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 micro-environment of tumors that are not epidemiologically related to inflammation. Recent studies have begun to unravel molecular pathways linking inflammation and cancer. In the tumor micro-environment, 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 'inflammation–cancer' 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

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)].
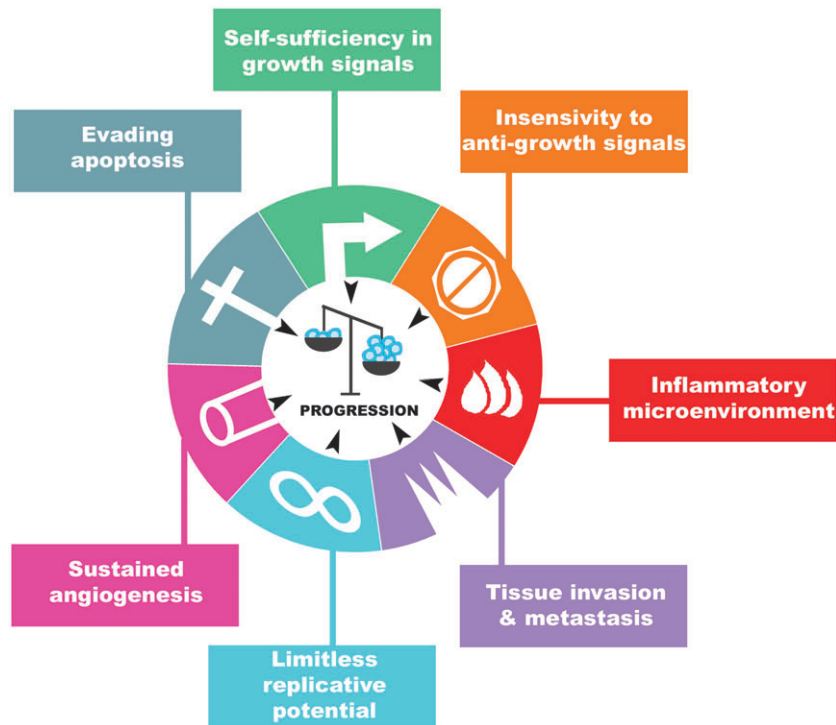
Masters and commanders in CRI

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-κB) and signal transducer activator of transcription-3 (Stat3) and primary inflammatory cytokines, such as IL-1β, 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-κB 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-κB 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-κB 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-κB in tumor-infiltrating leukocytes, by a strategy targeting IkappaB-kinase 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-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 E_2, 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-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-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-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-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-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 (3,4,61,62). 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 neo-angiogenesis (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. IFNα) (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-κB 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-κB activation (97) and substantial data suggest that p50 homodimers (acting as negative regulators of NF-κB) are responsible for the sluggish NF-κB 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 TGFβRII, 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).
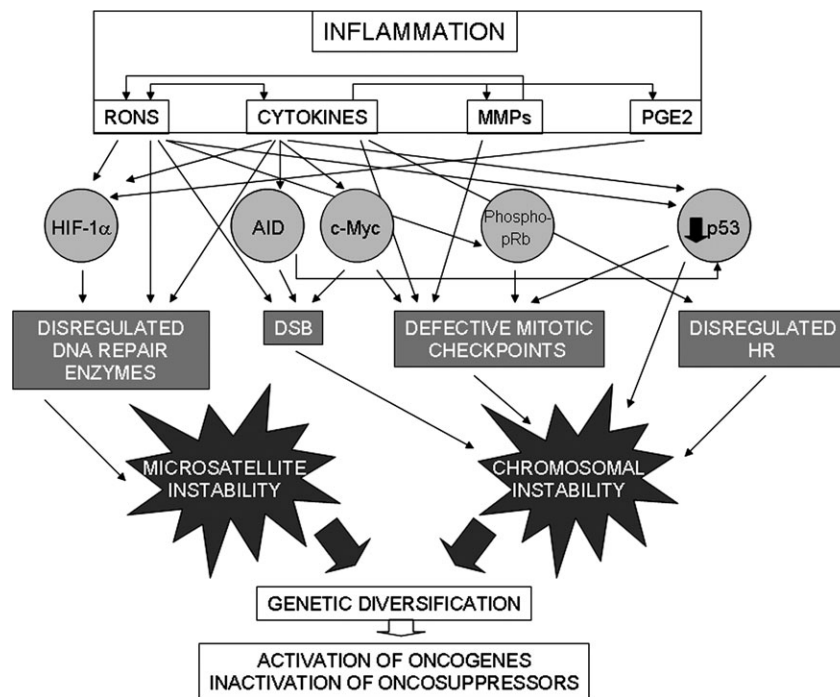
Fig. 2. Molecular pathways linking CRI and genetic instability. Schematic representation of inflammation-related pathways leading to microsatellite and CI in cancer cells. Inflammatory cells produce reactive oxygen and nitrogen species (RONS) and other mediators, including cytokines, metalloproteinases (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-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 noncancerous 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). 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 non-cancerous 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-1β (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 double-strand 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-1α, 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 4×10^{-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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References

