Chapter 13

Inflammation and oxidatively induced DNA damage: A synergy leading to cancer development

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List of abbreviations

AG	aminoguanidine
AID	activation-induced cytidine-deaminase
BER	base excision repair
COX2	cyclooxygenase 2
c-PTIO	2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide
CRT	calreticulin
CSFS	colony-stimulating factors
DAMPs	damage associated molecular patterns
DC	dendritic cell
DDR	DNA damage response
DMSO	dimethyl sulfoxide
DSB	double strand break
EGFR	epidermal growth factor receptor
HIF	hypoxia-inducible factor
HMGB1	high-mobility group-protein B1
IBD	inflammatory bowel diseases
ICAM	intercellular adhesion molecule
IFN-γ	interferon gamma
IL	interleukin
IR	ionizing radiation
JNK	c-Jun N-terminal kinase
L-NNA	N(omega)-nitro-L-arginine
MDSC	myeloid-derived suppressor cells
MHC	major histocompatibility complex
MIF 1	macrophage migration inhibitory factor
NADP	nicotinamide adenine dinucleotide phosphate
NER	nucleotide excision repair
NFκB	nuclear factor kappa beta
NO	nitric oxide
NSCLC	nonsmall cell lung cancer

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OCDLs oxidatively induced clustered DNA lesions
PTC papillary thyroid carcinoma
PTEN phosphatase and tensin homologue
RNS reactive nitrogen species
ROS reactive oxygen species
SABR stereotactic ablative body radiotherapy
SASP senescence-associated secretory phenotype
STAT signal transducer and activator of transcription
TAM tumor associated macrophages
TGFβ transforming growth factor-β
TLR4 toll-like receptor 4
TNF tumor necrosis factor
VHL von Hippel-Lindau

Introduction

Cancer is a very real threat to people of all ages and despite decades of research we have failed to conquer the disease. Oncogenic transformation is due to the accumulation of various mutations, whether acquired or inherited and caused by endogenous and/or exogenous agents. These bestow pro-survival capacities to the transformed cells and often allow them to evade and modify the immune response. Multiple genes in the human body precisely control cell growth. Errors in these genes lead to further alterations or mutations. Accumulation of many mutations over time usually leads to a malignant state manifested by high chromosomal instability.

The human body is under continuous attack from both external and internal insults which results in numerous DNA lesions per cell per day (10,000 – 100,000).[1] These lesions can block DNA replication and transcription leading to mutations and possibly transformation and carcinogenesis. Just one unrepaired double-strand break (DSB) can be lethal to the cell or highly mutagenic. Failure to repair any DNA damage leads to apoptotic or necrotic cell death. The DNA damage response (DDR) network detects DNA lesions, signals their presence, and promotes DNA repair. Defects in this pathway are often seen in cancer.

DNA damage can be induced by oxidation and this may eventually progress to carcinogenesis. In addition, cancer is considered a pro-inflammatory disease and a number of current therapies target this pro-inflammatory state within the tumor microenvironment. Thus, in this chapter, we discuss the role(s) of oxidatively induced DNA damage and inflammation in cancer. Overall, a better understanding of the synergy between oxidative DNA damage, inflammation, and cancer, that is, a "lethal triptych" will provide the center for future therapies.

Oxidative DNA damage

Oxidative DNA damage is an inevitable consequence of endogenous and exogenous events, such as cellular metabolism and toxic insults such as exposure to chemicals or IR. Oxidative stress has been associated with various serious diseases including cancer, Alzheimer's, arteriosclerosis diabetes, gastrointestinal disorders, and aging. Oxidative damage occurs when the body is exposed to excessive amounts of electrically charged, aggressive oxygen and nitrogen compounds: reactive oxygen and nitrogen species (ROS, RNS). Whether endogenous or exogenous, these compounds can modify major cellular components such as DNA but also proteins, lipids, and carbohydrates. The effects of free radicals on lipids and lipid components have been thoroughly studied until recently.[2, 3] Focusing on the DNA, purine and pyrimidine bases and sugar moieties can be affected by oxidation. Oxidatively induced DNA lesions, multiple DNA lesions in close proximity (clusters or OCDLs), play a critical role in carcinogenesis mainly due to their repair resistance.[4] DNA-protein crosslinks can also result from oxidation. Though oxidative modifications occur in proteins, lipids, and DNA, since proteins and lipids are readily degraded and resynthesized, the most significant consequence of the oxidative stress is the modifications to the DNA, which can cause mutations and lead to genomic instability (Fig. 1).

Mechanisms of induction

Oxidation is a critical component of energy production by mitochondria, the inflammatory response and, in general, by the cellular defense system. Acute inflammatory response recruits activated leukocytes that can cause extensive DNA damage by secreting various chemical mediators. Some of the oxygen-derived products include hydroxyl radical and superoxide

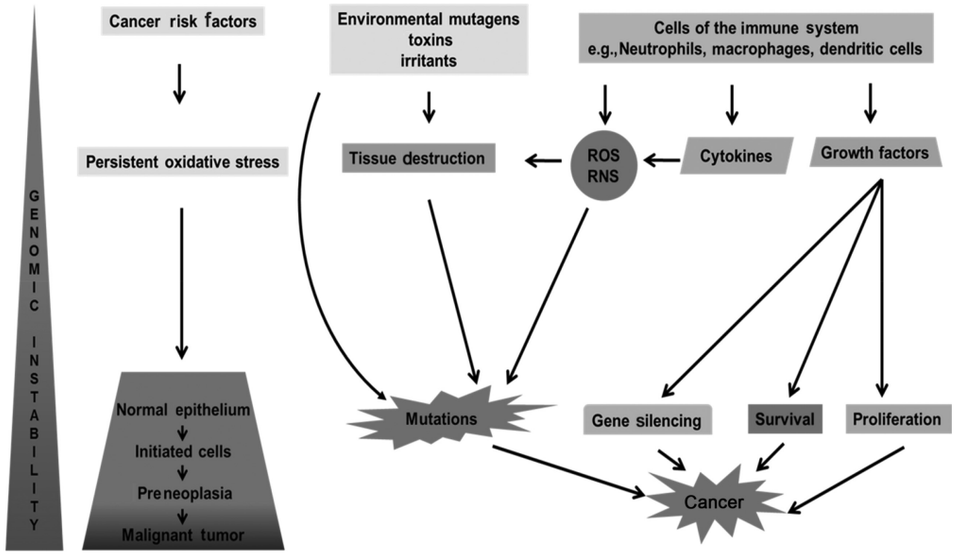

FIG. 1 Persistent oxidative stress leads to neoplastic transformation. The key steps are the production of reactive oxygen and nitrogen species (ROS/RNS) which can lead to the accumulation of mutations and therefore preneoplastic states. In this first initiation step, components of the immune system seem to play a pivotal role through their recruitment in these damage/mutation sites. The occurrence of a tissue malignancy seems always to coincide with genomic and chromosomal instability.

radical. The hydroxyl radical reacts with biological molecules such as DNA, causing damage to the heterocyclic DNA bases and the sugar moiety by a variety of mechanisms. Hydroxyl radical reacts with purines and pyrimidines of DNA by addition to double bonds and by the abstraction of an H from the methyl group of thymine and from each of the C—H bonds of 2′-deoxyribose leading to modifications.[5] This oxidative stress can also lead to DSBs. In order to cope with the oxidation damage cells, use several defense compounds such as antioxidants, antioxidant enzymes, and DNA repair mechanisms.

