

Roles of reactive oxygen species (ROS) in inflammation and cancer

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Abstract

Cancer can be caused by either endogenous (genetic disorders and impairments of the immune system) or exogenous (environmental, carcinogen, infection, and persistent inflammation) factors. Inflammation is an important physiological defense response of the biological system to protect cells or tissues from injury or infection. Acute inflammation occurs over a short duration as part of the normal defense response, while chronic inflammation is a prolonged reaction related to various diseases including carcinogenesis. Many types of immune cells are involved directly or indirectly in chronic inflammation in the production of inflammatory cytokines and it appears that chronic inflammation predisposes susceptible cells to mutation(s). Reactive oxygen species (ROS) are one of the mediators produced by inflammatory cells to eradicate invading pathogens. Persistent production of ROS during chronic inflammation can overcome antioxidant defenses leading to intense oxidative stress. Consequently, cellular structures and DNA become damaged, which is a critical aspect of carcinogenesis. Notwithstanding, ROS can induce apoptotic cell death when cells are exposed at optimum levels and time to ROS. ROS generation through radiotherapy and some types of chemotherapy is therefore a goal of cancer treatment. Care must be taken, however, as ROS can cause serious side-effects. This paradoxical effect of ROS—carcinogenesis vs. cancer therapy—depends on the level intracellular ROS and exposure time. In this review, we reported the associations of chronic inflammation, ROS and carcinogenesis as well as the role of ROS in cancer treatment. The mechanism of the association of ROS with inflammation and carcinogenesis remain inconclusive, so several research studies have focused upon investigating these phenomena.

Keywords: reactive oxygen species (ROS), inflammation, cancer, apoptosis

บทคัดย่อ

มะเร็งเกิดได้จากหลายปัจจัยทั้งจากปัจจัยภายใน เช่น ความผิดปกติทางกรรมพันธุ์และความบกพร่องของระบบภูมิคุ้มกัน และจากปัจจัยภายนอก เช่น สิ่งแวดล้อม สารก่อมะเร็ง การติดเชื้อ หรือการอักเสบ ซึ่งการอักเสบเป็นกระบวนการตอบสนองของร่างกายเมื่อได้รับการบาดเจ็บ ซึ่งจัดเป็นกลไกที่สำคัญในการป้องกันเซลล์หรือเนื้อเยื่อจากอันตรายหรือการติดเชื้อ การอักเสบเฉียบพลันเกิดขึ้นอย่างรวดเร็ว และจัดเป็นกลไกที่ช่วยกำจัดสิ่งแปลกปลอมที่เข้ามาในร่างกาย ในขณะที่การอักเสบเรื้อรังมักจะสัมพันธ์กับการเกิดมะเร็งในแต่ละขั้น อนุมูลอิสระเป็นสารตัวกลางตัวหนึ่งที่หลั่งโดยเซลล์อักเสบในระหว่างกระบวนการอักเสบ เพื่อช่วยในการกำจัดสิ่งแปลกปลอม แต่ในภาวะของการอักเสบเรื้อรังส่งผลให้อนุมูลอิสระหลั่งออกมาในปริมาณมาก และเมื่อกระบวนการต้านอนุมูลอิสระของร่างกายไม่สามารถรักษาสสมดุลไว้ได้จะทำให้เกิดภาวะเครียดออกซิเดชัน และมีการทำลายโครงสร้างของเซลล์รวมถึงดีเอ็นเอ ซึ่งเป็นจุดเริ่มต้นที่วิกฤตของกระบวนการเกิดโรคมะเร็ง แต่ในทางกลับกันพบว่าเมื่อเซลล์ได้รับอนุมูลอิสระในปริมาณและระยะเวลาที่เหมาะสมสามารถเหนี่ยวนำให้เซลล์เกิดการตายแบบอะพอพโทซิส จึงมีการพัฒนาวิธีในการรักษามะเร็งโดยการเหนี่ยวนำให้มีการสร้างอนุมูลอิสระในปริมาณที่สามารถฆ่าเซลล์มะเร็ง เช่น รังสีรักษาและการใช้ยาเคมีบำบัดบางชนิด อย่างไรก็ตามอนุมูลอิสระทำให้เกิดผลข้างเคียงที่รุนแรงได้เช่นกัน ซึ่งผลตรงกันข้ามกันของอนุมูลอิสระดังกล่าวขึ้นกับระดับความเข้มข้นของอนุมูลอิสระ และระยะเวลาที่เซลล์ได้รับอนุมูลอิสระ บทความปริทัศน์นี้ได้อธิบายถึงความสัมพันธ์ของอนุมูลอิสระในกระบวนการอักเสบ และการเกิดมะเร็ง แต่อย่างไรก็ตามความสัมพันธ์นี้ยังไม่มีข้อสรุปที่แน่ชัด จึงยังมีหลายการศึกษาที่กำลังดำเนินการเพื่ออธิบายปรากฏการณ์นี้ในปัจจุบัน

คำสำคัญ: อนุมูลอิสระ, การอักเสบ, มะเร็ง, อะพอพโทซิส

1. Introduction

Cancer is a group of diseases in which abnormal cells divide without control with consequent morphological cellular transformations, failure of apoptosis, invasion, angiogenesis and metastasis (Lin & Karin, 2007). Cancer is caused by both endogenous and exogenous factors: the former include genetic disorders and impairment of the immune system and the latter environmental factors (i.e., aflatoxin, nitrosamine and arsenic found in food and water; UV radiation; and benzo[a]pyrene in cigarette smoke). Infections from some strains of viruses and bacteria are considered exogenous (e.g., Hepatitis B (HBV) and C (HCV) viruses, human papilloma virus (HPV) and the gram-negative bacterium *Helicobacter pylori*)—a leading cause of hepatocellular carcinoma, cervical cancer and gastric adenocarcinoma (Macarthur, Hold, & El-Omar, 2004). Chronic inflammation is thus an underlying aspect associated with development of cancer.

