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 proinflammatory mediators participate in 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 patients with cancer 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 patients with pancreatic cancer and 60% of patients with lung cancer present at diagnosis with cachexia (9). Cachectic patients also have higher radiotherapy- 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 deaths of patients with cancer (11, 12). Although awareness is growing 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: precachexia, 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 focuses 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 radiotherapy.

Inflammation and Radiation Resistance

Radiotherapy 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 radiotherapy at some point in the course of their treatment (15). The technological sophistication of imaging, planning, and radiotherapy delivery has enabled more cancers to be treated with higher and more tumoricidal doses of ionizing radiation 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

Abstract

Dysregulated inflammatory responses are key contributors to a multitude of chronic ailments, including cancer. Evidence indicates that disease progression in cancer is dependent on the complex interaction between the tumor and the host microenvironment. Most recently, the inflammatory response has been suggested to be critical, as both the tumor and microenvironment compartments produce cytokines that act on numerous target sites, where they foster a complex cascade of biologic outcomes. Patients with cancer-associated cachexia (CAC) suffer from a dramatic loss of skeletal muscle and adipose tissue, ultimately precluding them from many forms of therapeutic intervention, including radiotherapy. The cytokines that have been linked to the promotion of the cachectic response may also participate in radiation resistance. The major changes at the cytokine level are, in part, due to transcriptional regulatory alterations possibly due to epigenetic modifications. Herein we discuss the role of inflammatory pathways in CAC and examine the potential link between cachexia induction and radiation resistance. Mol Cancer Res; 11(9): 967–72. ©2013 AACR.
that ionizing radiation not only damages cellular DNA but also affects disparate cellular components that collectively elicit the multilayered biologic 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 ionizing radiation (1–5 Gy) occurs at two distinct sites: (i) 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 (ii) cytoplasmic—signaling at the receptor level that is partly triggered by reactive oxygen species (ROS) inactivation of phosphatases and subsequent ligand-independent activation of receptor tyrosine kinases (RTK; 19, 20). Both of these events elicit prosurvival and antiapoptotic responses (the inducible radioresistance model) that increase overall tumor cell survival.

Signaling from the cell-surface receptors and from damaged DNA leads to downstream pathways that ultimately result in the activation of a variety of transcription factors important in governing gene expression patterns (21). Radiation-induced transcription factors chiefly include the dynamic NF-κB 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, matrix metalloprotease (MMP), and proinflammatory cytokines (23–25). The production of these growth factors and angiogenic factors in response to radiotherapy by activated transcription factors is the principal mechanism of inducible radioresistance, whereas constitutively activated NF-κB or STAT3 contributes to intrinsic radioresistance (Fig. 1; ref. 27).

Several studies have investigated the impact of NF-κB inhibition on radiosensitivity in different models (28). Clinical approaches for NF-κB 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 short hairpin RNA (shRNA) against STAT3 increased radiosensitivity of human squamous cell carcinoma (A431; ref. 29). The proteomic analysis of radioresistant prostate cancer cells confirmed that the radioresistant phenotype is the result of multiple mechanisms with radiation-induced activation of the Janus-activated kinase (Jak)-STAT pathway playing a significant role (30, 31).

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 ionizing radiation (32). Furthermore, studies using mice with germ-line deletions in TNF receptors (TNFR) 1 and 2 or TNF-α as well as treatment of wild-type mice with a soluble TNFR fusion protein (etanercept), revealed that radioresistance required intact TNF-α signaling. Radiation exposure upregulated VEGF in macrophages, and VEGF-neutralizing antibodies enhanced the antitumor response to ionizing radiation (32).

**Cytokines and Cancer-Associated Cachexia**

The production of chemical mediators associated with CAC can be divided into two categories: (i) those with the growing tumor as the source and (ii) humoral factors (mainly...
cytokines) secreted by the cells in the tumor microenvironment (5). The primary inflammatory cytokines that have been implicated in CAC are TNF-α and interleukin-6 (IL-6; refs. 33, 34). 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 biologic 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 (35, 36). During inflammatory processes, MCP-1 promotes the attraction of monocytes, T lymphocytes, and natural killer cells to the site of inflammation (37). 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-α has long been associated with muscle pathology and was originally named “cachectin” in recognition of its catabolic action (38). To date, evidence of increased TNF-α in plasma of patients with cancer is controversial, perhaps due to the different sensitivities of the assay methods, short half-life of TNF-α in vivo, or localized paracrine production of TNF-α. The mechanism of TNF-α action in vivo remains largely enigmatic, although it has long been recognized that TNF-α may stimulate catabolism via indirect mechanisms. TNF-α alters circulating levels of hormones that regulate muscle growth and affect tissue sensitivity to such factors. TNF-α also stimulates production of catabolic cytokines and induces anorexia (39). Any of these mechanisms could indirectly promote muscle wasting and fat loss. Mechanisms by which TNF-α 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 preadipocytes and adipocytes, and as a positive mediator of lipolysis (Fig. 1; refs. 40, 41). Multiple pathways mediate the cellular response to TNF-α with activation of NF-κB, a primary mediator of transcriptional control, being the major candidate for catabolic signaling (42). TNF-α is also known to induce the expression and production of MCP-1 in adipose tissue (43).

