Identifying and defusing weapons of mass inflammation in carcinogenesis

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Abstract

The continued cancer risks associated with chronic inflammation necessitate the identification of inflammatory molecules and the cancer pathways they affect. Evidence indicates that there are multiple mechanisms linking inflammation to cancer and that there are multiple targets for chemoprevention. Here, we review some of the key factors and the cancer pathways they disturb as a necessary prerequisite to the identification of targets for chemoprevention.

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Keywords: Inflammation; Cancer; Chemoprevention; COX-2; NF-kappa B; iNOS; Cytokine; Interleukin; Colitis; Crohn's; Hepatitis; Gastritis; Esophagitis; Barrett's; Pancreatitis; p53; Retinoblastoma; NSAID; Free radical

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1. Introduction

There is a close association between chronic inflammation and cancer. Evidence for this comes from epidemiological studies, linking reactive species overload diseases to high cancer risk (Table 1); clinical trials, showing decreased cancer risk with the use of anti-inflammatory drugs; and animal studies which show increased cancer incidence with genetically (knockout/transgenic animals) or environmentally (chemical/physical/biological) induced chronic inflammation. Epidemiological and cell culture studies demonstrate that there are diverse causes and mechanisms.

At the molecular level, free radicals and aldehydes, produced during chronic inflammation, can induce deleterious
gene mutation and post-translational modifications of key cancer-related proteins [3]. Other products of inflammation, including cytokines, growth factors and transcription factors such as nuclear factor-kappa B (NF-\(\kappa\)B), control the expression of cancer genes (e.g., suppressor genes and oncogenes) and key inflammatory enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). These enzymes, in turn, directly influence reactive oxygen species (ROS) and eicosanoid levels. The pro-cancerous outcome of chronic inflammation is increased DNA damage, increased DNA synthesis, cellular proliferation, the disruption of DNA repair pathways and cellular milieu, the inhibition of apoptosis, the promotion of angiogenesis and invasion. Chronic inflammation is also associated with immunosuppression which is a risk factor for cancer. Owing to these and other proposed mechanisms associated with inflammatory-mediated carcinogenesis, many potential targets for therapeutic intervention exist.

Current treatment strategies of reactive species overload diseases are frequently aimed at treating or preventing the cause of inflammation (Table 1). Although there has been progress in combating reactive species overload diseases and associated cancers by targeting the causes, often exposure is repeated following eradication or treatment to eradicate the cause fails and has long-term side-effects. Therefore, the identification of molecules and pathways involved in chronic inflammation and cancer is critical to the design of agents that may help in preventing reactive species overload disease progression and cancer associated with disease progression.

2. Targets for chemoprevention of reactive species overload diseases

2.1. The arachidonic acid cascade

Perhaps, the best known, oldest, and effective agent in combating inflammation is acetylsalicylic acid (ASA). With origins going back to Hippocrates at around 400 B.C., ASA is a non-steroidal anti-inflammatory drug (NSAID), first synthesized in the lab over 100 years ago, and effective in the prevention of many diseases, including colorectal and other cancers. Its main target is the cyclooxygenase (COX) pathway of the arachidonic acid cascade. By inhibiting enzymes in this pathway (COX-1 and COX-2), it reduces the eicosanoid and reactive species load, thereby reducing genomic damage and controlling cellular proliferation and apoptosis. NSAIDs can also protect against carcinogenesis by directly affecting cancer pathways, such as inducing SMAC/Diablo-dependent apoptosis [4].

There is some evidence that NSAIDs can protect against Barrett’s esophagus [5]. The strongest indication that specific NSAIDs can protect against reactive species overload diseases is the consistent association between 5-acetylsalicylic acid (5-ASA) use and its ameliorating effects on inflammatory bowel disease (IBD, especially ulcerative colitis) (reviewed in [6,7]). The mechanisms of 5-ASA are not fully understood, but it is a non-steroidal anti-inflammatory drug (NSAID), first synthesized in the lab over 100 years ago, and effective in the prevention of many diseases, including colorectal and other cancers. Its main target is the cyclooxygenase (COX) pathway of the arachidonic acid cascade. By inhibiting enzymes in this pathway (COX-1 and COX-2), it reduces the eicosanoid and reactive species load, thereby reducing genomic damage and controlling cellular proliferation and apoptosis. NSAIDs can also protect against carcinogenesis by directly affecting cancer pathways, such as inducing SMAC/Diablo-dependent apoptosis [4].