Rudolf Virchow first suggested the relationship between inflammation and cancer in 1863 (Balkwill & Mantovani, 2001). Virchow observed that inflammatory cells were present within tumors and tumors often occur at sites of chronic inflammation. Several epidemiological studies have confirmed that chronic inflammatory diseases are often associated with increased risk of cancers (Lu, Ouyang, & Huang, 2006; Philip, Rowley, & Schreiber, 2004). Chronic inflammation has been related to various steps of carcinogenesis; such as cellular transformation, promotion, proliferation, survival, angiogenesis, invasion and metastasis (Aggarwal, Shishodia, Sandur, Pandey, & Sethi, 2006; Mantovani, 2005). Much evidence supports this conclusion as up to 25% of all cancers are caused by chronic infections or chronic inflammation and several studies have demonstrated that anti-inflammatory therapies appeared to reduce the development of cancer (Hussain & Harris, 2007; Schetter, Heegaard, & Harris, 2010).

Inflammation is the physiological response of cells to injury caused by microbial and viral infection, exposure to allergens, radiation and toxic substances, alcohol consumption, cigarette smoking, and autoimmune disease. There are two types or stages of inflammation: acute and chronic. Acute inflammation is defined as the early stage of inflammation mediated through the activation of the immune system. This type of inflammation lasts for a short period and is beneficial to the host. At the cellular level, the activation of tissue-specific cell

types (e.g., mast cells for the intestinal epithelium) and non-tissue-specific cell types (e.g., macrophages, neutrophils, dendritic cells, T-cells, B-cells) of the immune system participate in the inflammation process. Macrophages and neutrophils are the first line of immunity against invading pathogens (de Visser, Eichten, & Coussens, 2006). If inflammation is prolonged, these cells release soluble factors (including cytokines and chemokines) in order to attract additional leukocytes into the site of damage. When inflammation becomes chronic, it predisposes the host to various chronic diseases (e.g., cardiovascular diseases, diabetes, arthritis, Alzheimer's disease and cancer (Lin & Karin, 2007; Reuter, Gupta, Chaturvedi, & Aggarwal, 2010).

The types of cancer associated with chronic inflammation and oxidative stress include colon cancer (associated with inflammatory bowel diseases), esophageal adenocarcinoma (associated with reflux esophagitis or Barrett's esophagus), and hepatocellular carcinoma (associated with chronic hepatitis C) (Federico, Morgillo, Tuccillo, Ciardiello, & Loguercio, 2007; Reuter et al., 2010). Although ROS are the cause of carcinogenesis, free radicals are also used in the cancer treatment to kill cancer cells. For example, ionizing radiation in cancer therapy and some types of chemotherapy can generate ROS, which are toxic to and kill cancer cells (Pelicano, Carney, & Huang, 2004). Notwithstanding, ROS can cause serious side-effects depending on the level of intracellular ROS and the duration of exposure. To elucidate the role of ROS in cancer development, we reviewed the associations between and among ROS, chronic inflammation, and carcinogenesis.

2. Reactive oxygen species (ROS)

Free radicals are defined as molecules or molecular fragments containing at least one unpaired electron in the outermost shell. This unpaired electron leads to highly reactive free radicals. The classification of the radical is based upon the major elemental composition (i.e., oxygen, nitrogen, carbon and sulfur-centered radicals). The most commonly found radical in the body is oxygen; the majority being singlet oxygen, superoxide radicals and/or hydroxyl radicals. These radicals are called reactive oxygen species (ROS). The ROS is also implicated in certain non-radical molecules with reactive properties like hydrogen peroxide (H_2O_2) (Droge, 2002; Kirkinezos & Moraes, 2001). ROS can be produced by endogenous and exogenous sources.

Endogenous sources of ROS are physiological processes, including aerobic metabolism, inflammatory cell activation to eliminate invading pathogenic microorganisms, or products from peroxisomes or cytochrome P450. Exogenous sources of ROS include ionizing radiation, redox cycling compounds and metal ions (Klaunig & Kamendulis, 2004).

During mitochondrial oxidative metabolism, the majority of the oxygen consumed is reduced to water; however, an estimated 4% to 5% of molecular oxygen is converted to reactive oxygen species, primarily superoxide anion, formed by an initial one-electron reduction of molecular oxygen (Table 1). Superoxide can be dismutated by

superoxide dismutase to yield H_2O_2 . In the presence of partially-reduced metal ions, in particular iron, H_2O_2 is subsequently converted through Fenton and Haber-Weiss reactions to a hydroxyl radical. The hydroxyl radical is highly reactive and can interact with nucleic acids, lipids, and proteins.

