Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability

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Inflammatory conditions in selected organs increase the risk of cancer. An inflammatory component is present also in the microenvironment of tumors that are not epidemiologically related to inflammation. Recent studies have begun to unravel molecular pathways linking inflammation and cancer. In the tumor microenvironment, smoldering inflammation contributes to proliferation and survival of malignant cells, angiogenesis, metastasis, subversion of adaptive immunity, reduced response to hormones and chemotherapeutic agents. Recent data suggest that an additional mechanism involved in cancer-related inflammation (CRI) is induction of genetic instability by inflammatory mediators, leading to accumulation of random genetic alterations in cancer cells. In a seminal contribution, Hanahan and Weinberg ([2000] Cell, 100, 57–70) identified the six hallmarks of cancer. We surmise that CRI represents the seventh hallmark.

Introduction
As early as in the 19th century it was perceived that cancer is linked to inflammation. This perception has waned for a long time. Recent years have seen a renaissance of the inflammation–cancer connection stemming from different lines of work and leading to a generally accepted paradigm (1–4).

Epidemiological studies have revealed that chronic inflammation predisposes to different forms of cancer. Usage of non-steroidal anti-inflammatory agents is associated with protection against various tumors, a finding that to a large extent mirrors that of in vitro studies (5). Inflammation is a non-specific response to tissue damage and of the inflammatory cytokines TNF-a, IL-1, IL-6, che-...
The NF-κB pathway is tightly controlled by inhibitors acting at different levels. There is now evidence that Tir8 (also known as SIGIRR), an orphan member of the IL-1R family highly expressed in intestinal mucosa, inhibits signaling from the IL-1R/TLR complexes, possibly by trapping IRAK-1 and TRAF-6. In a mouse model of intestinal carcinogenesis in response to dextran sulfate sodium salt and azoxymethane administration, Tir8-deficient mice exhibited a dramatic susceptibility to inflammation and showed increased colon carcinogenesis, associated to local production of prostaglandin E₂, proinflammatory cytokines (IL-1, IL-6) and chemokines (KC/CXC, JE/CCL2 and CCL3) (21,22). These mediators are downstream of NF-κB and have been shown to promote inflammation-propelled neoplasia (12). Thus, the lack of a checkpoint (Tir8) of NF-κB activation leads to increased carcinogenesis in the gastrointestinal tract, underlying once more the connection between chronic inflammation and cancer promotion. In addition, Tir8 gene deficiency is associated with B cell lymphoproliferation and autoimmunity (23).

Along with NF-κB, STAT3 is a point of convergence for numerous oncogenic signaling pathways (13). Lee et al. (24) recently showed that the maintenance of NF-κB activation in tumors requires STAT3. This transcription factor is constitutively activated both in tumors and in immune cells and plays a role in carcinogenesis, as well as in tumor immune evasion by hampering dendritic cells maturation (13,25). Studies on colon cancer revealed that STAT3 is a major controller of cell proliferation and survival, regulating the expression of c-Myc, Mcl-1, Cyclin D and Bcl-2 (26). In lung adenocarcinomas, constitutive STAT3 phosphorylation is downstream of activating mutations in epidermal growth factor receptor (27,28).

A major effector molecule of NF-κB activation and also linked to STAT3 is IL-6, a multi-functional cytokine with growth-promoting and antiapoptotic activity (29,30). Recent reports have provided evidence for the key role of the NF-κB–IL-6–STAT3 cascade. It was found that IL-6 produced by myeloid cells is a critical tumor promoter during intestinal carcinogenesis. IL-6 protects normal and premalignant intestinal epithelial cells from apoptosis and promotes the proliferation of tumor-initiating cells (31,32). Interestingly, STAT3 also regulates the balance between IL-12 and IL-23 in the tumor microenvironment and consequently the polarization of T-helper subsets (33). In multiple myeloma, a well-known IL-6-dependent neoplasia, it was described an alternative pathway of connection between IL-6 and NF-κB (34,35). Another link between IL-6 and cancer is in liver carcinogenesis. Naugler et al. have clarified the mechanisms underlying the increased susceptibility of male mice to hepatocellular carcinoma. Carcinogen-induced tissue injury activated, in liver macrophages of male mice, high levels of IL-6 in a TLR/MyD88-dependent manner. IL-6 promotes liver inflammation, injury, compensatory cell proliferation and carcinogenesis. In females, estrogen steroid hormones inhibit IL-6 production and so protect female mice from cancer (36,37).