Pathways of repair

Oxidative DNA damage is repaired by multiple, overlapping DNA repair pathways. Two major mechanisms exist to repair oxidatively induced DNA lesions: base-excision repair (BER) and nucleotide-excision repair (NER). In BER-mediated repair, DNA glycosylase usually detects the damaged base and mediates base removal prior to nuclease, polymerase, and ligase proteins bridging the gap and completing the repair process. On the other hand, NER-mediated repair recognizes base lesions that distort the helical structure. The damaged base is excised as a 22–30 base oligonucleotide resulting in single-stranded DNA that is repaired by proteins such as DNA polymerase before proceeding to ligation. There are two pathways that differ in the mechanism of helix recognition: transcription-coupled NER specifically targets lesions that transcription while global-genome NER covers the other lesions. Other repair pathways include mismatch repair, nonhomologous end joining, and homologous recombination all of which repair DSBs.[6]

Role of inflammation in the induction of oxidative stress and DNA damage leading to cancer

Inflammation is a key component of the tumor microenvironment and a recognized hallmark of cancer.[7, 8] The causal linkage between inflammation and cancer was initially suggested in the 19th century following the observation that tumors often developed in settings of chronic inflammation and that pro-inflammatory cells were present in biopsied tumor specimens.[9] Accumulating evidence shows that chronic inflammation is, in fact, associated with an increased risk of cancer development. Moreover, chronic inflammation is linked to between 15% and 20% of worldwide cancer deaths.[8]

The interlink between inflammation and cancer involves two major pathways which are interconnected: an extrinsic mechanism, where a constant inflammatory state (chronic inflammation) contributes to increased cancer risk; and an intrinsic mechanism, where genetic events (e.g., oncogenes) induce neoplastic transformation triggering the inflammatory cascade.[8] The relationship between cancer and inflammation is discussed in detail below and is summarized in Figs. 1–4.

Extrinsic pathway of carcinogenesis

Inflammatory or infectious conditions can increase cancer risk via the extrinsic pathway. Leukocytes producing inflammatory mediators are primarily responsible for triggering inflammation. Chronic inflammation can be induced by, among other sources, chronic infections, exposure to noxious agents that trigger inflammation (e.g., gastric acid reflux, tobacco, asbestos, and other chemicals) and autoimmune conditions.[8] Due to the presence of ROS and RNS different mutagenic lesions may occur such as 8-oxodG and 8-nitroguanine, with the second being most common between various types of inflammation-related carcinogenesis.[10] Pathogenic infections such as those due to Hepatitis B and C viruses, human papillomavirus (HPV) or *Helicobacter pylori* results in chronic inflammation that favors initiation and progression of tumors.[11] The formation of 8-oxodG at cancer sites of patients with these diseases has been strongly indicated.[12] In all cases, the production of DNA damage and the accumulation of mutations and epigenetic changes is considered critical. Autoimmune diseases such as inflammatory bowel disease for colon cancer and prostatitis for prostate cancer, mechanical, radiation, and chemical insults can also induce inflammation associated with human malignancy (Fig. 4).

The role of the tumor microenvironment (stroma) is being increasingly appreciated as being a critical part of carcinogenesis. Inflammatory cells are an important component of the stroma and milieu fosters proliferation, survival, and migration.[13] Figs. 2, 4, and 5 illustrate how chronic inflammation may contribute to carcinogenesis.

Chronic inflammation promotes the development of blood vessels and the remodeling of the extracellular matrix fostering the perfect environment in which a mutation bearing normal cell can turn potentially malignant. In addition, immune cells like neutrophils and macrophages produce ROS via a plasma membrane-bound nicotinamide adenine dinucleotide phosphate (NADP). Based on in vitro and in vivo data, ROS and RNS that play a vital role in normal cellular metabolism

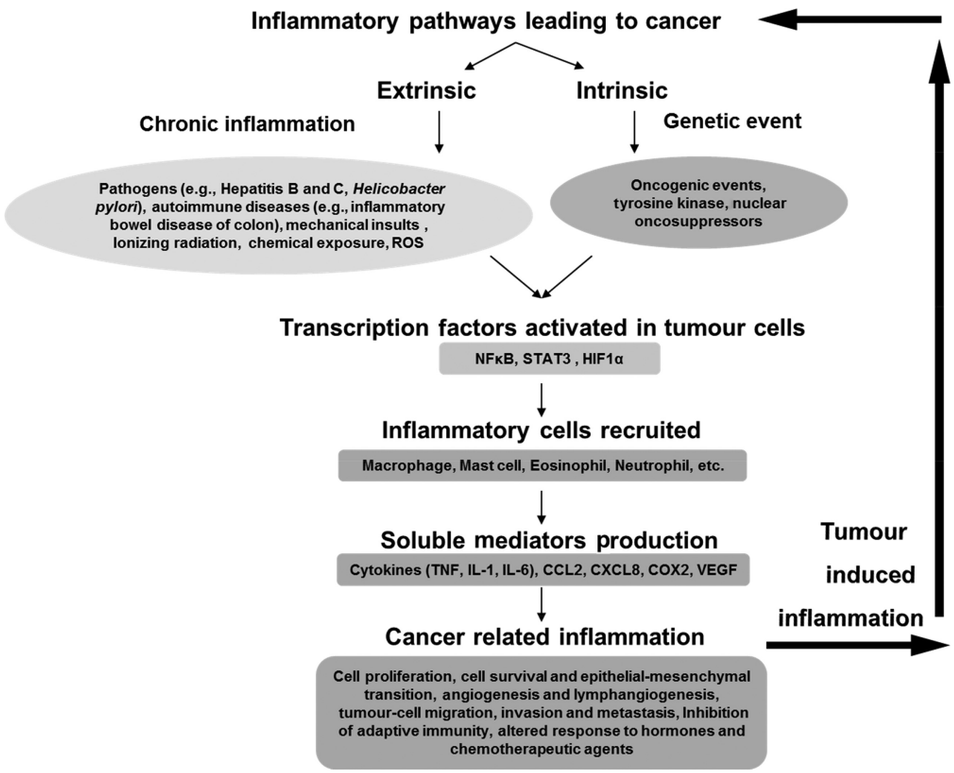

FIG. 2 Inflammatory pathways lead to cancer. As explained in the text the major events and sources contributing to persistent inflammation can be of extrinsic or intrinsic nature. The two pathways can be identified as major contributors to the inflammatory milieu: the intrinsic pathway where genetic events (e.g., mutations in oncogenes) induce neoplastic transformation triggering the inflammatory cascade and the extrinsic pathway where chronic inflammation (e.g., infections and low doses of IR) significantly increases the risk for different types of cancer. The two pathways converge, resulting in the activation of transcription factors (e.g., NFκB, STAT3, HIF) that coordinate the production of inflammatory mediators and the activation of various leukocytes generating an inflammatory microenvironment that nurtures cancer progression. The resulting activation of several transcription factors, inflammatory cells, and chemical mediators like cytokines has been closely bonded to the creation of uncontrolled cell proliferation, mitigation of apoptosis, abnormal angiogenesis, and other molecular changes leading to cancer. The production of DNA damage is considered a critical step. In addition, a high inflammatory response has been also related to tumor cell migration and metastatic ability.

