Journal of Cellular Biochemistry 93:871–877 (2004)

PROSPECTS

Tumor–Host Interactions

M.J. Tisdale*

Pharmaceutical Sciences Research Institute, Aston University, Birmingham, B4 7ET, United Kingdom

Abstract A number of malignant tumors interact with the host to cause a syndrome of cachexia, characterized by extensive loss of adipose tissue and skeletal muscle mass, but with preservation of proteins in visceral tissues. Although anorexia is frequently present, the body composition changes in cancer cachexia cannot be explained by nutritional deprivation alone. Loss of skeletal muscle mass is a result of depression in protein synthesis and an increase in protein degradation. The main degradative pathway that has been found to have increased expression and activity in the skeletal muscle of cachectic patients is the ubiquitin-proteasome proteolytic pathway. Cachexia-inducing tumors produce catabolic factors such as proteolysis-inducing factor (PIF), a 24 kDa sulfated glycoprotein, which inhibit protein synthesis and stimulate degradation of intracellular proteins in skeletal muscle by inducing an increased expression of regulatory components of the ubiquitin-proteasome proteolytic pathway. While the oligosaccharide chains in PIF are required to initiate protein degradation the central polypeptide core may act as a growth and survival factor. Only cachexia-inducing tumors are capable of elaborating fully glycosylated PIF, and the selectivity of production possibly rests with the acquisition of the necessary glycosylating enzymes, rather than expressing the gene for the polypeptide core. Loss of adipose tissue is probably the result of an increase in catabolism rather than a defect in anabolism. A lipid mobilizing factor (LMF), identical with the plasma protein Zn-α_2-glycoprotein (ZAG) is found in the urine of cachectic cancer patients and is produced by tumors causing a decrease in carcass lipid. LMF causes triglyceride hydrolysis in adipose tissue through a cyclic AMP-mediated process by interaction with a β3-adrenoreceptor. Thus, by producing circulating factors certain malignant tumors are able to interfere with host metabolism even without metastasis to that particular site. J. Cell. Biochem. 93: 871–877, 2004.

Key words: muscle atrophy; proteasome proteolysis; proteolysis-inducing factor; cancer cachexia; lipolysis; lipid-mobilizing factor; zinc-α_2-glycoprotein

There are numerous interactions between a malignant tumor and the host, which eventually leads to death of the cancer patient. Probably one of the most important of these in terms of survival is the syndrome of cachexia, characterized by progressive weight loss, involving substantial depletion of both adipose tissue and skeletal muscle mass even without tumor metastasis. Cachexia is responsible for the death of at least 20% of all cancer patients [Inagaki et al., 1974], while loss of skeletal muscle leads to weakness (asthenia) and immobility, lowering the quality of life of the cancer patient. Cachexia is also associated with a reduced responsiveness to chemotherapy, and an increased susceptibility to toxicity, resulting in the administration of lower dose levels of chemotherapeutic agents. Death of the patient normally occurs when the weight loss approaches 30%, providing a direct link between loss of body weight and an increased morbidity and mortality. Death of AIDS patients is also imminent when they have lost 34% of their ideal body weight. For many tumor types there is an inverse relationship between degree of weight loss and median survival time, and even small amounts of weight loss (less than 5% of body weight) may significantly worsen the prognosis [De Wys, 1986]. In patients with nonsmall cell lung carcinoma, men lost significantly more weight over their disease course than women, and experienced an eight-fold faster rate of initial weight loss, according for their shorter overall median survival time (40 weeks) compared with women (78 weeks) [Palomares et al., 1996].

While patients with lung cancer and 30% weight loss showed a 75% depletion of their

*Correspondence to: M.J. Tisdale, Pharmaceutical Sciences Research Institute, Aston University, Birmingham, B4 7ET, UK. E-mail: m.j.tisdale@aston.ac.uk

Received 24 June 2004; Accepted 25 June 2004

DOI 10.1002/jcb.20246

skeletal muscle protein reserves, non-muscle protein stores remained the same, and structural and visceral proteins were relatively well preserved [Fearon and Preston, 1990]. Loss of skeletal muscle accounts for the shorter survival of cachectic cancer patients, with impairment of respiratory muscle function resulting in the high incidence of hypostatic pneumonia as the terminal event [Inagaki et al., 1974]. Respiratory failure has been found to be responsible for the death of 48% of cancer patients. Since cachexia plays such an important part in the morbidity and mortality of the cancer patient there have been a number of studies to investigate the mechanisms involved, with the view to providing appropriate therapy.