1. Balkwill,F. *et al.* (2001) Inflammation and cancer: back to Virchow? *Lancet*, **357**, 539–545.
2. Coussens,L.M. *et al.* (2002) Inflammation and cancer. *Nature*, **420**, 860–867.
3. Balkwill,F. *et al.* (2005) Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell*, **7**, 211–217.
4. Mantovani,A. *et al.* (2008) Cancer-related inflammation. *Nature*, **454**, 436–444.
5. Borrello,M.G. *et al.* (2005) Induction of a proinflammatory program in normal human thyrocytes by the RET/PTC1 oncogene. *Proc. Natl Acad. Sci. USA*, **102**, 14825–14830.
6. Mantovani,A. (2009) Cancer: inflaming metastasis. *Nature*, **457**, 36–37.
7. Rius,J. *et al.* (2008) NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. *Nature*, **453**, 807–811.
8. Balkwill,F. (2009) Tumour necrosis factor and cancer. *Nat. Rev. Cancer*, **9**, 361–371.
9. Hanahan,D. *et al.* (2000) The hallmarks of cancer. *Cell*, **100**, 57–70.
10. Loberg,R.D. *et al.* (2007) Targeting CCL2 with systemic delivery of neutralizing antibodies induces prostate cancer tumor regression *in vivo*. *Cancer Res.*, **67**, 9417–9424.
11. Harrison,M.L. *et al.* (2007) Tumor necrosis factor alpha as a new target for renal cell carcinoma: two sequential phase II trials of infliximab at standard and high dose. *J. Clin. Oncol.*, **25**, 4542–4549.
12. Karin,M. (2006) Nuclear factor-kappaB in cancer development and progression. *Nature*, **441**, 431–436.
13. Yu,H. *et al.* (2007) Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. *Nat. Rev. Immunol.*, **7**, 41–51.
14. Voronov,E. *et al.* (2003) IL-1 is required for tumor invasiveness and angiogenesis. *Proc. Natl Acad. Sci. USA*, **100**, 2645–2650.
15. Langowski,J.L. *et al.* (2006) IL-23 promotes tumour incidence and growth. *Nature*, **442**, 461–465.
16. Carbia-Nagashima,A. *et al.* (2007) RSUME, a small RWD-containing protein, enhances SUMO conjugation and stabilizes HIF-1alpha during hypoxia. *Cell*, **131**, 309–323.
17. Taylor,C.T. (2008) Interdependent roles for hypoxia inducible factor and nuclear factor-kappaB in hypoxic inflammation. *J. Physiol.*, **586**, 4055–4059.
18. Greten,F.R. *et al.* (2004) IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell*, **118**, 285–296.
19. Pikarsky,E. *et al.* (2004) NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature*, **431**, 461–466.
20. Maeda,S. *et al.* (2005) IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. *Cell*, **121**, 977–990.
21. Garlanda,C. *et al.* (2007) Increased susceptibility to colitis-associated cancer of mice lacking TIR8, an inhibitory members of the IL-1 receptor family. *Cancer Res.*, **67**, 6017–6021.
22. Xiao,H. *et al.* (2007) The Toll-interleukin-1 receptor member SIGIRR regulates colonic epithelial homeostasis, inflammation, and tumorigenesis. *Immunity*, **26**, 461–475.
23. Lech,M. *et al.* (2008) Tir8/Sigirr prevents murine lupus by suppressing the immunostimulatory effects of lupus autoantigens. *J. Exp. Med.*, **205**, 1879–1888.
24. Lee,H. *et al.* (2009) Persistently activated Stat3 maintains constitutive NF-kappaB activity in tumors. *Cancer Cell*, **15**, 283–293.
25. Kortylewski,M. *et al.* (2005) Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. *Nat. Med.*, **11**, 1314–1321.
26. Becker,C. *et al.* (2004) TGF-beta suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. *Immunity*, **21**, 491–501.
27. Gao,S.P. *et al.* (2007) Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. *J. Clin. Invest.*, **117**, 3846–3856.
28. Grivennikov,S. *et al.* (2008) Autocrine IL-6 signaling: a key event in tumorigenesis? *Cancer Cell*, **13**, 7–9.
29. Lin,W.W. *et al.* (2007) A cytokine-mediated link between innate immunity, inflammation, and cancer. *J. Clin. Invest.*, **117**, 1175–1183.
30. Naugler,W.E. *et al.* (2008) The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. *Trends Mol. Med.*, **14**, 109–119.
31. Grivennikov,S. *et al.* (2009) IL-6 and stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell*, **15**, 103–113.
32. Bollrath,J. *et al.* (2009) gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. *Cancer Cell*, **15**, 91–102.
33. Kortylewski,M. *et al.* (2009) Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment. *Cancer Cell*, **15**, 114–123.
34. Annunziata,C.M. *et al.* (2007) Frequent engagement of the classical and alternative NF-kappaB pathways by diverse genetic abnormalities in multiple myeloma. *Cancer Cell*, **12**, 115–130.
35. Keats,J.J. *et al.* (2007) Promiscuous mutations activate the noncanonical NF-kappaB pathway in multiple myeloma. *Cancer Cell*, **12**, 131–144.
36. Naugler,W.E. *et al.* (2007) Gender disparity in liver cancer due to sex differences in MyD88-dependent-IL-6 production. *Science*, **317**, 121–124.
37. Rakoff-Nahoum,S. *et al.* (2007) Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. *Science*, **317**, 124–127.
38. Moore,R.J. *et al.* (1999) Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. *Nat. Med.*, **5**, 828–831.
39. Popivanova,B.K. *et al.* (2008) Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. *J. Clin. Invest.*, **118**, 560–570.
40. Kulbe,H. *et al.* (2007) The inflammatory cytokine tumor necrosis factor-alpha generates an autocrine tumor-promoting network in epithelial ovarian cancer cells. *Cancer Res.*, **67**, 585–592.
41. Oguma,K. *et al.* (2008) Activated macrophages promote Wnt signalling through tumour necrosis factor-alpha in gastric tumour cells. *EMBO J.*, **27**, 1671–1681.
42. Chang,Y.C. *et al.* (2008) Epigenetic control of MHC class II expression in tumor-associated macrophages by decoy receptor 3. *Blood*, **111**, 5054–5063.
43. Brown,E.R. *et al.* (2008) A clinical study assessing the tolerability and biological effects of infliximab, a TNF-alpha inhibitor, in patients with advanced cancer. *Ann. Oncol.*, **19**, 1340–1346.
44. Giavazzi,R. *et al.* (1990) Interleukin 1-induced augmentation of experimental metastases from a human melanoma in nude mice. *Cancer Res.*, **50**, 4771–4775.