Among proinflammatory cytokines, TNF plays a major role. Originally identified as a cytokine inducing hemorrhagic necrosis of tumors, TNF soon turned out to have also protumoral functions. The finding that TNF-deficient mice are protected from skin carcinogenesis offered genetic evidence linking TNF-mediated inflammation and cancer (8,38). Tumor promotion by this cytokine can involve different pathways: TNF enhances tumor growth and invasion, leukocyte recruitment, angiogenesis and facilitate epithelial to mesenchymal transition (8,39,40). TNF secreted by TAM promotes Wnt/beta-catenin signaling through inhibition of glycogen synthase kinase 3 beta, which may contribute to tumor development in the gastric mucosa (41). In addition, TNF family members contribute to immune suppression; the decoy receptor-3 has been involved in the downregulation of major histocompatibility complex class II in TAM (42). On the whole, these findings provide a rationale for the development of clinical protocols employing TNF antagonists in cancer therapy. Phase I and II clinical cancer trials with TNF antagonists are under way and showed some clinical activity (11,43).

Together with TNF and IL-6, also IL-1 has long been known to augment the capacity of cancer cells to metastatize, by affecting multiple steps of the CRI cascade (4,44,45). IL-1R1 gene-targeted mice have provided clear evidence for the protumor potential of IL-1 (14,46). In particular, in models of chemical carcinogen-induced tumors, IL-1β secreted by malignant cells or infiltrating leukocytes...
contributes to increased tumor adhesiveness and invasion, angiogenesis and immune suppression, whereas IL-1ra negatively controls these processes (47). In diethyl-nitrosamine-induced hepatocarcinoma, the unique membrane-associated form of IL-1α acts as protumorigenic mediator; diethyl-nitrosamine-induced hepatocyte death results in the release of IL-1α and activation of IL-1R signaling, leading to IL-6 induction and compensatory proliferation, critical for hepatocarcinogenesis (48). In a pancreatic islet tumor model, a first wave of myc-driven angiogenesis is induced by the inflammatory cytokine IL-1 (49). Polymorphisms of IL-1β are associated with an increased risk of gastric carcinoma (50). Stomach-specific expression of human IL-1β in transgenic mice lead to spontaneous gastric inflammation and cancer that correlated with early recruitment of myeloid-derived suppressor cells (MDSCs) (51). Recent studies have uncovered a novel relationship between sex steroid hormones, IL-1 and cancer. In carcinoma of the prostate, an androgen-dependent tumor sensitivity to hormonal stimulation is regulated by selective androgen receptor modulators. The inflammatory cytokine IL-1 produced by macrophages in the tumor microenvironment converts selective androgen receptor modulator from inhibitors to stimulators, thus inducing resistance to hormonal therapies (52).

On the other hand, IL-1α is possibly of importance in 3-methylcholanthrene-induced fibrosarcoma for its efficiency in activating antitumor innate and specific immune responses, by acting as a focused adjuvant, through binding to IL-1RI on cells deemed to immune surveillance (53,54). Moreover, small amounts of IL-1α, which is homeostatically expressed in cells but not secreted, can be poured out from necrotizing cells and serve as a 'danger signal' for mounting antitumor immunity (55). These findings call attention to the concept that inflammatory reactions can also trigger antitumor activity (4).

Significance of myeloid cell recruitment within tumors

Besides neoplastic cells, the ‘other half’ of the tumor is composed of a stroma containing fibroblasts, vessels and leukocytes. TAMs are the principal leukocyte subset driving an amplification of the inflammatory response in the tumor milieu. However, also mast cells, neutrophils and even effectors of the adaptive immunity (especially in the form of antibodies) may activate inflammatory reactions that promote cancer progression (56,57). Chemokines have long been associated with the recruitment of TAM in tumors (e.g. CCL2 and CCL5) (4,58). For their phenotypic and functional properties, TAM resembles M2-polarized macrophages, though there are some distinctive features (59,60). In most studies, accumulation of TAM has been associated with the angiogenic switch and poor prognosis (3,4,5,62). TAM assists tumor cell malignant behavior in many ways by releasing cytokines, growth factors and matrix-degrading enzymes (63–66) and a host of angiogenic mediators for DNA damage and evidence obtained (85).