CACHEXIA AND ANOREXIA

Cancer cachexia appears as a classic case of malnutrition, especially since most cachectic patients also suffer from anorexia. However, cachexia can occur even in the absence of anorexia, and the body composition changes, particularly the preservation of visceral protein mass are not seen in anorexia nervosa. Attempts to reverse the body weight loss in cachexia by dietary counselling, or by provision of extra nutrients through total parenteral nutrition (TPN) have little impact on overall weight stabilization, and any increase in body weight arises from an increase in fat and water, rather than lean body mass [Evans et al., 1985]. Similar changes in body composition are seen in patients administered appetite stimulants such as megestrol acetate or medroxyprogesterone acetate. Since these agents have no effect on muscle mass they do not improve the Karnofsky index. These results suggest that loss of muscle mass is a specific feature of cancer cachexia unrelated to the nutritional status of the patient.

EFFECT OF THE TUMOR ON SKELETAL MUSCLE

Atrophy of skeletal muscle is due to a combination of hypoanabolism, together with an increase in catabolism. Studies on whole-body protein turnover in cachectic subjects are complicated by protein synthesis in visceral tissue, which may actually increase because of hepatic production of acute phase proteins (APP), such as C-reactive protein (CRP) and fibrinogen [Preston et al., 1998]. Thus, in a group of weight-losing cancer patients no changes in total body protein synthesis or degradation were detected, but muscle protein synthesis only accounted for 8% of total body synthesis, compared with 53% for healthy controls, due to a two-fold increase in protein synthesis in visceral tissues [Rennie et al., 1983]. An increased rate of protein degradation was observed in muscle biopsies from 43 newly diagnosed cancer patients [Lundholm et al., 1982]. A change in muscle isoform expression has also been reported with a decrease in type I and an increase in type II (fast) isoform expression. Contractile proteins are more extensively depleted than sarcoplasmic proteins and white or phasic muscle tends to be lost faster than red or tonic muscle.

The mechanism for the depression in protein synthesis in skeletal muscle is not known, although tumor factors such as proteolysis-inducing factor (PIF) [Lorite et al., 1997] and cytokines such as tumor necrosis factor-α (TNF-α) [Garcia-Martinez et al., 1993] depressed protein synthesis in skeletal muscle of normal animals. Protein synthesis requires a correctly balanced mixture of the 20 amino acids together with a source of energy for peptide bond formation. The plasma concentration of amino acids was found to be lower in mice bearing a cachexia-inducing tumor, with most amino acids at only 60% of the levels found in non-tumor-bearing animals, and with a maximal decrease of 54% for valine and isoleucine [Beck and Tisdale, 1989]. Most investigators have also noted widespread decreases in plasma levels of free amino acids in patients with cachexia. Tumor factors such as PIF are also able to depress plasma amino acid levels, and this may contribute to the depression in protein synthesis [Lorite et al., 1997]. Increased ATP production in the liver may alter the supply of amino acids for protein synthesis in muscle, since acute phase proteins contain relatively high levels of sulfur amino acids [Reeds et al., 1994]. However, the situation is likely to be more complicated than this, since the provision of extra nutrients is unable to reverse the loss of muscle mass [Evans et al., 1985]. Also depression of CRP production by the anti-inflammatory drug indomethacin has no effect on lean body mass, although there was an increase in fat mass resulting from an increased food intake [Lundholm et al., 2004]. The most likely reason for the depression of plasma amino acids in

cancer cachexia is an increased utilization as a metabolic fuel. Thus, the carbon skeletons of branched-chain amino acids, arising from transamination provide a major source of fuel for skeletal muscle, while gluconeogenic amino acids such as alanine are channeled to the liver to supply the increased glucose demands of the tumor. Increased gluconeogenesis from amino acids would explain the elevation of nitrogen excretion in cancer cachexia [Beck and Tisdale, 1989].

