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# Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways

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**Chronic inflammation and infection are major causes of cancer. There are continued improvements to our understanding of the molecular connections between inflammation and cancer. Key mediators of inflammation-induced cancer include nuclear factor kappa B, reactive oxygen and nitrogen species, inflammatory cytokines, prostaglandins and specific microRNAs. The collective activity of these mediators is largely responsible for either a pro-tumorigenic or anti-tumorigenic inflammatory response through changes in cell proliferation, cell death, cellular senescence, DNA mutation rates, DNA methylation and angiogenesis. As our understanding grows, inflammatory mediators will provide opportunities to develop novel diagnostic and therapeutic strategies. In this review, we provide a general overview of the connection between inflammation, microRNAs and cancer and highlight how our improved understanding of these connections may provide novel preventive, diagnostic and therapeutic strategies to reduce the health burden of cancer.**

## Introduction

There is an undeniable link between inflammation and cancer. Virchow first noted that inflammatory cells are present within tumors and tumors arise at sites of chronic inflammation. This observation was made >150 years ago and led to the conclusion that inflammation significantly contributes to the development of cancer. Epidemiological evidence now supports this conclusion and suggests that up to 25% of all cancers are due to chronic infection or other types of chronic inflammation (1). The sources of inflammation are widespread and range from microbial and viral infections to exposure to allergens and toxic chemicals to autoimmune diseases and obesity. An acute inflammatory response is usually beneficial, especially in response to microbial infections and tissue damage. A well-regulated inflammatory response can also be anti-tumorigenic and have a role in tumor suppression (2). Chronic inflammation, however, is detrimental and, among other deleterious effects, will frequently predispose cells for an oncogenic transformation. Irrespective of its underlying cause, chronic inflammation can be oncogenic by various mechanisms. This includes induction of genomic instability, increasing angiogenesis, altering the genomic epigenetic state and increasing cell proliferation. Over-production of reactive oxygen and nitrogen species (RONS), aberrant inflammatory cytokine and chemokine expression, increased cyclooxygenase-2 (COX-2) and nuclear factor kappa B (NFκB) expression are just some of the molecular factors that contribute to inflammation-induced carcinogenesis. Inflammation can alter the expression of oncogenes and tumor suppressor genes (including both protein coding genes and non-coding microRNA genes) to promote neoplastic transformation.

While much remains to be deciphered, the understanding of the connection between inflammation and cancer is rapidly improving. Whether an inflammatory immune response is pro- or anti-tumorigenic is a delicate balance between the adaptive and innate immune system (Figure 1). A healthy and regulated adaptive immune response is regarded as anti-tumorigenic, whereas an unrestrained innate or inappropriate adaptive response may lead to chronic inflammation and a pro-tumorigenic environment. As our understanding of this balance grows, so does the potential of using this knowledge for medical intervention. Measuring the inflammatory state of a tissue may serve as a measure of diagnosis and provide information that can guide therapeutic decisions. Using anti-inflammatory drugs, one may develop chemoprevention strategies to reduce cancer incidences. Manipulation of the local inflammatory states surrounding tumors may also constitute a therapeutic option.

This review provides an overview of the interwoven pathways of inflammation and cancer. We discuss the role of chronic inflammation and infections in oncogenesis. Several of the key molecular components and pathways that connect chronic inflammation with inflammation-associated oncogenic transformation will be described. We emphasize how the increased understanding of cancer-related inflammation may provide novel preventive, diagnostic and therapeutic strategies to reduce the health burden of cancer.

**Abbreviations:** CLL, chronic lymphocytic leukemia; COX-2, cyclooxygenase-2; IFN, interferon; IL, interleukin; KRAS, kirsten rat sarcoma oncogene; LPS, lipopolysaccharide; NFκB, nuclear factor kappa B; NO, nitric oxide; NOS, nitric oxide synthase; NSAIDs, non-steroidal, anti-inflammatory drugs; p53, protein 53; PGs, prostaglandins; RAS, rat sarcoma oncogene; RISC, RNA-induced silencing complex; RONS, reactive oxygen and nitrogen species; TGFβ, transforming growth factor; TNF, tumor necrosis factor; UTR, untranslated region.

### *Epidemiology of the association of cancer with inflammation*

*Chronic inflammation and infections as causes of cancer.* Epidemiological data demonstrate a strong connection between chronic inflammation and developing cancer (Table I). Both endogenous (e.g. inherited diseases and obesity) and exogenous (acquired infections and noxious insults) inducers of inflammation contribute to chronic inflammation-associated cancer (3).

Several chronic inflammatory diseases lead to increased risk of cancer. Inflammatory bowel diseases, i.e. Crohn's disease and ulcerative colitis, are associated with increased rates of colon adenocarcinoma (4–6). In chronic pancreatitis, there is an increased rate of pancreatic cancer (7). Heavy alcohol consumption is the primary cause of chronic pancreatitis and hereditary pancreatitis also contributes with a 50-fold increased risk of pancreatic cancer (8). α-1-Antitrypsin deficiency can lead to inflammation and cirrhosis of the liver with increased risk of hepatocellular carcinoma (9). Gastroesophageal reflux disease and Barrett's esophagus result in a chronically inflamed esophagus with a tissue field effect of genetic and epigenetic changes that lead to increased rates of esophageal carcinoma (10). Chronic bronchitis and emphysema each carry an increased risk of lung cancer (11,12).

Chronic infections, whether microbial or parasitic, are also a major cause of cancer. Viral hepatitis B and C infections lead to chronic inflammation of the liver and are responsible for the majority of hepatocellular carcinomas worldwide (13). Bacterial infection and colonization of the stomach by *Helicobacter pylori* causes chronic gastritis and is associated with the majority of gastric cancers (14). Infestations of parasitic worms lead to chronic inflammation and increased risk of multiple cancers, including bladder cancer (by *Schistosoma hematobium*) (15) and cholangiocarcinoma (by *Opisthorchis viverrini*, *Opisthorchis felineus* or *Clonorchis sinensis*) (16).

Environmental and chemical exposures can cause chronic inflammation and contribute to inflammation-associated carcinogenesis. Tobacco smoke is a key example, where inhalation of >60 chemical carcinogens can cause both mutations in cancer-related genes and, along with other chemical irritants, contributes to inflammation of

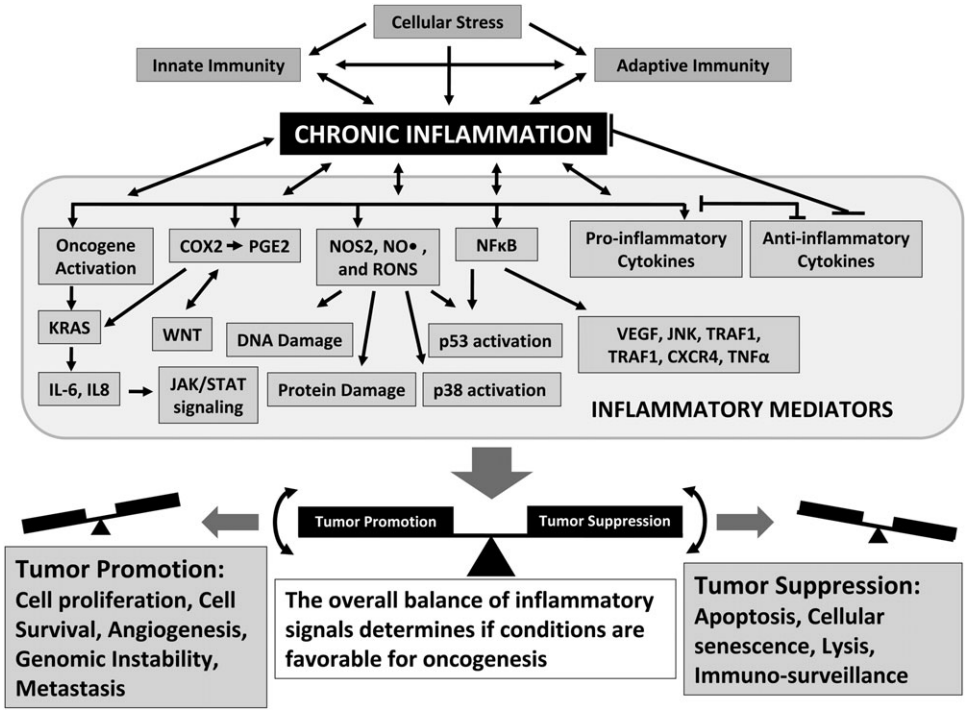


**Fig. 1.** Chronic inflammation alters the cellular levels of inflammatory mediators, including COX-2, RONS and inflammatory cytokines and activates proto-oncogenes. Depending on the collective functions and balance of inflammatory mediators, an inflammatory response may be either pro- or anti-tumorigenic.