Neutrophils, eosinophils, and macrophages are an additional endogenous source and are major contributors to the cellular reactive oxygen species. Activation of macrophages, through “respiratory burst,” elicits a rapid but transient increase in oxygen uptake that gives rise to a variety of reactive oxygen species, including superoxide anion, H_2O_2 , and nitric oxide (NO). In addition, peroxynitrite is formed from the coupling of NO and superoxide (Table 1).

Table 1 Pathways for intercellular ROS generation (Klaunig and Kamendulis, 2004)

Generation of reactive oxygen species via reduction of molecular oxygen
$O_2 + e^- \rightarrow O_2^{\bullet -}$ (superoxide anion)
$O_2^{\bullet -} + H_2O \rightarrow HO_2^{\bullet}$ (hydroperoxyl radical)
$HO_2^{\bullet} + e^- + H \rightarrow H_2O_2$ (hydrogen peroxide)
$H_2O_2 + e^- \rightarrow OH^- + \bullet OH$ (hydroxyl radical)
A series of reactive oxygen species generated by reduction of molecular oxygen. These ROS are highly reactive to reacts with biomolecules.
Production of reactive nitrogen species
$L\text{-arginine} + O_2 \rightarrow \bullet NO$ (nitric oxide) + L-citrulline
$O_2^{\bullet -} + \bullet NO \rightarrow ONOO^-$ (peroxynitrite)
$ONOO^- + CO_2 \rightarrow ONOOCO_2^-$ (nitrosoperoxy carbonate)
$ONOOCO_2^- \rightarrow \bullet NO_2$ (nitrogen dioxide) + $CO_3^{\bullet -}$ (carbonate anion radical)
Fenton reaction
$H_2O_2 + Fe^{2+} \rightarrow OH^- + \bullet OH + Fe^{3+}$
Hydroxyl radical is produced by Fenton reaction catalyzed by transition metals such as Cu(2+), Cr(V), and Ni, resulting in radical formation.

The pathways of intercellular ROS generation are presented in Table 1. A series of oxygen radicals are produced by the reduction of molecular oxygen. Of the radicals produced, the hydroxyl radical, hydroperoxyl radical, and the superoxide anion are sufficiently reactive and may interact with biomolecules. By NO synthase, nitric oxide is produced when molecular oxygen reacts with L-arginine. Following the production of this radical, and interaction with a superoxide anion, a number of oxidizing and reactive species are produced. The Fenton reaction describes the reaction of ferrous iron with H_2O_2 —named after H. H. Fenton (1894) who first observed the oxidation of tartaric acid by a mixture of iron sulfate and hydrogen peroxide acid. Fe (II) is known to decompose H_2O_2 to oxygen and water. The Fenton reaction can produce the hydroxyl radical. Transition metals such

as Cu(2+), Cr(V), and Ni can also catalyze this reaction and result in radical formation.

Since ROS are the most abundantly produced and have highly oxidative properties that can damage essential cellular components (Valavanidis, Vlachogianni, Fiotakis, & Loidas, 2013), the role of ROS are of interest and are the focus of this review. ROS have a dual role in the body—both useful and harmful aspects—depending on their concentration. At micromolar concentrations, ROS have beneficial effects vis-à-vis cellular responses against pathogens and cellular signaling systems (Gough & Cotter, 2011; Valko et al., 2007). The effect of free radicals is complicated depending on the local concentration, the microenvironment and the genetic background of the individual (Hussain, Hofseth, & Harris, 2003). The harmful effects of ROS arise due to an imbalance

between ROS and the antioxidant system; such as excessive generation of ROS or decrease in cellular antioxidant system to eliminate ROS. During inflammation, ROS are released for defense against pathogens but these are harmful if they persist in the long-term. In chronic inflammation, a large number of ROS are generated in inflammatory sites; causing oxidative stress and resulting in DNA damage, mitochondrial dysfunction, lipid peroxidation,

protein oxidation and apoptosis. Moreover, oxidative stress is associated with many diseases including acute respiratory distress syndrome, Alzheimer's disease, atherosclerosis, inflammation, rheumatoid arthritis, vascular disease, and cancer (Klaunig & Kamendulis, 2004; Reuter et al., 2010). The mechanism of ROS in cancer treatment is via either apoptosis or necrosis depending on its concentration (Figure 1).

