Systemic DNA Damage Related to Cancer

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Abstract

The importance of bystander effects is becoming more appreciated, as studies show they may affect the course of cancer and other chronic diseases. The term "bystander effects" refers to changes in naïve cells sharing the same milieu with cells that have been damaged. Bystander cells may be in contact with, or distant from, damaged cells. In addition, it has been shown in culture that not only physically damaged cells, but also cells that have become abnormal (i.e., cancerous or senescent) may induce bystander effects. Recently, we have shown a similar effect in animals. Mice harboring subcutaneous tumors exhibited elevated levels of DNA damage in distant organs. In contrast to cell culture, immune cells seemed to be involved in tumor-induced bystander effects in animals because CCL2-null tumor-bearing mice did not exhibit increased distant DNA damage. Here, we discuss some of the implications of these observations. Cancer Res; 71(10); 1–5. © 2011 AACR.

Bystander, Nontargeted, and Abscopal Effects

The terminology used to describe effects on cells sharing the same milieu with damaged or abnormal cells may be confusing. The term "bystander effect" was used to describe results obtained in cell cultures irradiated with alpha particles. When only 1% of the cells were traversed by alpha particles, 30% of the cells exhibited sister chromatid exchanges, indicating that many nontargeted cells also sustained damage (1). These "bystander" cells exhibit various types of genomic instability (2). The observation that the targeted cells release cell damage–inducing substances into the media was seen when media conditioned on irradiated cultures were shown to induce various types of damage in unirradiated cultures (2). This phenomenon was named the radiation-induced bystander effect.

In 1953, Mole (3) described "out-of-field," or abscopal effects, which they defined as "an action at a distance from the irradiated volume but within the same organism." For example, in mice, irradiation of the cranium or one side of the body led to genetic and/or epigenetic changes in shielded organs, such as skin and spleen (4, 5). Abscopal effects can have oncogenic consequences. DNA double-strand breaks (DSB), apoptosis, and, ultimately, tumors were induced in the shielded cerebella of Patched-1 (Ptch1), a radiosensitive strain of mice, after X-ray exposure of the remainder of the body (6).

Strikingly, abscopal effects can be transmitted to future generations. Although genomic instability was known to be elevated in offspring of parents whose germ cells were directly irradiated prior to conception as part of a medical procedure (7), Tamminga and colleagues showed, in male rats, that localized cranial irradiation resulted in an accumulation of unrepaired DNA damage in their sperm cells. This abscopal effect was manifested as epigenetic deregulation in the unexposed progeny conceived after paternal exposure (8).

Abscopal effects have been shown to result from a number of other localized stimuli, such as surgery, hyperthermia, and laser immunotherapy, among others (reviewed in ref. 9), leading to the proposal that the term "abscopal" be used interchangeably with "distant bystander effect."

Abscopal Effects and Cancer

The presence of a tumor has been shown to induce both proinflammatory and damage signals in cells in the immediate tumor microenvironment, possibly because of the production of reactive oxygen, nitrogen species (ROS/RNS), and/or cytokines (10).

However, results obtained in cell culture suggest that tumors may exert their influence far beyond the microenvironment to tissues distant from a tumor. Normal cell cultures sustained elevated levels of DNA damage when incubated with medium previously conditioned on tumor cell cultures (11). These results led us to hypothesize that the presence of a tumor in vivo may induce DNA damage in distant tissues, because blood or lymph might be "conditioned" by the tumor and then expose distant tissues. To test this hypothesis, we prepared several cohorts of mice implanted with a variety of s.c. tumors,
levels of DNA DSBs, as marked by γ-H2AX foci, were present not only in the tumor as expected (3.1- to 5.7-fold above those in normal skin), but also in several distant tissues. The duodenum and colon exhibited γ-H2AX foci incidences 2.3 to 3.7 fold elevated over controls; lesser, but still significant, elevations were found in the stomach and rectum.

Elevated levels of oxidative clustered DNA lesions (OCDL) were found not only in the tumor mass (1.7- to 4.4-fold over controls), but also in the gastrointestinal (GI) tract tissues, 1.6- to 3.8-fold over controls. OCDLs are hallmark oxidative DNA lesions, but it is quite likely that oxidized RNA, protein, and lipid molecules are also formed under these conditions. Their presence would also be expected to contribute to stress, as the cell attempts to repair and/or remove the damage. However, although damaged protein and lipid molecules can be replaced with nascent molecules, the damage to DNA is potentially more serious, because it can have long-term genotoxic consequences, as it may not be faithfully repaired.

In addition, 2 other tissues, ovary and lung, which did not exhibit significantly elevated γ-H2AX foci, did exhibit elevated OCDL levels. This wider incidence of elevated OCDLs versus γ-H2AX foci may be attributable to the mechanisms of lesion formation. γ-H2AX focus formation is favored in tissues with larger fractions of proliferating cells, such as those in the GI tract, in which replication forks may participate in DSB formation. OCDLs form equally well in highly proliferating and less proliferating tissues, which would include ovary and lung tissues. In addition to internal tissues, skin samples were taken at distances up to 2 cm from the tumor mass; these also exhibited elevated levels of both types of DNA lesions compared with controls.