**Table I.** Chronic inflammation or infection increases cancer risk

| Disease | Type of cancer | Increased risk |
|---|---|---|
| Auto-inflammatory/non-infectious | | |
| Crohn's disease | Colon cancer | 3 |
| Ulcerative colitis | Colon cancer | 6 |
| Chronic pancreatitis | Pancreatic cancer | 2–50 |
| Endometriosis | Endometrial cancer | 1.4 |
| Hemochromatosis | Liver cancer | 219 |
| Thyroiditis | Thyroid cancer | 3 |
| α-1-Anti-trypsin deficiency | Liver cancer | 20 |
| Acquired | | |
| Viral | | |
| Hepatitis B | Liver cancer | 88 |
| Hepatitis C | Liver cancer | 30 |
| Epstein–Barr virus | Hodkin's and Burkitt's lymphoma | 4 |
| Bacterial | | |
| *Helicobacter Pylori* | Gastric cancer | 11 |
| Pelvic inflammatory disease | Ovarian cancer | 3 |
| Chronic protatitis | Prostate cancer | 2–3 |
| Parasitic | | |
| *Schistosoma hematobium* | Bladder cancer | 2–14 |
| *Schistosoma Japonicum* | Colon cancer | 2–6 |
| Liver fluke | Cholangiocarcioma and liver cancer | 14 |
| Chemical/physical/metabolic | | |
| Alcohol | Multiple cancers (including liver, pancreas, head and neck cancer) | 2–7 |
| Asbestos | Mesothelioma | >10 |
| Obesity | Multiple cancers | 1.3–6.5 |
| Tobacco smoke and inhalation of other noxious chemicals | Lung cancer (and multiple other cancers) | >10 |
| Gastric reflux, Barrett's esophagus | Esophageal cancer | 50–100 |

the lung and chronic obstructive pulmonary disease. These are collectively responsible for the majority of lung cancer cases. Inhalation of asbestos fibers also causes chronic lung and pleural inflammation and increased rates of mesothelioma (17). Alcohol abuse can also cause chronic inflammation and subsequent cancers of the liver and pancreas.

Some of the most convincing data demonstrating the connection between inflammation and cancer are that certain anti-inflammatory drugs reduce the risk of various cancers. Cyclooxygenase inhibitors are non-steroidal, anti-inflammatory drugs (NSAIDs). They can be non-specific inhibitors (aspirin) or selective inhibitors (COX-2 inhibitors). Long-term use of NSAIDs has been associated with reduced risk of several different types of cancer (18). Randomized clinical trials have shown that NSAIDs are protective against colon adenomas, a precursor of colon cancer (19–21). This indicates that active inflammation can contribute to carcinogenesis and that inflammatory carcinogenesis is preventable. Also, the adaptive immune response may prevent tumor formation by inhibiting tumor growth and stimulating lysis of tumor cells. Accordingly, a key mechanism of immune evasion is the ability of some tumors to block activating receptors (such as natural killer group 2, member D) of the immune system by decoy ligands (22). Immunocompromised individuals have an increased risk of several types of cancers. Patients with acquired immunodeficiency syndrome have increased rates of Kaposi's sarcoma, non-Hodgkin's lymphoma and cervical cancer (23). Patients taking immunosuppressive drugs following transplant surgery have three times greater risk of developing various malignancies (24). Therefore, a balanced immune response is essential for the prevention and control of tumors.

*Oncogenic mechanisms in chronic inflammation.* A balanced immune system monitors tissue homeostasis. Perturbations of tissue homeostasis, such as tissue damage or infections, lead to an immune response. An imbalance of this response, i.e. a constitutively active innate immune response, can lead to chronic inflammation that favors neoplastic transformation as shown in mouse models (25). Modulation of the immune system in these models can affect

angiogenesis, cell proliferation, tumor volume and overall cancer incidence.

A prolonged inflammatory response can contribute to increased DNA mutation rates and overall genetic instability (1,26). Inflammation and free radicals can reduce expression and enzymatic activity of the DNA mismatch repair genes mutS homolog 2 and 6. It can also cause increased expression of DNA methyltransferases, leading to a global hypermethylation of the genome. This leads to promoter silencing of several genes, including the DNA mismatch repair gene *hMLH1* (27). Hypermethylation of tumor suppressor genes including *APC, CDKN2, BRCA1*, *Rb* and *MDM2* is thought to contribute to carcinogenesis (28). Increased DNA methylation is observed in a variety of chronic inflammatory diseases including ulcerative colitis and Barrett's esophagus. Colonization of *H.pylori* in the gastric mucosa also leads to increased DNA methylation of tumor suppressor genes (29) and this hypermethylation is associated with increased gastric cancer risk (30). These findings suggest that induced epigenetic changes and genomic instability are involved in inflammation-induced carcinogenesis.

*Molecular mediators common to inflammation and cancer*

*Oncogene-induced inflammation.* There are several mediators and mechanisms that contribute to inflammation-associated cancers (Figure 1, Table II). Both extrinsic and intrinsic inflammation pathways may be carcinogenic (31). In the extrinsic pathway, chronic inflammation and/or infection is the driving force that causes the increase in cancer risk. Alternatively, in the intrinsic pathway, genetic alterations of oncogenes and/or tumor suppressor genes are the primary cause of cancer. These genetic alterations affect the expression of various inflammatory genes and leads to recruitment of inflammatory cells. This is the reason why nearly all tumors have inflammatory cells present in the tumor or tumor microenvironment regardless of the underlying cause of the tumor. Common inflammatory mediators including cytokines, chemokines, RONS, COX-2 and NFκB can lead to cellular conditions favorable for tumor promotion.

As an example, dominant mutations of the *RAS* proto-oncogene induce an inflammatory response. This GTPase signaling molecule is mutationally activated in ~25% of all malignancies (32). Mutations in rat sarcoma oncogene (RAS) turn it into a dominant oncogene capable of inducing cell proliferation, tumor growth and angiogenesis. RAS activation results in a pro-tumorigenic microenvironment surrounding tumor cells. Activated RAS induces the expression of various inflammatory gene products, including the pro-inflammatory cytokines interleukin (IL)1, IL6 and IL11 and the chemokine IL8, which in turn are important for the oncogenic function of RAS (33). RAS induction of IL8 contributes to RAS-induced tumor growth and angiogenesis (34). Antibody inhibition or small interfering RNA knock down of IL6 can also abrogate RAS-induced tumorigenesis (35). Activation of other oncogenes (examples myelocytomatosis viral oncogene homolog and rearranged during transfection) or inactivation of tumor suppressor genes (examples phosphatase and tensin homolog and von Hippel-Lindau tumor suppressor) leads to similar inductions of inflammatory pathways that create an inflammatory microenvironment favorable for oncogenic transformation. These genetic mutations stimulate the tumor cells to produce inflammatory cytokines and free radicals, which in turn can create feedback loops in which inflammatory cells are recruited to the tumors where they produce additional cytokines and free radicals that favor carcinogenesis. This release of inflammatory molecules can also stimulate cellular senescence of suppressor T cells, causing reduction of cell-mediated cytotoxicity and potential immune evasion for tumors (36).