In addition to TNF-α, 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 in patients with pancreatic cancer. Increased levels of IL-6 are associated with large tumor size, leading to significantly greater weight loss and a poorer overall prognosis (44). 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 (NSCLC) displaying weight loss showed a significant increase in circulating IL-6 compared with weight-stable patients with NSCLC (45). However, another study did not find a correlation between TNF-α, IL-1, IL-6, and weight loss in 61 patients with terminal cancer (46). Interestingly, when tumors are removed from cachectic rodents, body mass can return to normal levels, correlating with a significant decrease in circulating IL-6 levels (47). In addition, clones of C26 adenocarcinoma cells that produced IL-6 caused cachexia when implanted into mice, whereas those that did not had no effect on body mass (48). 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 (49). However, recent trials of a monoclonal anti–IL-6 antibody in weight-losing patients with lung cancer have shown reversal of anorexia, fatigue, and anemia but no significant effect on loss of lean body mass (50). Furthermore, the factor causing depletion of adipose tissue in C26 tumor–bearing mice is different from IL-6 or TNF-α, suggesting that IL-6 cannot be the only factor causing cachexia in tumor–bearing mice (51). 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 nonreceptor protein tyrosine kinases called JAK (52). Chronic inflammatory conditions that drive carcinogenesis can also be attributed to genetic alterations that directly affect the STAT3 pathway (52). STAT3 can act in close liaison with NF-κB to mediate various steps involved in initiation, promotion, and development of cancer (53). Moreover, NF-κB and STAT3 control both distinct and overlapping groups of genes involved in CAC (52, 53). More recently, it was shown that 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 (54). In addition, inhibition of JAK/STAT3 signaling through pharmacologic or genetic means results in reduced muscle atrophy downstream of IL-6 or cancer (54). 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 proinflammatory pathways and downstream effectors involved in CAC and
radiation resistance. Through activation of NF-κB 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 proinflammatory networks. One of the main questions currently unaddressed in the field is how a tumor benefits 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 (55). 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 from muscle during CAC development. A rapidly proliferating and dividing tumor requires glucose, lactate, FFA, and amino acids to maintain its high energy demands and anabolic synthesis. It is thus possible that the tumor benefits from CAC by acquiring these factors (9, 55). Murine models of cancer have shown that dietary supplements significantly increase tumor growth (56, 57). Also, in murine models of acute fasting increased circulating FFA concentrations lead to increased tumor proliferation (56, 57). Using an enhanced proinflammatory 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 (58).

An additional link between precachexia 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 (59). The expression of G0S2 is tightly regulated by PPAR-γ and PPAR-α as well as being influenced by nutritional status, insulin, and TNF-α (60, 61). G0S2 interacts with cytosolic adipose triglyceride lipase (ATGL), that catalyzes the rate-limiting first step converting triacylglycerol into diacylglycerol and FFA. This interaction impedes its interaction with triacylglycerol (TAG), thus inhibiting TAG hydrolysis (60). Interestingly, TNF-α can reduce G0S2 expression and thus increase lipolysis via changes in ATGL activity (62). The potential effect of G0S2 on cell-cycle regulation and the DNA damage response has not been fully investigated (Fig. 1).

Separate from the proinflammatory induction of radiosensitivity, 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 have been identified as the cause of the disease (63). 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 (65). In addition, pioglitazone treatment leads to a reduction in IL-6 levels in patients with Werner syndrome (66). These findings further support a potential link between the DNA repair machinery and CAC.

Conclusions and Prospectives

The mechanisms of CAC are complex and multifactorial. Because 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 present, single agents or limited combinations of anti-inflammatory/immunomodulatory agents have been tested for halting or reversing the effects of CAC in patients with advanced cancer. Unfortunately, most of these trials have not shown any substantial benefits (67–70). In fact, one study showed an overall survival benefit for cachectic patients using a nonpharmacologic intervention: better palliative/supportive care (71). 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 that could be translated into more effective therapies. An understanding of 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. As CAC has recently been separated into three stages—precachexia, 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 future studies, there is a need for delineating the pathways shared by CAC and radiation resistance to develop potential pharmacologic interventions. As mentioned earlier, it would be of interest to determine whether 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 whether altering the inflammatory response can reduce both the severity of CAC and the 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 (72–75). 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 radioresistance, leading to improved local control and, potentially, overall improved survival.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: P. Iyengar, T.K. Pandita
Development of methodology: P. Iyengar
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P. Iyengar

References
7. Lees J. Incidence of weight loss in head and neck cancer patients on chemoradiation therapy (i.e., chemoradiation), treatment that overall improved survival.

Cachexia and Radiation Resistance

Writing, review, and/or revision of the manuscript: A. Laine, P. Iyengar, T.K. Pandita
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P. Iyengar

Acknowledgments
The authors thank the members of their laboratories for the discussions and their suggestions.

Grant Support
The research in authors’ laboratory is supported by NIH grants CA129537, CA154320, and U19AI091175 (to T.K. Pandita).

Received April 16, 2013; revised May 19, 2013; accepted June 11, 2013; published OnlineFirst June 20, 2013.

www.aacrjournals.org
Mol Cancer Res; 11(8) September 2013 971

Published OnlineFirst June 20, 2013; DOI: 10.1158/1541-7786.MCR-13-0189

Downloaded from mcr.aacrjournals.org on April 20, 2017. © 2013 American Association for Cancer Research.


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

Aaron Laine, Puneeth Iyengar and Tej K. Pandita


Updated version
Access the most recent version of this article at:
doi:10.1158/1541-7786.MCR-13-0189

Cited articles
This article cites 75 articles, 22 of which you can access for free at:
http://mcr.aacrjournals.org/content/11/9/967.full.html#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.