To gain insight into the nature of the signaling from tumor to distant tissues, we measured the levels of 56 cytokines in the blood from the different mouse cohorts, hypothesizing that blood could be a major route of transmission of DNA damage—inducing signals. Only 3 cytokines, CCL2/MCP-1, CXCL10/IP-10, and CCL7/MCP-3, were elevated more than 3-fold in the blood of tumor-bearing mice (12). CCL2 and CCL7 are 2 members of the monocyte chemoattractant protein (MCP-1 to MCP-5) family, each member attracting a different subset of leukocytes (13). CXCL10/IP-10 is also a chemoattractant for several types of immune cells, including monocytes (14).

These results indicate that monocytes and/or macrophages might play important roles in the induction of abscopal DNA damage present in the tumor-bearing mice. Upon examination, the tissues with elevated DNA damage levels in the tumor-bearing mice, including the gut and skin, were found to contain elevated numbers of activated macrophages. Interestingly, kidney tissue that did not exhibit increased levels of DNA damage also did not contain increased numbers of macrophages. These results suggest a mechanistic model, in which macrophage activation in the tumor induces the secretion of proinflammatory cytokines that affect distant tissues by inducing DNA damage in the cells of organs containing activated macrophages, indicating an association between inflammation and abscopal DNA damage responses in these tumor-bearing mice.

CCL2 was the first chemokine characterized. In addition to being an attractant for many immune cells, including monocytes, lymphocytes, natural killer cells, microglia, and immature dendritic cells to sites of injury, CCL2 seems to be involved in macrophage activation. Sources of CCL2 secretion include normal tissues, immune cells, and some cancer cells (15). On recipient cells, CCL2 binds to CCR2, a G-protein-coupled receptor that targets cellular pathways dependent on phosphoinositide 3-kinase and protein kinase C, among others. This binding leads to elevated COX-2 production and TGF-β upregulation, both major players in bystander signaling and carcinogenesis (reviewed in ref. 12).

CCL2 has been associated with autoimmune and other diseases related to chronic inflammation (16) and has been implicated in the progression and prognosis of several cancers (17). The role of CCL2 in cancer development has been controversial, with evidence of pro- and antitumorigenic effects. However, recent studies suggest that CCL2 contributes to cancer growth. Blockade of CCL2 action can inhibit tumor growth of primary and metastatic disease in animal models of non–small cell lung and prostate cancer. The mechanisms of retarded tumor growth include alteration of tumor macrophages to more antitumor phenotype and activation of cytotoxic CD8+ T lymphocytes, accompanied by a significant decrease in microvascular density (18).

Thus, CCL2 seems to play important roles in several disease-related biological processes. To examine whether the association between CCL2 and tumor-induced abscopal DNA damage was causal, we implanted our model tumors into syngeneic CCL2-null mice. Strikingly, there was no measurable increase in abscopal DNA damage in the tumor-bearing CCL2-null mice (12). These results indicate that CCL2 is essential in the tumor-induced abscopal genotoxic response in vivo, most likely through the activation of macrophages. Taken together, these findings suggest a model in which abscopal DNA damage in these tumor-bearing mice is due to the presence of activated macrophages in the distant tissues. CCL2 seems to play essential roles in this process, possibly as an activating cytokine for macrophages resident in the distant tissues, and/or by inducing macrophage transmigration to these tissues.

Roles of CCL2, Chronic Inflammation, Parainflammation, and Premetastatic Niches in Aging-Related Diseases and Oncogenesis

Although toxic in excess, ROS also function as important signaling molecules. Thus, several pathways help maintain ROS homeostasis. We observed abscopal DNA damage of ~1 γ-H2AX focus per cell in the GI tissues of tumor-bearing mice, approximately 2- to 3-fold over background. Therefore, an important question is whether this modest elevation of damage is part of the homeostatic mechanisms of the organism in the face of...
Figure 1. Chronic activation of the immune system is responsible for an abscopal or bystander effect related to cancer, in addition to other human diseases. Recurrent exposure to endogenous and exogenous stresses (psychological stress, infectious agents, oxygen metabolism, exposure to exogenous DNA damaging agents, etc.) promotes both changes in immune homeostasis and normal aging progression. Alteration in immune homeostasis leads to the activation of immune cells that result in the production of ROS, RNS, and cytokines, and, in turn, chronic inflammation. Senescent cells, which also arise from normal aging or genotoxic stresses, are another source of cytokines and ROS/RNS. Chronic inflammation is involved in age-related diseases and is also thought to be involved in tumorigenesis and cellular senescence. Although immune signaling and cellular senescence could act as tumor suppressors early in life (red), this function would decrease with age (blue). Tumor cells release cytokines and ROS/RNS, which perturb surrounding normal cells and can lead to cellular senescence. In addition to involvement in chronic diseases, we recently showed that activated immune cells are involved in a bystander effect related to cancer. In this study, we hypothesized that the presence of tumor-activated macrophages (or other activated immune cells) would contribute to increased CCL2 levels in the bloodstream. In turn, higher CCL2 levels would result in a parainflammation response leading to macrophage activation and ROS production in distant tissues, leading to both DNA oxidative damage and DNA DSB formation.
chronic irritation. Macrophages are differentially activated depending on whether the source of inflammation is transient or persistent. The acute response to transient damage leads to the classical macrophage response, M1. However, when an irritation is chronic, such as in the presence of a tumor, macrophages seem to be activated in an alternative mode, M2. Chronic inflammation is characterized by the continued presence of proinflammatory factors, including CCL2, at levels higher than baseline. Tissues targeted by chronic inflammation are characterized by macrophage and lymphocyte infiltration, development of abundant blood vessels, production of excess fibrous connective tissue, and frequently, tissue necrosis.