Although reduced protein synthesis and reduced uptake of amino acids contribute to the atrophy of skeletal muscle in cancer cachexia, increased protein degradation, in particular breakdown of the myofibrillar proteins actin and myosin, probably plays the major role. Of the three pathways for protein degradation, lysosomal, Ca^{2+}-dependent (calpain), and ubiquitin-proteasome proteolytic pathway, the latter has been found to be most important in muscle atrophy in cancer [Bossola et al., 2003]. However, lysosomal proteases may play a role in muscle wasting in the early stages of the disease, since mRNA levels for the lysosomal protease cathepsin B were found to be much higher in patients with a weight loss of 2.9%, compared with non-wasting controls, and there was no evidence for an increase in expression of components of the ubiquitin-proteasome pathway [Jagoe et al., 2002]. The functional role of the increased cathepsin B is not known, since lysosomes have been shown to play no role in the degradation of myofibrillar proteins, at least in the rat [Lowell et al., 1986]. There is evidence that Ca^{2+}-dependent proteases may be involved in the degradation of Z-bands with subsequent loss of actin and myosin from the sarcomeres, since the ubiquitin-proteasome system does not degrade intact myofibrils. There is an increase in Ca^{2+}-dependent proteolysis in skeletal muscle and heart in rats bearing a cachexia-inducing tumor, suggesting that it plays a role in muscle degradation.

The main function of the ubiquitin-proteasome system is to control the intracellular concentration of regulatory proteins, and it also degrades proteins damaged by oxidation or mutation, or that misfold or mislocalize. Proteins are marked for degradation in the multisubunit 26S proteasome by the attachment of a polyubiquitin chain. Only selected proteins are recognized by ubiquitin protein ligases (E3) and two of these enzymes Muscle Ring Finger 1 (MURF1) and Muscle Atrophy Fbox (MAFbx) are elevated in skeletal muscle under conditions of protein degradation. Muscle from MAFbx- and MURF1-deficient mice had decreased loss of muscle mass after denervation atrophy, which induces increased expression of ubiquitin-dependent proteolysis [Bodine et al., 2001]. The 26S proteasome consists of the 20S core proteasome and two 19S regulatory subunits. The 20S proteasome is a tube-like structure appearing as a stack of four rings, two outer α-rings and two inner β-rings in the order $\alpha\beta\beta\alpha$. The proteolytic sites are on the inner surface of the β rings. Skeletal muscle from cachectic patients shows an increased expression of proteasome subunits and functional enzymatic activity [Bossola et al., 2003]. Since the ubiquitin-proteasome proteolytic pathway is independent of the amount of protein consumed simple nutritional supplementation would not be expected to prevent muscle catabolism by this pathway, and reversal can only be achieved when the molecular switch is identified.

TUMOR FACTORS MEDIATING MUSCLE ATROPHY

Studies on the mechanism of protein degradation in mice bearing the cachexia-inducing MAC16 tumor showed an increased serum bioactivity of a factor capable of stimulating tyrosine release from isolated gastrocnemius muscle in weight losing animals [Smith and Tisdale, 1993]. Serum samples from patients with weight loss greater than 10% showed a similar bioactivity, suggesting that muscle atrophy may be mediated by circulating factors produced either by the tumor or by the host in response to the tumor [Belizario et al., 1991]. It was subsequently discovered that serum from mice bearing the MAC16 tumor contained antibodies reactive with a 24 kDa material on Western blots of tumor extracts, and from the urine of patients with cancer cachexia [McDevitt et al., 1995]. Such antibodies were absent from the serum of mice bearing a related tumor (MAC13), which does not induce cachexia. This suggested that the 24 kDa material was related to the cachectic state, and raised the possibility that cachexia in humans may be caused by the same species as in the mouse. The 24 kDa factor was subsequently purified using a monoclonal version of the antibody produced in mice bearing the

MAC16 tumor, and was shown to be a complex glycoprotein with a short polypeptide chain (Mr 4 kDa), to which was attached an O-linked sulfated oligosaccharide chain (Mr 6 kDa), and one N-linked sulfated oligosaccharide chain (Mr 10 kDa) [Todorov et al., 1997]. The factor produced weight loss in non-tumor-bearing mice (about 10% in 24 h), arising specifically through loss of lean body mass, particularly skeletal muscle, while visceral protein reserves were preserved as in cancer cachexia [Todorov et al., 1996]. The factor was also capable of inducing direct protein degradation in isolated skeletal muscle and for this reason was named PIF [Todorov et al., 1997]. Biological activity required intact N- and O-glycosidic chains and the free peptide core was devoid of biological activity. The peptide core has since been shown to be homologus with a peptide called dermicidin, first isolated as an antibiotic from human sweat, and which has been shown to be overexpressed in about 10% of primary invasive breast carcinomas with poor prognostic features, and is a growth and survival factor [Porter et al., 2003]. Overexpression and copy number gain of dermicidin is likely to confer a selective advantage for breast tumor cells, which is consistent with it being a putative oncogene. Since these workers did not establish whether there was glycosylation of the peptide core it is not certain whether these conclusions apply to dermicidin or PIF.

