The Role of Inflammatory Pathways in Cancer Associated Cachexia and Radiation Resistance

Aaron Laine, Puneeth Iyengar and Tej K. Pandita*

Department of Radiation Oncology, University of Texas Southwestern Medical Center, Dallas, Texas, 75390

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*Corresponding author:

Tej K. Pandita, Ph.D.
Department of Radiation Oncology
UT Southwestern Medical Center
5323 Harry Hines Blvd., NC7.126
Dallas, TX 75390
Tel: 214-648-1918
E-mail: tej.pandia@utsouthwestern.edu
Abstract

Dysregulated inflammatory responses are important in a multitude of chronic ailments including cancer. Disease progression in cancer is dependent on the complex interaction between the tumor and the host microenvironment including inflammatory responses, as both the tumor and microenvironment components can produce cytokines that act on multiple target sites where they promote a complex cascade of biological responses. Patients with cancer-associated cachexia (CAC) suffer from a dramatic loss of skeletal muscle and adipose tissue and are, therefore, precluded from many forms of therapeutic interventions, including radiotherapy. The cytokines that have been linked to the promotion of the cachectic response also play a role in radiation resistance. The major changes at the cytokine level are in part due to transcriptional regulatory alterations possibly due to epigenetic modifications. Here we summarize the role of inflammatory pathways in CAC and discuss the potential link between cachexia induction and radiation resistance.
Introduction

Controlled inflammatory responses are necessary for an array of protective processes including tissue repair, wound healing and defense against foreign pathogens. However, chronic, uncontrolled inflammation is harmful and has been linked to a number of human ailments (1, 2) including cancer (2, 3). Virchow postulated that an inflammatory milieu promotes a cellular environment that drives the initiation and development of carcinogenesis (1, 2). Recent studies have confirmed that within the tumor microenvironment, a network of pro-inflammatory mediators participate in a complex signaling processes that promote tumor progression (4).

Cancer associated cachexia (CAC) is a term that indicates marked and rapid decrease in body weight, characterized by depletion of skeletal muscle and white adipose tissue mass. CAC affects approximately 50% of cancer patients and is present in the vast majority of patients with advanced cancer (5). For example, more than 50% of patients with advanced head and neck cancer have significant weight loss and possible cachexia (6-8). More than 80% of pancreatic cancer patients and 60% of lung cancer patients present at diagnosis with cachexia (9). Cachectic patients also have higher radio- and chemotherapy related morbidity and often have decreased performance status that precludes them from receiving optimal therapeutic interventions (10). This devastating condition is estimated to contribute up to 15% of cancer patient deaths (11, 12). While there is a growing awareness about CAC related treatment effects; the cause and potential interventions to reverse these adverse effects are largely unexplored (13). A major hindrance to the development of effective approaches for managing CAC has been lack of an exact definition of what constitutes CAC and
reproducible diagnostic criteria. Recently a consensus definition of CAC that recognized cachexia as a progressive disorder rather than a single event was published that classified the manifestation of cachexia into three stages: pre-cachexia, cachexia and refractory cachexia (14). This framework should in the future allow a more thorough understanding of the metabolic and inflammatory mechanisms that lead to the occurrence and progression of cachexia, potentially leading to more effective therapies to prevent or ameliorate CAC. This review will focus on the evidence that inflammatory signaling pathways promote the development and progression of CAC and that these same mechanisms may also modulate the response of tumors to radiation therapy (RT).