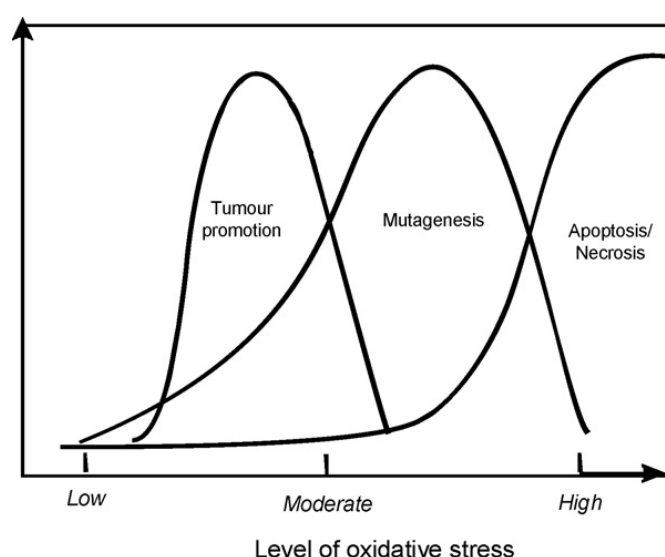


Figure 1 Dose-dependent effect of oxidative stress on tumor promotion, mutagenesis as well as apoptosis/necrosis (Valko et al., 2007)

An optimum concentration of ROS can result in apoptosis. H_2O_2 at 9-30 μM induces apoptosis whereas $H_2O_2 \geq 100 \mu M$ induces necrosis in leukemia cells (Wagner, Buettner, Oberley, Darby, & Burns, 2000). The ROS triggering apoptosis has been used in cancer treatment such as radiotherapy and some types of chemotherapy (i.e., mitomycin c and doxorubicin). The overproduction ROS in cancer cells exhausts the capacity of antioxidant defenses resulting in cancer cell death. Thus, ROS not only act as carcinogens but they are also useful for cancer treatment.

3. The relationship of chronic inflammation and cancer

Rudolph Virchow first suggested the relationship between chronic inflammation and cancer in 1863. This relationship was then supported by epidemiologic and clinical studies. For instance,

gastric chronic inflammation caused by *Helicobacter pylori* infection was associated with adenocarcinoma and mucosal-associated lymphoid tissue lymphoma. Chronic hepatitis, caused by hepatitis B and C viral infections, was associated with hepatocellular carcinoma. Chronic inflammation in the bile tract, caused by *Chlonorchis sinensis* infection, predisposes persons to cholangiocarcinoma. Chronic inflammation caused by non-infection may also be a cause of cancer. For example inflammatory bowel disease (i.e., ulcerative colitis and Crohn's disease) was associated with the risk of colorectal cancer and cigarette smoking was related to bronchitis and leading to lung cancer (Lu et al., 2006; Philip et al., 2004). The relationship between inflammation and predisposition to cancer is summarized in Table 2: this evidence of an association between chronic inflammation and cancer strongly supports the hypothesis.

Table 2 Relationship between chronic inflammation and cancer (Aggarwal et al., 2006)

Inducer	Inflammation	Cancers	% Predisposed that progress to cancer
Tobacco smoke	Bronchitis	Lung cancer	11-24
<i>Helicobacter pylori</i>	Gastritis	Gastric cancer	1-3
Human Papilloma virus	Cervicitis	Cervical cancer	<1
Hepatitis B & C virus	Hepatitis	Hepatocellular carcinoma	10
Bacteria, Gall bladder stones	Cholecystitis	Gall bladder cancer	1-2
Gram-uropathogens	Cystitis	Bladder cancer	<1
Tobacco, genetics	Pancreatitis	Pancreatic cancer	≤10
Gastric acid, alcohol, tobacco	Esophagitis	Esophageal cancer	15
Asbestos fibers	Asbestosis	Mesothelioma	10-15
Epstein-Barr virus	Mononucleosis	Burkitt's lymphoma	<1
	Hodgkin's disease		
Gut pathogens	IBD	Colorectal cancer	1
Ultraviolet light	Sunburn	Melanoma	≤9

In chronic inflammation, various types of leukocytes and other inflammatory cells are activated and recruited to inflammatory sites via a signaling network involving growth factors, cytokines (i.e., tumor necrosis factor- α (TNF- α)) and chemokines (i.e., NF- κ B and STAT 3) (Coussens & Werb, 2002; Lu et al., 2006; Kamp, Shacter, & Weitzman, 2011). The key molecular linkages between chronic inflammation and cancer are shown in Table 3. Inflammatory cells secrete pro-inflammatory cytokines (i.e., TNF- α , interleukin-1 β (IL-1 β)) and interferon- γ (IFN- γ) during the inflammation process. TNF- α and IL-1 β are secreted by macrophages while IFN- γ is secreted by lymphocytes. These pro-inflammatory cytokines stimulate ROS production via various mechanisms (Oh et al., 1999; Yang et al., 2007). For example, IL-1 stimulates superoxide anion production via activation of the phagocytic NADPH oxidase in macrophages and neutrophils (Brigelius-Flohe, Banning, Kny, & Bol, 2004). Two transcription factors, NF- κ B and STAT3, have recently been demonstrated as major factors linking inflammation to cancer (Fan, Mao, & Yang, 2013).

NF- κ B is a redox-sensitive transcription factor that regulates the expression of genes involved in inflammation, immune modulation and apoptosis, as well as in various stages of carcinogenesis (i.e., proliferation, invasion, angiogenesis, and metastasis) (Chen et al., 2011; Wang et al., 2011). Activation of NF- κ B is associated with the production of ROS. The ROS-producing enzyme is the phagocyte-derived NADPH oxidase (Nox), which plays an

important role during bacterial infection and inflammation (Gloire, Legrand-Poels, & Piette, 2006; Quinn, Ammons, & Deleo, 2006). STAT3 (Signal Transducer and Activator of Transcription) regulates the expression of a variety of genes in response to cellular stimuli, and thus plays a pivotal role in cell growth and apoptosis. Persistent activation of STAT3 in tumor cells activates cytokines, chemokines and growth factors, which in turn activate STAT3 in stromal cells—the main resource of inflammation mediators (Fan et al., 2013). The activation and interaction between STAT3 and NF- κ B play vital roles in the control of communication between cancer and inflammatory cells. NF- κ B and STAT3 are thus the two major factors controlling the ability of pre-neoplastic and malignant cells to resist apoptosis-based cancer-surveillance and regulating angiogenesis and invasiveness (Fan et al., 2013).