PIF has been detected in the urine of patients with carcinoma of the breast, ovary, lung, colon, rectum, and liver when the rate of weight loss is greater than or equal to about 1.2 kg month^{-1}. PIF has been detected in the urine of 80% of patients with pancreatic carcinoma and these patients had a significantly greater total weight loss and rate of weight loss than patients whose urine did not contain PIF. There is a correlation between detection of PIF in the urine, weight loss and PIF expression in tumors for patients with gastrointestinal cancer, providing direct evidence that tumors are the source of PIF in humans [Cabal-Manzano et al., 2001]. Prostate cancer patients with disseminated disease often suffer from severe cachexia, and Western blot analysis showed PIF to be present in 9 of 19 urine samples from cachectic patients, but to be absent from the urine of noncachectic patients [Wang et al., 2003]. In situ hybridization showed mRNA for the peptide core to be localized only in the epithelial cells of the prostatic carcinoma, in the metastatic foci in bone, liver, and lymph node, but not in the stromal cells or in normal prostate tissues. These results confirm that tumor cells express the gene for the peptide core of PIF and that this is associated with the development of cachexia.

Studies in mice showed that PIF caused depletion of lean body mass through a depression (by 50%) in protein synthesis and an increase (by 50%) in protein degradation [Lorite et al., 1997]. The mechanism of the effect on protein synthesis is not known, although in vitro studies [Smith et al., 1999] show that it is attenuated by insulin at physiological concentrations and below, suggesting an effect on translation. The effect of PIF on protein degradation is mediated through an increased expression and activity of the ubiquitin-proteasome-proteolytic pathway [Lorite et al., 2001] specifically in skeletal muscle, with no evidence for upregulation in visceral tissues, such as heart. The effect of PIF on increased expression of proteasome subunits and the ubiquitin conjugating enzyme ($E2_{14k}$) was also demonstrated in vitro, confirming a direct effect of PIF on the proteolytic pathway. The action of PIF on protein degradation was mediated by the phospholipase A_2 catalyzed release of arachidonic acid from membrane phospholipids and its conversion to the lipoxygenase product 15-hydroxyeicosatetraenoic acid (15-HETE) [Smith et al., 1999]. Other lipoxygenase products were found to be ineffective in inducing protein degradation in skeletal muscle, as were the prostaglandins E_2 and $F_{2\alpha}$. The action of PIF on protein degradation, but not protein synthesis was attenuated by the polyunsaturated fatty acid eicosapentaenoic acid (EPA), which has been shown to be effective in increasing lean body mass in cachectic patients with advanced pancreatic cancer [Barber et al., 1999]. The effect of EPA on preserving lean body mass is mediated by down-regulation of the increased expression and activity of the ubiquitin-proteasome proteolytic pathway, through a direct effect on PIF signaling, by attenuating the production of 15-HETE [Smith et al., 1999], and by inhibiting nuclear translocation of the transcription factor nuclear factor-κB (NF-κB), by preventing degradation of the inhibitor protein I-κB in the cytosol [Whitehouse and Tisdale, 2003]. These results suggest that NF-κB is involved in induction of gene expression of proteasome subunits and enzymes involved in ubiquitin conjugation.

EFFECT OF THE TUMOR ON ADIPOSE TISSUE

Cachexia results in large losses of adipose tissue in cancer patients with 85% depletion occurring when the weight loss reaches 30% [Fearon and Preston, 1990]. Loss of triglycerides has been suggested to be due to an increased lipolysis, decreased lipogenesis, and decreased lipoprotein lipase (LPL), which would prevent synthesis of lipids from circulating triglycerides. Cancer patients have a high turnover of both glycerol and free fatty acids (FFA) and the elevated mobilization of fatty acids is often evident before weight loss becomes established. Fasting plasma glycerol concentrations are much higher in weight losing subjects compared with weight stable individuals suggesting an increase in lipolysis. Adipose tissue from cancer patients shows a two-fold increase in the relative level of mRNA for hormone-sensitive lipase involved in triglyceride hydrolysis which is activated by the cyclic AMP-dependent protein kinase, but no change in the total LPL enzyme activity, or the relative levels of the mRNA's for LPL and fatty acid synthase [Thompson et al., 1993]. Thus, the observed two-fold elevation of serum triacylglycerol and FFA levels probably reflects an increase in lipolysis.