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Another group of drugs that were developed to reduce the gastrointestinal damage associated with classical NSAID use are COX-2-specific inhibitors. Although COX-2 inhibitors appear to reduce the frequency of familial colorectal cancers [8], the benefits of these drugs in IBD remain controversial. Some studies show both genetic or pharmaceutical ablation of COX-2 exacerbates colitis in animals [9]. Others have shown a
protective effect on animal colitis and subsequent colon carcinogenesis [10]. Similar contradicting studies have been done in models of pancreatitis [11,12] and gastritis [13]. With the finding that COX-2 inhibitors reduce esophagitis-associated cancer in animals [14], it will be interesting to see the results of an ongoing prospective chemoprevention trial to evaluate the effects of COX-2 inhibition on the progression of Barrett's esophagus in humans [15]. Based on the findings that COX-2 inhibitors increase the risk of cardiovascular disease [16] and that there is contradicting evidence for a beneficial effect on high cancer risk, reactive species overload diseases, these drugs are not recommended at this time for treatment of these diseases.

### 2.2. Nitric oxide

Although nitric oxide (NO) has both protective and destructive effects in carcinogenesis [17], as briefly mentioned, NO-releasing mesalamine or aspirin have been used successfully to treat reactive species overload diseases such as experimental colitis [18] and gastritis [19]. In addition to their gastrointestinal-sparing effects, they have nonselective COX inhibition, they can suppress cytokines by S-nitrosilation of interleukin-1β converting enzyme (ICE/caspase 1) [20], and they can inhibit β-catenin/T-cell factor signaling pathways, NF-κB, and iNOS [21]. In this way, they can inhibit the propagation of inflammation. Caution, however, should be used when extrapolating these positive outcomes in reactive species overload diseases to cancer associated with these diseases. NO is elevated in many reactive species overload diseases [3]. NO and its by-products also have the capability of damaging DNA and post-translationally modify cancer proteins [17]. Therefore, although the immediate suppressive effects on inflammation appear to be beneficial, continued DNA damage and cancer protein modification by NO may have deleterious effects. So, although the pharmacotherapeutical possibilities of NO are extensive, tracking the long-term outcomes from stimulation and/or inhibition of NO is required in order to determine usefulness of the NO pathway in cancer chemotherapy. This is especially important when evaluating carcinogenesis in reactive species overload diseases.

### 2.3. Free radicals

A critical mechanism toward cancer associated with reactive species overload diseases is the attack of cancer genes, cancer proteins, RNA, and lipids by reactive nitrogen and oxygen species [3]. By these mechanisms, cancer pathways such as p53, retinoblastoma, repair, pro-cancerous kinase, apoptosis, SMAD, APC, and hypoxia inducible factor-1 can be altered [3,22]. Therefore, to diminish these pro-cancerous mechanisms, a key treatment strategy is to reduce the free radical load. Vitamins, trace minerals, and anti-oxidant enzymes [e.g., catalase, MnSOD, glutathione, glutathione peroxidase] are often used for this purpose.

Antioxidant vitamins reduce cancer risk. Unfortunately, because of the time, man-power and money involved, relatively few large-scale studies have prospectively examined their long-term impact on carcinogenesis associated with high cancer risk, reactive species overload diseases. Table 2 summarizes prospective studies examining the influence of vitamins on cancer risk. Perhaps, specific inflammatory biomarkers that accurately predict carcinogenesis will improve the evaluation strategies of these and many other chemopreventive agents.

Vitamin C, either alone, or in combination with other antioxidants has been shown to be beneficial against IBD (Crohn’s disease) and pancreatitis [23–26]. The specific mechanisms remain unresolved. Vitamin C is called an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized. Although by this mechanism, vitamin C becomes oxidized (into ascorbyl radical), this is...
relatively stable and unreactive. Formation of ascorbyl radical is mediated by a wide variety of free radicals, including molecular oxygen, superoxide, hydroxyl radical, hypochlorous acid, nitric oxide, reactive nitrogen species, iron, and copper [27]. Depending on the location of vitamin C, then, it can protect against lipid peroxidation, prevent cancer protein modification, and protect against DNA adduct formation and mutation to cancer genes. Because vitamin C can increase iron bio-availability, it is not recommended for iron overload diseases, such as hemochromatosis.

