

Review

Mechanisms of skeletal muscle degradation and its therapy in cancer cachexia

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Summary. Severe or chronic disease can lead to cachexia which involves weight loss and muscle wasting. Cancer cachexia contributes significantly to disease morbidity and mortality. Multiple studies have shown that the metabolic changes that occur with cancer cachexia are unique compared to that of starvation. Specifically, cancer patients seem to lose a larger proportion of skeletal muscle mass. There are three pathways that contribute to muscle protein degradation: the lysosomal system, cytosolic proteases and the ubiquitin (Ub)-proteasome pathway. The Ub-proteasome pathway seems to account for the majority of skeletal muscle degradation in cancer cachexia and is stimulated by several cytokines including tumor necrosis factor- α , interleukin-1 β , interleukin-6, interferon- γ and proteolysis-inducing factor.

Cachexia is particularly severe in pancreatic cancer and contributes significantly to the quality of life and mortality of these patients. Several factors contribute to weight loss in these patients, including alimentary obstruction, pain, depression, side effects of therapy and a high catabolic state. Although no single agent has proven to halt cachexia in these patients there has been some progress in the areas of nutrition with supplementation and pharmacological agents such as megestrol acetate, steroids and experimental trials targeting cytokines that stimulate the Ub-proteasome pathway.

Key words: Cancer cachexia, Skeletal muscle degradation

Introduction

Cachexia can be described as weight loss, muscle wasting, loss of appetite and general debility occurring with a chronic disease. This condition can be seen in patients with acquired immune deficiency syndrome, sepsis, renal failure, burns, trauma and cancer. Cachexia is present in up to 50% of cancer patients and accounts for at least 30% of cancer-related deaths overall (Palesty and Dudrick, 2003). The wasting of respiratory muscles eventually causes these patients to succumb to pneumonia (Windsor and Hill, 1988).

The body composition changes that occur with cancer cachexia are unique compared to those for starvation. For equivalent amounts of weight loss, there is a greater degree of muscle mass lost in cancer cachexia (Heymsfield and McManus, 1985). In patients with anorexia, the majority of weight lost is from fat, whereas lung cancer patients who had lost 30% of their baseline weight, demonstrated an 85% decrease in total body fat and a 75% decrease in skeletal muscle protein mass (Fearon, 1992; Moley et al., 1987). This demonstrates that both fat stores and muscle stores are significantly reduced in cancer cachexia. There is also a preferential loss of skeletal muscle versus visceral organ muscle in response to acidosis, infection or cancer (Mitch and Goldberg, 1996). Baracos et al. demonstrated that rats implanted with Yoshida ascites hepatoma (YAH), showed a rapid and selective loss of skeletal muscle protein due mainly to a marked increase (63-95%) in the rate of protein degradation (Baracos et al., 1995). However, in this study there was no change in weight or mRNA content of liver, kidney, heart or brain.

Skeletal muscle protein catabolism

Muscle protein degradation occurs through three pathways: the lysosomal system, a group of calcium activated cytosolic proteases, and the ubiquitin (Ub)-

proteasome pathway (Lecker et al., 1999). The lysosomal system accounts for the degradation of endocytosed proteins and phagocytosed bacteria. Lysosomes contain several acid optimal proteases such as cathepsins B, H, and D. Lysosomal degradation of proteins is accelerated by glucagon in the liver and the lack of insulin or essential amino acids (Gronostajski et al., 1984). The use of lysosomal protease and acidification inhibitors demonstrated that the lysosomal pathway is mostly to degrade surface membrane proteins and endocytosed, extracellular proteins rather than influencing the normal turnover of cytosolic proteins (Furano and Goldberg, 1986; Lowell et al., 1986). The second pathway for protein degradation is via calpains which are calcium activated cytosolic cysteine proteases. These proteases are ATP-independent and are activated by an increase in cytosolic calcium, indicating that they are important in tissue injury, necrosis and autolysis (Murachi et al., 1980; Waxman, 1981; Mellgren, 1987; Gikk et al., 1992). The ATP-ubiquitin dependent proteolytic pathway which is responsible for the majority of skeletal muscle protein catabolism (Lecker et al., 1999). This pathway likely accounts for the advanced proteolysis seen in wasting conditions such as fasting, sepsis, metabolic acidosis, acute diabetes, weightlessness and cancer cachexia (Goll et al., 1992).