TUMOR FACTORS MEDIATING LIPID CATABOLISM

There is evidence dating back over 40 years that certain tumors contain substance(s) capable of inducing fat depletion, and that this is present in the circulation of tumor-bearing animals. The lipid mobilizing factor (LMF) was subsequently purified from the MAC16 tumor and from the urine of cachectic cancer patients using a combination of ion exchange, exclusion and hydrophobic interaction chromatographies to yield a glycoprotein of apparent Mr 43 kDa, the amino acid sequence of which was identical with human plasma Zn-α_2-glycoprotein (ZAG) [Todorov et al., 1998]. Only tumors which produced a decrease in carcass lipid expressed mRNA for ZAG. Both LMF and ZAG were shown to directly induce lipolysis in isolated murine adipocytes and to cause selective loss of adipose tissue in exbreeder male mice, without a change in body water or nonfat carcass mass, and without a drop in food or water intake [Hirai et al., 1998]. Induction of lipolysis was mediated through the classical system used by lipolytic hormones, through stimulation of plasma membrane adenylate cyclase, with increases in intracellular cyclic AMP, which stimulated cyclic AMP-dependent protein kinase. Stimulation of adenylate cyclase was mediated through a β3-adrenoreceptor with a Kd value of 78 ± 45 nM [Russell et al., 2002]. The ability of LMF to interact with a β3-adrenoreceptor could explain the ability of the non-specific β-blocker, propranolol, to significantly reduce resting energy expenditure, whole body oxygen uptake and carbon dioxide production in weight-losing cancer patients, since LMF has been shown to increase expression of uncoupling proteins (UCP)-1,-2, and-3 in brown adipose tissue (BAT) and UCP-2 in both skeletal muscle and liver [Bing et al., 2002]. Recent studies indicate that ZAG is produced not only by certain tumors, but also by BAT and white adipose tissue (WAT) [Bing et al., 2004]. Moreover, in weight losing mice bearing the MAC16 tumor there was a 10-fold increase in ZAG mRNA and protein in WAT and a three to four-fold elevation in BAT. Increased production of ZAG in BAT and WAT in cancer cachexia may have important implications for lipid mobilization and utilization within the tissue. In 3T3-L1 adipocytes ZAG expression was markedly increased by the β3-agonist BRL 37344 suggesting that the sympathetic system may play a role in the regulation of ZAG expression. Glucocorticoids were also shown to stimulate ZAG gene expression suggesting that increased cortisol levels seen in cachectic patients may lead to an increased lipolysis through increased ZAG expression.

CONCLUSIONS

Unlike benign tumors, certain malignant tumors secrete catabolic factors that have profound effects on host metabolism resulting in depletion of both lipids and skeletal muscle proteins. In the case of PIF this is most likely due to the resurgence of an embryonic protein, since PIF expression peaks during the embryonic period E8 to E9, a stage that is crucial in the development of skeletal muscle and liver [Watchorn et al., 2001]. It is likely that PIF production in normal tissues is switched-off in the adult state, but that certain tumors, possibly due to aberrant gene expression, regain the ability to glycosylate the core peptide. Although the main effect of PIF is on skeletal muscle PIF also activates the transcription factors NF-κB

and STAT3 in the liver resulting in the increased production of interleukin (IL)-6 and-8 and CRP, and the decreased production of transferrin [Watchorn et al., 2001]. It is thus likely that PIF is responsible for the APR seen in cancer patients with cachexia. In contrast with PIF, ZAG appears to be a normal lipolytic factor, the expression of which is elevated in certain tumors and adipose tissue, either by increased cortisol production, or through a cascade effect by which ZAG induces its own production through the intervention of a β3-adrenoreceptor. The end result of this tumor–host interaction is to debilitate the patient and hasten the onset of death. Further studies are required to elucidate signaling pathways for these agents and to identify the mechanism of transcriptional activation in order to develop effective pharmacological agents for the treatment of cancer cachexia.