Prospective studies have also shown vitamin E supplementation is associated with an improved viral hepatitis B and C condition [28,29]. The mechanisms remain unresolved, but in vitro studies have shown that vitamin E can reduce cytochrome p450 induction associated with induced hepatitis [30], reduce DNA damage [31], inhibit lipid peroxidation, restore antioxidant balance [32], and modify molecular pathways that influence cell death, cell cycle, and free radical production in hepatocytes [33,34].

Vitamin E in combination with vitamin A and 
\(-\)carotene may also decrease the risk of progression of chronic esophagitis [35]. Two groups so far have shown vitamin C and 
\(-\)-carotene are effective in reducing the cancer risk associated with gastritis [36,37]. Vitamin A (retinoids) are thought to exert most of their effects by regulating gene expression primarily through two classes of nuclear receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs). RARs and RXRs modulate the expression of target genes by interacting as either homodimers or heterodimers with the retinoic acid response element located in the promoter regions of target genes [38]. In this fashion, retinoids can decrease proliferation and induce apoptosis by modifying the expression of cell cycle genes [e.g., retinoblastoma and its pathway proteins] and apoptosis genes [39,40]. They can also decrease mutagenesis [41] and inhibit COX-2 expression [42] in esophageal cells. The mechanisms of 
\(-\)-carotene are complex and few studies have examined the mechanisms in gastric epithelial cells. Mannick et al. showed 
\(-\)-carotene can reduce H. pylori-induced iNOS expression and DNA damage [43].

Trace minerals also have free radical scavenging properties and are quality candidates for prevention of cancer associated with reactive species overload diseases. In prospective human trials, selenium has been shown to protect against hepatitis B virulence [44]. As mentioned above, it has also been shown to be useful as a part of an antioxidant cocktail in the prevention of pancreatitis [24,26,45]. Selenium primarily works through the reduction of free radicals and propagation of inflammation by inhibiting NF-\(\kappa\)B activation and activating anti-oxidant enzymes [46]. Selenium can also directly interact with cancer pathways by stimulating cell cycle arrest, promoting apoptosis, activating p53, and modulating p53-dependent DNA repair mechanisms [47].

Zinc is a prevalent trace element in the body and is a part of the structure and function of many proteins. Zinc has been shown to enhance the response to interferon-\(\alpha\) (IFN-\(\alpha\)) therapy in hepatitis C patients [48]. Perhaps, the ability of IFN-\(\alpha\) to increase the activity of zinc-dependent antioxidant enzymes (e.g., superoxide dismutate) in hepatocytes provides insight into the mechanism [49].

Metals, especially iron and copper, can also play a key role in free radical generation and propagation. Therefore, metal chelators are a potential source of high cancer risk, reactive species overload disease prevention. The copper chelating agent, tetrathiomolybdate, has been shown to protect against animal hepatitis associated with copper overload [50]. Because the metal binding protein, metallothionein, is upregulated in high cancer risk, reactive species overload diseases (perhaps as an adaptive response), this could prove to be a beneficial molecule for treatment and chemoprevention. Mice deficient in metallothionein are more susceptible to gastritis [51] and hepatitis [52]. Copper chelation therapy is a useful strategy for the treatment of the high liver cancer risk, copper overload disease, Wilson’s disease. Iron chelators have also been useful in disease treatment, including the iron overload disease, hemochromatosis (Table 1; [53]).

