REVIEW

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 inflammation as a risk factor for certain cancers. The 'inflammationcancer' connection is not restricted to increased risk for a subset of tumors. An inflammatory component is present in the microenvironment of most neoplastic tissues, including those not causally related to an obvious inflammatory process. Key features of cancer-related inflammation (CRI) include the infiltration of white blood cells, prominently tumor-associated macrophages (TAMs); the presence of polypeptide messengers of inflammation [cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, chemokines such as CCL2 and CXCL8] and the occurrence of tissue remodeling and angiogenesis.

Recent efforts have shed new light on molecular and cellular circuits linking inflammation and cancer (4). Two pathways have been

Abbreviations: AID, activation-induced cytidine deaminase; BER, base excision repair; CI, chromosomal instability; CRI, cancer-related inflammation; DSB, double-strand break; HIF, hypoxia inducible factor; HR, homologous recombination; IL, interleukin; MDSC, myeloid-derived suppressor cell; MMP, matrix metalloproteinase; MMR, mismatch repair; MSI, microsatellite instability; NF-κB, nuclear factor-kappaB; NO, nitric oxide; ROS, reactive oxygen species; TAM, tumor-associated macrophage; TLR, toll-like receptor; TNF, tumor necrosis factor; UC, ulcerative colitis; VEGF, vascular endothelial growth factor.

schematically identified; in the intrinsic pathway, genetic events causing neoplasia initiate the expression of inflammation-related programs that guide the construction of an inflammatory microenvironment [e.g. RET oncogene in papillary carcinoma of the thyroid (4–6)]. Oncogenes representative of different molecular classes and mode of action (tyrosine kinases, ras–raf, nuclear oncogenes and tumor suppressors) share the capacity to orchestrate proinflammatory circuits (e.g. angiogenetic switch; recruitment of myelo-monocytic cells). In the extrinsic pathway, inflammatory conditions facilitate cancer development. The triggers of chronic inflammation that increase cancer risk or progression include infections (e.g. *Helicobacter pylori* for gastric cancer and mucosal lymphoma; papilloma virus and hepatitis viruses for cervical and liver carcinoma, respectively), autoimmune diseases (e.g. inflammatory bowel disease for colon cancer) and inflammatory conditions of uncertain origin (e.g. prostatitis for prostate cancer).

Key orchestrators at the intersection of the intrinsic and extrinsic pathway include transcription factors and primary proinflammatory cytokines (7,8). Thus, CRI is a key component of tumors and may represent the seventh hallmark of cancer (Figure 1) (6). Here, we will review molecular links connecting inflammation and cancer and their mutual influence. We will emphasize in particular emerging evidence suggesting that CRI may contribute to the genetic instability of cancer cells. Thus, CRI represents a target for innovative therapeutic strategies and prevention. These results provide further impetus for studies targeted to the inflammatory microenvironment of tumors [e.g. (10,11)].

Masters and commanders in CRI

Transcription factors and primary inflammatory cytokines

In the panoply of molecular players involved in CRI, one can identify prime movers (endogenous promoters). These include transcription factors such as nuclear factor-kappaB (NF- κ B) and signal transducer activator of transcription-3 (Stat3) and primary inflammatory cytokines, such as IL-1 β , IL-6 and TNF- α (12–15).

NF-κB is a key orchestrator of innate immunity/inflammation and aberrant NF-κB regulation has been observed in many cancers (12). In both tumor and inflammatory cells, NF-κB is activated downstream of the toll-like receptor (TLR)-MyD88 pathway (sensing microbes and tissue damage) and of the inflammatory cytokines TNF-α and IL-1β. In addition, NF-κB activation can be the result of cell-autonomous genetic alterations (amplification, mutations or deletions) in cancer cells. Interestingly, NF-κB can be activated in response to hypoxia, though to a lesser extent than hypoxia inducible factor (HIF)-1α (7,16,17). Accumulating evidence suggests that intersections and compensatory pathways may exist between the NF-κB and HIF-1α systems linking innate immunity to the hypoxic response.