45. Luo,J.L. *et al*. (2007) Nuclear cytokine-activated IKKalpha controls prostate cancer metastasis by repressing Maspin. *Nature*, **446**, 690–694.

46. Dinarello,C.A. (2006) The paradox of pro-inflammatory cytokines in cancer. *Cancer Metastasis Rev.*, **25**, 307–313.

47. Krelin,Y. *et al*. (2007) Interleukin-1beta-driven inflammation promotes the development and invasiveness of chemical carcinogen-induced tumors. *Cancer Res.*, **67**, 1062–1071.

48. Sakurai,T. *et al*. (2008) Hepatocyte necrosis induced by oxidative stress and IL-1 alpha release mediate carcinogen-induced compensatory proliferation and liver tumorigenesis. *Cancer Cell*, **14**, 156–165.

49. Shchors,K. *et al*. (2006) The Myc-dependent angiogenic switch in tumors is mediated by interleukin 1beta. *Genes Dev.*, **20**, 2527–2538.

50. El-Omar,E.M. *et al*. (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature*, **404**, 398–402.

51. Tu,S. *et al*. (2008) Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *Cancer Cell*, **14**, 408–419.

52. Zhu,P. *et al*. (2006) Macrophage/cancer cell interactions mediate hormone resistance by a nuclear receptor derepression pathway. *Cell*, **124**, 615–629.

53. Marhaba,R. *et al*. (2008) Opposing effects of fibrosarcoma cell-derived IL-1 alpha and IL-1 beta on immune response induction. *Int. J. Cancer*, **123**, 134–145.

54. Elkabets,M. *et al*. (2009) Host-derived interleukin-1α is important in determining the immunogenicity of 3-methylcholantrene-tumor cells. *J. immunol.*, **182**, 4874–4881.

55. Chen,C.J. *et al*. (2007) Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat. Med.*, **13**, 851–856.

56. de Visser,K.E. *et al*. (2006) Paradoxical roles of the immune system during cancer development. *Nat. Rev. Cancer*, **6**, 24–37.