Tumor progression is largely mediated by the host inability to mount a protective antitumor immune response. TAM and MDSC express a large immunosuppressive repertoire. In addition to inhibit CD8 T cell activation by the expression of NOS2 and Arg1, MDSC induce the development of CD4+ FOXP3+ T-regulatory cells and an M2 polarization of TAM (87,91–93). Indoleamine 2,3 dioxygenase is a key immunosuppressive factor. Skin application of phorbol myristate acetate provoked a chronic inflammation and release of indoleamine 2,3 dioxygenase that facilitated tumor progression (94).

The immunoregulatory activity of TAM is mostly influenced by cues encountered locally in tissues. In the tumor milieu, a number of immunosuppressive factors (e.g. IL-10 and transforming growth factor-β) have been described to affect the differentiation of incoming monocytes toward M2 macrophages (59,62,64,95). NF-kB has also been recently involved in driving the M2 polarization of TAM (96). In established advanced tumors, where inflammation is typically smoldering (4), TAM have defective and delayed NF-kB activation (97) and substantial data suggest that p50 homodimers (acting as negative regulators of NF-kB) are responsible for the sluggish NF-kB activation in TAMs and for their protumor phenotype. Metabolic changes in the tumor milieu, in addition to provide growth and survival advantages for cancer cells, may also influence infiltrating leukocytes (98). It was found that lactic acid secreted by tumor cells promotes the IL-23/IL-17 axis in TAM (99). Thus, lactic acid is a proinflammatory stimulus inducing the IL-23 IL-17 pathway to the expenses of the immunoprotective IL-12-inducible Th1 pathway. Also, components of the extracellular matrix may constitute a link between tumor cells and macrophages. Kim et al. (100) have recently reported that versican, by triggering the innate receptors TLR2/TLR6 on TAM, amplifies an inflammatory cascade leading to enhanced metastasis.

The perfect storm: CRI and tumor cell genetic instability

In the extrinsic pathway, it remains uncertain whether chronic inflammation per sé is sufficient for carcinogenesis. Reactive oxygen and nitrogen intermediates are obvious inflammation-generated candidate mediators for DNA damage and evidence obtained in vitro and in vivo is consistent with this view (4). Hereafter, we summarize data suggesting that inflammatory cells and mediators can destabilize the cancer cell genome by a variety of mechanisms either directly inducing DNA damage or affecting DNA repair systems and altering cell cycle checkpoints (Figure 2). These emerging data suggest that an additional mechanism by which inflammation can contribute to cancer initiation and progression is genetic destabilization of cancer cells.

An unstable genome is a hallmark feature of nearly all solid tumors and hematopoietic leukemias (101,102). Cancer genetic instability through accelerated somatic evolution leads to a genomically heterogeneous population of expanding cells naturally selected for their ability to proliferate, invade distant tissues and evading host defenses (103). Genetic instability in cancer reflects an increased rate of DNA alterations in tumor cells, which may arise either from increased rates of damage or defective mechanisms that maintain genetic integrity within cells (101,102). Such systems recognize and correct damaged DNA, regulate the proper timing and accuracy of the genetic material duplication and faithfully segregate chromosomes into the daughter cells (104).

Inflammation and microsatellite instability

Mismatch repair (MMR) family members repair base–base mispairs and larger insertions/deletions (104). Mutations or epigenetic silencing of MMR members is associated with increased genetic instability termed as microsatellite instability (MSI), shown as increased rates of DNA replication errors throughout the genome. These errors preferentially affect genes such as TGFβR1, IGF-2R and BAX that contain in their coding regions microsatellites (short repetitive nucleotide sequences in DNA) that are intrinsically unstable and therefore prone to be copied incorrectly during DNA replication (101).
Inflammation downregulates MMR proteins by a variety of mechanisms. HIF-1α, which is induced in cancer cells by inflammatory cytokines (TNF and IL-1β), PGE2 (105) and reactive oxygen and nitrogen species (106) downregulate MMR proteins MSH2 and MSH6 by displacing c-Myc from MSH2/MSH6 promoters (107). Hydrogen peroxide inactivates MMR members by damaging the enzymes at the protein level (108). Direct evidence for the role of oxidative stress in carcinogenesis via MMR inactivation comes from experiments that induce frameshift mutations in a reporter gene after exposure to hydrogen peroxide (109). NO-induced upregulation of DNA methyltransferase results in extensive methylation of the cytosine bases, which is associated with promoter silencing and loss of gene expression of the MMR member hMLH1 (110). By immunohistochemistry, decreased levels of hMLH1 proteins are seen in gastric epithelial cells in H. pylori-positive patients (111). In colitis-associated cancers, hMLH1 hypermethylation is observed in a substantial proportion of patients (110). MSI can be detected early in premalignant tissues without dysplasia of patients with ulcerative colitis (UC), suggesting that inactivation of the MMR system is an early event in colon carcinogenesis in UC (112,113). In an in vitro model, exposure to activated neutrophils, which accumulate within crypts in UC, increases the number of replication errors in colonic cells (114).