The Ub-Proteasome Pathway

Most cellular proteins are degraded by the ATP-dependent Ub-proteasome pathway. This entails proteins being identified for degradation by the addition of multiple ubiquitin molecules and subsequent recognition and degradation by the 26S proteasome. Proteins are initially marked for degradation by binding ubiquitin, a small protein cofactor (Mitch and Goldberg, 1996). Ubiquitin is activated by an activating enzyme (E1) in a two step process. Firstly, an intermediate is formed by ATP hydrolysis connecting adenosine monophosphate (AMP) with the carboxy-terminal carboxyl group of glycine in ubiquitin. This then forms a thioester linkage with a cysteine residue in E1 (Tisdale, 2005). The ubiquitin carrier protein (E2) then accepts this ubiquitin to its active site at a cysteine residue. Next, the E2 carrier protein recognizes the Ub protein ligase (E3). The E3 ligase transfers ubiquitin from the E2 thioester intermediate either to a specific ubiquitin binding site or to an isopeptide linkage with some degree of substrate specificity (Lecker et al., 1999). Multiple rounds of E3 ubiquitin ligation create a polyubiquitin chain on the substrate.

Once the proteins are marked with a polyubiquitin chain, they are degraded into oligopeptides by the 26S proteasome. This molecule is comprised of a 20S proteasome in the center with a 19S particle on each end. The 19S particles unfold proteins to be denatured by the 20S proteasome via at least six different ATPases. The 20S proteasome appears as a stack of four rings with two outer α rings and two inner β rings. This protein has

three specific proteolytic actions: "chymotrypsin-like," "trypsin-like," and cleavage after acidic residues making it "caspase-like" (Tisdale, 2005). Once proteins are processed, short oligopeptides comprised of six to nine amino acid residues are released and further degraded into tripeptides by tripeptidylpeptidase II and then into single amino acids by aminopeptidases. It is important to understand the components of the ubiquitin-proteasome pathway as they are key targets in regulating the skeletal muscle degradation seen in cancer cachexia.

The ubiquitin-proteasome pathway in catabolic states

The function of the ubiquitin-proteasome pathway is to degrade defective protein products produced from errors in translation or from oxidative stress (Schubert et al., 2000; Tisdale, 2005). This pathway is activated in catabolic states resulting in muscle atrophy. Studies of *in vitro* atrophying muscles have demonstrated that inhibition of lysosomal proteases or calcium-activated proteases does not change the rate of proteolysis. However, with inhibitors of ATP production, the rate of proteolysis decreases to that of control muscles, indicating that the ATP-dependent Ub-proteasome pathway is primarily responsible for skeletal muscle degradation (Wing and Goldberg, 1993; Mitch et al., 1994). Muscle protein degradation in Yoshida Ascites Hepatoma (YAH) bearing rats was not inhibited by the removal of calcium or by blocking the calcium-dependent proteolytic system. The inhibition of lysosomal function reduced proteolysis by 12% in muscles from YAH tumor-bearing rats. However, when ATP production was inhibited, the remaining accelerated proteolysis in muscles of tumor-bearing rats fell to that of control levels. This study also revealed that while muscles of YAH-bearing rats showed a total decrease in total RNA content (by 20-30%), there was a significant increase in ubiquitin mRNA (590-880%), the level of ubiquitin-conjugated proteins, and of mRNA for multiple proteasome subunits (100-215%) (Baracos et al., 1995). These studies support the concept that accelerated muscle proteolysis is primarily due to the activation of the ATP-dependent pathway. In addition, at least three specific E3 ubiquitin ligases have been identified. The E3 α II ligase has been shown to be more specifically expressed in muscle tissues and is also differentially activated by the cytokines tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) (Beutler and Cerami, 1988; Matthys and Billiau, 1997; Moldawer and Copeland, 1997; Tisdale, 2002; Kwak et al., 2004).

Stimulators of the ubiquitin proteasome pathway in catabolic states

Multiple cytokines including TNF- α , interleukin 1 β (IL-1 β), IL-6, interferon γ (IFN- γ) and proteolysis inducing factor (PIF) have been shown to stimulate protein degradation in models of cancer cachexia

(Fig. 1).

