteasome components mediated protein degradation were completely attenuated by low dose of curcumin (50 microM); however, higher dose of curcumin (150 and 300 mg kg(-1) had no effect [65].

Thalidomide: It is reported to reduce the levels of TNFα, by enhancing the rate of degradation of TNF-alpha mRNA. This also reduces skeletal muscle wasting in cancer patients by blocking of the NF-kB-regulated genes through suppression of IKK activity [78]. Previous study in patients of esophageal cancer have shown that when patients received an isocaloric diet only, for 2 weeks, had lost both body weight and lean body mass; however, reverse was observed in the patients who received thalidomide [79]. Another study in patients with advanced pancreatic cancer confirmed that patients with placebo group had lost approximately 10% of their body weight while those who received thalidomide had stabilized arm muscle mass and did not lose their body weight [80]. Recent published data also suggests that thalidomide may attenuate the signaling cascade of ANG II, PIF, or TNF-alpha, and down regulate the increased expression of the ubiquitin-proteasome dependent pathway by inhibition of NF-kB activation [6].

Double-stranded oligodeoxynucleotides:

Double-stranded oligodeoxynucleotides (ODN) transfection strategy is based on the principle that 'decoy' cis-elements are responsible for blocking of binding of nuclear transcription factors to promoter regions of targeted genes, and influencing the inhibition of gene transactivation. Previous study in tumors of adenocarcinoma colon26 mice had shown that targeting of NF-kappa B (NF kappa B) binding cis-elements with the transfection of synthetic double-stranded oligodeoxynucleotides decoy resulted in attenuation of the reduction in body weight, epididymal fat, gastrocnemius muscle mass that was induced by the presence of tumor [45]. Moreover, authors suggested that adenocarcinoma colon26 induced cachexia could be inhibited by the blocking of NF kappa B activation by the application of molecular decoy strategy. Recent studies demonstrated that inhibitor of double stranded RNA-dependent protein kinase (PKR) play major role in attenuation of NF-kB activation as well as in expression of the 20S proteasome in weight loosing mice bearing the MAC16 tumor [43,44,81]. Furthermore these authors suggested that inhibitor of PKR may be useful therapeutic target against the tumor showing higher expression of PKR and increased NF-kB activation.

NBD peptide: NBD peptide is a competitive inhibitor of the IKK complex. It is reported to have therapeutic potential to ameliorate tumor-induced muscle wasting. Recently, a study by Wysong et al. (2011) have shown that when tumor bearing mice were treated with NBD peptide (80, 200, 500 microgram), a dose dependent increase in lean body mass, and fat mass was recorded as compared to non treated tumor bearing mice [81]. Furthermore it was demonstrated that tumor bearing mice had significant hind limb atrophy as compared to non-tumor bearing mice. However, in NBD peptide (200, 500 microgram) treated mice, complete reversal of hind limb wasting and reduction of size of tumor volume were observed as compared to untreated mice. Moreover, they further demonstrated that NBD untreated mice had increased NF-kB activity (increased phospho-p65 but no change in p65) in cachextic gastrocnemius muscle, which was reversed with NF-kB inhibition by treatment with NBD peptide. Same group also confirmed that NBD treated tumor-challenged mice showed decreased expression of MuRF1, and MAFbx/ Atrogin-1 genes as compared to non tumor bearing controls mice, suggesting that inhibition of NF-κB by the NBD peptide is involved in inhibition of skeletal muscle atrophy through the decreased expression of enzymes involved in ubiquitin pathways [82].

Conclusion and Future Prospective

Cancer cachexia is a type of cancer metabolic syndrome characterized by irreversible erosion of body cell mass, progressive weakness and muscle atrophy in response to malignant growth. The mechanism of development of cachexia has remained illusive so far but two widely accepted theories include the deregulation of leptin feedback loop control and the proteolysis-inducing and lipid mobilising factors (PIF and LMF) [1,4,6]. Whereas the pathologic deregulation of leptin loop control is implicated in altering the neuropeptidergic control cycles leading to decreased energy intake but high metabolic demand for nutrients, the PIF and LMF which are present in the urine of cachectic patients are closely related to weight loss in cachexia. LMF produces a significant increase in mitochondrial uncoupling proteins (UCPs) 1, 2, and 3 which are involved in the control of energy metabolism through thermogenesis in brown adipose tissue as well as in skeletal muscles and liver[5]. Another view elucidating the mechanism of cancer cachexia is based upon induction by proinflammatory cytokines.