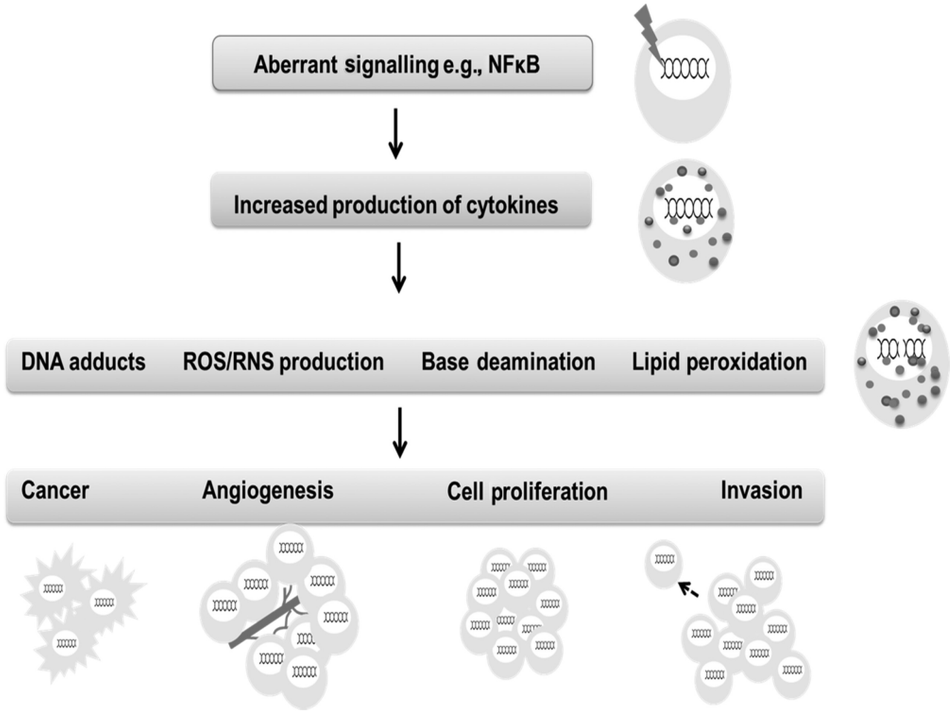

FIG. 3 Signaling of the inflammatory pathways leading to tissue abnormal changes. Aberrant signaling can lead to increased angiogenesis, cell proliferation, and invasion which, in turn, can lead to abnormal growth premalignant and finally malignant states.

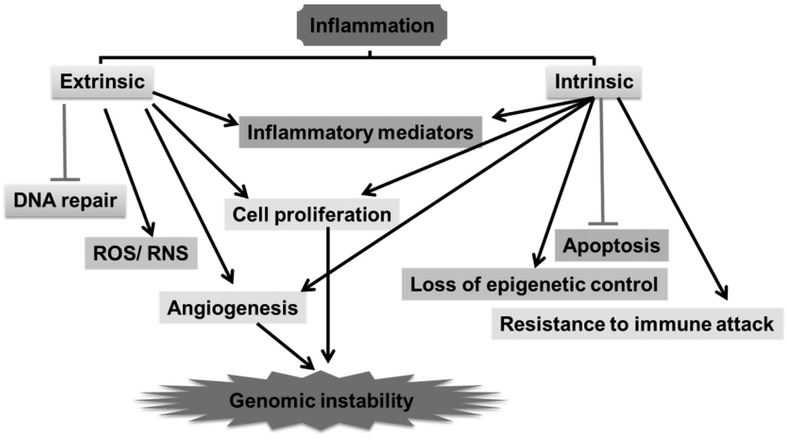

FIG. 4 Overview of pathways leading to cancer. As described in the text, the association of inflammation with the generation of ROS/RNS and DNA damage induces several relating pathways of DNA damage response (DDR) like signaling and induction of DNA repair proteins, cell cycle arrest, and proliferation changes. In every step, there are two major safeguarding mechanisms and these are DNA repair and apoptosis assuming they work properly. A premalignant state will be characterized by loss of all these control mechanisms due to the accumulation of mutations, epigenetic changes, and finally genomic and chromosomal instability.

are inflammation-generated mediators of DNA damage. An increase in oxidative stress leads to a spike in ROS/RNS formation.[14] These highly reactive species can easily bind to proteins, lipids, and DNA. Since proteins and lipids are usually turned over, damage to these macromolecules is usually not detrimental to the cell. However, damage to the DNA can lead to cancer.[4] Interestingly, tumor promoters are able to recruit inflammatory cells and stimulate them to generate ROS/RNS which, in turn, generate DNA lesions and lead to mutations. Numerous reports suggest that tumor growth in vivo, inflammation, and OCDLs are interconnected. Moreover, tumors can induce their DNA damaging effects in distant tissues and organs.[15] Since clustered DNA lesions (both DSBs and OCDLs) are highly mutagenic, these results are biologically relevant.[16] Reports also suggest that OCDL scan is induced by a cytokine CCL2-based mechanism.[17] Researchers are actively pursuing these avenues of inflammation-induced carcinogenesis.

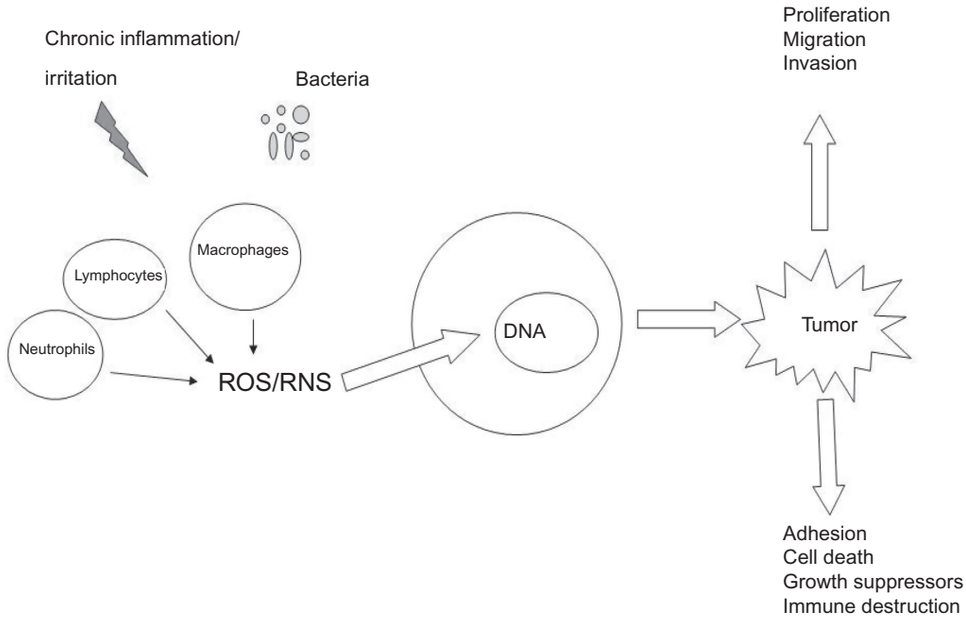

FIG. 5 An overview of the suggested interplay between inflammation and associated oxidative stress. This interplay can affect key aspects of tumorigenesis, angiogenesis, and metastasis. In this model, the generation of oxidative stress and potentially DNA damage through the inflammatory responses involving macrophages and different cytokines like the MCP-1/IL-6 are considered critical.[13]

Several recent reports support the involvement of ROS in cancer-related processes. For instance, ROS production has been demonstrated to be required for mediating K-ras-induced lung cancer in mice.[18] Moreover, ROS released by damaged cells can induce inflammation and trigger the production of pro-inflammatory cytokines by functioning as signaling molecules. New molecular pathways involving mitochondrial damage and ROS production are being actively investigated. These not only play a significant role in DNA damage and activation of oncogenes but also in different aspects of inflammation. This suggests that ROS play an important role in the promotion of inflammation and tumorigenesis by modulating cancer-related signaling pathways.