Tumor Necrosis Factor- α (TNF- α)

TNF- α is a cytokine produced primarily by macrophages in response to invasive stimuli and has effects on growth, differentiation and immune system functions (Evans et al., 1989). TNF- α has long been thought to play a significant role in disease resulting in cachexia. Recombinant human TNF- α (rTNF α) was given intravenously to patients as part of an anti-neoplastic trial resulted in dose-related metabolic effects of enhanced energy expenditure with elevated CO₂ production, increased protein catabolism, peripheral efflux of amino acids, decreased total arterial amino acid levels, and an increase in plasma cortisol (Starnes et al., 1988). TNF α treatment also resulted in elevated serum triglycerides, as well as increased glycerol and free fatty acid turnover, suggesting that TNF α increased lipolysis and fat utilization. The above metabolic derangements are similar to the findings in patients with end stage cancer cachexia.

In an attempt to mimic the apparent increase in TNF- α production in cancer patients, multiple *in vivo* models have been studied. Oliff et al. transfected CHO cells with a vector containing TNF- α /cachectin gene (Oliff et al., 1987). Nude mice injected intraperitoneally with CHO/TNF-20 cells died more quickly than controls and 87% of the animals injected intramuscularly developed severe cachexia and weight loss (Oliff et al., 1987). In another study, mice with methylcholanthrene-induced sarcoma or Lewis lung adenocarcinoma were given a rabbit immunoglobulin against murine cachectin/TNF- α (Sherry et al., 1989). TNF- α passive immunization reduced carcass protein and fat loss in mice with sarcoma and diminished carcass lipid depletion in mice with lung cancer (Sherry et al., 1989). Despite these findings, cachexia was not a completely reversed, suggesting that other factors contribute to the weight loss in these animal models of cancer cachexia. A similar experiment was carried out in YAH tumor-bearing rats that exhibit enhanced protein degradation in gastrocnemius muscle, heart and liver. This hypercatabolic pattern is associated with the presence of TNF- α in the circulation. The daily administration of a

goat anti-murine TNF- α immunoglobulin (IgG) to these rats decreased the rate of protein degradation in skeletal muscle, heart, and liver compared with tumor-bearing rats receiving a non-immune goat IgG. However, this treatment did not prevent the reduction in body weight (Costelli et al., 1993).

Multiple studies have been designed to investigate the direct effects of TNF- α on skeletal muscle. TNF- α injection in low doses in animals increases the metabolic rate secondary to an increase in blood flow and thermogenic activity which correlates with an increase in an uncoupling protein (UCP1) in brown adipose tissue. Uncoupling proteins function as mitochondrial protein carriers that stimulate heat production by dissipating the proton gradient generated during respiration across the inner mitochondrial membrane and thus uncouple respiration from ATP synthesis. The mRNA of two other uncoupling proteins UCP2 (expressed ubiquitously) and UCP3 (expressed in human skeletal muscle and rodent brown adipose tissue) are elevated in skeletal muscle during tumor growth. Furthermore, TNF- α induces UCP2 and UCP3 gene expression (Argiles et al., 2003). Acute intravenous administration of recombinant TNF- α also resulted in a time-dependent increase in the levels of ubiquitin mRNA in rat skeletal muscle (Garcia-Martinez et al., 1994). In a similar study, intravenous administration of recombinant TNF- α doubled the expression of both the 2.4 and 1.2 kb transcripts of the ubiquitin genes (Llovera et al., 1997, 1998). Acute treatment of rats with recombinant TNF- α enhanced proteolysis and decreased protein synthesis in soleus muscle (Garcia-Martinez et al., 1993). Human recombinant TNF- α treatment of isolated rat soleus muscles resulted in more than a 50% increase in ubiquitin gene expression (Llovera et al., 1997). Mouse-derived C2C12 muscle cells and primary cultures from rat skeletal muscle that were treated with TNF- α demonstrated time- and concentration-dependent reductions in total protein content and loss of adult myosin heavy chain (MHCf) content that was not associated with a decrease in MHCf synthesis (Li et al., 1998). This study also demonstrated that TNF- α induced binding of nuclear factor κ B (NF- κ B) to its DNA target sequence and stimulated degradation of the NF- κ B inhibitory protein, I- κ B α . Finally, TNF- α stimulated