*NFκB.* NFκB is a transcription factor and key mediator of inflammation-induced carcinogenesis (37). NFκB has a variety of

**Table II.** Functional features of molecular mediators of inflammation and oncogenesis

| Mediators | Effect on inflammation | Role in tumorigenesis |
|---|---|---|
| Activated oncogenes | Pro-inflammatory, induce inflammatory gene expression, increase RONS | Oncogenic |
| Inactivated tumor suppressors | Pro-inflammatory, IL6/TNFα/IFNβ-induced oncogenic miRNAs inactivate tumor suppressors | Oncogenic, accelerates activated RAS tumorigenesis |
| Toll-like receptor gene polymorphisms | Activating, sustain inflammation | Pro-neoplastic drive caused by chronic inflammation |
| Cytokine/cytokine receptor gene polymorphisms | Increased/sustained inflammation | Same |
| MicroRNA/microRNA-binding site gene polymorphisms | | Oncogenic |
| Changes in microRNA expression patterns | Induced or repressed by inflammation<br>Pro- or anti-inflammatory actions | Pro- or anti-tumorigenic actions |
| Oncogenic microRNA expression (e.g. miR-21 and miR-155) | Induced by inflammation | Oncogenic |
| Activated transcription factor NFκB | Inflammatory gene activation → acute inflammatory responses, including induction of NO• synthase; in itself activated by pro-inflammatory cytokines, e.g. TNFα | Oncogenic through anti-apoptotic effects, cell cycle and angiogenesis activation, promotion of tumor metastasis and through chronic inflammation |
| IκB gene mutations/activation of IκB kinase | Pro-inflammatory by allowing nuclear translocation of NFκB; activated by infections and by pro-inflammatory cytokines | Oncogenic through NFκB effects |
| RONS, including NO• | Microbial killing in acute inflammation<br>Tissue damage if sustained/unregulated; NO• important signaling molecule in immunity | Anti- or pro-tumorigenic depending on cellular context; oxidative stress lead to DNA damage, genomic instability affecting tumor suppression and oncogenic activation; may also induce apoptosis, cellular senescence (anti-tumorigenic) |
| NO• and p53 | | Cooperate to inhibit tumorigenesis |
| Cytokines | Depends on balance between pro- and anti-inflammatory cytokines; tumors recruit inflammatory cells through secretion of chemokines | Sustained high-level expression of pro-inflammatory cytokines is oncogenic; anti-inflammatory cytokines (e.g. IL10 and TGFβ) are anti-tumorigenic |
| PGE2 and cyclooxygenases | Increased concentration and activity in chronic inflammation | Oncogenic; increased COX-2 expression found in all tumors; PGE2 induces cellular proliferation |

IκB, inhibitor of kappa B.

pro-tumorigenic activities. Under normal cellular conditions, NFκB binds to and is negatively regulated by inhibitor of kappa B in the cytoplasm. Following an inflammatory stimulus, inhibitor of kappa B is phosphorylated and undergoes proteosomal degradation. This allows an activated NFκB to translocate to the nucleus where it activates the transcription of target genes, including inflammation-related genes [e.g. cytokines and chemokines, nitric oxide synthase (*NOS*)*2*, *Cox-2* and tumor necrosis factor (TNF)α]. By increasing the expression of several cell cycle genes, activated NFκB leads to increased cell proliferation, i.e. a pro-tumorigenic environment. NFκB activation also leads to other pro-tumorigenic changes, including stimulation of angiogenesis by activating vascular endothelial growth factor and angiopoetin, and making cells more resistant to necrosis and apoptosis through direct and indirect regulation of a variety of genes, including c-Jun-N-terminal kinase, inhibitors of apoptosis proteins 1 and 2, TNF receptor-associated factors 1 and 2, protein 53 (p53) and B-cell CLL/lymphoma 2 family members. NFκB can also promote tumor metastasis through a number of mechanisms, including the activation of chemokine (C-X-C motif) receptor 4 (38).

NFκB plays an integral role for inflammation-induced cancers in several mouse models. Mutations in inhibitor of kappa B lead to increased activity of NFκB, which results in increased tumor size and vascularization in xenograft models (39). Inhibition of NFκB leads to reduced tumor incidence in inflammation-induced models of liver (40) and colon cancer (41). Suppression of the NFκB pathway can also reduce metastasis in mouse models of breast cancer (42). While the majority of evidence suggests that NFκB activity is associated with pro-tumorigenic effects, there is also evidence in certain cell types that NFκB can have an anti-tumorigenic role, indicating a complex role for NFκB in carcinogenesis (37).

*Reactive oxygen and nitrogen species.* An inflammatory stimulus leads to the recruitment and activation of various immune cells (including macrophages, neutrophils and dendritic cells), which release an accumulation of reactive oxygen species and reactive nitrogen species. Reactive oxygen species and reactive nitrogen species (collectively RONS) are highly reactive radicals that contain unpaired valence shell electrons. These radicals have an important role in the microbicidal activity of the innate immune response. In response to a stimulus, phagocytic cells release RONS and non-phagocytic cells are stimulated to produce RONS by pro-inflammatory cytokines (43). Proper regulation of RONS is vital for an efficient immune response and for limiting tissue damage.

Chronic inflammation causes elevated levels of RONS to be sustained for extended periods of time. The role for RONS in carcinogenesis is complex. Depending on the concentration of these free radicals and the cellular context in which they are expressed, they can be either pro-tumorigenic or anti-tumorigenic (44). Most cancer types have increased RONS, creating increased oxidative and nitrosative stress, which is thought to contribute to carcinogenesis (43,45,46). Increased RONS lead to DNA strand breaks, point mutations and aberrant DNA cross-linking, thereby causing genomic instability. This contributes to carcinogenesis by mutating proto-oncogenes and tumor suppressor genes, which has been demonstrated by mouse models (47). RONS can cause lipid peroxidation that generates other reactive molecules, such as malondialdehyde and 4-hydroxynonenal, which can form DNA adducts that, if not adequately repaired, can lead to point mutations in tumor suppressor genes (48). These reactive molecules may also generate inflammatory stimuli to propagate the effect (49). RONS can post-translationally modify various proteins, rendering them auto-antigenic (i.e. inflammatory), and may also increase phosphorylation and inactivate the retinoblastoma 1 tumor suppressor protein and thus lead to cell proliferation (50). Furthermore, elevated RONS can increase angiogenesis and transcriptional activation of proto-oncogenes (51) and increase the metastatic potential of tumors (52). Oncogene activation can also increase RONS. For example, activated RAS signaling leads to the over-production of RONS and this is thought to contribute to RAS-induced carcinogenesis.

While these examples suggest a pro-tumorigenic effect of RONS, RONS can also inhibit tumor formation. As an example, in certain cellular contexts, RONS will cause inflammation-induced cellular senescence in epithelial cells, which is thought to be anti-tumorigenic (53). Through p38, RONS can negatively regulate cell proliferation and tumorigenesis. Additionally, RONS can also activate apoptosis signal-regulating kinase 1 leading to apoptosis and decreased tumorigenicity (54).

*Nitric oxide and p53.* Nitric oxide (NO)• is one type of reactive nitrogen species. It is a highly reactive signaling molecule that is an important regulator of cellular functions. A family of enzymes called NOS converts arginine into citrulline and NO•. NO• is more stable and diffusible (through several cellular diameters) than hydroxyl radicals (OH•) (55). The three isoforms of NOS include NOS1 (neuronal NOS), NOS2 (inducible NOS) and NOS3 (endothelial NOS). NOS1 and NOS3 are constitutively expressed and are responsible for producing low levels of NO• in the picomolar range in normal tissues. The inducible NOS2 enzyme is capable of producing higher levels of NO• as a response to inflammatory stimuli. NOS2 can also bind and *S*-nitrosylate the pro-inflammatory COX-2 protein to increase its activity (56). NOS2 can be induced by various factors including inflammatory cytokines, NFκB, hypoxia, wingless-type MMTV integration site family-signaling or microbial endotoxins (1). Induction of NOS2 in phagocytic cells (such as monocytes, macrophages and neutrophils) leads to the over-expression of NO•, which is a key mediator in the immunoinflammatory response. NO• levels in both the tumor cells and the tumor microenvironment determine cellular response. Invading immune cells, tumor-associated fibroblasts and the tumor cells themselves can be stimulated to produce high levels of NO• through NOS2, which is thought to contribute to cancer progression (55).