Clinical data indicate that chronic inflammation promotes carcinogenesis. For instance, patients with inflammatory bowel diseases (IBD, ulcerative colitis, and Crohn's disease) have five- to sevenfold increased risk of developing colorectal cancer. Alarmingly, 43% of patients with ulcerative colitis develop colorectal cancer after 25–35 years.[19] Chronic airway inflammatory conditions such as asbestosis, silicosis, exposure to airborne particulate matter, idiopathic pulmonary fibrosis, and tuberculosis have been reported to trigger nonsmoking related cancer development.[20] Another form of lung disease is mesothelioma which is caused by exposure to asbestos and asbestos-induced chronic inflammation, and subsequent production of ROS and DNA damage.

Chronic inflammation also leads to gastric cancer. Aberrant expression of activation-induced cytidine-deaminase (AID), a member of the cytidine-deaminase family that acts as a DNA- and RNA-editing enzyme, is induced by *H. pylori* and is observed in this malignancy.[21] *H. pylori*-mediated upregulation of AID results in accumulation of nucleotide alterations in gastric cells which ultimately leads to the development of gastric cancer.[21] Moreover, tumor necrosis factor (TNF) stimulation in human bile-duct cells induces ectopic AID production, which results in chronic biliary inflammation and the development of cholangiocarcinoma. Therefore, AID may be the link between chronic inflammation and DNA damage in these tumors.

Oxidative DNA damage and inflammation is also implicated in schistosomiasis,[22] lung, liver, and breast cancers. Elevated levels of DNA damage is seen upon urine analysis of patients with schistosomiasis.[22] The cells are more prone to DNA damage induced by the ROS/RNS produced by activated inflammatory cells. This, in turn, leads to an increased risk for bladder cancer in adults.[22] Chronic infection with hepatitis B or C viruses or ingestion of aflatoxin that causes ROS and subsequent DNA damage production leads to hepatocellular carcinoma and is considered as a significant cause of cancer-associated mortality in Asia and Africa.[23] Oxidative DNA damage may be involved in the development of breast cancer as well. Increased steady-state levels of DNA damage and ROS have been reported in invasive ductal carcinoma.[23] Whether the changes are due to decreased DNA repair and/or increased oxidative DNA damage remains to be confirmed.

Intrinsic pathway

The intrinsic pathway is induced by genetic events such as activation of various types of oncogenes by mutation, chromosomal rearrangement or amplification, and the inactivation of tumor-suppressor genes. These cells produce inflammatory mediators which creates an inflammatory microenvironment in tumors without prior underlying inflammatory condition.[24]

Results reported in the literature show that various oncogenic mechanisms are involved in cancer-related inflammation pathways[8] (Figs. 2 and 3). For example, inhuman papillary thyroid carcinoma (PTC), a tumor characterized by the presence of chemokine-guided macrophage and dendritic cell infiltration, rearrangements in the protein tyrosine kinase RET play a key role in the pathogenesis.[25] One of the signaling molecules in PTC colony-stimulating factors (CSFS) promotes leukocyte recruitment and survival. Interleukin 1β (IL-1β) is also secreted and is one of the main inflammatory cytokines. Cyclooxygenase 2 (COX2) is frequently expressed in cancer and is involved in the synthesis of prostaglandin E(2) which can promote tumor growth by binding its receptors and activating signaling pathways which regulate cell proliferation, migration, apoptosis, and angiogenesis. Chemokines attract monocytes and dendritic cells leading to secretion of CCL2 and CCL20 and, as expected, these molecules have been reported to be pro-tumorigenic. Angiogenic chemokines such as CXCL8 coordinate induction and inhibition of matrix-degrading enzymes which promote tumor progression and survival. Upregulation of L-selectin and expression of the chemokine receptor CXCR4 that promote metastasis are also observed in these cells.[8, 25] Thus, an early, causative and sufficient genetic event promotes an inflammatory microenvironment which, in turn, leads to tumor formation. The inflammatory cascade and tumor progression can be triggered by the activation of oncogenes or inactivation of tumor suppressors. For example, in the *ras* family oncogenes activation induces expression and production of inflammatory mediators. Expression of *ras* in a cervical carcinoma cell line induces the production of CXCL8 which promotes angiogenesis and tumor progression.[8] Moreover, mild chronic pancreatitis and K-ras mutation induce pancreatic intraepithelial neoplasia and invasive ductal carcinoma.[26] Similarly, *Braf*, which is frequently activated in malignant melanoma, induces cytokines which create a pro-tumorigenic microenvironment.[27]

The *myc* oncogene encodes a transcription factor that is overexpressed in many human tumors. Deregulation of *myc* is important in the initiation and maintenance of key aspects of the tumor phenotype. In association with inflammatory cells and mediators, *myc* promotes cell proliferation and remodeling of the extracellular milieu. *Myc*-mediated alterations include secretion of chemokines which recruit mast cells and help sustain the formation of new vessels and tumor growth.[8] The epidermal growth factor receptor (EGFR) family plays an important role in cancer. EGFR activation in glioma induces COX2 expression which is involved in the synthesis of prostaglandin E(2) which can promote tumor growth by binding its receptors and activating signaling pathways which regulate cell proliferation, migration, apoptosis, and angiogenesis. COX2 expression is an independent prognostic factor in glioma.

Production of inflammatory mediators can also be regulated by tumor suppressor proteins such as von Hippel-Lindau/hypoxia-inducible factor (VHL/HIF), transforming growth factor-β (TGF-β) and phosphatase and tensin homologue (PTEN). The chemokine receptor CXCR4 is frequently expressed on malignant cells and has been implicated in cell survival and metastasis. CXCR4 and TNF-α lie downstream of the VHL/HIF axis in human renal-cell carcinoma.[8] Mutation of PTEN in nonsmall cell lung cancer (NSCLC) results in upregulation of HIF-1 activity and subsequent HIF-1-dependent transcription of the CXCR4 gene. CXCR4 regulates migration of lung cells through activation of Rac1 and matrix metalloproteinases. CXCR4 also modulates the action of ERK, IKK, NFκB, and integrins which promote metastasis of the lung cancer.[28] Data from breast carcinoma suggests that inactivation of the gene encoding the type II TGF-β receptor stimulates the production of CXCL5 and CXCL12, which draws myeloid-derived suppressor cells (MDSC). CXCL5 induces Raf/MEK/ERK activation, Elk-1 phosphorylation, and Snail upregulation. Activation of Elk-1 facilitates recruitment of phosphorylated mitogen- and stress-activated protein kinase 1, which in turn enhances histone H3 acetylation and phosphorylation of Snail promoter, resulting in Snail enhancement and E-cadherin downregulation. This facilitates metastasis of breast cancer.[8, 29] Thus, oncogenes and tumor suppressor genes can induce inflammation.