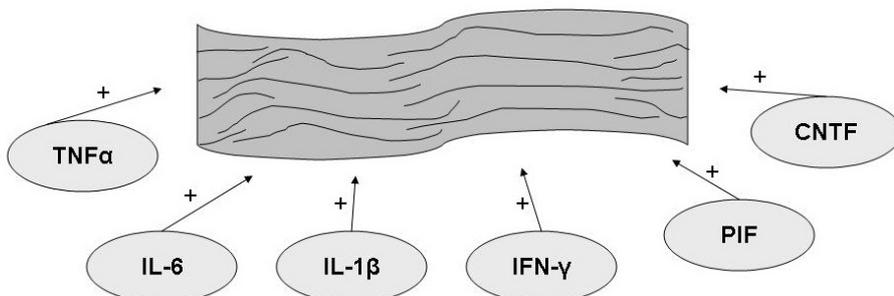


Fig. 1. Activators of the ubiquitin-proteasome pathway in skeletal muscle. TNF- α : tumor necrosis factor- α ; IL-6: interleukin-6; IL-1 β : interleukin-1 β ; IFN- γ : interferon- γ ; PIF: proteolysis-inducing factor; CNT: ciliary neurotrophic factor.

ubiquitin conjugation, while a 26S proteasome inhibitor blocked TNF- α activation of NF- κ B. This data supports the concept that TNF- α directly induces skeletal muscle protein loss, and that NF- κ B is activated by TNF- α in differentiated skeletal muscle cells. However, these findings indicate that TNF- α plays a significant role in increasing muscle catabolism in multiple models of cancer cachexia, but it is not the sole mediator of this process.

Interleukin-6 (IL-6)

IL-6 is a pleotropic cytokine with varied systemic functions including a major role in the inflammatory process. Its role in cancer cachexia has mostly been demonstrated in *in vivo* models. Studies with a drug (suramin) that interferes with secretion of IL-6 and binding of IL-6 to its cell surface receptors, partially reduced the catabolism seen in colon-26 (C26) adenocarcinoma-bearing mice (Strassman et al., 1993a). However, suramin has also been shown have effects on various growth factors in cell culture studies and this may play a role in the reduction of tumor associated cachexia. In a similar model, an anti-IL-6 receptor antibody decreased muscle atrophy in C-26 bearing mice (Fujita et al., 1996). Seventeen days after tumor inoculation, the gastrocnemius muscle weight of C-26 bearing mice significantly decreased to 69% of control and this was associated with increased mRNA levels of cathepsins B and L, poly-ubiquitin (Ub) and proteasome subunits in the muscles. The enzymic activity of cathepsins B+L in the muscles also increased compared with control. Administration of anti-murine IL-6 receptor antibody to C-26 bearing mice reduced, but did not completely prevent the weight loss in the gastrocnemius muscle (Fujita et al., 1996). In yet another experiment using the C-26 inoculated mice, a novel IL-6 inhibitor, 20S,21-epoxy-resibufogenin-3 acetate (ERBA) markedly inhibited body weight loss (Enomoto et al., 2004).

Despite the work in these animal models, there is still not adequate conclusive data to attribute cancer cachexia and skeletal muscle degradation to IL-6 alone. Studies have shown that while acute administration of IL-6 to rats induces both total and myofibrillar degradation in muscle, mice receiving murine IL-6 over a 7-day period showed no decrease in body weight or food intake (Goodman, 1994). However, these animals did demonstrate a hepatic acute phase response to IL-6. In another experiment, a human-mouse chimeric IL-6 monoclonal antibody (CNTO 328) that inhibits IL-6 function was administered to nude mice with cachexia induced by either human melanoma or prostate cancer. In both models, the cachexia was reversed, and the mice carrying the human prostate tumors actually gained weight after treatment with CNTO 328 (Zaki et al., 2004). Finally, IL-6 has been shown to up-regulate the ubiquitin ligase E3 α -II. As noted above, E3 ubiquitin ligases control polyubiquitination which is a rate-

limiting step in the ubiquitin-proteasome system. E3 α -II is highly enriched in skeletal muscle and is markedly up-regulated by IL-6, indicating that the cytokine plays a significant role in the muscle protein catabolism that occurs with cancer cachexia (Kwak et al., 2004). In a study to evaluate the role of host cytokines on tumor growth and cachexia, methylcholanthrene tumors were injected subcutaneously into both wild-type and mice with gene knockouts of either IL-6, IL-12, IFN, TNFR1 or TNFR2. The only gene knockout that attenuated both tumor growth and cachexia was IL-6 knockouts, indicating it plays a significant role in this model of tumor induced cachexia (Cahlin et al., 2000).