REFERENCES

Barber MD, Ross JA, Voss AC, Tisdale MJ, Fearon KCH. 1999. The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer. Br J Cancer 81:80–86.

Beck SA, Tisdale MJ. 1989. Nitrogen excretion in cancer cachexia and its modification by a high fat diet in mice. Cancer Res 49:3800–3804.

Belizario JE, Katz M, Raw CI. 1991. Bioactivity of skeletal muscle proteolysis-inducing factors in the plasma proteins from cancer patients with weight loss. Br J Cancer 63:705–710.

Bing C, Russell ST, Beckett EE, Collins P, Taylor S, Barraclough R, Tisdale MJ, Williams G. 2002. Expression of uncoupling proteins-1,-2, and-3 mRNA is induced by an adenocarcinoma-derived lipid-mobilizing factor. Br J Cancer 86:612–618.

Bing C, Bao Y, Jenkins J, Sanders P, Manieri M, Cinti S, Tisdale MJ, Trayhum P. 2004. Zinc-α_2-glycoprotein, a lipid mobilizing factor, is expressed in adipocytes and is up-regulated in mice with cancer cachexia. Proc Natl Acad Sci USA 101:2500–2505.

Bodine SC, Latres E, Baumhueter SI, Lai VK, Nunez L, Clarke BA, Poueymirou WT, Panora FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuela DM, DeChiara TM, Stitt TN, Yancopoulos GD, Glass DJ. 2001. Identification of ubiquitin ligases required for skeletal muscle atrophy. Science 294:1704–1708.

Bossola M, Muscaritoli M, Costelli P, Grieco G, Bonelli G, Pacelli F, Fanelli FR, Doglietto GB, Baccino FM. 2003. Increased muscle proteasome activity correlates with disease severity in gastric cancer patients. Ann Surg 237: 384–389.

Cabal-Manzano R, Bhargava P, Torres-Duarte A, Marshall J, Bhargava P, Wanier IW. 2001. Proteolysis-inducing factor is expressed in tumors of patients with gastrointestinal cancers and correlates with weight loss. Br J Cancer 84:1599–1601.

De Wys WD. 1986. Weight loss and nutritional abnormalities in cancer patients: incidence, severity and significance. In: Calman KC, Fearon KCH, editors. Nutritional support for the cancer patient. London: W.B. Saunders. pp 251–261.

Evans WK, Makuch R, Clamon GH, Feld K, Weiner RS, Moran E, Blum R, Shepherd FA, Jeejeebhoy KW, De Wys WD. 1985. Limited impact of total parenteral nutrition on nutritional status during treatment for small cell lung cancer. Cancer Res 45:3347–3353.

Fearon KC, Preston T. 1990. Body composition in cancer cachexia. Infusionstherapie 17(Suppl 3):63–66.

Garcia-Martinez C, Lopez-Soriano FJ, Argiles JM. 1993. Acute treatment with tumour necrosis factor-α induces changes in protein metabolism in rat skeletal muscle. Mol Cell Biochem 125:11–18.

Hirai K, Hussey HJ, Barber MD, Price SA, Tisdale MJ. 1998. Biological evaluation of a lipid-mobilizing factor isolated from the urine of cancer patients. Cancer Res 58:2359–2365.

Inagaki J, Rodriguez V, Bodey GP. 1974. Causes of death in cancer patients. Cancer 33:568–571.

Jagoe RT, Redfern CPF, Roberts RG, Gibson GJ. 2002. Skeletal muscle mRNA levels for cathepsin B but not components of the ubiquitin-proteasome pathway, are increased in patients with lung cancer referred for thoractomy. Clin Sci 102:353–361.

Lorite MJ, Cariuk P, Tisdale MJ. 1997. Induction of muscle protein degradation by a tumour factor. Br J cancer 76: 1035–1040.

Lorite MJ, Smith HJ, Arnold JA, Morris A, Thompson MG, Tisdale MJ. 2001. Activation of ATP-ubiquitin-dependent proteolysis in skeletal muscle in vivo and murine myoblasts in vitro by a proteolysis-inducing factor (PIF). Br J Cancer 85:297–302.