**Inflammation and Radiation Resistance**

Radiation therapy (RT) remains an integral part of modern cancer management in both benign and malignant diseases. More than 50% of the newly diagnosed cancer patients worldwide receive RT at some point in the course of their treatment (15). The technological sophistication of imaging, planning and RT delivery has enabled more cancers to be treated with higher and more tumoricidal doses of ionizing radiation (IR) with curative intent (16). As the understanding of radiobiology has improved, investigators are seeking the basis for tumor cell radioresistance (both inherent and acquired) that is the underlying cause of tumor, recurrence and treatment failure (17). It is now recognized that IR not only damages cellular DNA, but also affects disparate cellular components that collectively elicit the multilayered biological response in the irradiated tumor cell (18).
The concept of intrinsic tumor radiosensitivity as governed by the balance between DNA damage and DNA repair following irradiation has prevailed in the field for some time. However, recent data indicate that this may not be the sole factor defining tumor radiosensitivity as the cascade of radiation-induced cytoplasmic signaling events may be an equally important determinant of tumor radiosensitivity (19). Cellular signaling triggered by low doses of IR (1-5Gy) occur at two distinct sites: 1) nuclear – signaling events initiated by damaged DNA, leading to cell cycle progression cessation and a DNA damage response to allow repair of damaged DNA; and 2) cytoplasmic – signaling at the receptor level which is partly triggered by reactive oxygen species (ROS) inactivation of phosphatases and subsequent ligand-independent activation of receptor tyrosine kinases (RTKs) (19, 20). Both these events elicit pro-survival and anti-apoptotic responses (the inducible radioresistance model) that increase overall tumor cell survival.

Signaling from the cell-surface receptors and from damaged DNA lead to downstream pathways that ultimately result in activation of a variety of transcription factors (TFs) important in governing gene expression patterns (21). Radiation induced TFs chiefly include the dynamic NF-kappaB family of proteins and STAT3 (22). Both are of prime importance and have been linked to chemoresistance and radioresistance due to production of a variety of proteins including cyclin D1, VEGF, MMPs, and pro-inflammatory cytokines (23-25). The production of these growth factors and angiogenic factors in response to radiotherapy by activated TFs is the principal mechanism of inducible radioresistance while constitutively activated NF-kappaB or STAT3 contributes to intrinsic radioresistance (Fig. 1) (26).
Several studies have investigated the impact of NF-kappaB inhibition on radiosensitivity in different models (27). Clinically approaches for NF-kappaB inhibition to induce tumor radiosensitization include a wide range of agents such as corticosteroids, phytochemicals, proteasome inhibitors and synthetic peptides (16) and have provided promising results that deserve further investigation.

The role of STAT3 in radioresistance has recently been established. Stable expression of shRNA against STAT3, increased radioresistance of human squamous cell carcinoma (A431) (28). The proteomic profiles of radioresistant prostate cancer cells confirm that the radioresistant phenotype is the result of multiple mechanisms with radiation-induced activation of the Jak-STAT pathway playing a significant role (29, 30).

Recent investigations have also focused on the tumor stroma as a target for radiosensitization. Depletion of tumor-associated macrophages by systemic or local injection of the macrophage-depleting liposomal clodronate before radiotherapy can increase the antitumor effects of IR (31). Furthermore, studies using mice with germline deletions in tumor necrosis factor receptors 1 and 2 or TNF-alpha as well as treatment of wild-type mice with a soluble TNF receptor fusion protein (Enbrel®), revealed that radioresistance required intact TNF-alpha signaling. Radiation exposure up-regulated vascular endothelial growth factor (VEGF) in macrophages and VEGF-neutralizing antibodies enhanced the antitumor response to IR (31).

**Cytokines and Cancer Associated Cachexia**
The production of chemical mediators associated with CAC can be divided into two categories: 1) those whose source is the growing tumor and 2) humoral factors (mainly cytokines) secreted by the cells in the tumor microenvironment (5). The primary inflammatory cytokines that have been implicated in CAC are tumor necrosis factor alpha (TNF-alpha) and interleukin 6 (IL-6) (32, 33). Although many inflammatory mediators may play a role in CAC, these cytokines have emerged as critical factors related to the loss of body mass during disease (32).

Activated macrophages (M0) secrete cytokines that modulate a complex cascade of biological responses. The infiltrating M0 in cachectic rats can, in part, favor the establishment of an inflammatory milieu, similar to that observed in obesity. In this manner, infiltrating M0 may contribute to metabolic disturbances, thereby worsening cachexia and depleting fat deposits. The chemokine, monocyte chemotactic protein-1 (MCP-1), is believed to be responsible for the migration of monocytes to adipose tissue in systemic chronic inflammation (34, 35). During inflammatory processes, MCP-1 promotes the attraction of monocytes, T lymphocytes, and natural killer cells to the site of inflammation (36). Despite the importance of M0 in the modulation of adipocyte function through a balance in the production of pro- and anti-inflammatory cytokines, the underlying mechanisms of how this process takes place during the development of CAC are not fully elucidated. The number or polarity of the activated M0 cells could potentially lead to the differences seen in obesity versus CAC.