Interleukin-1 β (IL-1 β)

The data for the role of IL-1 β in cancer cachexia is controversial. Like TNF- α , chronic treatment of rats with recombinant IL-1 β resulted in a body protein redistribution and a significant decrease in muscle protein content associated with a coordinated decrease in muscle mRNA levels of myofibrillar proteins (Fong et al., 1989). Intratumoral injection of soluble IL-1 β receptors caused a significant decrease in cachexia in C-26 bearing mice, but did not prevent tissue depletion or protein hypercatabolism in rats with the Yoshida ascites hepatoma (Strassman et al., 1993b; Costelli et al., 1995). In a methylcholanthrene sarcoma model in Fischer 344 rats, the expression of anorexigenic cytokines, IL-1 β , TNF- α , and IFN- γ messenger RNA were examined in the tumor tissue, liver and brain. This model revealed that in the brain tissue, anorexia is associated with the up-regulation of IL-1 β and its receptor mRNA, suggesting that it may play a significant role in cancer anorexia (Turrin et al., 2004). Similar to TNF- α , intravenous administration of IL-1 β in rats caused an increase in the expression of the 2.4 and 1.2kb transcripts of ubiquitin genes in skeletal muscle (Llovera et al., 1998). IL-1 β obtained from human monocytes was able to stimulate muscle protein degradation and was inhibited by lysosomal thiol proteases however, this effect was not reproducible with recombinant human IL-1 β (Baracos et al., 1983; Goldberg et al., 1988). Intravenous injection of IL-1 β or TNF- α had no effect on muscle protein metabolism in rats with Yoshida sarcoma (Ling et al., 1991). Although IL-1 β may play some synergistic role with the other cytokines to create an environment for the muscle breakdown seen with cancer cachexia, the data available at present assign it a less prominent role in this phenomena.

Interferon- γ (IFN- γ)

Interferon (IFN- γ) is produced by activated T and natural killer cells and has many similar activities to TNF- α . Monoclonal anti-IFN- γ antibodies markedly decrease the cachexia seen in mice bearing Lewis lung tumors (Matthys, 1991). In another experiment nude mice inoculated with CHO-IFN- γ cells exhibited severe

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cachexia. In contrast, cachexia did not occur in mice given monoclonal Ab against IFN- γ prior to injection of tumor cells (Matthys et al., 1991). IFN- γ up-regulated the 2.4 and 1.3 kb transcripts of ubiquitin gene expression in rat skeletal muscle in a similar manner to TNF- α and IL-1 β (Llovera et al., 1998). In other models of cancer cachexia, myotubes and mouse muscles treated with TNF- α together with IFN- γ exhibited a significant reduction in myosin expression through an RNA-dependent mechanism indicating that these two cytokines are complementary in muscle degradation (Acharyya et al., 2004). Serum levels of cytokines, including IFN- γ , TNF- α , IL-1 β , and IL-6 are poorly correlated with weight loss and cachexia in cancer patients (Maltoni et al., 1997).

Proteolysis Inducing Factor (PIF)

This proteoglycan was discovered as an antigen that was reactive with murine monoclonal antibody isolated from the cachexia-inducing tumor (MAC 16) and induced *in vitro* muscle protein degradation of isolated mouse soleus tissue. Administration of PIF to mice caused a significant decrease in body weight that was inhibited when pretreated with the monoclonal antibody (Todorov et al., 1996; Lorite et al., 1997). The antibody to this proteoglycan was also reactive to a similar material detectable in the urine of cachectic cancer patients with a variety of solid tumors and absent in non-cachectic patients (Cariuk et al., 1997). Skeletal muscle of mice treated with PIF and murine myotubes treated *in vitro* demonstrated an increased activity and expression of the ubiquitin-proteasome proteolytic pathway components (Lorite et al., 2001). PIF has also been shown to induce the NF- κ B and STAT3 pathways in isolated human hepatocytes. These are two independent pathways responsible for expression of proinflammatory cytokines, adhesion molecules and acute phase proteins (Watchorn et al., 2001). These mechanisms may account for the effect of PIF on skeletal muscle degradation in cancer patients with cachexia.