In many chronic inflammatory conditions, there is evidence of compromised and imbalanced endogenous antioxidant defense enzymes, leading to reactive species overload, tissue damage, and perhaps carcinogenesis. Therefore, replenishing these enzymes and restoring their balance may have beneficial effects on disease progression (Table 3). The specific free radical each antioxidant enzyme depletes has been described elsewhere [54,55], and therefore will not be reviewed here. The primary cellular antioxidant, glutathione, and its precursor (N-acetylcysteine), has been used successfully to ameliorate animal pancreatitis, and cancer associated with animal colitis [56,57]. Because glutathione S transferase levels or activity are reduced in Barrett’s esophagus, IBD, gastritis, and hepatitis, elevating these levels may be a useful strategy for the prevention of cancer associated with these diseases. Catalase and/or superoxide dismutate have been shown to inhibit experimental pancreatitis, colitis, and esophagitis [58–60]. Finally, xanthine oxidase inhibitors have been used successfully to treat experimental prostatitis and pancreatitis [58,61]. In human studies, these inhibitors can ameliorate prostatitis symptoms [62] and in combination with 5-ASA, are useful for combating ulcerative colitis (UC) [63].

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Glutathione</th>
<th>Catalase</th>
<th>Superoxide dismutate</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD</td>
<td>N-acetylcysteine precursor inhibits (^a)</td>
<td>Inhibits (^a)</td>
<td>Inhibits (^a)</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>N-acetylcysteine does not appear to have an effect</td>
<td>Not examined</td>
<td>Not examined (^a)</td>
</tr>
<tr>
<td>Gastritis</td>
<td>Not examined</td>
<td>Not examined</td>
<td>Not examined</td>
</tr>
<tr>
<td>Pancreatititis</td>
<td>Glutathione and N-acetylcysteine inhibit (^a)</td>
<td>Inhibits (^a)</td>
<td>Inhibits (^a)</td>
</tr>
<tr>
<td>Esophagitis</td>
<td>Not examined</td>
<td>Not examined</td>
<td>Not examined</td>
</tr>
<tr>
<td>Prostatitis</td>
<td>Not examined</td>
<td>Not examined</td>
<td>Not examined</td>
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<tr>
<td>Cystitis</td>
<td>Not examined</td>
<td>Not examined</td>
<td>Not examined</td>
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</table>

\(^a\) Animal studies.

\(^b\) Although a superoxide dismutate mimetic has been shown to inhibit FAS-induced liver failure in mice [132].
2.4. Nuclear factor-kappa B

Nuclear Factor-kappa B (NF-κB) is an inducible and ubiquitously expressed transcription factor discovered by Sen and Baltimore almost 20 years ago [64]. Research over the last few years has revealed that NF-κB is a key molecular node in inflammatory-mediated carcinogenesis. In unstimulated conditions, NF-κB proteins bind to IκB. Upon stimulation, IκB proteins are phosphorylated and degraded through the proteasome. This liberates NF-κB which then translocates to the nucleus and binds to its promoter elements (κB sites) and regulates the expression of many genes. Because (i) NF-κB is upregulated in reactive species overload diseases, and (ii) many of the genes targeted by NF-κB are involved in the inflammatory cascade (iNOS, COX-2, cytokines and matrix metalloproteinases), anti-apoptotic events (e.g., the pro-survival Bcl-2 homolog Bfl-1/A1) and cell cycle events (e.g., cyclin D1), it is emerging as one of the more promising targets in the chemoprevention of these diseases.

NF-κB is a target of many antioxidants associated with chemoprevention (e.g., NSAIDs, butyrate, curcumin, triterpenoids, gliboixin and other sponge compounds, black tea, leptons, caffeic acid). Therefore, its usefulness in taming high cancer risk, reactive species overload diseases has been shown indirectly. More direct genetic and pharmacological inhibition of the NF-κB pathway has been shown to inhibit experimental colitis [65,66]. A recent study has shown NOD2 mutations – that play a key role in Crohn’s disease – can lead to NF-κB activation [67]. Karin’s group also showed [68] that the genetic deletion of a functional NF-κB pathway in epithelial cells protected from tumorigenesis associated with experimental colitis. Deletion of functional NF-κB pathway in myeloid cells resulted in diminished activation (and thus propagation of inflammation) and reduced tumor size, indicating a protection from inflammatory-driven tumor progression. NF-κB inhibition has also been shown to protect from pancreatitis, cystitis, and hepatitis progression to liver cancer [69–71].