TNF-alpha has long been associated with muscle pathology and was originally named ‘cachectin’ in recognition of its catabolic action. To date, evidence of increased TNF-alpha in plasma of cancer patients is controversial, perhaps due to the different
sensitivities of the assay methods, short half-life of TNF-alpha in vivo or localized paracrine production of TNF-alpha. The mechanism of TNF-alpha action in vivo remains largely enigmatic, though it has long been recognized that TNF-alpha may stimulate catabolism via indirect mechanisms. TNF-alpha alters circulating levels of hormones that regulate muscle growth and affects tissue sensitivity to such factors. TNF-alpha also stimulates production of catabolic cytokines and induces anorexia (37). Any of these could indirectly promote muscle wasting and fat loss. Mechanisms by which TNF-alpha might directly lead to catabolism are less clear. Potential mechanisms are by inhibiting myoblast differentiation that could limit the regenerative response of satellite cells to muscle injury, regulating insulin resistance, as an inducer of apoptosis in pre-adipocytes and adipocytes, and as a positive mediator of lipolysis (Fig. 1) (38, 39). Multiple pathways mediate the cellular response to TNF-alpha with activation of NF-kappaB, a primary mediator of transcriptional control, being the major candidate for catabolic signaling (40). TNF-alpha is also known to induce the expression and production of MCP-1 in adipose tissue (41).

In addition to TNF-alpha, IL-6 is also a major mediator of the hepatic acute phase response in CAC. IL-6 inhibits hepatic albumin production and correlates positively with serum levels of C-reactive protein (CRP) in pancreatic cancer patients. Increased levels of IL-6 are associated with large tumor size, leading to significantly greater weight loss and a poorer overall prognosis (42). IL-6 is considered a prime regulator of the acute phase response in cachectic patients. In one study, patients with non-small cell lung cancer displaying weight loss showed a significant increase in circulating IL-6 compared with weight-stable NSCLC patients (43). However, another study did not find
a correlation between TNF-alpha, IL-1, IL-6 and weight loss in 61 terminal cancer patients (44). Interestingly, when tumors are removed from cachectic rodents, body mass can return to normal correlating with a significant decrease in circulating IL-6 levels (45). In addition, clones of C26 adenocarcinoma cell that produced IL-6 caused cachexia when implanted into mice, whereas those that did not had no effect on body mass (46). When IL-6 producing human melanoma or prostate cancer cells are implanted into mice, cachexia is induced, and that administration of an IL-6 neutralizing antibody to human IL-6 prevented body mass loss (47). However, recent trials of a monoclonal anti-IL-6 antibody in weight-losing lung cancer patients has shown reversal of anorexia, fatigue, and anemia, but no significant effect on loss of lean body mass (48). Furthermore, the factor causing depletion of adipose tissue in C26 tumor bearing mice is different from IL-6 or TNF-alpha, suggesting that IL-6 cannot be the only factor causing cachexia in tumor bearing mice (49). Although IL-6 has been postulated to be a tumor growth factor, the functional role of tumor-produced IL-6 in modulating skeletal muscle and adipose mass has not been elucidated.

Another important downstream effector of IL-6 signaling is STAT3. STAT3 phosphorylation is mediated through the activation of non-receptor protein tyrosine kinases called JAKs (50). Chronic inflammatory conditions that drive carcinogenesis can also be attributed to genetic alterations that directly affect the STAT3 pathway (50). STAT3 can act in close liaison with NF-kappaB to mediate various steps involved in initiation, promotion and development of cancer (51). Moreover, NF-kappaB and STAT3 control both distinct and overlapping groups of genes involved in CAC (50, 51). More recently, it was shown IL-6/STAT3 activation, induced skeletal muscle to
synthesize acute phase proteins, thus establishing a molecular link between the observations of high IL-6, increased acute phase response proteins and muscle wasting in cancer. These results suggest a mechanism by which STAT3 might causally influence muscle wasting by altering the profile of genes expressed and translated in muscle such that amino acids liberated by increased proteolysis in cachexia are synthesized into acute phase proteins and exported into the blood (52). Additionally, inhibition of JAK/STAT3 signaling through pharmacological or genetic means results in reduced muscle atrophy downstream of IL-6 or cancer (52). These results indicate that STAT3 is a primary mediator of muscle wasting in cancer cachexia and other conditions of high IL-6 family signaling.