NF-kB induces the expression of inflammatory cytokines, adhesion molecules, key enzymes in the prostaglandin synthase pathway (COX-2), nitric oxide (NO) synthase and angiogenic factors. In addition, by inducing antiapoptotic genes (e.g. Bcl2), it promotes survival in tumor cells and in epithelial cells targeted by carcinogens. A number of studies provided unequivocal evidence that NF-KB is involved in tumor initiation and progression in tissues in which CRI typically occurs (such as the gastrointestinal tract and the liver) (12,18,19). NF-kB gene targeting in epithelial cells can have divergent effects in different models of carcinogenesis, possibly depending on the balance between promotion of apoptosis in initiated cell and triggering of compensatory cell proliferation (18-20). Specific inactivation of NF-KB in tumor-infiltrating leukocytes, by a strategy targeting IkappaBkinase beta, inhibited colitis-associated cancer, thus providing unequivocal genetic evidence for the role of NF-κB and inflammatory cells in intestinal carcinogenesis (18).



Fig. 1. Inflammation as the seventh hallmark of cancer. An integration to the six hallmarks of cancer [modified from Hanahan and Weinberg (9) and Mantovani (6)].

The NF- κ B 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-kB and have been shown to promote inflammation-propelled neoplasia (12). Thus, the lack of a checkpoint (Tir8) of NF-kB 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- κ B, STAT3 is a point of convergence for numerous oncogenic signaling pathways (13). Lee *et al.* (24) recently showed that the maintenance of NF- κ B 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-kB 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-kB–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-kB (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-1 β 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-1 a acts as protumorigenic mediator; diethyl-nitrosamine-induced hepatocyte death results in the release of IL-1 α 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-1 β are associated with an increased risk of gastric carcinoma (50). Stomach-specific expression of human IL-1 β 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-1 α is possibly of importance in 3-methylcholantrene-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 deputed to immune surveillance (53,54). Moreover, small amounts of IL-1 α , 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 (3,4,61,62). 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 factors (e.g. vascular endothelial growth factor (VEGF), platelet-derived growth factor, fibroblast growth factor and CXCL8) (1,67-77). It is well known that TAM accumulates in hypoxic regions of tumors and hypoxia triggers a proangiogenic program in these cells (67). Recent results suggest that TAM promotes tumor angiogenesis also via Semaphorin 4D (78). Monocytes express VEGF receptors and VEGF is a known chemoattractant of myeloid cells in tumors (79). VEGF1R⁺ hematopoietic cells home to tumor-specific premetastatic sites that favors secondary localization of cancer (80,81).

Recently, new evidence was provided that a distinct subset of monocytes expressing the Tie2 receptor (TEM) has a major role in tumor angiogenesis (82–84). Conditional deletion of Tie2+ myeloid cells in mice resulted in significant reduction of transplanted tumor mass and vasculature, demonstrating the importance of TEM in neoangiogenesis (82). Like TAM, TEM are clustered in hypoxic areas of solid tumors, in close proximity to nascent tumor vessels. The tumor-homing ability of TEM has been suggested as a potential vehicle for antitumor gene delivery (e.g. IFN α) (85).

Chemokines (e.g. CXCL5 and CXCL12) are also involved in the attraction of MDSCs (86,87). MDSCs, like TAM, are important effectors in tumor angiogenesis (88,89) and Gr^+Mac1^+ cells, presumably MDSC, have been shown to mediate resistance to antiangiogenic therapy (90).

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-кB 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- κ B 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 adult-onset leukemias (101,102). Cancer genetic instability through accelerated somatic evolution leads to a genomically heterogenous 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 TGF β RII, 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).



Fig. 2. Molecular pathways linking CRI and genetic instability. Schematic representation of inflammation-related pathways leading to microsatellite and CI in cancer cells. Inflammatory cells produce reactive oxygen and nitrogen species (RONS) and other mediators, including cytokines, metalloproteinases (MMPs) and PGE2, which, in turn, amplify and perpetuate the inflammatory cascade (e.g. MMPs induce reactive oxygen intermediates, cytokines induce PGE2). Inflammatory mediators by a variety of mechanisms (see text) downregulate DNA repair pathways (e.g. MMR system) inducing MSI. Inflammatory mediators affect also CI by inducing either directly or indirectly DSB, defective mitotic checkpoints and disregulated HR pathway of DSB repair. The inflammatory cytokine TNF also upregulates the AID that, in addition to hypermutate Ig loci, can also induce point mutations and translocations (through DSB). MSI and CI induce random genetic diversification of expanding cancer cells. Cancer clones that randomly acquired the right combination of activated oncogenes and inactivated oncosuppressors will display the six hallmarks of cancer phenotype described by Weinberg and Hanahan.