NO• has a complicated and sometimes contradictory role in cancer. NO• expression can either inhibit or promote tumor development, depending on the cellular context and concentration of NO• (57). Treatment of cells with NO• inhibits cell proliferation and promotes apoptosis in certain cell lines (58) while promoting cell proliferation and inhibiting apoptosis in others (59). In its pro-tumorigenic role, NO• induces DNA damage, increases angiogenesis through vascular endothelial growth factor stimulation and increases tumor growth and cell invasion properties. Further, genetic deletion of NOS2 can lead to reduced tumorigenicity in certain mouse models (60) and inhibition of NOS2 activity can reduce tumor burden in mouse models of inflammation-associated colon cancer (61). Conversely, increased NO• levels have also been demonstrated to have anti-tumorigenic effects (62). NO• can trigger cytotoxic cell death of malignant cells and also modulate tumor immunity to allow the immune system to eliminate cancer cells.

Whether increased NO• has a pro-tumorigenic or anti-tumorigenic effect may in part depend on the status of the p53 tumor suppressor gene (Figure 2). There is a negative feedback loop between NO• and p53 where NO• causes the stabilization and accumulation of p53, and activated p53 will then repress NOS2 (63,64). Therefore, NO• leads to increased p53 activity, which in turn promotes apoptosis, cell cycle arrest or senescence in damaged cells. In this context, NO• can have anti-tumorigenic properties (65). In the absence of p53, cells are not as sensitive to NO•-induced apoptosis or cell cycle arrest and instead NO• can lead to genotoxic stress and cell proliferation. In mice lacking p53, NOS2 deletions reduce sarcomas and lymphoma, consistent with the idea that p53 and NO• cooperate to regulate tumorigenesis (62,66).

*Cytokines and chemokines.* Cytokines are signaling molecules that are key mediators of inflammation or an immune response. These signaling molecules have a wide variety of cellular functions and are stimulated when tissue homeostasis is altered. Cytokines can be generally classified as pro-inflammatory (including IL1, IL6, IL15, IL17, IL23 and TNFα) or anti-inflammatory [including IL4, IL10, IL13, transforming growth factor (TGFβ) and interferon (IFN)α]. Depending on the balance of cytokines, their collective effect can

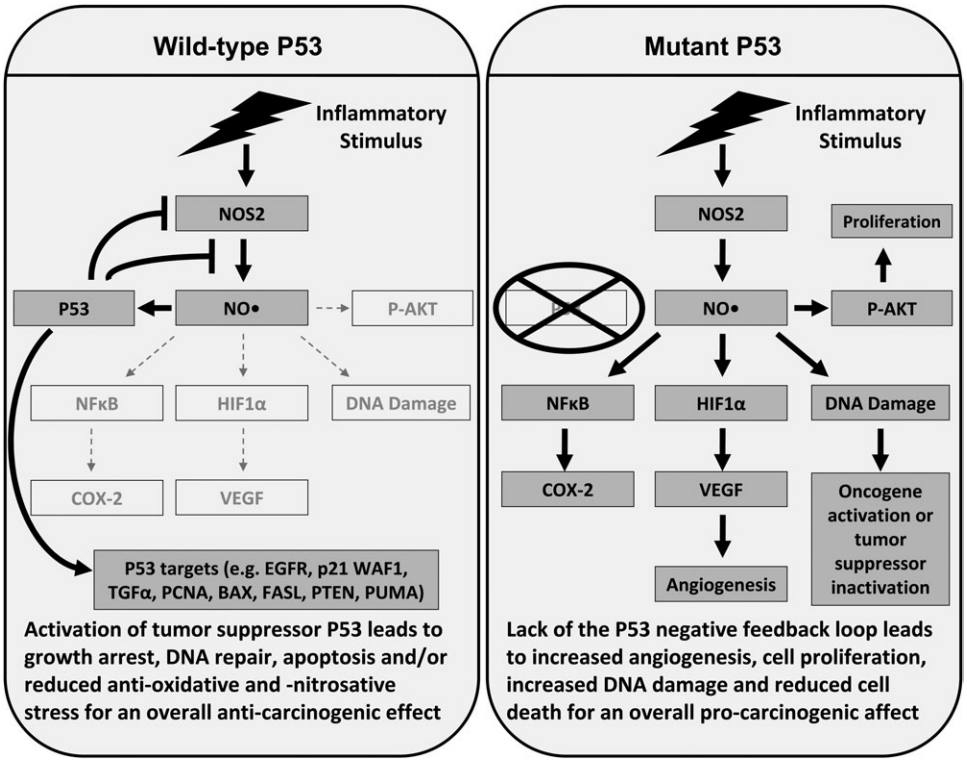


**Fig. 2.** Inflammation-induced NO• production can be pro- or anti-tumorigenic, depending on the functional status of the tumor suppressor p53. Under conditions with functional p53, NO• promotes the stabilization of p53 and p53 then acts in a negative feedback loop to reduce NOS2 and NO•. Elevated p53 levels then lead to an overall tumor-suppressive effect. In the absence of functional p53, an inflammatory stimulus can lead to the over-production of NO•. Without p53, NO• levels remain high leading to pro-tumorigenic conditions. This includes activating proto-oncogenes, inactivating tumor suppressor genes, increased cell proliferation and increased angiogenesis.

be either pro- or anti-tumorigenic. Upon binding their membrane receptor, cytokines activate signal transduction pathways that lead to apoptosis, cell proliferation, angiogenesis and cellular senescence. In general, constitutive exposure to high levels of pro-inflammatory cytokines is thought to be pro-tumorigenic. For example, TNFα is a pro-inflammatory cytokine for which there is convincing evidence of a role in tumor promotion (67). Originally identified as a factor that caused tumor necrosis at high concentrations, TNFα activity at moderate levels can increase tumor growth, stimulate angiogenesis, cause DNA damage and increase the metastatic potential of tumors in animal models. One of the key functions for TNFα is to activate the pro-inflammatory transcription factor NFκB. TNFα knockout mice are more resistant to certain tumors (68) and inhibition of TNFα with anti-TNFα antibodies reduces tumor burden and metastasis, demonstrating a causal role for TNFα in tumorigenesis. IL6 is another pro-inflammatory cytokine that promotes tumor formation. Upon binding the gp130/IL6R heterodimeric receptor, IL6 activates the Janus kinase/signal transducer and activator of transcription signaling pathway, which leads to the increased expression of multiple oncogenes. IL6 has been implicated in pro-tumorigenic activity for many cancers and has been found to be required for colitis-associated colon cancer in mouse models (69).

IL10 and TGFβ are examples of anti-inflammatory cytokines, which have a general role in tumor suppression. A main function of IL10 is to suppress NFκB, which leads to reduced levels of the pro-inflammatory cytokines TNFα, IL6 and IL12 (70). Consistent with its anti-tumorigenic function, *IL10* deletions in mice exposed to *Helicobacter hepaticus* lead to increased chronic inflammation and colitis-associated adenocarcinoma (71). Treatment of these animals with IL10 prevented both chronic inflammation and colon adenocarcinomas, demonstrating an inhibitory effect of IL10 in inflammation and inflammation-associated cancer (71). Most human colon tumors express reduced levels of IL10, indicating that the data from mouse models may have some clinical relevance. TGFβ also acts as an anti-inflammatory and anti-tumorigenic factor. TGFβ signaling activates SMAD transcription factors and mitogen-activated protein kinase activation. TGFβ signaling prevents IL6 release from T helper cell inflammatory cells, which is in part responsible for its anti-inflammatory/anti-tumorigenic properties. Similar to IL10, deletions of *TGFβ* in mice exposed to *H.hepaticus* lead to increased chronic inflammation and colitis-associated colon adenocarcinomas (72). Inactivating mutations in the TGFβ receptor are also frequently found in human colon tumors, indicating that the TGFβ pathway is important for carcinogenesis (73).

Chemokines are a subgroup of cytokines that recruit leukocytes to sites of inflammation by chemotaxis. Chemokines are released from various cells after stimulation by pro-inflammatory cytokines. Tumors usually have increased expression levels of chemokines, resulting in the recruitment of leukocytes to those tumors. IL8 (or CXCL8) is a pro-inflammatory chemokine that can be stimulated by various pro-inflammatory cytokines, including TNFα and IL1 (74). IL8 or IL6 can stimulate several signal transduction pathways and, depending on the cellular context, lead to cell proliferation, cell survival, tumor invasion or angiogenesis.