The number of known diseases related to chronic inflammation in which CCL2 may be involved is increasing sharply. Neurodegenerative diseases (Alzheimer and Parkinson), liver diseases, type II diabetes, joint damage, cardiovascular diseases, periodontal disease, ulcerative colitis, and pulmonary diseases, among others (Fig. 1), all seem to involve proinflammatory factors. Studies in humans and primates have shown that CCL2 is present in macrophage-rich atherosclerotic plaques, is upregulated in the intestinal mucosa of inflammatory bowel diseases such as ulcerative colitis, and plays a role in nephropathies and corneal inflammatory diseases (reviewed in refs. 16, 20) and Alzheimer disease (21). Yet, the immune system may not be the only source of proinflammatory factors. Senescent cells (fibroblast and epithelial cells losing their ability to divide because of exposure to toxins and DNA damage accumulation) were shown to secrete proinflammatory factors including CCL2 (22).

There is mounting evidence linking proinflammatory processes and cancer (16). ROS and RNS are secreted from activated immune cells and/or stressed epithelial cells, resulting in DNA damage and genomic instability that may contribute to carcinogenesis. Cellular senescence and apoptosis are 2 powerful anticancer mechanisms, both removing cells from the pool of proliferating cells. But unlike apoptotic cells, senescent cells remain metabolically active and may secrete proinflammatory factors into their surrounding microenvironment. These factors may function deleteriously on neighboring cells by promoting both cell transformation and malignancy (Fig. 1; ref. 23). Senescent cells increase in number with age and could be involved in age-related pathologies, exhibiting their beneficial action only early in life (24).

In addition to stimulating oncogenesis and tumor progression, systemic inflammation may also play a role in determining where metastases occur. In 1989, Paget (25) proposed that cancer "seeds" would only colonize receptive "soils." Recently, this notion has been extended at the cellular and molecular level in the concept of the "premetastatic niche" (26). In this model, molecular factors secreted by the tumor cells stimulate normal fibroblasts in future metastatic sites to produce proteins that bind cells to the extracellular matrix, creating a favorable environment for bone-marrow cells. Named "premetastatic niches," these microenvironments present a favorable "soil" for the pioneer disseminating tumor cell "seeds." These cells activate resident fibroblasts and macrophages at metastatic sites and interact with the components of the premetastatic niche, paving the way for future tumor cell arrival (reviewed in refs. 26, 27).

Targeting CCL2-CCR2 in Therapeutic Treatments

Because CCL2 has been implicated not only in many inflammatory diseases and cancer, but also in autoimmune illnesses (16), modulating the immune response by specifically targeting the CCL2-CCR2 partnership presents an attractive therapeutic strategy in several clinical settings. Many CCR2 antagonists and CCL2-blocking antibodies have been described, and some are undergoing clinical trials for the treatment of various diseases (16, 28). One, bindarit, a CCL2 inhibitor, has been shown to prevent severe disease in mice treated with a compound known to induce ulcerative colitis (29).

However, despite promising results with animal models, human clinical trials for the treatment of autoimmune diseases have been disappointing. Nevertheless, other approaches are being tried. CCL2 production may be targeted in chronically inflamed tissues by using RNA interference technology to silence the CCL2 gene (reviewed in ref. 16), and a combination of inhibitors directed against different cytokine receptors may be used to overcome the redundancy of the cytokine response. Because inhibiting cytokines may compromise their beneficial effects, making the elderly more vulnerable to neurologic disorders (16), future insights on the role(s) of CCL2 as well as the other proinflammatory cytokines may help clinicians develop treatments to control the unwanted effects of chronic inflammation. Finally, because CCL2 could promote tumorigenesis, targeting this cytokine with monoclonal antibodies has also been shown to be an effective therapeutic approach in preclinical trials in prostate cancer (30).

Thus, our recent work shows that tumor-induced abscessal DNA damage is dependent on the presence of CCL2, connecting this process with the much larger field of CCL2-related signaling. These observations show that the presence of a tumor has proinflammatory effects with consequences for the overall homeostasis and genomic stability of the organism. What these consequences are and their relevance to overall organismal health await future studies.

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