57. Galli,S.J. *et al*. (2008) Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nat. Rev. Immunol.*, **8**, 478–486.

58. Balkwill,F. (2004) Cancer and the chemokine network. *Nat. Rev. Cancer*, **4**, 540–550.

59. Mantovani,A. *et al*. (2002) Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.*, **23**, 549–555.

60. Sica,A. *et al*. (2006) Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. *Eur. J. Cancer*, **42**, 717–727.

61. Bingle,L. *et al*. (2002) The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J. Pathol.*, **196**, 254–265.

62. Pollard,J.W. (2004) Tumour-educated macrophages promote tumour progression and metastasis. *Nat. Rev. Cancer*, **4**, 71–78.

63. Wyckoff,J.B. *et al*. (2007) Direct visualization of macrophage-assisted tumor cell intravasation in mammary tumors. *Cancer Res.*, **67**, 2649–2656.

64. Mantovani,A. *et al*. (2006) Role of tumor-associated macrophages in tumor progression and invasion. *Cancer Metastasis Rev.*, **25**, 315–322.

65. Condeelis,J. *et al*. (2006) Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell*, **124**, 263–266.

66. Yang,L. *et al*. (2008) Abrogation of TGFbeta signaling in mammary carcinomas recruits Gr-1+CD11b+ myeloid cells that promote metastasis. *Cancer Cell*, **13**, 23–35.

67. Murdoch,C. *et al*. (2008) The role of myeloid cells in the promotion of tumour angiogenesis. *Nat. Rev. Cancer*, **8**, 618–631.

68. Du,R. *et al*. (2008) HIF1alpha induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. *Cancer Cell*, **13**, 206–220.

69. Seandel,M. *et al*. (2008) A catalytic role for proangiogenic marrow-derived cells in tumor neovascularization. *Cancer Cell*, **13**, 181–183.

70. Ahn,G.O. *et al*. (2008) Matrix metalloproteinase-9 is required for tumor vasculogenesis but not for angiogenesis: role of bone marrow-derived myelomonocytic cells. *Cancer Cell*, **13**, 193–205.

71. Dineen,S.P. *et al*. (2008) Vascular endothelial growth factor receptor 2 mediates macrophage infiltration into orthotopic pancreatic tumors in mice. *Cancer Res.*, **68**, 4340–4346.

72. Kusmartsev,S. *et al*. (2008) Oxidative stress regulates expression of VEGFR1 in myeloid cells: link to tumor-induced immune suppression in renal cell carcinoma. *J. Immunol.*, **181**, 346–353.

73. Duluc,D. *et al*. (2007) Tumor-associated leukemia inhibitory factor and IL-6 skew monocyte differentiation into tumor-associated macrophage-like cells. *Blood*, **110**, 4319–4330.

74. Morandi,F. *et al*. (2007) Human neuroblastoma cells trigger an immunosuppressive program in monocytes by stimulating soluble HLA-G release. *Cancer Res.*, **67**, 6433–6441.

75. Robinson-Smith,T.M. *et al*. (2007) Macrophages mediate inflammation-enhanced metastasis of ovarian tumors in mice. *Cancer Res.*, **67**, 5708–5716.

76. Stearman,R.S. *et al*. (2008) A macrophage gene expression signature defines a field effect in the lung tumor microenvironment. *Cancer Res.*, **68**, 34–43.

77. Lewis,C.E. *et al*. (2006) Distinct role of macrophages in different tumor microenvironments. *Cancer Res.*, **66**, 605–612.

78. Sierra,J.R. *et al*. (2008) Tumor angiogenesis and progression are enhanced by Sema4D produced by tumor-associated macrophages. *J. Exp. Med.*, **205**, 1673–1685.

79. Fischer,C. *et al*. (2007) Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. *Cell*, **131**, 463–475.

80. Kaplan,R.N. *et al*. (2005) VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature*, **438**, 820–827.

81. Witz,I.P. (2008) Tumor-microenvironment interactions: dangerous liaisons. *Adv. Cancer Res.*, **100**, 203–229.

82. De Palma,M. *et al*. (2005) Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. *Cancer Cell*, **8**, 211–226.

83. De Palma,M. *et al*. (2003) Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. *Nat. Med.*, **9**, 789–795.

84. De Palma,M. *et al*. (2007) Tie2-expressing monocytes: regulation of tumor angiogenesis and therapeutic implications. *Trends. Immunol.*, **28**, 519–524.

85. De Palma,M. *et al*. (2008) Tumor-targeted interferon-alpha delivery by Tie2-expressing monocytes inhibits tumor growth and metastasis. *Cancer Cell*, **14**, 299–311.

86. Sawanobori,Y. *et al*. (2008) Chemokine-mediated rapid turnover of myeloid-derived suppressor cells in tumor-bearing mice. *Blood*, **111**, 5457–5466.

87. Sica,A. *et al*. (2007) Altered macrophage differentiation and immune dysfunction in tumor development. *J. Clin. Invest.*, **117**, 1155–1166.