In addition to cytokines and chemokines, ROS are also produced by either inflammatory/immune cells and act as the central endogenous carcinogens, driving cancer-promoting signaling pathways in inflammation-associated cancer (Figure 2). ROS is one of the major causes of DNA damage, followed by cancer initiation. The major sources of ROS in inflammation-associated cancer include NADPH oxidase (present in phagocytes and other cells) and mitochondria (Kamp, Shacter, & Weitzman, 2011); however, it is unclear whether ROS/RNS produced by neutrophils and macrophages are sufficient to induce the specific kinds of epithelial cell DNA damage and tumorigenesis of inflamed cells.

Table 3 Key molecular players linking cancer to inflammation (Lu et al., 2006)

Potential Linkers	Functions in linking inflammation to cancer
Cytokines	
IL-6	Promote tumor growth
TNF- α	Induce DNA damage and inhibit DNA repair
	Promote tumor growth, Induce angiogenic factor
Chemokines	Promote tumor cell growth
	Facilitate invasion and metastasis by directing tumor cell migration and Promoting basement membrane degradation
NF- κ B	Mediate inflammation progress, promoting chronic inflammation
	Promote the production of mutagenic reactive oxygen species
	Protect transformed cell from apoptosis
	Promote tumor invasion and metastasis
iNOS	Feedback loop between pro inflammatory cytokines
	Downstream of NF- κ B and pro inflammatory cytokines
	Induce DNA damage and disrupt DNA damage response
COX-2	Regulate angiogenesis and metastasis
	Produce inflammation mediator prostaglandins
HIF-1 α	Promote cell proliferation, antiapoptotic activity, angiogenesis, and metastasis
	Promote chronic inflammation
	Induced by proinflammatory cytokines through NF- κ B
	Enhance the glycolytic activity of cancer cell
STAT3	Contribute to angiogenesis, tumor invasion, metastasis by transactivating VEGF
	Activated by proinflammatory cytokines
	Promote proliferation, apoptosis resistance, and immune tolerance
Nrf2	Anti-inflammatory activity
	Protect against DNA damage
NFAT	Regulate proinflammatory cytokine expression
	Required in cell transformation

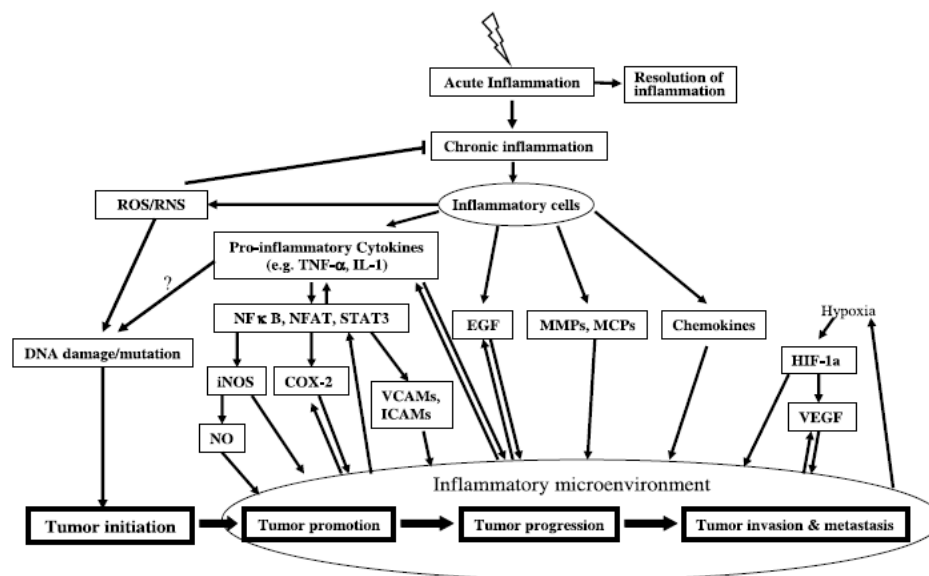


Figure 2 Schematic diagram representing the mechanisms involved in inflammation and cancer development. Inflammation involved in tumor initiation then develops to tumor promotion and progression—the steps involved in benign tumors progressing to malignant carcinomas (Lu et al., 2006)

Chronic inflammatory conditions that promote tumor formation can also be attributed to genetic alterations that directly affect the STAT3 pathway (Yu, Pardoll, & Jove, 2009). STAT3 signaling is a major intrinsic pathway for cancer inflammation because it is frequently activated in malignant cells and capable of inducing a large number of genes that are crucial for inflammation (Yu et al., 2009). STAT3 gene ablation in diverse tumor models results in the inhibition of tumor growth; therefore, the development of small-molecule STAT3 inhibitors is an alternative approach to overcoming chronic inflammation-associated cancer. The inhibition of STAT3 was used to try and convert inflammation in the tumor microenvironment from a tumor promoting to a tumor suppressing. It was proposed that the STAT3 inhibitors might work via the disruption of protein-protein or protein-DNA interactions. Moreover, tyrosine kinase inhibitors that modulate the tumor immunological environment by inhibiting STAT3 activity, combined with immunotherapeutic approaches, may lead to increased anti-tumor immune responses (Lee, Pal, Reckamp, Figlin, & Yu, 2011).