Some independent clinical studies have supported a role for of PIF in cachexia while others have not. One study showed a correlation between expression of PIF in tumors, its detection in urine and weight loss of patients with gastrointestinal malignancies (Cabal-Manzano et al., 2001). A longitudinal study also established a relationship between urinary PIF excretion and weight loss over time (Williams et al., 2004). However, a recent prospective study in of patients with metastatic gastric and esophageal cancer showed no correlation between urinary PIF and weight loss, anorexia, tumor response or patient survival (Jatoi et al., 2006). Stable forced expression of human PIF in multiple murine and human cell lines resulted in secretion of PIF but not glycosylation of the peptide (Monitto et al., 2004). Furthermore, tumor xenografts of cells engineered to express PIF do not induce cachexia *in vivo* (Monitto et al., 2004). Hopefully, further investigation will resolve

these apparent discrepancies and establish how important PIF is in cancer cachexia.

Ciliary Neurotrophic Factor (CNTF)

Ciliary neurotrophic factor (CNTF) is produced primarily by glial cells in the peripheral nervous system and in skeletal muscle. In mice implanted with C6 glioma cells, this cytokine is secreted and induces acute-phase proteins as well as significant cachexia (Henderson et al., 1996). However, the effect of CNTF on muscle degradation *in vitro* has not been consistent in concentration and time course treatments of cultured rat skeletal muscle cells (Wang and Forsberg, 2000).

Muscle catabolism in pancreatic cancer

Despite work that has been done thus far, cancer cachexia continues to be a significant cause of morbidity and mortality. Cancer cachexia is a particular problem in pancreatic cancer with grave implications in the quality of life of these patients. Unfortunately pancreatic cancer prognosis and survival continue to be poor with the available surgical and adjuvant therapies. In 2006, there will be an estimated 33,730 cases of pancreatic cancer in the United States and 32,300 estimated deaths from the disease (American Cancer Society, 2006). Pancreatic cancer is currently the fourth leading cause of cancer-related deaths in the United States, with less than 5% of patients alive at 5 years after diagnosis (Society, 2006). The high mortality rate of pancreatic cancer is due to metastatic disease present at the time of diagnosis, rapid progression and inadequate systemic therapies. Due to the debilitating metabolic effects of unrestrained growth, the actual median survival rate for patients with advanced disease is only 3-6 months (Gold and Goldin, 1998). The incidence of cachexia in these patients can be as high as 80% (Ryan and Grossbard, 1998; Splinter, 1992). The etiology of cachexia in pancreatic cancer is multifactorial. Factors that contribute to weight loss in this disease can include alimentary obstruction, pain, depression, side-effects of therapy and a generalized catabolic state that may account for the high amounts of skeletal muscle degradation (Table 1) (Uomo et al., 2006). Obstructive symptoms can be accounted for by duodenal stenosis secondary to tumor burden, early satiety from lack of gastric accommodation, gastroparesis or delayed antropyloric emptying that leads

Table 1. Factors contributing to the severe cachexia seen in pancreatic cancer.

Increased resting energy expenditure
Mechanical obstruction of the gastrointestinal tract
Pain
Depression
Side effects of therapy
Nausea

to early postprandial bloating and intractable nausea. A great deal of the obstructive symptoms are also accompanied by pain that is exacerbated with food intake. Pancreatic cancer patients also frequently suffer from severe depression that may affect appetite. The toxic effects of chemotherapy and radiation also play a significant role in both appetite suppression, pain with oral intake and nausea. Lastly, is the complex catabolic state that accompanies the latter phases of this disease. In a study done by Falconer et al. it was determined that resting energy expenditure (REE) is increased by 33% in cachectic patients with pancreatic cancer (Falconer et al., 1994). The REE was also significantly greater in cancer patients with an acute phase response (C-reactive protein >10 mg/L) than those who did not have such a response. Interestingly, there was no correlation in IL-6 levels between cachectic patient with and without an acute phase response. In contrast, spontaneous production of TNF- α and IL-6 by isolated peripheral blood mononuclear cells was significantly greater in cancer patients with an acute-phase response than in those without. This may indicate that in pancreatic cancer cachexia, local rather than systemic cytokine production may be important in regulating the acute-phase response.