Inflammation downregulates MMR proteins by a variety of mechanisms. HIF-1 α , which is induced in cancer cells by inflammatory cytokines (TNF and IL-1 β), 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-1 α 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). In

UC, p53 mutations occur early and are often detected in mucosa that is non-dysplastic (134,135).

The DNA/RNA editing enzyme activation-induced cytidine deaminase (AID) induces hypermutation of the immunoglobulin loci in B cells. AID is overexpressed in human lymphoid malignancies (136,137) and, ectopically, in cholangiosarcoma biopsies (8,137), gastric epithelial cells of H.pylori-positive chronic gastritis and cancer (138), inflamed colonic mucosa of UC and in colitis-associated cancer (139), in human hepatocellular carcinoma and surrounding noncancerous liver tissue with underlying chronic inflammation (140) and in human liver with chronic hepatic inflammation caused by hepatitis C virus infection (140). AID is induced by the inflammatory cytokines TNF and IL-1 β (139,141), by the T-helper cell 2-driven cytokines IL-4 and IL-13 (142) and by transforming growth factor- β (140). In addition to targeting immunoglobulin loci in B cells, AID produces mutations and translocations [through induction of doublestrand breaks (DSBs), see below] in a number of other genes, including p53, c-Myc and BCL6 (143-145).

Apart from its peroxidase activity that would increase oxidative stress in cells, COX-2 overexpression in breast cancer cells was associated with a significant increase in chromosomal aberrations (fusions, breaks and tetraploidy), possibly due to COX-2-mediated activation of AKT-induced inhibitory phosphorylation of CHK1 (146), whose haploinsufficiency induces accumulation of DNA damage by failure to restrain mitotic entry in the presence of a damaged S-phase (147).

Malignant cells employ matrix metalloproteinases (MMPs) to penetrate the extracellular matrix and basement membrane and to invade distant tissues. Recent data suggest that MMPs may also function as oncogenes by promoting CI. MT1-MMP, which is present also in the pericentrosomal compartment, compromises normal cytokinesis inducing aneuploidy. Overexpression of MT1-MMP caused increased chromosome numbers and multipoles along with misaligned mitotic spindle formation (148). A potential target of MT1-MMP is pericentrin, an integral centrosomal/midbody protein required for centrosome performance and chromosome segregation (149). Endogenous pericentrin is cleaved in different cell types transfected with MT1-MMP (149). MMP-3, which is upregulated in many breast cancers (150), also mediates CI in cultured cells and in transgenic mice (151,152). Expression of MMP-3 in cells stimulates the production of Rac1b (153), an hyperactive alternative splicing form of Rac1, which stimulates ROS production which can cause oxidative DNA damage and CI (154).

The retinoblastoma protein is hyperphosphorylated in both mouse and human colitis (155). NO induces hyperphosphorylation of retinoblastoma protein (156). In its hyperphosphorylated form, retinoblastoma protein releases the E2 promoter-binding factor-1 (E2F1) (155), leading to CI by upregulation of Mad2 that is overexpressed in several tumor types (157). Elevated Mad2, a key component of the spindle checkpoint, can produce a hyperactive spindle checkpoint and thereby altering the sequence of mitotic events and the accuracy of chromosome segregation (157).

Mad2 is also involved in FAT10-induced CI. FAT10, a member of the ubiquitin-like modifier family of proteins, is overexpressed in 90% of hepatocellular carcinoma and in >80% of colon, ovary and uterus carcinomas (158). FAT10 impairs Mad2 during mitosis, inducing an abbreviated mitotic phase and CI (159). IFN- γ and TNF- α synergistically upregulate FAT10 expression in liver and colon cancer cells 10- to 100-fold (160). FAT10 expression in malignancies is also attributed to transcriptional upregulation upon the loss of p53 (161).