2.5. Cytokines

Associated with inflammation is a ‘cytokine storm’. Cytokines are key molecules that can inhibit or propagate inflammation, and activate or deactivate cancer genes and their pathways. Knocking out specific cytokines can predispose animals to various types of cancer (reviewed in [72]). These observations, in addition to the growing body of evidence that specific cytokine polymorphisms are associated with increased chronic inflammatory diseases, make them attractive targets for the treatment of these diseases. To target cytokines, the methods of choice are biological therapies [monoclonal antibodies and recombinant cytokines/cytokine inhibitors].

Key proinflammatory cytokines include interleukin- (IL-) 1, 6, 12, and 18, tumor necrosis factor alpha (TNF-α), and macrophage migration inhibitory factor (MIF). Since these molecules propagate inflammation, their inhibition is an attractive approach to containing inflammation. Anti-inflammatory cytokines include IL-4 and 10, IFN-α and IFN-β. Stimulating these molecules may enhance their ability to halt inflammation. Table 4 summarizes the reactive species overload diseases ameliorated by targeting selected cytokines.

In the context of tumorigenesis, many cytokines can directly affect cancer pathways (Table 4). A well-known function is the ability of some cytokines to induce iNOS and COX-2 [3]. They can also induce pro-cancerous kinase pathways [73] and

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Disease</th>
<th>Examples of cancer pathways affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>Colitis, Pancreatitis, Hepatitis, Cystitis</td>
<td>Induces nitric oxide synthase (iNOS); inhibits COX-2, inhibits DNA repair</td>
</tr>
<tr>
<td>IL-6</td>
<td>(Crohn’s in humans)</td>
<td>Antagonizes p53; pRb hyperphosphorylation; induces Bcl-2 and Bcl-XL; inhibits apoptosis</td>
</tr>
<tr>
<td>IL-7</td>
<td>Colitis</td>
<td>Induces myc transcription</td>
</tr>
<tr>
<td>IL-12</td>
<td>(Crohn’s in humans), Hepatitis</td>
<td>Inhibits apoptosis and induces nucleotide exCISION repair; induces iNOS and NF-κB</td>
</tr>
<tr>
<td>IL-16</td>
<td>Colitis, (Crohn’s in Humans)</td>
<td>Induces kinase signaling cascades</td>
</tr>
<tr>
<td>IL-18</td>
<td>Colitis, Hepatitis</td>
<td>Induces NF-κB</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Colitis, (Crohn’s in humans), Hepatitis, Cystitis</td>
<td>Induces NF-κB, iNOS and COX-2</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Hepatitis</td>
<td>Activates fas-mediated apoptosis; induces iNOS and COX-2, induces DNA repair</td>
</tr>
<tr>
<td>MIF</td>
<td>Colitis</td>
<td>Antagonizes p53</td>
</tr>
<tr>
<td>Chemokines</td>
<td>CCR1/5 Colitis,</td>
<td>Affects proliferation in a p53-dependent manner</td>
</tr>
<tr>
<td></td>
<td>IP-10 Hepatitis</td>
<td>Unknown in hepatocytes</td>
</tr>
<tr>
<td></td>
<td>Mig Hepatitis</td>
<td>Unknown in hepatocytes</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>IL-2 Colitis</td>
<td>Induces fas-mediated apoptosis</td>
</tr>
<tr>
<td></td>
<td>IL-4 Gastritis</td>
<td>Induces pRb hypophosphorylation; decreases cyclin D1 and myc</td>
</tr>
<tr>
<td></td>
<td>IL-10 Colitis, (Crohn’s in humans), Hepatitis, Pancreatitis, Gastritis</td>
<td>Inhibits proliferation</td>
</tr>
<tr>
<td></td>
<td>IL-11 Colitis, (Crohn’s in humans)</td>
<td>Promotes pRb dephosphorylation</td>
</tr>
<tr>
<td></td>
<td>IFN-α Hepatitis, Colitis, (UC in humans)</td>
<td>Activates p53, inhibits proteasomal degradation of survivin; promotes TRAIL and fas-mediated apoptosis; inhibits proliferation</td>
</tr>
<tr>
<td>IFN-β</td>
<td>Colitis, (UC in humans), Hepatitis</td>
<td>Induces apoptosis</td>
</tr>
</tbody>
</table>