Therapeutics and cancer cachexia

It is well established that cancer cachexia leading to weight loss and malnutrition is associated with adverse outcomes. In pancreatic cancer, the ideal therapy would be a curative resection. However, at the present time, few patients are resection candidates and most patients are ultimately failed by radiation and chemotherapy. As a result, palliation is a significant therapeutic target in this population. The cancer cachexia seen in pancreatic cancer is a significant contributor to the diminished quality of life in this patient population. There have been multiple attempts at therapeutics to target symptoms and quality of life in patients with cancer cachexia (Table 2).

Nutritional supplementation

The first category of intervention is nutrition. Two meta-analyses evaluating prospective randomized clinical trials studying the role of preoperative nutrition in patients with either a variety of gastrointestinal cancers or pancreatic cancer alone concluded that there was no reduction in morbidity or mortality using either

total parenteral nutrition (TPN) or enteral nutrition (Detsky et al., 1987; Heys et al., 1999). In a prospective randomized clinical trial, postoperative TPN provided no therapeutic benefit in 117 patients who had undergone major pancreatic resections (Brennan et al., 1994). Surprisingly in this study, the rates of major complications in these patients was actually higher. A caveat in this study was that the patients had only lost an average of 6% total body weight preoperatively and, therefore, may not necessarily be identifiable as cachectic. In another attempt to address nutritional supplementation and outcome, Daly et al. evaluated the role of immune enhancing enteral formulas (arginine, RNA and omega-3 fatty acids) in two prospective randomized clinical trials (Daly et al., 1992, 1995). This group found that immune enhancing enteral formulas decreased both morbidity (infectious and wound-related complications) and length of hospital stay. In contrast, another group found no differences in morbidity and length of stay in a similar population given an early postoperative immune-enhancing enteral formula (arginine, RNA, omega-3 fatty acids, vitamins and minerals) (Hesli et al., 1997). Unfortunately, none of the studies is ideal for addressing nutrition in pancreatic cachexia as there was no absolute indication in either of these studies that the population was cachectic.

In another attempt to positively impact cachexia and quality of life, Fearon et al. conducted a randomized double blind trial to assess the effect of a protein and energy dense n-3 fatty acid enriched oral supplement on the loss of weight and lean tissue in cancer cachexia (Fearon et al., 2001). At enrollment, patient's mean rate of weight loss was 3.3 kg/month and were included only if they had lost more than 5% of their pre-illness stable weight over the previous six months. Over the course of eight weeks, both groups stopped losing weight given either an isocaloric isonitrogenous control supplement or an energy dense supplement enriched with n-3 fatty acids and antioxidants. The limitation in this study was that there was non-compliance in both groups and at the mean dose taken in both groups, there was no therapeutic advantage. However, with correlation analyses, if taken in sufficient quantity, only the n-3 fatty acid enriched energy and protein dense supplement results in net gain of weight, lean tissue, and improved quality of life. The potential benefit of omega-3 fatty acids, such as eicosapentaenoic acid (EPA) in reducing cancer cachexia was derived from evidence that EPA had been shown to have anti-tumor and anti-cachectic effects in the murine MAC-16 colon adenocarcinoma model (Beck et al., 1991). In addition, EPA has been shown to antagonize the loss of skeletal muscle proteins in cancer cachexia associated with this model by down-regulation of proteasome expression (Whitehouse et al., 2001).

More recently a group looked at the affect of n-3 fatty acids on total energy expenditure (TEE), resting energy expenditure (REE) and physical activity in cachectic patients with pancreatic cancer given a energy and protein dense oral supplement with or without the n-3 fatty acid eicosapentaenoic acid (EPA) (Moses et al.,

Table 2. Attempts at therapy of cancer cachexia.

Total parenteral nutrition
Immune enhancing formulas
Omega-3 fats
β -hydroxy β -methylbutyrate
Megesterol acetate
Pentoxifylline
Thalidomide

2004). Their findings were that after 8 weeks, TEE and physical activity was significantly increased in the group receiving the EPA enriched supplement, whereas there was no difference in REE between the two groups. The findings implied that EPA played a role in decreasing the hypermetabolism associated with cancer cachexia and that an increase in physical activity is reflective of an improved quality of life. Unfortunately, this study did not specifically look at the effects of EPA on lean body mass or composition to assess if EPA was able to specifically decrease muscle degradation.