*Prostaglandin pathway molecules.* Cyclooxygenases are enzymes required for the production of prostaglandins (PGs) from fatty acids. PGs (especially PGE2) are produced by cyclooxygenases and are key mediators in inflammation and inflammation-associated cancers. There are two cyclooxygenase isoforms, COX-1 and COX-2. COX-1 is constitutively expressed at relatively low levels, whereas COX-2 is the inducible form of the enzyme and is primarily responsible for the increased cyclooxygenase activity due to chronic inflammation. While usually undetectable in tissues, COX-2 expression can

dramatically increase after a variety of stimuli, including inflammation, hypoxia or wingless-type MMTV integration site family-signaling (75,76). Initially found to be highly expressed in colon adenocarcinoma (77), COX-2 has now been found to be highly expressed in nearly every tumor type examined. Notably, pre-malignant lesions, early stages and late stages of cancer express increased COX-2, suggesting that COX-2 has an important role in tumor initiation and maintenance. Expression of COX-2 is necessary and sufficient to cause a malignant transformation in multiple *in vitro* and animal models. COX-2 activity and the subsequent increase in PGE2 can affect cell proliferation, DNA mutation rates, angiogenesis, apoptosis and metastasis (78). Metabolism and transport of PGE2 may also contribute to colon carcinogenesis (71). The use of NSAIDs that inhibit COX-2 activity is associated with reduced risk of malignancies such as colorectal, esophageal, stomach, lung, breast and ovarian cancers (18). Randomized clinical trials demonstrate that NSAIDs prevent formation of colon adenomas and thus agree with the notion that COX-2 functions at an early stage of development of colon adenocarcinoma (19–21).

*MicroRNAs as emerging regulatory molecules in inflammation and cancer*

*MicroRNAs as mediators of inflammation.* MicroRNAs are a recently discovered class of regulatory molecules that have a convincing role in inflammation and cancer. MicroRNAs are small, non-coding RNAs that regulate the translation of specific genes. The first microRNA, *lin-4*, was discovered in *Caenorhabditis elegans* in 1993 and was found to regulate developmental timing and cell fate specification (79). In 2000, microRNAs were found to be conserved in vertebrates (80) and over the last decade microRNAs have been shown to be involved in nearly every cellular and development processes examined.

We know much about the biogenesis of microRNAs and how they function. Long primary transcripts (pri-microRNAs) are processed by a protein complex consisting of Drosha and DiGeorge syndrome critical region 8 into 60–80 nucleotide stem-loop products called precursor microRNAs. P53 aids in the assembly of the Drosha complex and therefore can modulate microRNA processing (81). Precursor microRNAs are translocated to the cytoplasm to be further processed by Dicer into a short, 18–24 bp RNA duplex. This duplex is unwound and is incorporated into the RNA-induced silencing complex (RISC) in a sequence-specific manner. This short RNA is referred to as a mature microRNA and can guide the RISC complex to the 3′ untranslated region (UTR) of target mRNA genes in a sequence-specific manner. MicroRNA target sequences are complementary to the mature microRNA, but they can be imperfectly matched. MicroRNA-binding sites are usually in the 3′ UTR of target transcripts. When the microRNA guides RISC to the target mRNA, RISC will repress its translation or degrade it. While there are exceptions to these general features of microRNA function, microRNAs are usually expected to reduce protein expression and/or mRNA levels of the target gene.

MicroRNA expression can be induced or expressed by a variety of mechanisms. These stimuli include direct transcriptional activation or repression from transcriptional enhancers, epigenetic modifications of the genome, genomic amplification or deletion, cellular stress and inflammatory stimuli. The effect of inducing or repressing microRNA expression can influence most biological processes, including cell fate specification, cell proliferation, DNA repair, DNA methylation and apoptosis and provide pro-inflammatory or anti-inflammatory stimuli. The biological effect of a specific microRNA will depend on the cellular environment in which it is expressed, its turnover rate and the target sequences that the microRNA can bind. Since a single microRNA can bind its target sequence with imperfect complementarity, a specific microRNA can have many potential targets. This allows a single type of microRNA to simultaneously regulate the translation of many genes in multiple pathways. In this manner, relatively small changes in microRNA expression can lead to modest changes in the levels of multiple proteins and collectively these can add up to large changes in biology (82,83).

MicroRNAs also have an essential role in both the adaptive and innate immune system. Proper microRNA expression is required for correct differentiation of immune cells (84). In an innate immune response, specific microRNAs can be regulated by inflammatory stimuli and certain microRNAs can act as mediators of inflammatory stimuli. Expression profiling experiments reveal that lipopolysaccharide (LPS)-induced inflammation causes altered expression of several microRNAs (85,86) (Figure 3). Specifically, LPS stimulated the expression of *miR-146a*, *miR-132* and *miR-155* in a human acute monocytic leukemia cell line. Treatment with either of the pro-inflammatory cytokines, IL1β or TNFα, also stimulated the expression of *miR-146a*. The promoter region for *miR-146a* contains NFκB-binding sites, indicating that NFκB was probably responsible for driving the expression of *miR-146a* (86). Interestingly, *miR-146a* expression can inhibit interleukin-1 receptor-associated kinase and TNF receptor-associated factor 6, both of which are downstream factors involved in IL1 receptor signaling, demonstrating the first negative feedback loop involving microRNAs in an inflammatory response (86). Confirmatory studies found IL6 and IL8 to be negatively regulated by *miR-146a* through this feedback loop (87,88). Similar experiments were performed with *miR-155* and demonstrated that this microRNA is induced by LPS or *H.pylori* infection (89,90) and this is mediated by NFκB and activator protein-1. Increased *miR-155* expression had a negative effect on IL8 signaling, indicating a role for *miR-155* in an inflammatory negative feedback loop. Using a variety of inflammatory stimuli, many additional microRNAs have been shown to be linked to an inflammatory response, including *miR-21*, *let-7*, *miR-9*, *miR-98*, *miR-214*, *miR-223*, *miR-224* and *miR-513* (85,91–93).

Because inflammatory signals lead to altered microRNA expression, it is not surprising that microRNA expression patterns are associated with chronic inflammatory diseases and other inflammatory conditions. As an example, psoriasis is a chronic inflammatory skin disease that affects 1–3% of the population (94). Psoriasis-affected skin has increased expression of *miR-203*, *miR-146a* and *miR-21* with reduced expression of *miR-125b* (95). Increased *miR-203* expression was concurrent with reduced levels of suppressor of cytokine signaling 3, which is a predicted target for *miR-203*. These results suggest that increased *miR-203* expression contributes to psoriasis pathogenesis by regulating inflammatory gene levels. In the same study, atopic eczema was examined and found *miR-21* levels to be elevated, whereas *miR-125b* levels were reduced in regions of the skin that were inflamed (95). Lung inflammation due to allergic reactions also increased *miR-21* expression (96). Rheumatoid arthritis is a chronic inflammatory autoimmune condition affecting joints and tissues and elevated levels of *miR-146a* were found in the synovial tissue of rheumatoid arthritis patients (97). MicroRNAs are also altered in inflammatory conditions that are associated with increased risk of cancer. Primary biliary cirrhosis is a chronic inflammatory autoimmune condition of the bile duct that carries an increased risk of liver cancer. In this condition, *miR-122a* and *miR-26a* were reduced and *miR-328* and *miR-299-5p* were increased (98) and these microRNAs are thought to have roles in cell proliferation, apoptosis, inflammation and oxidative stress, indicating that the alterations of these microRNAs may contribute to the condition. Similarly, active inflammation in ulcerative colitis leads to increased expression of several microRNAs, including *miR-21* (99). These changes in microRNA expression levels may contribute to both the active inflammation and the increased risk of cancer associated with these diseases.