The link between extrinsic and intrinsic pathways

Some of the molecules involved in both the intrinsic and extrinsic pathways include transcription factors, such as nuclear factor-κB (NF-κB), signal transducer and activator of transcription 3 (STAT3) and hypoxia-inducible factor 1α (HIF1α).[30] These transcription factors modulate the inflammatory response and promote tumorigenesis via soluble mediators including cytokines for example, IL-1, IL-6, and IL-23, chemokines CCL2 and CXCL8, and other cellular components, for example, tumor-associated macrophages.[8] These factors recruit and activate various leukocytes, mainly of

myelomonocytic lineage. The cytokines also activate key transcription factors in various cell types such as inflammatory, stromal, and tumor cells. This results in a cascade where even more inflammatory mediators are generated and a cancer-related inflammatory milieu is created.[24]

NFκB is a key transcription factor that potentially is a link between tumor cells and inflammatory cells. NFκB has a variety of roles including facilitating proliferation and survival of malignant cells by activating genes that regulate cell cycle progression (e.g., cyclin D and c-myc) and apoptosis (e.g., cIAPs, A1/BFL1, Bcl2, c-Flip), promoting angiogenesis and metastasis, disrupting adaptive immunity, and altering responses to hormones and chemotherapeutic agents.[8] In this respect, NFκB induces the expression of inflammatory cytokines such as key enzymes in the prostaglandin synthase pathway (COX2), adhesion molecules, nitric oxide (NO) synthase, and angiogenic factors which promote inflammation as well as tumorigenesis. Hepatocarcinogenesis is substantially reliant on NFκB activation in both parenchymal (hepatocytes) and nonparenchymal cells of the liver.[8]

STAT3 is also implicated in both extrinsic and intrinsic pathway.[30] Constitutively activated STAT3 increases tumor cell proliferation, survival and invasion, and subdues antitumor immunity. Persistent activation of STAT3 leads to inflammation which promotes tumor formation. This dual role of STAT3 in tumor inflammation and immunity involves upregulation of pro-oncogenic inflammatory pathways, including NFκB and IL-6-gp130-JAK pathways, and downregulation of STAT1 and NFκB-mediated Th1 antitumor immune responses.

Soluble mediators and cellular components

Inflammation is sustained by molecules such as TNF-α. Tumor-derived TNF-α supports the growth and development of skin, pancreatic, liver, and bowel tumors.[31] Constitutively produced TNF-α is associated with increased release of chemokines such as CCL2, CXCL12, CXCL8, CXCL1, CXCL13, CCL5, CCL17, and CCL22, IL-1, IL-6, VEGF, and macrophage migration inhibitory factor (MIF-1).[8]

Tumor-associated macrophages (TAM) represent the major inflammatory component of the stroma of many tumors and affect different aspects of the tumor.[8] TAM accumulation has been reported to promote angiogenesis via production VEGF and platelet-derived endothelial cell growth factor.[32] Moreover, myeloid cells in the tumor milieu also play a role as an angiogenic switch at different levels. Mast cells, eosinophils, neutrophils, and effectors of the adaptive immune response are capable of tolerating the inflammatory reactions that lead to cancer.

Tissue injury

There are significant overlaps between key features of wound healing and tumor development. These include stem cell and myofibroblast activation, enhanced cell proliferation, inflammation, and neoangiogenesis. Chronic injury results in an aberrant healing and regenerative response that ultimately stimulates the growth and development of initiated cells. Indeed, in the initial phase, the body interprets tumors as wounds and similar to healing tissues, activated platelets are present in tumors. This phase of tumor growth is governed by the actions of the stroma which is similar to physiologic tissue repair.[4] However, during late tumor growth, the tumor becomes independent of stromal signaling for progression and survival. So far we have focused on the interaction of extrinsic and intrinsic mechanisms of inflammation and their role in the induction of carcinogenesis. It should be noted though that the inflammatory response is also critical in other aspects of tumor progression as well such as tissue invasion and metastasis. Angiogenesis significantly augments vascular invasion of migrating cells. Matrix metalloproteases and their inhibitors are essential for angiogenesis and remodeling of the extracellular matrix. Similar to cancer, cell proliferation is enhanced in a wound which results in tissue regeneration. However, unlike cancer, cell proliferation and inflammation subside after the foreign particle is removed or the repair is complete.[4]

Chronic tissue damage and inflammation can indeed promote the growth and progression of cancer. For instance, *v-Src* oncogene cannot induce cancer unless supplemented by tissue injury and ensuing tissue renewal.[33] Similarly, pancreatic insult is required to unravel the oncogenic potential of activated *K-Ras*. Finally, tissue injury inflicted by tobacco smoke influences lung cancer development, and suppression of cell death-related pathways (c-Jun N-terminal kinase and JNK) or death-induced pro-inflammatory cytokines (TNF and IL-6) reduces tumor development. Taken together, these results support the notion that a substantial fraction of all cancer cases is likely to be initiated and promoted by chronic tissue injury. Given that persistent inflammation promotes genetic instability,[8] targeting cancer-related inflammation is a possible treatment strategy that can minimize normal tissue injury. The global inflammation that prevails in cancer can be targeted to restore normal tissue homeostasis and, perhaps, can be used in cancer prevention.

Nontargeted effects, inflammation, oxidative stress, and DNA damage

Bystander and abscopal effects

The term "bystander" effect was first used in radiation biology to explain the results obtained in cell cultures irradiated with α-particles (energetic helium nuclei with the short range of absorption which can be produced by cyclotrons or synchrotrons). Although only a few cells were traversed by α-particles, many more exhibited sister chromatid exchanges, indicating that nontargeted cells also sustained damage.[34] Subsequently, the term has been used in various scenarios to describe the ability of cells affected by an agent to convey manifestations of damage to other cells not directly targeted by the agent or necessarily susceptible to it themselves.[35] These indirectly affected, bystander cells exhibited various types of genomic destabilization such as altered clonogenic survival, changed the frequency of gene mutations, induction of apoptosis and micronuclei, altered expression of stress-related genes, elevated frequencies of malignant transformation of mammalian cells in vitro, altered DNA damage and repair and senescence arrest, and various epigenetic changes[36] (reviewed in Refs. 35, 37). All these indirect (systemic) consequences in bystander cells are delayed effects, and though similar to the direct effects of radiation, can follow different kinetics. For instance, elevated levels of phosphorylated histone H2AX (γ-H2AX) have been found in both irradiated and bystander cultures indicating the presence of DNA DSBs.[38, 39]

In contrast to direct effects of radiation, bystander effects show no true radiation dose response[40] and are detectable at doses as low as 10 mGy.[41] The nonlinear dose-response was first demonstrated in vitro[34, 42] and recently in an in vivo study involving various synchrotron radiation settings including different doses and irradiation field sizes.[43] The results of this study indicated that radiation settings did not substantially influence the persistent biological effects observed in out-of-field tissues following synchrotron irradiation of the right hind leg of mice.