4. The relationship of chronic inflammation and ROS generation

During inflammation, the inflammatory cells (e.g., macrophage and neutrophils or leukocytes) are recruited to the site of inflammation. These inflammatory cells increase uptake of oxygen that leads to the release of free radicals in the site to eradicate pathogens that cause inflammation (Hussain et al., 2003). The inflammatory cells also produce soluble mediators including metabolites of arachidonic acid, cytokines and chemokines (i.e. IL-1, IL-6, TNF- α)—whose role includes further recruiting inflammatory cells to the site of damage and producing more ROS. The primary free radical-generating system is the phagocytic oxidase system. Phagocytic oxidase is induced and activated by many stimuli, including IFN- γ and signals from TLRs. The function of this enzyme is to reduce molecular oxygen into ROS such as superoxide radicals, with the reduced form of NADPH acting as a cofactor (Abbas, Lichtman, & Pillai, 2010). The process by which ROS are produced is called the respiratory burst (Hussain et al., 2003). In addition to ROS, macrophages produce reactive nitrogen intermediates, mainly NO, by the action of an

enzyme called inducible nitric oxide synthase (iNOS). iNOS catalyzes the conversion of arginine to citrulline, and freely diffusible NO gas is released. Within phagolysosomes, NO may combine with H₂O₂ or superoxide, generated by phagocyte oxidase, to produce highly reactive peroxynitrite radicals that can kill microbes (Abbas, Lichtman, & Pillai, 2010).

The phagocytic oxidase via the NADPH oxidase pathway is not a unique function of phagocytes, as it has been detected in various cell types, including cancer cells. NADPH oxidases (Nox) family is a complex of membrane-bound components (cytochrome b558: gp91phox (Nox2), p22phox, the GTP-binding protein Rap) and cytosolic components (p47phox, p67phox, the GTP-binding protein Rac) (Manda, Nechifor, & Neagu, 2009). Nox are reactive oxygen species, generating enzymes that widely and specifically regulate redox-sensitive signaling pathways involved in cancer development and progression. There are seven isoforms in the Nox family including Nox1, Nox2, Nox3, Nox4, Nox5, Duox1, and Duox2—which account for the differences in generating ROS, as observed in various normal or pathological cell types, besides phagocytes. These enzymes share the capacity to transport electrons across the plasma membrane and to generate superoxide and other downstream ROS. Among the Nox isoforms, Nox2 is the most widely distributed for examples in thymus, small intestine, colon, spleen, pancreas, ovary, placenta, prostate, and testis. Nox2 was first described in neutrophils and macrophages; and referred to as the phagocyte NADPH oxidase (Bedard & Krause, 2007). Besides Nox, Dual oxidase (Duox)2 also plays a role in the generation of H₂O₂ for host defense against a variety of pathogens. Recently the Duox2 over-expression was found to link with the pancreatic inflammation and pancreatic cancer cells as a result of the induction of IFN- γ -related activation of Stat1 that collaboratively mediated NF- κ B-related up-regulation of Duox2 (Wu et al., 2013).

If there is an inefficiency of the biological mechanisms to address inflammation, chronic inflammation will ensue, resulting in persistent production of ROS at the site of inflammation (Hussain et al., 2003; Reuter et al., 2010). When more ROS are produced than eliminated, oxidative stress occurs, resulting in protein damage, lipid peroxidation, DNA damage and mutation (Figure 3).