a Indicates the use of recombinant antagonists or antibodies are used to block activity of the cytokine of interest.
b Indicates data from animal studies, models of which are difficult to discern between UC and Crohn’s, and therefore the word ‘colitis’ is used in this context.
c Indicates although IFN-γ is considered pro-inflammatory, it has anti-virulence properties, and may have clinical use against viral hepatitis B when used in combination with other interferons.
d Indicates recombinant cytokines or methods of stimulating the anti-inflammatory cytokine are used for treatment.
directly influence tumor suppressor protein function and oncogene induction. For example, MIF and IL-6 can antagonize p53 function, and therefore favor target-cell survival in foci of chronic inflammation [74,75]. IL-6 can also mediate the hyperphosphorylation of the retinoblastoma protein (pRb) [76]. IL-7, which exacerbates experimental colitis, induces transcription of the myc oncogene [77]. IL-11 promotes pRb dephosphorylation in intestinal epithelial cells [78], and therefore its usefulness in ameliorating colitis may extend to protection from colon cancer associated with colitis.

Cytokines can also affect the cell death and cell cycle machinery. Both IL-2 and TNF-α induce apoptosis in colon cancer cells [79,80]; IL-4 inhibits proliferation, induces pRb hypophosphorylation, and promotes decreases in myc and cyclin D1 in gastric cancer cells [81]; and IL-6 can induce the anti-apoptotic genes Bcl-2 and Bcl-XL in pancreatic cells [82]. Although the pro-apoptotic cytokine, TNF, can induce apoptosis in liver cells [83], its co-induction of NF-κB survival signaling during this process can feed back and inhibit this process [84]. Thus, one mechanism by which anti-TNF antibodies may inhibit carcinogenesis is through their inhibition of NF-κB activation. Another mechanism is through inhibition of stimulated proliferation [85]. The recent finding that TNF-α causes free radical generation [86] suggests still another mechanism as a free radical generation blocker. NF-κB can also be induced by IL-18 in liver cells [87]. Finally, IFNs have been shown to induce apoptosis and/or have anti-proliferative effects in gastric, pancreatic, and liver cells [88–90].

2.6. Growth factors

Patients with UC frequently have microsatellite instability [91], which is often associated with inactivation of transforming growth factor-β (TGF-β) signaling. Various animal models of colitis have shown TGF-β and in particular, TGF-β1, protects against experimental colitis [92]. Although the use of TGF-β in the inhibition of colon cancer associated with colitis has yet to be published, it has been shown to inhibit sporadic colon cancer progression through the inhibition of IL-6 signaling [93]. As mentioned, IL-6 can inhibit p53 and mediate pRb hyperphosphorylation [75,76]. TGF-β3, also inhibits proliferation and carcinogenesis in TGFβRII functional colon cells, and promotes colon cancer cell–cell adhesion [94,95]. This growth factor may have proliferation regulatory functions through its stimulation of proliferation, stimulating carcinogenesis (in TGFβRII functional colon cells, and promotes colon cancer cell–cell adhesion [94,95]. This growth factor may have proliferation regulatory functions through its stimulation of proliferation, stimulating carcinogenesis (in TGFβRII functional colon cells, and promotes colon cancer cell–cell adhesion [94,95]. This growth factor may have proliferation regulatory functions through its stimulation of proliferation, stimulating carcinogenesis (in TGFβRII functional colon cells, and promotes colon cancer cell–cell adhesion [94,95]. This growth factor may have proliferation regulatory functions through its stimulation of proliferation, stimulating carcinogenesis (in TGFβRII functional colon cells, and promotes colon cancer cell–cell adhesion [94,95]. This growth factor may have proliferation regulatory functions through its stimulation of proliferation, stimulating carcinogenesis (in TGFβRII functional colon cells, and promotes colon cancer cell–cell adhesion [94,95]. This growth factor may have proliferation regulatory functions through its stimulation of proliferation, stimulating carcinogenesis (in TGFβRII functional colon cells, and promotes colon cancer cell–cell adhesion [94,95]. This growth factor may have proliferation regulatory functions through its stimulation of proliferation, stimulating carcinogenesis (in TGFβRII functional colon cells, and promotes colon cancer cell–cell adhesion [94,95].