*MicroRNAs as mediators of inflammation-induced carcinogenesis.* Inflammatory conditions lead to alterations in microRNA expression levels and these changes are thought to contribute to inflammation. It is likely that the changes in microRNA expression can act as mediators in inflammation-associated carcinogenesis.

There is convincing evidence for a causal role for microRNAs in cancer. This connection was first suggested in 2002 by Croce *et al.* (100) with the discovery that *miR-15* and *miR-16* were located on

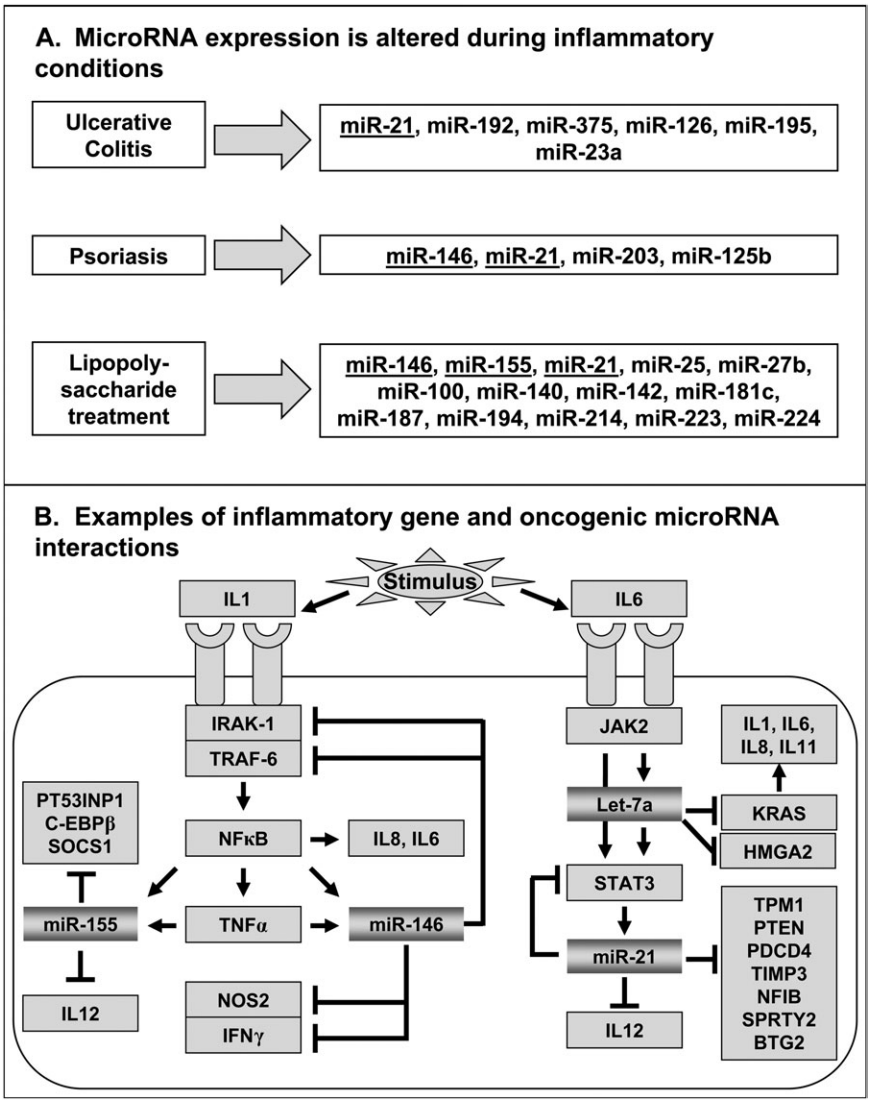


**Fig. 3.** MicroRNAs are inflammatory mediators. (**A**) MicroRNA expression levels are altered in a variety of inflammatory conditions. Underlined microRNAs are highlighted in panel (**B**). (B) The expression of oncogenic microRNAs is induced by inflammatory cytokines. These microRNAs can then target inflammatory mediators, tumor suppressor genes or oncogenes to have a role in inflammation-induced carcinogenesis.

chromosome 13q14, a region frequently deleted in chronic lymphocytic leukemia (CLL). Upon examining the expression levels of these microRNAs, *miR-15* and *miR-16* were reduced or eliminated in 68% of CLL cases. They also noted that the 13q14 deletion was frequently the only genetic abnormality in patients and thus the deletion of *miR-15/16* may be a direct cause of CLL. Upon examination of genomic locations of microRNAs, they reported that many microRNA-coding regions are located in fragile regions of the genome that are frequently amplified or deleted in many cancers, arguing that gain or loss of microRNAs were selected for and are important in tumorigenesis (101). Subsequently, mouse models demonstrated that over-expression of a single microRNA, *miR-155*, could cause B-cell tumors (102) and over-expression of *let-7* could decrease Kirsten rat sarcoma oncogene (KRAS) expression and reduce lung tumors (103), indicating that altered microRNA expression can play a causal role in carcinogenesis. Global expression profiling revealed alterations of microRNA expression patterns first in CLL (104) and then in every malignancy evaluated (105–107). These studies showed that the expression of several microRNAs (*miR-17-5p*, *miR-20a*, *miR-21*, *miR-92*, *miR-106a* and *miR-155*) was increased in the majority of tumor types, arguing that these may be common oncogenic microRNAs. It was also noted in these studies that microRNA expression patterns could distinguish tumors and tissue types, indicating that microRNA expression levels may be useful biomarkers for cancer. MicroRNA expression patterns were then found to be associated with poor prognosis of CLL and lung cancer to offer further demonstration of this potential (108–110). Subsequent mechanistic studies demonstrated that alteration of specific microRNAs could affect cell proliferation, apoptosis, tumor growth and angiogenesis in mouse models (111–114). Altogether, the evidence is convincing that alterations of microRNAs occur during and are contributing to carcinogenesis.

Inflammatory stimuli alter the expression of microRNAs while specific microRNAs have oncogenic or tumor-suppressive activity; therefore, microRNAs may be mediators of inflammation-induced carcinogenesis. Although many microRNAs fit this description, only *miR-21* and *miR-155* are discussed below.

Inflammatory stimuli can increase the expression of *miR-21*. It has been shown that IL6, a pro-inflammatory cytokine, can induce the expression of *miR-21* in a STAT3-dependent manner (115). The EGFR pathway has also been shown to increase *miR-21* expression (116). As mentioned earlier, increased levels of *miR-21* are also found in several chronic inflammatory diseases, including at sites of active inflammation in ulcerative colitis (99). The elevated levels of *miR-21* in these tissues may be in part responsible for inflammation-associated

cancers. Increased levels of *miR-21* are found in nearly every malignancy examined and this increase is thought to be oncogenic (117). *MiR-21* targets a number of tumor suppressor genes, including programmed cell death 4 (118), tropomyosin 1 (119), phosphatase and tensin homolog (120) and BTG family member 2 (121). Increased *miR-21* expression can increase cell proliferation and inhibit apoptosis, whereas the inhibition of *miR-21* can cause tumor regression in xenograft models (122). Recently, *miR-21* was found to directly target and repress IL12-p35 expression in mouse models (96). The *miR-21*-binding site in the 3′ UTR of IL12-p35 is conserved in humans. We have recent data from human tissues indicating that *miR-21* expression negatively correlates with IL12-p35 expression, consistent with the idea that IL12-p35 is a target for *miR-21*, and positively correlates with IL6 expression in human colon cancer tissues, consistent with IL6 driving the expression of *miR-21* (123). This suggests that the *in vitro* data demonstrating the connections between IL6, IL12-p35 and *miR-21* are probably relevant in the context of human colon cancer and that *miR-21* may contribute to inflammation-induced carcinogenesis.

*MiR-155* is another oncogenic microRNA (124) that is stimulated during pro-inflammatory conditions. Infections by *H.pylori* (89) and Epstein–Barr Virus (125) or LPS treatment lead to increased *miR-155* expression. Inflammatory mediators, including TNFα and IFNβ, can stimulate the expression of *miR-155* (126,127). *MiR-155* can target suppressor of cytokines 1 and lead to the induction of NOS2 (128), demonstrating that it is a mediator of inflammatory signaling. Increased *miR-155* expression is found in the bone marrow of leukemic patients and over-expression of *miR-155* in mouse models causes hyperproliferation of B cells, a common hallmark of leukemia and lymphoma (102). Over-expression of *miR-155* also causes the repression of tumor p53-induced nuclear protein 1, which is a pro-apoptotic gene downstream of p53 signaling (129). The suppression of tumor p53-induced nuclear protein 1 is a likely mechanism for pro-tumorigenic functions of *miR-155* and a possible mediator of inflammation-induced carcinogenesis.