Chronic systemic inflammatory response and the acute phase proteins are elevated as evident from high serum levels of IL-1, IL-6 and TNF in cancer patients [4, 6, 7, 10]. These cytokines also stimulate expression of leptin and influence satiety resulting in long-term inhibition of food intake.

With recent accumulating evidence, mediator based myriad of signaling pathways such as ubiquitin-proteasome and NF-kB signaling pathways are suggested to be the part of the mechanisms by which cancer induces adipose tissue and skeletal muscle loss. Upon interaction with their unique receptors on skeletal muscle, these mediators activate NF-kappaB transcription factor which is essential for atrophy related degradation of muscle proteins to occur. The mechanisms by which NF-kB promotes muscle wasting in cancer cachexia remain poorly understood but this could be possibly due to its ability to inhibit myogenesis [4, 27,38,43] or to induce protein turnover through the ubiquitin proteasome pathway [3, 6,7, 11, 25, 44, 65]. Previous studies related to discovery that NF-kB activation is sufficient to cause skeletal muscle atrophy and that blockade of the NF-kB pathway can ameliorate skeletal muscle atrophy are suggestive of a new set of drug targets for clinical intervention in cancer cachexia. Recently various therapeutic NF-kB blockers such as Resveratrol, Epigallocatechin-3-gallate, Beta-hydroxy-beta-methylbutyrate, Dehydroepiandrosterone, Eicosapentaenoic acid-enriched oral supplements, Pyrrolidine dithiocarbamate, Dehydroxymethylepoxyquinomicin, Indomethacin, NBD peptide, indomethacin, Ghrelin, Thalidomide have been identified which are effective in treatment of cancer cachexia [6, 24,49, 50, 66, 67,70,76,81, 82]. Although the mechanisms of action of these drugs in prevention of cancer cachexia is not well understood; but many of these drugs have been shown to attenuate cancer cachexia by inhibition of NF-κB activation in various cell types. This is particularly significant, as of now there are no drugs approved for the treatment of skeletal muscle atrophy in cancer cachexia that could demonstrate significant improvement in treatment outcome, functional status and quality of life. Therefore, future studies should be directed towards better defining the mechanism involved to inhibit cancer cachexia by NF-kB blockade and determining the potential of these therapeutic agents through potential clinical trials in animal models and in human cancer cachexia patients.

Authors' contributions

Dr. Daya Shankar Lal Srivastava participated in designing, drafting and finalization of the manuscript. Dr D.B. Dhaulakhandi has helped in reviewing and finalizing the article. Both the authors have read and approve the final manuscript.

Abbreviations

NF-κB: Nuclear factor-kappa-B;
IκB: inhibitory kappa-B;
NIK: NF-kB inducing kinase;
IKK: IκB kinase; PIF: proteolysis-inducing factor;
MAC16 tumor: Cachectic mice bearing murine adenocarcinoma cells;
MMPs: Matrix metalloproteinases;
TWEAK:Tumor necrosis factor-like weak inducer of apoptosis;
LLC: Lewis lung carcinoma;
TRAF: TNF receptor associated factor;
EGCG: Epigallocatechin-3-gallate;
HMB: Beta-hydroxy-beta-methylbutyrate;
PDTC: Pyrrolidine dithiocarbamate;
IND: Indomethacin;

ODN: Oligodeoxynucleotides;
PKR: Double stranded RNA-dependent protein kinase;
LMF: lipid mobilising factors;
UCPs: Uncoupling proteins;
DHMEQ: Dehydroxymethylepoxyquinomicin;
EPA: Eicosapentaenoic acid;
MIKK: activated IkB kinase beta

Acknowledgements

Authors wish to thank Dr Neetu Tyagi (Assistant Professor, Department of Physiology and Biophysics, University of Louisville, Louisville, Kentucky-USA) for giving the valuable suggestion. The authors are highly thankful to Pt. B. D. Sharma University of Health Science, Rohtak for scientific encouragement & required support.

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