**Cancer Associated Cachexia and Radiation Resistance**

Significant overlap is present in the pro-inflammatory pathways and downstream effectors involved in CAC and radiation resistance. Through activation of NF-kappaB and STAT3, similar transcriptional programs can be initiated for both processes. It is possible that cachexia-inducing tumors are more radioresistant due to modulation of pathways stimulated by the upregulation of pro-inflammatory networks. One of the main questions currently unanswered in the field is how does a tumor benefit from the induction of cachexia? It is possible that the induction of cachexia is a stochastic process attributable to random mutations occurring in the tumor (53). However, this would make it hard to explain why CAC occurs with relatively high frequency for certain tumor types. Another hypothesis is that the tumor benefits from the release of substances, such as free fatty acids (FFA) from adipose tissue or amino acids (AA) from muscle, during CAC development. A rapidly proliferating and dividing tumor requires
glucose, lactate, FFA and AA to maintain its high energy demands and anabolic synthesis. It is thus possible that the tumor benefits from CAC by acquiring these factors (9, 53). Murine models of cancer have demonstrated that dietary supplements significantly increase tumor growth (54, 55). Also, in murine models of acute fasting increased circulating FFA concentrations lead to increased tumor proliferation (54, 55). By utilizing an enhanced pro-inflammatory state to promote the release of ‘building blocks’ from target organs, cachexia-inducing tumors could additionally develop increased radioresistance. Potentially, some cachectic tumors could rely more on fatty acid oxidation for their metabolism and less on glycolysis under certain conditions, thereby generating a hypoxic state, a situation that would also contribute to increased radioresistance (56).

An additional link between pro-cachexia cytokines and radiation resistance is the recent finding of the role for G0/G1 switch gene 2 (G0S2) in regulating lipolysis. G0S2 was first described in lymphocytes as a protein highly expressed between the G0 and G1 phases of the cell cycle (57). The expression of G0S2 is tightly regulated by PPAR-gamma and PPAR-alpha, as well as being influenced by nutritional status, insulin, and TNF-alpha (58, 59). G0S2 interacts with cytosolic adipose triglyceride lipase (ATGL), that catalyzes the rate-limiting first step converting triacylglycerol into diacylglycerol and free fatty acid. This interaction impedes its interaction with triacylglycerol (TAG), thus inhibiting TAG hydrolysis (58). Interestingly, TNF-alpha can reduce G0S2 expression and thus increase lipolysis via changes in ATGL activity (60). The potential effect of G0S2 on cell cycle regulation and the DNA damage response has not been fully investigated (Fig. 1).
Separate from the pro-inflammatory induction of radioresistance, another mechanism of interest is the status of the DNA repair machinery in cachexia-inducing tumors. Werner syndrome is a rare autosomal recessive disorder known for its premature aging phenotype including loss of hair, cataracts, atrophy of peripheral soft tissue, diabetes mellitus, and atherosclerosis. Mutations in a DNA helicase gene has been identified as the cause of the disease (61). One common feature of Werner syndrome is insulin resistance, but how such resistance occurs in this syndrome is unknown. It has been previously observed that visceral fat accumulation is strongly associated with insulin resistance in Werner syndrome (62). Administration of pioglitazone, a thiazolidinedione, improves insulin sensitivity, glucose tolerance, lipid metabolism, and abdominal fat distribution (63). Additionally, pioglitazone treatment leads to a reduction in IL-6 levels in Werner patients (64). This further supports a potential link between the DNA repair machinery and CAC.