88. Yang,L. *et al*. (2004) Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell*, **6**, 409–421.

89. Noonan,D.M. *et al*. (2008) Inflammation, inflammatory cells and angiogenesis: decisions and indecisions. *Cancer Metastasis Rev.*, **27**, 31–40.

90. Shojaei,F. *et al*. (2007) Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. *Nat. Biotechnol.*, **25**, 911–920.

91. Huang,B. *et al*. (2006) Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. *Cancer Res.*, **66**, 1123–1131.

92. Sinha,P. *et al*. (2007) Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. *J. Immunol.*, **179**, 977–983.

93. Nagaraj,S. *et al*. (2008) Tumor escape mechanism governed by myeloid-derived suppressor cells. *Cancer Res.*, **68**, 2561–2563.

94. Muller,A.J. *et al*. (2008) Chronic inflammation that facilitates tumor progression creates local immune suppression by inducing indoleamine 2,3 dioxygenase. *Proc. Natl Acad. Sci. USA*, **105**, 17073–17078.

95. Allavena,P. *et al*. (2008) The Yin-Yang of tumor-associated macrophages in neoplastic progression and immune surveillance. *Immunol. Rev.*, **222**, 155–161.

96. Hagemann,T. *et al*. (2008) "Re-educating" tumor-associated macrophages by targeting NF-kappaB. *J. Exp. Med.*, **205**, 1261–1268.

97. Biswas,S.K. *et al*. (2006) A distinct and unique transcriptional programme expressed by tumor-associated macrophages: defective NF-kB and enhanced IRF-3/STAT1 activation. *Blood*, **107**, 2112–2122.

98. Tennant,D.A. *et al*. (2009) Metabolic transformation in cancer. *Carcinogenesis*, in press.

99. Shime,H. *et al*. (2008) Tumor-secreted lactic acid promotes IL-23/IL-17 proinflammatory pathway. *J. Immunol.*, **180**, 7175–7183.

100. Kim,S. *et al*. (2009) Carcinoma produced factors activate myeloid cells via TLR2 to stimulate metastasis. *Nature*, **457**, 102–106.

101. Loeb,L.A. *et al*. (2008) DNA polymerases and human disease. *Nat. Rev. Genet.*, **9**, 594–604.

102. Rajagopalan,H. *et al*. (2002) Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. *Nature*, **418**, 934.

103. Nowell,P.C. (1976) The clonal evolution of tumor cell populations. *Science*, **194**, 23–8.

104. Hakem,R. (2008) DNA-damage repair; the good, the bad, and the ugly. *EMBO J.*, **27**, 589–605.

105. Jung,Y.J. *et al.* (2003) IL-1beta-mediated up-regulation of HIF-1alpha via an NFkappaB/COX-2 pathway identifies HIF-1 as a critical link between inflammation and oncogenesis. *FASEB J.*, **17**, 2115–2117.

106. Sandau,K.B. *et al.* (2000) Induction of hypoxia-inducible-factor 1 by nitric oxide is mediated via the PI 3K pathway. *Biochem. Biophys. Res. Commun.*, **278**, 263–267.

107. Koshiji,M. *et al.* (2005) HIF-1alpha induces genetic instability by transcriptionally downregulating MutSalpha expression. *Mol. Cell*, **17**, 793–803.

108. Chang,C.L. *et al.* (2002) Oxidative stress inactivates the human DNA mismatch repair system. *Am. J. Physiol. Cell Physiol.*, **283**, C148–C154.

109. Gasche,C. *et al.* (2001) Oxidative stress increases frameshift mutations in human colorectal cancer cells. *Cancer Res.*, **61**, 7444–7448.

110. Fleisher,A.S. *et al.* (2000) Microsatellite instability in inflammatory bowel disease-associated neoplastic lesions is associated with hypermethylation and diminished expression of the DNA mismatch repair gene, hMLH1. *Cancer Res.*, **60**, 4864–4868.

111. Mirzaee,V. *et al.* (2008) *Helicobacter pylori* infection and expression of DNA mismatch repair proteins. *World J. Gastroenterol.*, **14**, 6717–6721.

112. Brentnall,T.A. *et al.* (1996) Microsatellite instability in nonneoplastic mucosa from patients with chronic ulcerative colitis. *Cancer Res.*, **56**, 1237–1240.

113. Hussain,S.P. *et al.* (2000) Increased p53 mutation load in noncancerous colon tissue from ulcerative colitis: a cancer-prone chronic inflammatory disease. *Cancer Res.*, **60**, 3333–3337.