Several agents induce DSBs in cancer cells, including reactive oxygen and nitrogen species (162,163), irradiation and chemotherapeutic agents. Moreover, ROS induce DSB increasing (163) by increasing telomere erosion due to loss of recognition of these sites by telomere protective proteins such as telomere repeat binding factors 1 and 2 (164,165). There are two major mechanisms for DSB repair, homologous recombination (HR) (166) and non-homologous end joining (167). Induction of DSB impairs genome integrity since the non-homologous end-joining pathway is intrinsically error prone, re-

sulting in small regions of non-template nucleotides around the DNA break. Moreover, a very precise regulation of the error-free HR mechanisms is also essential for genome stability since uncontrolled HR excess promotes CI as well as HR deficiency.

Growth factors and chemokines produced by inflammatory cells in tumor microenvironment induce overexpression of structurally normal c-Myc in cancer cells. c-Myc alters the expression of hundreds of target genes related to cell growth, apoptosis and invasion. However, c-Myc also accelerates the intrinsic mutation rate in cancer cells. c-Myc induces DSB, as well as activated Ras (168), by production of ROS (169) (see above) and utilization of cryptic replication origins leading to aberrant and incomplete DNA synthesis (170). In addition, c-Myc drives aberrant DNA synthesis as a result of upregulation of cyclin B1, particularly when coupled with p53 deficiency (171). Finally, c-Myc delays prometaphase inducing chromosomal missegregation by direct transactivation of the spindle checkpoint proteins Mad2 and BubR1 (172) and mitigates p53 function (169).

Inflammatory mediators affect the expression and activity of DSB repair mechanisms. Bcl-2 is overexpressed in cancer cells by a variety of stimuli from the tumor microenvironment through NF-kB activation (12). The oncogenic role of Bcl-2 might extend well beyond the inhibition of apoptotic death. Bcl-2 inhibits DSB repair resulting in elevated frequencies of inducible and spontaneous mutagenesis by posttranslational modification (173) and inhibition the HR member RAD51 (104). Several cytokines and growth factors activate the signal transducer JAK-2. Mutated JAK-2 and, to a lesser extent, wild-type JAK-2 increase the HR pathway inducing CI (174). HIF-1 α , which is upregulated by inflammatory cytokines, induces miR-210 and miR-373 that in turn downregulate expression of the HR member RAD52 (119).

Is inflammation associated with genetic instability in non-cancer conditions?

The concept that an inflammatory microenvironment contributes to genome destabilization in cancer is in keeping with findings of MSI and CI also in non-cancer-related inflammatory conditions. The mutation rate in the inflamed microenvironment is higher than in normal tissues, with a mutation frequency of 4 $\,\times\,10^{-8}$ and ${<}1\,\times\,10^{-8}$ per base pair, respectively (175). MSI and a high frequency of p53 mutations are detected in pancreatitis and in UC patients whose colonic mucosa was negative for dysplasia (112,113,134,135). MSI associated with MMR deficiency, p53 mutations and chromosomal alterations are described in atherosclerotic plaques and synovia of rheumatoid arthritis patients (176,177). AID, which produces mutations and translocations in a number of genes (see above), is overexpressed in non-cancer-related chronic gastritis (138), inflamed mucosa of UC (142) and inflamed liver (140). Moreover, loss of heterozygosity, changes in gene copy number of certain loci and somatic mutations in key tumor suppressor genes, including PTEN and p53, are found in the genomes of tumor-associated stromal cells (178-180). In contrast, a recent report has failed to document genetic alterations in fibroblasts in carcinoma-associated fibroblasts of breast and ovarian tumors (181). Since technical differences may account for these contrasting results, more in depth and well-controlled studies are required to confirm the hypothesis of somatic mutations and coevolution of stromal cells in the tumor microenvironment (178).

Concluding remarks

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 heterogenous population of expanding cells naturally selected for their ability to proliferate, invade and evade host defenses (103).

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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