Conclusions and prospectives

The mechanisms of cancer associated cachexia are complex and multifactorial. Since metabolic alterations often appear soon after the onset of tumor growth, the scope of appropriate treatment could influence the course of the patient’s clinical state or, at least, prevent the decreased performance status often associated with CAC. A better understanding of the molecular mechanisms involved could potentially contribute to improved patient outcomes. At the present time, single agents or limited combinations of anti-inflammatory/immune modulatory agents have been tested for halting or
reversing the effects of CAC in advanced cancer patients. Unfortunately, most of these trials have not demonstrated any substantial benefits (65-68). In fact, one study demonstrated an overall survival benefit for cachectic patients utilizing a non-pharmacological intervention: better palliative/supportive care (69). However, a better understanding of the role of cytokines, both host- and tumor-derived and how they affect the molecular mechanisms accounting for protein wasting in skeletal muscle and lipolysis of adipose tissue is essential for the design of future strategies and that could translate into more effective therapies. Understanding the intracellular signaling mechanisms, particularly transcription factors, may also be very important for the design of effective therapeutic approaches. An additional aspect is the severity of CAC symptoms. Since CAC has recently been separated into three stages: pre-cachexia, cachexia and refractory cachexia, it could be that patients with more advanced cachexia symptoms will be less responsive to any intervention. One could make the argument that future CAC clinical trials should be designed with appropriate and specific eligibility criteria and include patients with earlier stages of CAC.

In subsequent future studies, there is a need for delineating the pathways shared by CAC and radiation resistance in order to develop potential pharmacological interventions. As mentioned above, it would be of interest to determine if there is an inherent radioresistance of cachexia-inducing tumors in vitro/in vivo, the status of the DNA repair machinery in cachexia-inducing tumors, the role the cachectic tumor microenvironment plays on radiation resistance, and if altering the inflammatory response can reduce both the severity of CAC and degree of radioresistance. These mechanisms could contribute to the observed radioresistance in pancreatic and head
and neck cancers, both of which are associated with CAC (70-73). Potentially by attenuating one process, it may lead to increased benefit for the other. For patients who are able to receive combined modality therapy (i.e. chemoradiation), treatment that inhibits cachexia could also increase the tumor’s radiosensitivity, leading to improved local control and potentially, overall improved survival.
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Contributors

T.K.P. initiated the strategy for this current review. A.L and P.I. wrote the main review. All three authors went through current literature and shaped the manuscript.
Figure 1. Multiple downstream effects of TNF-alpha signaling in radiation resistance and cancer-associated cachexia (CAC). Cachexia-inducing tumors promote the circulation of multiple pro-inflammatory cytokines, either by directly releasing these factors or by promoting the host cells to release these cytokines. One of the more important cytokines implicated is tumor necrosis factor-alpha (TNF-a). Treatment with IR can elicit cellular signaling by two distinct mechanisms: 1) nuclear activation – the damaged DNA activates the DNA repair machinery which arrest the cell cycle; 2) cytoplasmic activation – IR can generate reactive oxygen species (ROS) which can activate receptor tyrosine kinases (RTKs), which initiate downstream pro-survival signaling pathways. These signaling cascades then converge on transcription factors such as NF-kappaB. In addition, TNF-a is released by the tumor and can act through the TNF receptor (TNFR) and downregulate G0/G1 switch gene 2 (G0S2), which binds to and negatively regulates adipose triglyceride lipase (ATGL) activity. ATGL is important in catalyzing the conversion of triacylglycerol (TAG) into diacylglycerol (DAG) and free fatty acid (FFA). Therefore, TNF-a can increase ATGL activity and promote increased release of FFA. G0S2 could potentially play a role in IR mediated DNA damage response/cell cycle arrest. It would be of interest to analyze the DNA damage response in adipose tissue and its possible effect on lipid metabolism.


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Ionizing Radiation → RTK

Tumor Cell

Cytoplasm

Nucleus

AKT

JAK

DNA-PKcs

ATM

NF-κB

STAT3

DNA Damage

DNA-PKcs

ATM

NF-κB

STAT3

TNF-α

TNFR

G0S2

ATGL

TAG

FFA

DAG

Lipid Droplet

White Adipose Tissue

Figure 1
Molecular Cancer Research

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