Another promising supplement for cancer cachexia is the leucine metabolite, β -hydroxy β -methylbutyrate (HMB). Stage IV weight losing cancer patients were treated with either placebo or with HMB supplementation combined with arginine and glutamine. Body mass increased significantly in the HMB group while the patients receiving placebo continued to lose weight (May et al., 2002). The increase in body weight was attributed to an increase in fat-free mass in keeping with the known effects of HMB on muscle tissue. Even more impressive increases in body weight and lean body mass were seen in weight losing HIV-AIDS patients who received the same supplement containing HMB (Clark et al., 2000).

Pharmacological agents

The next major area for therapeutic intervention against cancer cachexia are pharmacologic agents. The two major agents used in the clinics today are megestrol acetate and corticosteroids. There have been at least 5 randomized trials demonstrating that megestrol acetate versus placebo provides a benefit in cancer cachexia, however none specifically to look at the effects of megestrol acetate on skeletal muscle degradation in cancer cachexia (Bruera et al., 1990; Loprinzi et al., 1990; Tchekmedian et al., 1990; Feliu et al., 1992; Vadell et al., 1998). The mechanism of action of megestrol is believed to involve stimulation of appetite by both direct and indirect pathways and antagonism of the metabolic effects of the principal catabolic cytokines (Femia and Goyette, 2005). The second major group of therapeutics used against cancer cachexia are corticosteroids. There have been several randomized, placebo-controlled trials demonstrating a limited benefit of corticosteroids for up to one month in appetite, nausea, caloric intake, pain control and the sensation of well being (Moertel et al., 1974; Willox et al., 1984; Bruera et al., 1985; Popiela et al., 1989). Unfortunately, these benefits are short-lasting and do not result in increased body weight. Treatment for a longer duration leads to all the well-described side effects of corticosteroids including immunosuppression, weakness, delirium and osteoporosis and there is no reduction in mortality (Argiles et al., 2001).

Another potential target against skeletal muscle degradation and the loss of lean body mass in cancer cachexia are the cytokines discussed previously in the sections above. They serve as potent mediators that can

account for a multitude of the metabolic derangements leading to skeletal muscle degradation. The most widely studied of these in humans is TNF- α . In humans with cancer anorexia and/or cachexia, a randomized, double-blind, placebo-controlled study was conducted administering pentoxifylline (Goldberg et al., 1995). Pentoxifylline inhibits TNF- α synthesis by decreasing gene transcription (Argiles et al., 2001). However this study failed to demonstrate any benefit of pentoxifylline as a therapy for cancer anorexia and/or cachexia (Goldberg, 1995). Another potential anti-TNF- α agent is thalidomide. Thalidomide (α -N-phthalimido-glutaramide) has been shown to decrease TNF production by monocytes *in vitro* by selectively inducing TNF- α mRNA degradation (Siampaio et al., 1991; Moreira et al., 1993). In 2005, Gordon et al. conducted a randomized placebo controlled trial to assess the safety and efficacy of thalidomide in attenuating weight loss in patients with cachexia secondary to advanced pancreatic cancer (Gordon et al., 2005). Fifty patients with advanced pancreatic cancer with a minimum of 10% body weight lost were randomized to thalidomide vs. placebo. At eight weeks, body weight remained stable in the thalidomide group, while the placebo group had a mean weight loss of nearly 4 kg. The authors concluded that thalidomide was well tolerated and effective at attenuating weight loss and lean body mass in patients with cachexia due to advanced pancreatic cancer. Unfortunately, limitations in this study were the small sample size and the relatively short term follow up of only 8 weeks.

IL-6, IL-1 β and IFN- γ are also additional cytokine targets for therapeutics against skeletal muscle degradation seen in cancer cachexia. The administration of anti-IL-6 monoclonal antibody to patients with AIDS and lymphoma resulted in positive effects on fever and cachexia (Emilie et al., 1994). However, this potential therapy has not been evaluated in cachectic pancreatic cancer patients. Similarly, there is little data on either antibodies or IL-1 β or IFN- α inhibitors in human studies of cancer cachexia and skeletal muscle degradation.

Conclusion

It is clear that there is a multitude of both host and tumor factors that contribute to the skeletal muscle degradation seen in the context of cancer cachexia. These cytokines create a complex milieu that function synergistically to create the metabolic derangements leading to the loss of lean body mass. Therapeutics that target these factors must be sought out to improve both the longevity and the quality of life of these patients, as cachexia continues to be a significant burden to patients with advanced cancer.

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