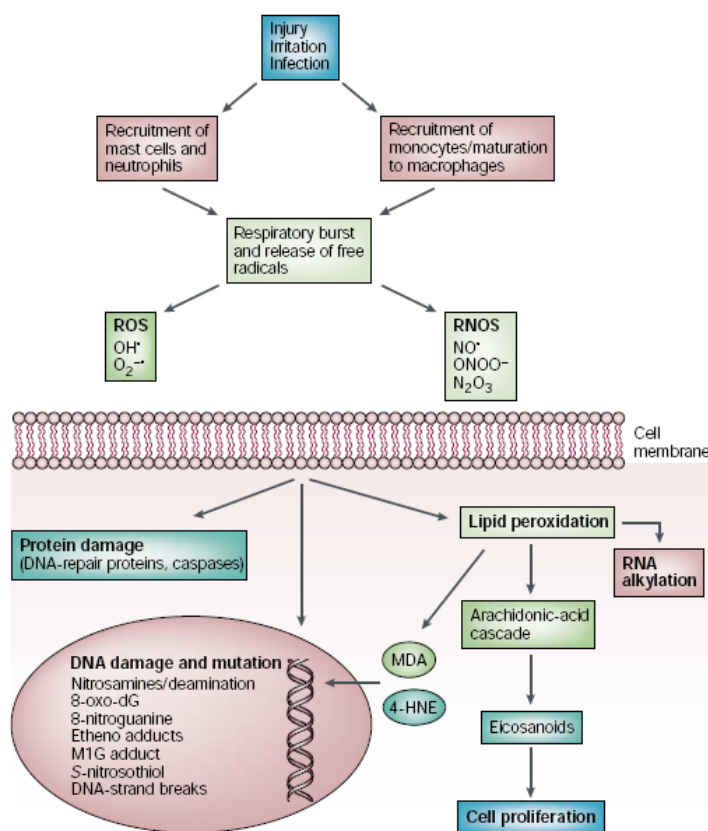


Figure 3 Impact of free radicals released at sites of inflammation on cellular molecules (Hussain et al., 2003)

5. ROS roles in carcinogenesis

Cancer development is a multi-stage process characterized by cumulative action of multiple events occurring in a single cell. There are three processes in carcinogenesis including initiation, promotion and progression (Berenblum & Schubik, 1949).

(1) Initiation is the first process in carcinogenesis, induced by carcinogen exposure resulting in DNA damage and gene mutation. A continuing active inflammation response leads to cell damage or cellular hyperplasia, following ROS overproduction from the inflammatory cells. Cytokines produced by inflammatory cells can elevate intracellular ROS and reactive nitrogen intermediates (RNI) in premalignant cells. During inflammation ROS can interact with DNA in mitotic cells resulting in permanent genomic mutation (i.e., point mutations, gene deletions, or gene rearrangement) (Khansari, Shakiba, & Mahmoudi, 2009). In addition,

inflammation can result in epigenetic change that favors tumor initiation. Tumor-associated inflammation contributes to further ROS and RNI, and cytokine production. Initiation involves a non-lethal mutation in DNA that produces an altered cell followed by at least one round of DNA synthesis to fix the damage (e.g. 8-OH-G). If dividing cells are damaged, cells are able to temporarily interrupt their cell cycle at stage G1, S, or G2 ("check-points"), repair the damage, and resume division (Loft & Poulsen, 1996). If cells fail to repair themselves and there is accumulation of gene mutation, then the promotion process will occur.

(2) The promotion stage is characterized by the clonal expansion of initiated cells by the induction of cell proliferation and/or inhibition of programmed cell death (apoptosis). This process results in the formation of an identifiable focal lesion. This stage dose-dependently requires the continuous presence of the tumor promotion stimulus and thus it is a

reversible process (Loft & Poulsen, 1996). Many factors are involved in the promotion process (i.e., cytokines, lipid metabolites, and certain phorbol esters), which have an affect on the patterns of specific gene expression. This may lead to an enhancement of cellular growth potential and interference of cellular communication, which functionally restricts cellular autonomy and coordinates tissue maintenance and development. Cytokines produced by tumor infiltrating immune cells activate key transcription factors (i.e., NF- κ B or STAT3) in pre-malignant cells to control different stages of carcinogenesis, including survival, proliferation, growth, angiogenesis, and invasion. Next, NF- κ B and STAT3 induce the production of chemokines—as part of a positive feed-forward loop—which attract additional immune/inflammatory cells to maintain tumor-associated inflammation (Grivennikov, Greten, & Karin, 2010).

(3) Progression involves cellular and molecular changes that occur from the pre-neoplastic to the

neoplastic stage. This process may be accelerated by repeated exposure to carcinogens, leading to rapidly increasing and uncontrollable cell growth. In addition, when tumor progression advances, the tumor cells can invade other tissues and organs via blood circulation and lymphatic systems: metastasis (Figure 4). This stage is irreversible and is characterized by accumulation of additional genetic damage, leading to the transition of the cell from benign to malignant and generation of new blood supplies that feed the malignant cells (viz., angiogenesis). Increased angiogenesis was reported triggered and promoted by tumor hypoxia, which increases the probability of metastasis. It is not certain whether hypoxia directly affected tumor angiogenesis or whether hypoxic stimuli generated inflammatory signals that drove angiogenesis. However, it was evident that the inactivation of NF- κ B or STAT3 resulted in disrupted angiogenesis and decreased tumor growth, which in turn defined their role as inflammatory mediators in tumor angiogenesis (Grivennikov et al., 2010).