Not all cell types and not every cell in a bystander culture are equally responsive.[44] For example, rapidly proliferating cancer cells in culture and, generally, proliferating cells in S-phase appear to be highly susceptible.[45] However, other data suggest that this is not the only factor determining bystander vulnerability.[46] In such cells, ROS generated directly or indirectly as a result of cell damage interact with bystander cell DNA, producing lesions ranging from base or sugar modifications to abasic sites and single-stranded breaks. ROS-induced DNA damage can interfere with both replication and transcription in proliferating cells and transcription in nonproliferating cells, leading to DSB formation. Bystander effects have been noted in response to a number of cellular stresses including UV exposure and nonradiation sources of cellular damage such as media from tumor and aging cells.[46] Therefore, bystander effects can be generalized as an overall cell population response to the presence of cells undergoing stresses of various types.[47]

Of particular interest in regards to human health are the nontargeted or systemic effects that have been reported by radiation oncologists for decades—reactions in normal unirradiated tissues after radiation therapy of a particular part of the body. These out-of-field or abscopal effects have been described as an action at a distance from the irradiated volume but within the same organism.[48] Abscopal effects have been also shown to be a general phenomenon; they result from a number of other localized stimuli, for example, surgery, hyperthermia, and laser immunotherapy.[37] The discovery of the radiation-induced bystander effect has prompted the description of abscopal effects as distant in vivo bystander effects, and their further investigation. Several studies reported in vivo radiation-induced bystander effects in animal models. Using strategies that involve partial head or body 1 Gy X-ray irradiation, profound genetic and epigenetic changes were identified in shielded organs, such as skin and spleen.[49, 50] The results of the in vivo RIBE can be transmitted to future generations. The radiation-induced bystander effect is a potential contributor to the well-documented clinical phenomenon of secondary cancers,[51] a major concern in cancer RT, affecting more than 1% of patients.[37] The frequency of secondary malignancies that arise as a function of distance from the irradiated area,[52] and irradiation volume and doses[53] have been investigated. Typically at distances closer to the irradiated area (at least 5 cm) 22% of tumors arise,[52] while primary tumors that receive doses of less than 2.5 Gy were shown to be associated with the development of 31% secondary malignancy outside the irradiated area.[53] A recent review compiled a list of inhibitors that have been shown to minimize or completely abrogate bystander effects, which could ultimately curb the manifestation of radiation-associated secondary cancers.[54] These inhibitors interrupt well-known mechanisms that drive bystander effects and can be categorized into three main groups, (1) inhibitors of intercellular gap junction communication, (2) detoxifiers of reactive species, and (3) agents with antiinflammatory properties.[54]

Bystander signaling in vitro

Two major mechanisms have been identified as playing a role in the transmission of bystander responses in cellular models, physical contact, via gap junction mediation, and soluble factors, such as ROS, NO, and cytokines often referred as Damage Associated Molecular Patterns (DAMPs) (reviewed in Ref. 37). DAMPs, also known as alarmins, are molecules released by

stressed (damaged) cells undergoing apoptosis or necrosis that act as endogenous danger signals to promote and intensify the inflammatory response. Some of the most well-known DAMPs are high mobility group box-1 (HMGB1), S100A8 (MRP8 and calgranulin A) and S100A9 (MRP14 and calgranulin B), and serum amyloid A (SAA). High serum levels of these DAMPs have been associated with many inflammatory diseases, including sepsis, arthritis, atherosclerosis, lupus, Crohn's disease, and cancer. Therapeutic strategies are being developed to modulate the expression of these DAMPs for the treatment of these diseases. For the bystander signaling, in the absence of gap junctions (e.g., in the medium transfer experiments), several studies found suppression of bystander responses when various inhibitors and ROS and NO scavengers were added to the media of donor cells and recipient cells. The use of 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO) as a NO-specific scavenger, the incidence of bystander micronuclei yields reduced, which indicated that NO contributes to the bystander effect.[55]

Various inflammation-related cytokines that have been found at elevated levels in medium conditioned by irradiated cells. Notably, stress events other than IR, such as UVC, UVA, and unirradiated cancer cells release similar to irradiated cultures cytokines in the culture medium.[46] They can target bystander cells directly; cytokine TGF-β, when added to cell cultures, induces elevated levels of DSBs similar to those induced by the conditioned medium, and addition of the blocking anti-TGF-β antibody reversed the effect.[46, 56] Indirectly, through activation of cytokine receptor-mediated pathways, bystander cells also start expression and production of IL-8, IL-6, IL-33, RGE2, and other factors.[52] When gene expression profile was compared between unirradiated and bystander normal human fibroblasts, the transcription level of COX-2 was found consistently upregulated in bystander cells by more than threefold. Addition of COX-2 inhibitor NS-398 suppressed COX-2 activity and decreased bystander mutagenesis.[57] Recently several publications addressed the role of the stimulatory neurotransmitter 5-hydroxytryptamine (serotonin) which is a serum component in a cell culture medium, on the radiation-induced bystander effect. Some publications report a trend for increasing bystander response with increasing serotonin concentration, while others found no effect.[58–60]

Role of cytokines for bystander signaling

The cell-cell communication in vivo is mediated by the immune system and is more complex. Recently, the abscopal DNA damage response in normal tissues has been described which was influenced by early-stage tumors growing in mice.[61] The presence of a tumor has been shown to induce inflammatory and DNA damage responses in the immediate tumor microenvironment, possibly due to the production of ROS and cytokines, similar to the radiation-induced bystander effect signaling. Results obtained in cell culture indicate that tumors could influence normal cell cultures; normal cells sustain elevated levels of DNA damage when incubated with medium previously conditioned on tumor cells, and similar cytokines released into the medium of unirradiated tumor and irradiated normal cells.[46] Syngeneic tumors (B16 melanoma, reticulum cell sarcoma, and colon adenocarcinoma) were implanted subcutaneously into mice, and 2 weeks later, the levels of two types of DNA damage in different tissues were measured. Elevated levels of DSBs, as marked by γ-H2AX foci, and OCDLs, were present in several distant tissues, such as duodenum, colon, stomach, rectum, and skin.[15] Both DSBs and OCDLs are potentially serious lesions and lead to genome instability if not fully repaired.[16] Ovary and lung did not exhibit elevated γ-H2AX foci, but had elevated OCDL levels. This wider incidence of elevated OCDLs versus γ-H2AX foci may be attributable to the mechanisms of lesion formation. γ-H2AX foci form in tissues with larger fractions of proliferating cells, such as those in the gastrointestinal tract, in which replication forks may participate in DSB formation, while OCDLs form equally well in every tissue. Out of 59 cytokines measured in mouse serum, CCL2/MCP-1, CCL7/MCP-3, and CXCL1/IP-10, were over threefold elevated in tumor-bearing mice. Elevated numbers of activated macrophages were found in gastrointestinal tract organs and skin. This suggested that macrophages in these distant tissues secrete ROS that induces DNA damage in the cells of the host organs. This also substantiated an association between inflammation and bystander DNA damage responses in these tumor-bearing mice. Interestingly, this systemic oxidative DNA damage in normal tissues both neighboring to and distant from injected tumors can be abrogated by feeding mice with the antioxidant tempol-rich food suggesting that the endogenous antioxidant systems could be efficiently boosted by a well-designed antioxidant therapy to suppress the oxidative load in the organism.[62]

The role of CCL2 in cancer development has been controversial, with evidence of pro- and antitumorigenic effects. Recent studies suggest that CCL2 contributes to cancer growth.[63, 64] There is mounting evidence linking inflammation and cancer. ROS are secreted from activated immune cells and stressed epithelial cells, resulting in DNA damage and genomic instability that may contribute to carcinogenesis.[65–67] In a study of patients treated with radiation therapy for non-small cell lung cancer, accumulation of unrepaired DNA damage in out-of-fields normal tissues was associated with changes in CCL2 plasma levels,[68, 69] which also were associated with high-grade lung toxicity.[69] To examine whether the association between CCL2 and tumor-induced bystander DNA damage was causal, the tumors were implanted into CCL2 knockout mice.