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>Disease</th>
<th>Inhibits/stimulates</th>
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<tbody>
<tr>
<td>TGF-β</td>
<td>Colitis</td>
<td>Inhibits</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Pancreatitis</td>
<td>Stimulates</td>
</tr>
<tr>
<td></td>
<td>Hepatitis</td>
<td>Stimulates</td>
</tr>
<tr>
<td>TGF-β2</td>
<td>Pancreatitis</td>
<td>Inhibits</td>
</tr>
<tr>
<td></td>
<td>Hepatitis</td>
<td>Inhibits</td>
</tr>
<tr>
<td>HGF</td>
<td>Pancreatitis</td>
<td>Inhibits</td>
</tr>
<tr>
<td></td>
<td>Hepatitis</td>
<td>Inhibits</td>
</tr>
<tr>
<td>EGF</td>
<td>Pancreatitis</td>
<td>Inhibits</td>
</tr>
<tr>
<td></td>
<td>Hepatitis</td>
<td>Inhibits</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Pancreatitis</td>
<td>Inhibits</td>
</tr>
<tr>
<td>bFGF</td>
<td>Pancreatitis</td>
<td>Inhibits</td>
</tr>
<tr>
<td>CSF</td>
<td>Hepatitis</td>
<td>Inhibits</td>
</tr>
<tr>
<td>Keratinocyte GF-2</td>
<td>Colitis (UC in humans)</td>
<td>Inhibits</td>
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<td></td>
<td>Cystitis</td>
<td>Inhibits</td>
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* Indicates data from animal studies, models of which are difficult to discern between UC and Crohn’s, and therefore the word ‘colitis’ is used in this context.

2.7. Matrix metalloproteases

Matrix metalloproteases (MMPs) are zinc- and calcium-dependent endopeptidases that degrade many components of the extracellular matrix. They are regulated by specific tissue inhibitors of MMP (TIMPs) and are secreted as zymogens that require proteolytic cleavage for activation. Because of their close association with the degree of reactive species overload-associated tissue damage, they have received increasing attention as targets for therapy. Studies in the past 5 years have shown inhibition of MMPs leads to a protection from experimental hepatitis, pancreatitis, and colitis [101–103]. Although MMP inhibitors are well tolerated in humans (musculoskeletal pain is the most common side effect), they have met little success in clinical trials [104]. The observation that MMPs function in earlier stages of carcinogenesis makes them attractive candidates to inhibit for chemoprevention from long term reactive species overload diseases. For example, MMP-2 can promote proliferation of hepatic stellate cells through the activation of the discoidin domain tyrosine kinase receptor 2 (DDR2) [105]. MMPs can also be immunomodulatory, stimulate angiogenesis, inhibit cell adhesion, and inhibit apoptosis [101,104,106].

2.8. Peroxisome proliferator-activated receptor ligands

Peroxisome proliferator-activated receptor (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor family. PPAR-γ ligands have been shown to attenuate experimental colitis [107] and a small prospective study showed a beneficial effect of these ligands in human UC [108]. PPAR-γ ligands have also been shown to protect against pancreatitis and gastritis [109,110]. The mechanisms of PPAR protection against inflammation remain to be fully elucidated. They can inhibit NF-κB, COX-2 and MMPs [111–113]. These ligands also can stimulate apoptosis and inhibit cell proliferation in multiple cell...
types. For example, PPAR-γ has been shown to upregulate of the cdk inhibitor, p27\(^{\text{Kip1}}\), and activate caspases [114,115].