114. Campregher,C. *et al.* (2008) Activated neutrophils induce an hMSH2-dependent G2/M checkpoint arrest and replication errors at a (CA)13-repeat in colon epithelial cells. *Gut*, **57**, 780–787.

115. Guo,H.H. *et al.* (2003) Tumbling down a different pathway to genetic instability. *J. Clin. Invest.*, **112**, 1793–1795.

116. Hofseth,L.J. *et al.* (2003) The adaptive imbalance in base excision-repair enzymes generates microsatellite instability in chronic inflammation. *J. Clin. Invest.*, **112**, 1887–1894.

117. Ramana,C.V. *et al.* (1998) Activation of apurinic/apyrimidinic endonuclease in human cells by reactive oxygen species and its correlation with their adaptive response to genotoxicity of free radicals. *Proc. Natl Acad. Sci. USA*, **95**, 5061–5066.

118. Hodge,D.R. *et al.* (2005) Interleukin 6 supports the maintenance of p53 tumor suppressor gene promoter methylation. *Cancer Res.*, **65**, 4673–4682.

119. Crosby,M.E. *et al.* (2009) MicroRNA regulation of DNA repair gene expression in hypoxic stress. *Cancer Res.*, **69**, 1221–1229.

120. Rajagopalan,H. *et al.* (2003) The significance of unstable chromosomes in colorectal cancer. *Nat. Rev. Cancer*, **3**, 695–701.

121. Roschke,A.V. *et al.* (2008) Chromosomal instability is associated with higher expression of genes implicated in epithelial-mesenchymal transition, cancer invasiveness, and metastasis and with lower expression of genes involved in cell cycle checkpoints, DNA repair, and chromatin maintenance. *Neoplasia*, **10**, 1222–1230.

122. Tomasini,R. *et al.* (2008) The impact of p53 and p73 on aneuploidy and cancer. *Trends Cell Biol.*, **18**, 244–252.

123. Baek,K.H. *et al.* (2003) p53 deficiency and defective mitotic checkpoint in proliferating T lymphocytes increase chromosomal instability through aberrant exit from mitotic arrest. *J. Leukoc. Biol.*, **73**, 850–861.

124. Talos,F. *et al.* (2007) p73 suppresses polyploidy and aneuploidy in the absence of functional p53. *Mol. Cell*, **27**, 647–659.

125. Hudson,J.D. *et al.* (1999) A proinflammatory cytokine inhibits p53 tumor suppressor activity. *J. Exp. Med.*, **190**, 1375–1382.

126. Calmels,S. *et al.* (1997) Nitric oxide induces conformational and functional modifications of wild-type p53 tumor suppressor protein. *Cancer Res.*, **57**, 3365–3369.

127. Cobbs,C.S. *et al.* (2003) Inactivation of wild-type p53 protein function by reactive oxygen and nitrogen species in malignant glioma cells. *Cancer Res.*, **63**, 8670–8673.

128. Ambs,S. *et al.* (1999) Cancer-prone oxyradical overload disease. *IARC Sci. Publ.*, **150**, 295–302.

129. Marshall,H.E. *et al.* (2000) Nitrosation and oxidation in the regulation of gene expression. *FASEB J.*, **14**, 1889–1900.

130. Jaiswal,M. *et al.* (2001) Nitric oxide in gastrointestinal epithelial cell carcinogenesis: linking inflammation to oncogenesis. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **281**, G626–G34.

131. Wink,D.A. *et al.* (1994) The Fpg protein, a DNA repair enzyme, is inhibited by the biomediator nitric oxide *in vitro* and *in vivo*. *Carcinogenesis*, **15**, 2125–2129.

132. Hmadcha,A. *et al.* (1999) Methylation-dependent gene silencing induced by interleukin 1beta via nitric oxide production. *J. Exp. Med.*, **190**, 1595–1604.

133. Goodman,J.E. *et al.* (2004) Nitric oxide and p53 in cancer-prone chronic inflammation and oxyradical overload disease. *Environ. Mol. Mutagen.*, **44**, 3–9.

134. Brentnall,T.A. *et al.* (1994) Mutations in the p53 gene: an early marker of neoplastic progression in ulcerative colitis. *Gastroenterology*, **107**, 369–378.

135. Burmer,G.C. *et al.* (1992) Neoplastic progression in ulcerative colitis: histology, DNA content, and loss of a p53 allele. *Gastroenterology*, **103**, 1602–1610.

136. Greeve,J. *et al.* (2003) Expression of activation-induced cytidine deaminase in human B-cell non-Hodgkin lymphomas. *Blood*, **101**, 3574–3580.

137. Lossos,I.S. *et al.* (2004) AID is expressed in germinal center B-cell-like and activated B-cell-like diffuse large-cell lymphomas and is not correlated with intraclonal heterogeneity. *Leukemia*, **18**, 1775–1779.