Lowell BB, Ruderman NB, Goodman MN. 1986. Evidence that lysosomes are not involved in the degradation of myofibrillar proteins in rat skeletal muscle. Biochem J 234:237–240.

Lundholm K, Bennegard K, Eden E, Svanringer G, Emery PW, Rennie MJ. 1982. Efflux of 3-methylhistidine from the leg in cancer patients who experience weight loss. Cancer Res 42:4807–4811.

Lundholm K, Daneryd P, Körner U, Hyltander A, Bosaeus I. 2004. Evidence that long-term COX treatment improves energy homeostasis and body composition in cancer patients with progressive cachexia. Int J Oncol 24:505–512.

McDevitt TM, Todorov PT, Beck SA, Khan SH, Tisdale MJ. 1995. Purification and characterization of a lipid-mobilizing factor associated with cachexia-inducing tumours in mice and humans. Cancer Res 55:1458–1463.

Palomares MR, Sayre JW, Shekar KC, Lillington LM, Chlebowski RT. 1996. Gender influence on weight-loss pattern and survival of nonsmall cell lung carcinoma patients. Cancer 78:2119–2126.

Porter D, Weremowicz S, Chin K, Seth P, Keshaviah A, Lahti-Domenici J, Bae Y-K, Monitto CL, Merlos-Suarez A, Chan J, Hulette CM, Richardson A, Morton CC, Marks J, Duyao M, Hruban R, Gabrielson E, Gelman R, Polyak K. 2003. A neural survival factor is a candidate oncogene in breast cancer. Proc Natl Acad Sci USA 100:10931–10936.

Preston T, Slater C, McMillan DC, Falconer JS, Shenkin A, Fearon KC. 1998. Fibrinogen synthesis is elevated in fasting cancer patients with an acute phase response. J Nutr 128:1355–1360.

Reeds PJ, Fjeld CR, Jahoon F. 1994. Do the differences between the amino acid composition of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? J Nutr 124:906–910.

Rennie MJ, Edwards RHT, Emery PW, Halidey D, Lundholm K, Millward DJ. 1983. Depressed protein synthesis is the dominant characteristic of muscle wasting and cachexia. Clin Physiol 3:387–398.

Russell ST, Hirai K, Tisdale MJ. 2002. Role of β3-adrenergic receptors in the action of a tumour lipid mobilizing factor. Br J Cancer 86:424–428.

Smith KL, Tisdale MJ. 1993. Mechanism of muscle protein degradation in cancer cachexia. Br J Cancer 68:314–318.

Smith HJ, Lorite MJ, Tisdale MJ. 1999. Effect of a cancer cachectic factor on protein synthesis/degradation in murine C_2C_{12} myoblasts: Modulation by eicosapentaenoic acid. Cancer Res 59:5507–5513.

Thompson MP, Cooper ST, Parry BR, Tuckey JA. 1993. Increased expression of the mRNA for the hormone-sensitive lipase in adipose tissue of cancer patients. Biochem Biophys Acta 1180:236–240.

Todorov P, Cariuk P, McDevitt T, Coles B, Fearon K, Tisdale M. 1996. Characterization of a cancer cachectic factor. Nature 379:739–742.

Todorov PT, Deacon M, Tisdale MJ. 1997. Structural analysis of a tumor-produced sulfated glycoprotein capable of initiating muscle protein degradation. J Biol Chem 272:12279–12288.

Todorov PT, McDevitt TM, Meyer DJ, Ueyama H, Ohkubo I, Tisdale MJ. 1998. Purification and characterization of a tumor lipid-mobilizing factor. Cancer Res 58:2353–2358.

Wang Z, Corey E, Hass GM, Higano CS, True LD, Wallace D, Jr., Tisdale MJ, Vessella RL. 2003. Expression of the human cachexia-associated protein (HCAP) in prostate cancer and in a prostate cancer animal model of cachexia. Int J Cancer 105:123–129.

Watchorn TM, Waddell ID, Dowidar N, Ross JA. 2001. Proteolysis-inducing factor regulates hepatic gene expression via the transcription factors NF-κB and STAT3. FASEB J 15:562–564.

Whitehouse AS, Tisdale MJ. 2003. Increased expression of the ubiquitin-proteasome pathway in murine myotubes by proteolysis-inducing factor (PIF) is associated with activation of the transcription factor NF-κB. Br J Cancer 89:1116–1122.