Strikingly, there was no measurable increase in distant DNA damage in the tumor-bearing CCL2 KO mice, suggesting that CCL2 is essential in the tumor-induced genotoxic response in vivo.[15] The proposed model states that the bystander DNA damage in the tumor-bearing mice is due to the presence of activated macrophages in the distant tissues.[61] These activated macrophages at the irradiation site of injured tissue secrete CCL2 and TGFβ into the extracellular environment, which then bind to their respective receptors in out-of-field tissues, including CCL receptor type-2 (CCR2)[70] and TFGβ receptor 1 (TGFβR1).[71] These receptors can interact with various cellular pathways consequently leading to TGFβ upregulation and increased COX-2 expression. Increased expression of COX-2 is linked to biochemical failure, distant metastasis and radiation toxicity in RT patients with prostate cancer.[72] Therefore, CCL2, TGFβ, and COX-2 are critical factors in bystander signaling and carcinogenesis,[37] and are attractive targets to manipulate abscopal effects. In support of this notion, therapies targeting these factors have already been considered in the clinical setting to block TGFβ signaling in RT breast cancer patients[73] and inhibit metastatic cascade in glioblastoma[74] and nonsmall cell lung cancer patients[75] (by using a CCL2-neutralizing antibody or small molecule to inhibit CCR2 reviewed in Refs. 14, 70, 76).

Another good example of systemic cytokine-mediated intercellular communication is the relationship between senescent cells and surrounding normal tissues in an organism. Cellular senescence is a part of normal aging, as well as a preventive strategy to stop the proliferation of cells undergoing malignant transformation.[77] This antiproliferative response can be driven by oncogene activation or loss of tumor suppressor signaling. A direct connection between cellular senescence and inflammation was established recently indicating a crucial role in oncogene-induced cellular senescence the senescence-associated secretory phenotype (SASP), a cross talk between senescent cells and their environment by secretion of numerous cytokines, chemokines, growth factors, and proteases. For example, IL-6 and IL-8, two well-known pro-inflammatory cytokines, seem to play a central role in premature cellular senescence induction. CCL2 appears as the most upregulated factor and a critical component in the SASP from melanoma cells.[78] Moreover, the SASP from senescent melanoma cells or recombinant CCL2 induces DNA damage in naïve melanoma cells, another indication that CCL2 triggers bystander effects.[78] On the detrimental side of the SASP effects, the chronic presence of senescent cells secreting numerous proteins has been predicted to significantly alter normal tissue structure and functions, not only in the local milieu but in the whole organism.

In the absence of a tumor, synchrotron X-ray irradiation is capable of inducing persistent abscopal effects to normal out-of-field tissues in mice. A recent report demonstrated that a short pulse of synchrotron X-ray irradiation on the right hind leg (200 and 810 ms for 10 and 40 Gy) was sufficient to induce significant and persistent DNA damage (DSBs and OCDLs), apoptosis, and local and systemic immune responses in out-of-field tissues.[43] Direct irradiation of skin tissue induced an innate immune response (due to increases in macrophages/DC and neutrophils) while in out-of-field duodenum both the innate and adaptive immune response (macrophages/DC, neutrophils, and T-cells) was activated.[43] In addition to these persistent immune responses in out-of-field duodenum, increases in oxidative stress, inflammation and senescent cells, and decrease in proliferation were observed in the same tissues. This report also showed significant alterations in a range of plasma cytokines including CSF1R, IL-10, TIMP1, VEGF, TGFβ1, and TGFβ2, representing a misbalance in the cellular microenvironment in the irradiated area, which likely triggered activation of other factors responsible for the propagation of the systemic effects observed in this study. A mechanism to explain the widespread and persistent abscopal effect observed in out-of-field tissues was proposed.[43] At the irradiated site, macrophages and neutrophils become activated via phagocytosis of radiation-induced apoptotic cells[79] and secrete cytokines.[80] Either directly or by triggering activation of other factors, cytokines, in turn, activate distant tissue-associated macrophages (and other immune cells) that generate free radicals and lead to persistent oxidative stress,[37, 80] resulting in OCDL formation in out-of-field tissues. In highly proliferative tissues such as intestine, oxidative DNA lesions can develop into DSBs, which can lead to apoptotic cell death.

To identify which components of the immune response drive abscopal effects a recent study used synchrotron-X-ray-irradiated immune-deficient mice with a range of immune system abnormalities to tease out which immune system components were essential abscopal effect propagators.[81] Contrary to healthy wild type mice, little or no change in DNA damage and apoptosis was observed in out-of-field tissues of immune-deficient mice, indicating that the abscopal effect relies on a functional immune response for its propagation to occur. Since no change in DNA damage and apoptosis was observed in CCL2 knockout mice, which lack the ability to recruit monocytes, macrophages/DC, and memory T-cells to sites of inflammation in damaged tissues,[70, 82] NSG (NOD SCID gamma) mice with severe immune deficiencies,[83, 84] and C57BL6/J mice treated with anti-CSF1R neutralizing antibody which renders mice macrophage-depleted,[83] it indicates that macrophages and CCL2 play key roles in initiating and propagating abscopal effects in out-of-field tissues following localized synchrotron radiation.[81] Therefore, targeting the innate immunity via CSF1R in macrophages and/or blocking TGFβ/CCL2 can potentially simultaneously protect out-of-field tissues, inhibit metastasis and primary tumor growth.[85, 86] Tumor suppression can potentially be further enhanced by using a CSF1R inhibitor which can reduce the secretion of radiation-induced CSF1R from tumors, subsequently decreasing the level of immunosuppressive myeloid cells.[87]

Radiation-induced inflammation

The concept that IR (IR) as a stress factor interferes with both targeted and nontargeted tissues is supported by multiple sources evidence of systemic response to radiation. As we already mentioned free radicals play an important role in cellular metabolism and cell signaling. However, after exposure to IR a redundant amount of ROS and NO is formed which can damage cellular components and genome. Chronic inflammation is strongly connected with oxidative damage after exposure to IR. After IR an increased number of immune system cells such as macrophages and T-cells may occur which can lead to the accumulation of several inflammatory mediators (NF-kB and SMAD2/3, cytokines, TNF-α, TGF-β, and IFN-γ). An increased number of these mediators are connected to ROS and NO.[88] Radiation induces cellular oxidative stress that results in damage of not only nuclear DNA but also mitochondrial DNA leading to a decrease in respiratory chain activity and loss of mitochondrial function. The outcome is persistent metabolic oxidative stress that could continue to cause further oxidative damage to critical biological structures after long radiation exposure.[89] This radiation-induced damage to mitochondrial DNA in directly targeted or bystander tissues could become heritable and contribute to radiation-induced genomic instability. Genomic instability in nonirradiated normal tissues has been reported to be mediated by late cytokine response, as in case of long-lived COX-2 pathway cytokine-dependent DNA damage and apoptosis response in nonirradiated mouse bone marrow cells after bone marrow was retrospectively irradiated. Such mechanistic studies provide insight into the nature of signaling molecules participating in targeted and nontargeted effects that potentially can be manipulated to increase therapeutic gain in radiotherapy.