### *Genes and gene products as cancer biomarkers and therapeutic targets*

*Polymorphisms in inflammatory genes and microRNAs associated with cancer risk.* Identifying polymorphisms associated with cancer risk and prognosis may help guide health decisions. If someone is genetically predisposed to developing cancer, appropriate preventive strategies can be taken. DNA mutations and polymorphisms may help stratify patients for more effective therapies. Polymorphisms in various inflammatory genes have been identified that are associated with cancer risk and prognosis for many cancer types. For the purpose of this review, we will only focus on IL10 as an example of this. Polymorphisms in the anti-inflammatory cytokine, IL10, can lead to reduced function of this gene, higher levels of inflammation and increased cancer risk. Several studies have demonstrated that specific polymorphisms in IL10 are associated with increased rates of non-Hodgkin lymphoma, melanoma, gastric cancer, multiple myeloma, lung cancer, cervical cancer, hepatocellular carcinoma, prostate cancer, colorectal cancer and renal cancer (130–134). This highlights the importance of immune system molecules in carcinogenesis and suggests that increased pro-inflammatory signaling can be oncogenic. Several studies have also shown that *IL10* polymorphisms are associated with prognosis in multiple cancers. In a recent study of 432 women from Austria, the IL10-592C > A polymorphism predicted shorter disease-free survival for breast cancer (135). In another study of 472 patients from Spain, the IL10-3575T > A polymorphism was associated with improved survival in an unstratified group of lymphoma patients composed of non-Hodgkin lymphoma, CLL, multiple myeloma and Hodgkin lymphoma patients (136). Other studies found that IL10 polymorphisms were associated with prognosis in melanoma (137), T-cell non-Hodgkin lymphoma (138) and diffuse large B-cell non-Hodgkin lymphoma (139). These findings indicate that polymorphisms in inflammatory genes may help stratify cancer patients into clinically relevant subgroups.

Emerging evidence also suggests that polymorphisms in microRNAs or their binding sites are associated with increased cancer risk. Inherited *miR-15* and *miR-16* polymorphisms have been found in breast cancer and CLL patients (109), providing the first evidence that polymorphisms in microRNAs may lead to increased risk of cancer. *MiR-196a* is thought to be an oncogenic microRNA that can regulate cell proliferation and target annexin A1, a mediator of apoptosis. In a study including 1058 cases and 1035 controls, a polymorphism in *miR-196a2* was associated with increased lung cancer risk in a Chinese population (140). It was also found that an *miR-196a2* polymorphism was associated with prognosis in lung cancer patients (141). *MiR-146a* is thought to be an oncogenic microRNA and can be induced by pro-inflammatory signals. This microRNA is found elevated in several cancers. In a study of 608 cases and 901 controls, a polymorphism in *miR-146a* is associated with thyroid cancer risk (142). This polymorphism was then found to be associated with early diagnosis of breast cancer (143) and increased risk of developing hepatocellular carcinoma (144). These findings indicate that polymorphisms in *miR-196a2* and *miR-146a* influence risk for multiple types of cancer.

Polymorphisms in microRNA-binding sites within target genes have also received some attention in evaluating cancer risk. KRAS is a proto-oncogene that can be regulated by *let-7*. A polymorphism disrupting the *let-7*-binding site in *KRAS* will inhibit the ability of *let-7* to negatively regulate *KRAS*, leading to the over-production of this proto-oncogene. This polymorphism has been associated with increased lung cancer risk (145) and poor prognosis of oral cancers (146). Polymorphisms in microRNA-binding sites of other genes have been reported to be associated with risk of breast cancer and colon cancer (147–149).

*Circulating cytokines and microRNAs as diagnostic biomarkers.* Early detection remains the strongest predictor of survival for nearly all cancer types. Therefore, accurate, non-invasive screening tests have the potential of reducing the burden of many different tumor types. Inflammatory mediators may constitute useful biomarkers for cancer detection. Altered levels of circulating inflammatory cytokines have been found in cancer patients for nearly every cancer examined (130), indicating that immune response has an important role during carcinogenesis. Unfortunately, the sensitivity and specificity is not high enough to use cytokine measurements as a cancer screening technique; but there have been some favorable results when using circulating inflammatory cytokines for prognostic purposes. For example, elevated levels of circulating IL6 have been associated with decreased overall survival or disease-free recurrence for breast, pancreatic, gastric, prostate and lung cancer (150–154). Levels of additional inflammatory markers have also been associated with prognosis, indicating that circulating inflammatory markers may be useful cancer biomarkers.

The potential of using circulating microRNA expression levels for detecting cancer has been reported for prostate cancer (155), large B-cell lymphoma, ovarian cancer (156), non-small cell lung cancer (157,158), breast cancer (159) and colon cancer (160). All these studies compared the expression levels of microRNAs in plasma or serum from cancer cases to healthy control populations. Notably, *miR-17-3p* and *miR-92* expression levels in plasma were found to be elevated in colorectal cancer patients (160). In a cohort of 90 patients and 50 controls, *miR-92* expression levels in plasma could distinguish colorectal cancer patients from healthy controls with 70% specificity and 89% sensitivity. While it is unclear if any of these circulating biomarkers are accurate enough on their own to use as a screening technique in healthy populations or among individuals at risk, it may be possible to improve these tests with technological advancements in the detection methodology or combine these tests with other non-invasive tests.

*Inflammatory genes and microRNAs as prognostic and therapeutic biomarkers.* The expression patterns of inflammatory genes and presence of inflammatory cells within the tumor and surrounding

microenvironment are potential prognostic biomarkers for cancer. An analysis of infiltrating immune cells in colon tumors found that the type, location and density of the infiltrating cells were strong predictors of prognosis (161). Similar studies found that the presence of infiltrating mast cells or macrophages were associated with poor prognosis in lung cancer (162), melanoma (163) and bladder cancer (164). The expression of inflammatory genes within tissues may also serve as clinically useful biomarkers. For example, levels of inflammatory cytokines in tumors have been associated with tumor node metastasis staging and prognosis in colon cancer (165,166). A recent study, using non-cancerous tissue adjacent to tumors, identified a unique expression signature composed of a panel of 17 inflammatory/immune response genes that predict metastatic progression and survival of patients with hepatocellular carcinoma (167). A similar study was performed in lung adenocarcinoma using the expression pattern of 18 inflammatory or immune response genes in both tumors and adjacent non-cancerous tissue to develop a signature that could predict prognosis in tumor node metastasis stage I lung adenocarcinoma patients (168).

Biomarkers for early clinical stages of cancer are especially useful to help guide therapeutic decisions. At early stages, metastases are not evident and surgery is potentially curative; therefore, additional adjuvant therapy to those patients will harm their quality of life with little therapeutic benefit. But in many cases, undetectable micro-metastases are present and for those instances adjuvant therapy will be beneficial. The previously described inflammatory gene signature could predict prognosis in tumor node metastasis stage I lung cancer; therefore, it may be useful in deciding which early stage lung cancer cases are at higher risk for metastases and disease progression (168). An inflammatory gene expression signature for colon adenocarcinoma was also recently reported to predict poor prognosis in stage II colon adenocarcinoma patients (123). Similar to biomarkers for early stage lung cancer, this biomarker signature may be useful in identifying high-risk, early stage patients who should receive adjuvant therapy.