While the MMR pathway has frequently been the focus of MSI studies, also the base excision repair (BER) pathway, which deals with base damage (104), may be implicated (115). In tissues from non-cancerous colons of UC patients, two BER enzymes (AAG and APE1) are significantly increased with a positive correlation with MSI (116). Mechanistic studies have shown that overexpression of these BER enzymes enhances MSI (116). This finding must be considered also in the view that reactive oxygen species (ROS) induce BER members (116,117) and that the BER enzyme APE1 promoter contains the consensus sequence for binding NF-kB (117).

The nucleotide excision repair pathway, which serves to repair a variety of DNA lesions caused by UV radiation, mutagenic chemicals and chemotherapeutic agents (104), appears to be affected by IL-6 that in multiple myeloma cells induces hypermethylation, and thus defective function, of the key nucleotide excision repair component hHR23B (118). HIF-1α induces the microRNA-373 that downregulates the expression of the nucleotide excision repair component RAD23B (119).

**Inflammation and chromosomal instability**

Chromosomal instability (CI) results in abnormal segregation of chromosomes and aneuploidy (120). Molecular mechanisms underlying CI are only partially described. In most cancers with CI, proteins of the mitotic checkpoints are disregulated (120). As a consequence, cancer cells fail to halt the cell cycle until DNA repair can be executed. Recently, a CI signature associated with cancer has been described in which 29 of 70 genes included in the signature are mitotic regulators (121).

Inactivation of p53 may play a role in CI (122). The p53 pathway protects cells from transformation by inducing apoptosis upon DNA damage and CI, p53 deficiency and a defective mitotic checkpoint in T lymphocytes increase CI through aberrant exit from mitotic arrest (123). Loss of p53 and p73 are associated with increased aneuploidy in mouse embryonic fibroblasts (124). The proinflammatory cytokine migration inhibitory factor suppresses p53 activity as a transcriptional activator (125). NO and its derivatives inhibit the function of p53 (126,127) and are associated with p53 mutations (113,128–131). NO (132) and the inflammatory cytokine IL-6 (118) increase the activity of DNA methyltransferase, resulting in CpG island methylation. Most of the p53 mutations in UC-associated cancers are G:C to A:T transitions at two hot spot CpG dinucleotide sites (113,133).
Inflammation is a key component of the tumor microenvironment. Recent efforts have shed new light on molecular and cellular pathways linking inflammation and cancer (4). Schematically, two pathways have emerged: in the intrinsic one, activation of different classes of oncogenes drives the expression of inflammation-related programs that guide the construction of an inflammatory milieu. In the extrinsic pathway, inflammatory conditions promote cancer development. Key orchestrators of the inflammation-mediated tumor progression (the
dark side of the force) are transcription factors, cytokines, chemokines and infiltrating leukocytes.

The high degree of genetic heterogeneity in tumors suggests that genetic instability is an ongoing process throughout tumor development. Accumulating evidence supports the view that inflammatory mediators, some of that are direct mutagens, also directly or indirectly downregulate DNA repair pathways and cell cycle checkpoints, thus destabilizing cancer cell genome and contributing to the accumulation of random genetic alterations. These in turn accelerate the somatic evolution of cancer to a genomically heterogeneous population of expanding cells naturally selected for their ability to proliferate, invade and evade host defenses (105).

CRI represents a target for innovative therapeutic strategies. For many years, all efforts to treat cancer have concentrated on the destruction/inhibition of tumor cells. Strategies to modulate the host microenvironment offer a complementary perspective. Primary proinflammatory cytokines represent prime targets and ongoing results in this direction justify continuing efforts (10,11).

Finally, inflammatory reactions can also result in antitumor activity (the bright side of the force) (4,95,182). This dual function of inflammatory cells and mediators is reflected by studies on correlations between parameters of CRI and clinical behavior in different contexts (183–188). The challenge now is to identify the mechanisms triggering a ‘bright’ inflammatory response leading to tumor inhibition, whereas neutralizing the protumor actions of the dark side (4).

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