138. Matsumoto,Y. *et al.* (2007) *Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. *Nat. Med.*, **13**, 470–476.

139. Endo,Y. *et al.* (2007) Expression of activation-induced cytidine deaminase in human hepatocytes via NF-kappaB signaling. *Oncogene*, **26**, 5587–5595.

140. Kou,T. *et al.* (2007) Expression of activation-induced cytidine deaminase in human hepatocytes during hepatocarcinogenesis. *Int. J. Cancer*, **120**, 469–476.

141. Komori,J. *et al.* (2008) Activation-induced cytidine deaminase links bile duct inflammation to human cholangiocarcinoma. *Hepatology*, **47**, 888–896.

142. Endo,Y. *et al.* (2008) Activation-induced cytidine deaminase links between inflammation and the development of colitis-associated colorectal cancers. *Gastroenterology*, **135**, 889–898;898, e1–e3.

143. Chesi,M. *et al.* (2008) AID-dependent activation of a MYC transgene induces multiple myeloma in a conditional mouse model of post-germinal center malignancies. *Cancer Cell*, **13**, 167–180.

144. Robbiani,D.F. *et al.* (2008) AID is required for the chromosomal breaks in c-myc that lead to c-myc/IgH translocations. *Cell*, **135**, 1028–1038.

145. Shen,H.M. *et al.* (1998) Mutation of BCL-6 gene in normal B cells by the process of somatic hypermutation of Ig genes. *Science*, **280**, 1750–1752.

146. Singh,B. *et al.* (2007) Cyclooxygenase-2 expression induces genomic instability in MCF10A breast epithelial cells. *J. Surg. Res.*, **140**, 220–226.

147. Lam,M.H. *et al.* (2004) Chk1 is haploinsufficient for multiple functions critical to tumor suppression. *Cancer Cell*, **6**, 45–59.

148. Golubkov,V.S. *et al.* (2005) Membrane type-1 matrix metalloproteinase (MT1-MMP) exhibits an important intracellular cleavage function and causes chromosome instability. *J. Biol. Chem.*, **280**, 25079–25086.

149. Golubkov,V.S. *et al.* (2007) Proteolysis-driven oncogenesis. *Cell Cycle*, **6**, 147–150.

150. Sternlicht,M.D. *et al.* (2001) How matrix metalloproteinases regulate cell behavior. *Annu. Rev. Cell Dev. Biol.*, **17**, 463–516.

151. Sternlicht,M.D. *et al.* (1999) The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. *Cell*, **98**, 137–146.

152. Lochter,A. *et al.* (1997) Misregulation of stromelysin-1 expression in mouse mammary tumor cells accompanies acquisition of stromelysin-1-dependent invasive properties. *J. Biol. Chem.*, **272**, 5007–5015.

153. Matos,P. *et al.* (2003) Tumor-related alternatively spliced Rac1b is not regulated by Rho-GDP dissociation inhibitors and exhibits selective downstream signaling. *J. Biol. Chem.*, **278**, 50442–50448.

154. Samper,E. *et al.* (2003) Mitochondrial oxidative stress causes chromosomal instability of mouse embryonic fibroblasts. *Aging Cell*, **2**, 277–285.

155. Ying,L. *et al.* (2005) Chronic inflammation promotes retinoblastoma protein hyperphosphorylation and E2F1 activation. *Cancer Res.*, **65**, 9132–9136.

156. Ying,L. *et al.* (2007) Nitric oxide inactivates the retinoblastoma pathway in chronic inflammation. *Cancer Res.*, **67**, 9286–9293.

157. Hernando,E. *et al.* (2004) Rb inactivation promotes genomic instability by uncoupling cell cycle progression from mitotic control. *Nature*, **430**, 797–802.

158. Lee,C.G. *et al.* (2003) Expression of the FAT10 gene is highly upregulated in hepatocellular carcinoma and other gastrointestinal and gynecological cancers. *Oncogene*, **22**, 2592–2603.

159. Ren,J. *et al.* (2006) FAT10 plays a role in the regulation of chromosomal stability. *J. Biol. Chem.*, **281**, 11413–11421.

160. Lukasiak,S. *et al.* (2008) Proinflammatory cytokines cause FAT10 upregulation in cancers of liver and colon. *Oncogene*, **27**, 6068–6074.

161. Zhang,D.W. *et al.* (2006) p53 negatively regulates the expression of FAT10, a gene upregulated in various cancers. *Oncogene*, **25**, 2318–2327.

162. Mills,K.D. *et al*. (2003) The role of DNA breaks in genomic instability and tumorigenesis. *Immunol. Rev.*, **194**, 77–95.