MicroRNAs may also be clinically useful as prognostic biomarkers. The first report on associations with cancer prognosis found that a microRNA expression signature composed of the *let-7* family of microRNAs could predict survival in lung cancer patients (108). A microRNA gene expression signature was then found to predict prognosis and disease progression of CLL (109). We first reported that the expression of individual microRNAs could act as independent prognostic biomarkers in lung cancer (110). This study found that increased *miR-155* or decreased *let-7a* was associated with poor prognosis, independent of all clinical covariates and could also predict prognosis in stage I lung cancer. We then published a similar study in colon cancer which found that high expression of *miR-21* in tumors could predict poor survival and poor therapeutic outcome and in two independent cohorts was significantly associated with cancer-specific mortality in stage II colon cancer (169). *MiR-21* is expressed at high levels in nearly every tumor type examined and has now been shown to be associated with prognosis in pancreatic cancer, lung cancer, breast cancer and tongue cancer (110,170–173). These findings indicate that *miR-21* may be a useful general prognostic biomarker for several types of cancer. Genome-wide microRNA expression studies have identified additional microRNA expression profiles that can predict prognosis in other cancer types including liver cancer (174), esophageal cancer (175,176) and ovarian cancer (177).

While single prognostic biomarkers can be clinically useful, a combination of multiple validated biomarkers may provide more information and give better risk stratification of patients (Figure 4). Combining microRNA biomarkers and inflammatory gene biomarkers may serve this purpose. We have recently reported that the combination of an inflammatory gene signature with *miR-21* expression data predicts cancer-specific mortality better than either alone (123).

*Inflammatory mediators and microRNA as therapeutic targets.* Inflammatory genes and microRNAs are associated with and contribute to the clinical outcome of cancer. Since they have causative roles in carcinogenesis, they are ideal candidates for therapeutic targets. Whether it is the activity of inflammatory cytokines RONS, NO•, NFκB and COX-2 or microRNAs, all are potential targets for therapeutic intervention in cancer. There are already drugs on the

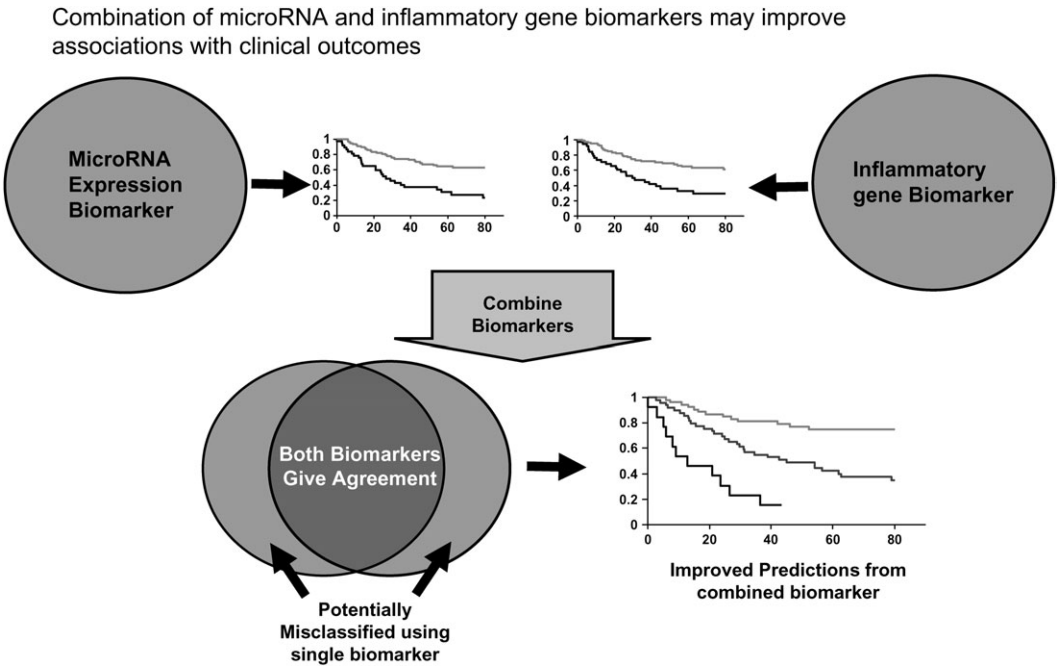


**Fig. 4.** The combination of multiple, validated biomarkers may improve predictions of clinical outcomes. In this example, microRNA expression biomarkers and inflammatory gene biomarkers are combined to improve associations with prognosis. Theoretical Kaplan–Meier plots are shown with time on the *X* axis and survival probability on the *Y* axis. Each biomarker misclassifies a different subset of patients and combining these biomarkers provides a more accurate prediction. This proof of principle of this strategy has recently been shown in colon cancer patients to improve predictions of cancer-specific mortality by combining inflammatory gene and microRNA biomarkers (123).

market or under evaluation for cancer treatment that target these pathways (178,179). Some examples are provided below. Treatment with IFNα is being used therapeutically to treat renal cell carcinoma, ovarian cancer, Kaposi's sarcoma, non-Hodgkin lymphoma, malignant melanoma, hepatocellular carcinoma and leukemia. IL2 therapy is used to treat T-cell lymphoma, malignant melanoma and kidney cancers. Granulocyte monocyte colony-stimulating factor is a cytokine that has been successfully used to treat malignant melanoma. Inhibitors of NFκB are in clinical trials to determine if inhibitors alone or in combination with other therapeutics will be useful to treat various cancers (37). As mentioned previously, COX-2 inhibitors have demonstrated great potential in both the treatment and prevention of cancers (78). COX-1 and -2 inhibitors reduce the risk of colon cancer, breast cancer, prostate cancer and lung cancer. A recent finding also demonstrates that taking aspirin (a COX-2 inhibitor) after diagnosis of colon cancer leads to improved survival, especially in individuals with tumors over-expressing COX-2 (180).

The potential to therapeutically regulate microRNA levels may offer new avenues for cancer treatment and possibly in regulating the immune system. Inhibiting oncogenic microRNAs or reintroduction of tumor suppressor microRNAs may serve as useful strategies to treat cancer. MicroRNAs can be inhibited by anti-sense oligonucleotides with various chemical modifications. Inhibition of specific microRNAs has been achieved *in vivo*, first in mice (181) and then in non-human primates (182). Other technologies are being developed to reintroduce microRNAs back into cells to mimic their function. Reintroduction of tumor suppressor microRNAs can cause apoptosis or senescence in malignant cells and provide new avenues for developing cancer therapeutics. A recent study clearly demonstrated this potential in mouse models of hepatocellular carcinoma (183). *MiR-26a* is a tumor suppressor microRNA that is reduced in hepatocellular carcinoma. Decreased levels of *miR-26a* have been associated with poor prognosis and predictive to the therapeutic response to IFNα in hepatocellular carcinoma patients (184). In an MYC-inducible model of liver cancer, animals were treated systemically with *miR-26a* (or with control microRNA) using adeno-associated virus for delivery (183). Treated animals showed significant tumor regression without toxicity, indicating that reintroduction of *miR-26a* may be an effective strategy to treat cancer. Currently, there are clinical trials evaluating therapy based on microRNA inhibition or over-expression. Estimates from the developers of microRNA-based therapeutics set target dates of 2011 for clinical trials evaluating efficacy for cancer treatments. Within the next several years, we will know if microRNA-based therapeutics, alone or in combination with other modalities, will be clinically useful treatments for various cancers and immune system disorders.

## Concluding statements

While much remains to be deciphered, we are beginning to understand the mechanistic connections between inflammation, inflammation-induced microRNAs and cancer development. As our understanding of these processes increases, the potential to use this knowledge to intervene in cancer also increases. Polymorphisms in inflammatory genes, microRNAs or the 3′ UTR of microRNA target genes may lead to genetic susceptibility of individuals to certain cancers. Identification of these polymorphisms may influence decisions about routine cancer screening. It may soon be possible to use the levels of circulating inflammatory proteins, alone or in combination with levels of circulating microRNAs, as diagnostic biomarkers to screen for cancers. Once cancer is detected, similar biomarkers may be developed that predict prognosis or therapeutic outcome to help guide therapeutic decisions. A mechanistic understanding for the role of inflammatory genes and microRNAs as in carcinogenesis may lead to the identification of promising therapeutic avenues to treat cancer. As new biomarkers and therapeutics based on this knowledge are developed, the long-term goal, a decrease in the overall cancer health burden, may be closer to realization.

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