Exposure to IR has long been known to modulate the immune capacity of irradiated subjects, with a recognized dose/effect relationship.[90] Radiation exposure directly damages hematopoietic stem cells and alters the capacity of bone marrow stromal elements to support and maintain hematopoiesis. Data from the atomic bomb victims suggest a threshold dose to the acute radiation hematological syndrome characterized by severe immune-compromise and subsequent death. In solid tumors these forms of unscheduled cell death can lead to a pro-inflammatory environment and an increase in cell-to-cell signaling. In this scenario, the innate immune system is important in mediating the antitumor effects of localized IR. For example, a preclinical murine study in as early as 1979 demonstrated that in vivo tumor control probability to radiation was profoundly influenced by the host immune-competence in a transplanted murine fibro-sarcoma model.[91] However, even in the presence of a competent immune system an established tumor system is usually adapted to avoid immune recognition in the absence of additional antitumor stimulus. This section focuses on the complex induced immune response of the tumor and host secondary to radiotherapy.

Local tumor environment and radiation

Immune cells associated with the complex tumor microenvironment can function to promote or suppress the adaptive immune response. Tumor-associated macrophages which are consistently colocated within the tumor microenvironment are pro-angiogenic and can assist in tumor growth. In established and advanced neoplasia, when persistent tumor cells have escaped the immune attack, M2-polarized macrophages predominate the tumor microenvironment and suppress adaptive immunity. The response to IR can trigger inflammation; however, the interpretation of this process by the innate immune system appears to be dependent on a variety of factors. In tumor cells, doses of <0.5 Gy (which are generally too low to directly induce cell death) results in the release of oxygen and nitrogen radicals that activate innate immune cells, such as macrophages, to release cytokines. Depending on the environment and genetic background, this process can result in chronic inflammation that causes genetic alterations and cell death as a secondary event. It is in this setting that the immune-modulating effects of radiation promote mostly a pro-tumorigenic role of the immune system. Conversely, at doses sufficient to directly provoke significant cell death, inflammatory cell signaling can result in an adaptive immune response. This inflammatory signaling cascade can promote antitumor immunity, for example, through activation of M1-polarized macrophages. These M1 macrophages have antitumor activity, mediated directly by the ability to kill tumor cells, as well as indirectly by the activation of adaptive antitumor immunity.

Radiation exposure and the immunogenic effect

Conventional radiotherapy comprises of doses of 1.8–2 Gy per fraction, delivered 5 days a week, for several weeks. In vitro, when mouse B16 melanoma cells were exposed to multiple daily doses of 2 Gy to a total dose of 50 Gy, mimicking clinical protocols, MHC-I expression was increased after the second week, when the total dose amounted to 20 Gy.[92] This expression profile was stable for greater than 5 weeks after the last radiation fraction. While immunogenic signaling may occur in a cumulative fashion during conventional radiotherapy, pro-inflammatory cytokines generally are produced

by higher doses than are conventionally used in RT. This has particular importance as the role of stereotactic ablative body radiotherapy (SABR) has emerged.

Recent technological advances in precision radiotherapy delivery have allowed the safe clinical application of high-dose per fraction SABR.[93] Typical dose/fractionation schedules are in the ablative spectrum, and potentially augment the tumoricidal properties of radiation through some proportion of vascular damage, ceramide-induced endothelial cell damage and increased apoptosis of tumor cells. Recent evidence also suggests that ablative doses of radiation evoke a particularly strong immune response. Lugade et al.[94] showed that cross-priming of T-cells against tumor antigens were induced by both 3 Gy by 5 fractions and a single dose of 15 Gy in the draining lymph nodes. Using the B16 mouse melanoma model, Lee et al.[95] showed that retardation of tumor was more pronounced with a single dose of 20 Gy or 3 fractions of 15 Gy were comparable. This effect was markedly reduced by host CD8+ T-cell depletion, suggesting that both regimens can promote cross-priming of antitumor T-cells. In contrast, a nonablative dose of 5 Gy by 4 fractions delivered over 2 weeks showed inferior tumor growth inhibition. These results suggest that RT induced adaptive immune response can be a dose dependent phenomenon and may result in additional tumor cell kill beyond direct DNA damage.

Radio-immunotherapeutic approaches present promising new anticancer treatments due to reports that support its immunosuppressive abilities[96] and more recent findings that indicate that RT has the capacity to engage host immune effector mechanisms that contribute to control and/or ablation of cancer.[73, 97, 98] In-depth reviews of the relationship between RT and immune response was reported in Ref. 99, 100, which includes the changes in immune response following various doses of irradiation. At doses as low as 0.05 Gy an immunosuppressive effect was reported, indicating that the level and type of DNA damage dictates the type of immune response that is activated.[101] Doses less than 2 Gy typically promote antiinflammatory responses by reducing the production of nitric oxide and IL-1β and increase release of IL-10 and TGFβ.[102, 103] Low doses are also able to stimulate angiogenesis and/or vasculogenesis in tumors, which occurs due to the infiltration of systemic endothelial cells or progenitor cells (that originated from bone marrow) into tumors,[102] this, in turn, can enhance oxygenation resulting in increased tumor radiosensitivity.[104, 105] Conversely, at doses above 15Gy the vascular network is damaged due to apoptosis of endothelial cells leading to tumor cell starvation.[106] High radiation doses can also increase the hypoxic state of tumors, which consequently contributes to radioresistance.[107] Therefore low doses of radiation can prime tumor cells to be more susceptible to direct cytotoxic and immunological effects of subsequent high dose radiation therapy.[99]

Conclusion

Persistent stress is induced by self-perpetuating inflammatory processes resulting in the buildup of DNA damage in target tissues. The resulting genetic changes act as a driving force in chronic inflammation-associated human disease pathogenesis. Therefore, increased steady-state levels of DNA damage due to pro-inflammatory molecules provide promising molecular signatures for predicting disease risk and may be potential targets and biomarkers for precancerous lesions and cancerous development.

Summary points

- DNA damage can be induced by oxidative stress and radiation and is resolved by DNA repair and activation of cell cycle checkpoints to arrest the cell to allow time for repair. If not properly repaired, it causes a threat to the maintenance of genomic stability and may progress to carcinogenesis.
- Oxidative stress, DNA damage, and inflammatory responses are interconnected.
- Inflammatory responses are critical at different phases of tumor development including initiation, promotion, malignant conversion, invasion, and metastasis.
- Cell-signaling systemic effects such as bystander and abscopal effects are mediated by inflammatory factors.
- Inflammation affects immune surveillance in response to cancer therapy. That result in antitumor immune activation and systemic effects which are indicative of the potential efficacy of radiation as a cancer therapy that extends beyond classical direct DNA damage.

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