163. Karanjawala,Z.E. *et al*. (2002) Oxygen metabolism causes chromosome breaks and is associated with the neuronal apoptosis observed in DNA double-strand break repair mutants. *Curr. Biol.*, **12**, 397–402.

164. Opresko,P.L. *et al*. (2005) Oxidative damage in telomeric DNA disrupts recognition by TRF1 and TRF2. *Nucleic Acids Res.*, **33**, 1230–1239.

165. von Zglinicki,T. *et al*. (2005) Telomeres as biomarkers for ageing and age-related diseases. *Curr. Mol. Med.*, **5**, 197–203.

166. San Filippo,J. *et al*. (2008) Mechanism of eukaryotic homologous recombination. *Annu. Rev. Biochem.*, **77**, 229–257.

167. Lieber,M.R. (2008) The mechanism of human nonhomologous DNA end joining. *J. Biol. Chem.*, **283**, 1–5.

168. Halazonetis,T.D. *et al*. (2008) An oncogene-induced DNA damage model for cancer development. *Science*, **319**, 1352–1355.

169. Vafa,O. *et al*. (2002) c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability. *Mol. Cell*, **9**, 1031–1044.

170. Dominguez-Sola,D. *et al*. (2007) Non-transcriptional control of DNA replication by c-Myc. *Nature*, **448**, 445–451.

171. Taylor,W.R. *et al*. (1999) Mechanisms of G2 arrest in response to over-expression of p53. *Mol. Biol. Cell*, **10**, 3607–3622.

172. Menssen,A. *et al*. (2007) c-MYC delays prometaphase by direct trans-activation of MAD2 and BubR1: identification of mechanisms underlying c-MYC-induced DNA damage and chromosomal instability. *Cell Cycle*, **6**, 339–352.

173. Saintigny,Y. *et al*. (2001) A novel role for the Bcl-2 protein family: specific suppression of the RAD51 recombination pathway. *EMBO J.*, **20**, 2596–2607.

174. Plo,I. *et al*. (2008) JAK2 stimulates homologous recombination and genetic instability: potential implication in the heterogeneity of myeloproliferative disorders. *Blood*, **112**, 1402–1412.

175. Bielas,J.H. *et al*. (2006) Human cancers express a mutator phenotype. *Proc. Natl Acad. Sci. USA*, **103**, 18238–18242.

176. Firestein,G.S. *et al*. (1997) Somatic mutations in the p53 tumor suppressor gene in rheumatoid arthritis synovium. *Proc. Natl Acad. Sci. USA*, **94**, 10895–10900.

177. Andreassi,M.G. *et al*. (2003) Genetic instability, DNA damage and atherosclerosis. *Cell Cycle*, **2**, 224–227.

178. Weinberg,R.A. (2008) Coevolution in the tumor microenvironment. *Nat. Genet.*, **40**, 494–495.

179. Danish,M. *et al*. (2008) Genetic stability of tumor microenvironment. *Cancer Biol. Ther.*, **7**, 331–332.

180. Polyak,K. *et al*. (2009) Co-evolution of tumor cells and their microenvironment. *Trends Genet.*, **25**, 30–38.

181. Qiu,W. *et al*. (2008) No evidence of clonal somatic genetic alterations in cancer-associated fibroblasts from human breast and ovarian carcinomas. *Nat. Genet.*, **40**, 650–655.

182. Apetoh,L. *et al*. (2007) Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat. Med.*, **13**, 1050–1059.

183. Shabo,I. *et al*. (2008) Breast cancer expression of CD163, a macrophage scavenger receptor, is related to early distant recurrence and reduced patient survival. *Int. J. Cancer*, **123**, 780–786.

184. Tamimi,R.M. *et al*. (2008) Circulating colony stimulating factor-1 and breast cancer risk. *Cancer Res.*, **68**, 18–21.

185. Taskinen,M. *et al*. (2007) A high tumor-associated macrophage content predicts favorable outcome in follicular lymphoma patients treated with rituximab and cyclophosphamide-doxorubicin-vincristine-prednisone. *Clin. Cancer Res.*, **13**, 5784–5789.

186. Byers,R.J. *et al*. (2008) Clinical quantitation of immune signature in follicular lymphoma by RT-PCR-based gene expression profiling. *Blood*, **111**, 4764–4770.

187. Liu,R. *et al*. (2007) The prognostic role of a gene signature from tumorigenic breast-cancer cells. *N. Engl. J. Med.*, **356**, 217–226.

188. Seike,M. *et al*. (2007) Use of a cytokine gene expression signature in lung adenocarcinoma and the surrounding tissue as a prognostic classifier. *J. Natl Cancer Inst.*, **99**, 1257–1269.