### 2.9. Kinase pathways

Mitogen-activated protein (MAP) kinases are widely expressed serine-threonine kinases that are activated in reactive species overload diseases and mediate important regulatory signals in the cell. Because these pathways are implicated in the regulation of pro-inflammatory pathways (e.g., activation of cytokines) and cancer, they are promising targets for the prevention of high cancer risk, reactive species overload diseases. Inhibiting MAP kinase pathways has been shown to ameliorate experimental colitis, pancreatitis, and gastritis [116–118]. Prospective human trials have shown promising results for the use of MAP kinase inhibitors (CNI-141) in the treatment of Crohn’s disease pathology [119]. The mechanisms of kinases as mediators of reactive species overload carcinogenesis are not well understood. Kinases can be activated by free radicals in vitro and in turn mediate the transcriptional activation and post-translational phosphorylation of key cancer proteins [3]. C-myc, for example, is activated through MAP kinase pathways [120]. Key cancer proteins, including p53 and pRb, are also post-translationally phosphorylated. Therefore, the finding that both p53 [121] and pRb [133] are hyperphosphorylated in epithelial cells in chronic inflammation makes kinase pathway inhibition an intriguing target for chemoprevention.

### 3. Conclusions and perspectives

Chronic activation of the inflammatory cascade can be procarcinogenic. Advances in our knowledge of key cancer-causing free radicals, NF-κB, PPAR, cytokines, growth factors, and kinase pathways will lead to advances in treatment strategies for high cancer risk, reactive species overload diseases. Certainly, scavengers of free radicals, inhibitors of enzymes in the arachidonic acid cascade, and inhibitors of inflammatory causes (e.g., stomach acids, microbes) have led to an attenuation of chronic inflammation. The use of 5-ASA and IFN-α and the protection against their respective IBD and hepatitis-associated cancers are good examples of our advances toward winning the war against these cancer types. Toward that end, it is unclear which agents will be better in inhibiting inflammatory-mediated carcinogenesis: those acting ubiquitously against inflammatory pathways (e.g., 5-ASA) or specific inhibitors of key inflammatory molecules and pathways (e.g., NF-κB antisense). Perhaps, advances in our knowledge of factors that selectively control RNA expression (e.g., through the use of microRNAs and small modulatory RNAs) and their ability to bind a broad spectrum of distinct mRNAs [122,123] will advance our therapeutic potential for reactive species overload diseases and associated cancers. Although these would equate to a multi-targeted biological “smart-bomb”, a better understanding of the efficacy of these molecules in an inflammatory microenvironment is warranted. Multi-targeting might also be accomplished by combination treatment strategies. Future studies should also be aimed at identifying new key molecular targets, molecular signatures in inflammation, and intermediate biomarkers of inflammatory-mediated carcinogenesis. The latter, combined with identifying polymorphisms in key inflammatory genes, will help identify specific populations at risk for free radical-driven cancers and provide insight into individuals sensitive or resistant to anti-inflammatory treatment.

Finally, understanding the mechanisms by which key cancer genes and proteins are altered in reactive species overload diseases will provide important insight into the protection against cancer associated with reactive species overload (Fig. 1). p53 is mutated in UC [124] and hemochromatosis [125]. The p53 pathway is also activated in UC [121]. Other cancer pathway molecules such as HIF-1α, signal transducer, and activator of transcription (STAT), heme-oxygenase-1, poly (ADP-ribose) polymerase (PARP), extracellular matrix molecules (integrins), and cyclins D1 and E are upregulated during free radical stress. Although the specific consequences of this upregulation are not fully understood, targeting these molecules holds promise for future research into chemoprevention in reactive species overload diseases. Inhibiting PARP has been shown to ameliorate experimental colitis [126]; inhibiting integrins improves Crohn’s [127] and UC [128]; germline p53 disruption inhibits helicobacter-induced premalignant lesions and invasive gastric carcinoma [129]; and c-myc induces free radical stress, and disables a p53 DNA damage response, implicating this oncogene as a potential target for treatment of chronic inflammation and associated cancers [130]. Toward this end, it is also clear that emphasis should be placed on examining the influence of promising drugs in patients with high cancer risk, reactive species overload diseases in prospective randomized control trials. Only then will we know the true value of targeting inflammation and affected cancer pathways for chemoprevention.

![Fig. 1. Some key pathways toward carcinogenesis in high cancer risk, reactive species overload diseases.](Image)
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Due to constraints on the number of references, we have not included all original data publications. However, the reader is encouraged to search indicated key words in *PubMed* in order to find original papers from where the data was drawn. Finally, we would like to guide the reader to a recent complimentary paper that appeared in the literature during processing of our review [131].

References