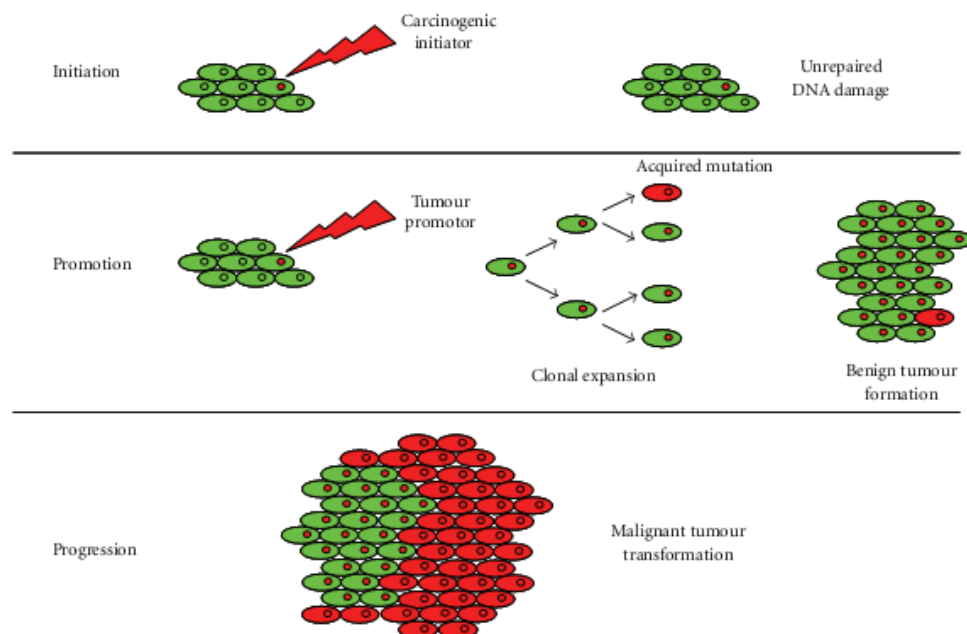


Figure 4 Cancer development (Centelles, 2012)

ROS can act at all stages of carcinogenesis. For instance, ROS such as hydroxyl radicals formed through the Fenton-type mechanism were reported to cause the oxidative DNA damage and gene mutations that initiate cancer development. The hypothesis vis-à-vis the role of ROS in carcinogenesis comes from *in vitro* studies which refer to their role in DNA damage and protein modifications. In 1956, Philips found that H₂O₂ in the presence of Fe₂(SO₄)₃—a peroxidation activator—induced chromosome breakage. From that time, many other studies have reported on the association of other free radicals causing DNA damage and protein modifications (Hussain et al., 2003). The destruction by ROS is the most common

type of DNA damage and ROS can attack various components of DNA resulting in single- or double-stranded DNA breaks, purine, pyrimidine or deoxyribose modifications and DNA cross-links (Federico et al., 2007). DNA damage can cause either arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, which are associated with carcinogenesis (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006). DNA mutation is a critical step of carcinogenesis and increased levels of oxidative DNA damage. It has been noted in various types of cancer that such damage is strongly related to the etiology of cancer. Various types of cancer associated with ROS are presented in Table 4.

Table 4 ROS associated diseases and cancer risk (Hussain, Hofseth, & Harris, 2003)

Disease	Cancer	Risk*
Inherited		
Haemochromatosis	Liver	219
Crohn's disease	Colon	3
Ulcerative colitis	Colon	6
Acquired: viral		
Viral hepatitis B	Liver	88
Viral hepatitis C	Liver	30
Human papillomavirus infection	Cervix	16
Acquired: bacterial		
<i>Helicobacter pylori</i> infection	Gastric	10
Urinary bladder catheterization	Bladder	5-28
Prostatitis	Prostate	2
Acquired: parasitic		
<i>Schistosoma hematobium</i>	Bladder	2-14
<i>Schistosoma japonicum</i>	Colon	1.2-6.0
Acquired: chemical/physical		
Barrett's oesophagus	Oesophageal	50-100
Pancreatitis	Pancreatic	2-3

*Relative risk or odd ratio.

Cancer promotion can be described as a consequence of extensive and continued free-radical-related damage. Free radicals can change cell growth and tumor promotion by activating signaling pathways, which result in the induction of growth stimulatory the proto-oncogenes, like *c-fos*, *c-jun*, and *c-myc*. It has been shown that phosphorylation and poly-ADP-ribosylation of chromosomal proteins are involved in the transcription of *c-fos* by oxidants, and that a pro-oxidant state can promote neoplastic growth (Khansari et al., 2009). In the promotion stage, the cellular antioxidant defense systems (i.e., superoxide dismutase (SOD), catalase, and glutathione) were strongly inhibited by many tumor promoters. It should be noted that a high level of oxidative stress is cytotoxic and pauses proliferation

by inducing apoptosis or even necrosis. By contrast, a low level of oxidative stress can actually stimulate cell division in the promotion stage and stimulate the promotion of tumor growth (Dreher & Junod, 1996).

Accordingly, it is interesting that increasing cellular ROS leads to various biological responses including cellular adaptation, increased cellular proliferation, DNA damage, cell injury and cell death via apoptosis and necrosis. The specific effects on cells depend on their genetic background, the type of ROS, and the level and duration of ROS stress. Since ROS have a direct effect on DNA, measurement of the product(s) resulting from DNA damage is useful as byproducts of DNA damage can be used as markers to assess overall exposure of the body to oxidative stress; including 8-hydroxyguanine

(8-OH-Gua), 8-hydroxydeoxyguanosine (8-OHdG), 8-nitro-soguanosine, and exocyclic etheno- and malondialdehyde-DNA adducts in leukocytes or target tissue (Hussain & Harris, 2007).

Yamaguchi, Hirano, Asami, Sugita and Kasai (1996) studied the involvement of oxygen radicals in ferric nitrilotriacetate (Fe-NTA) carcinogenesis. Fe-NTA is known to induce renal oxidative stress, enhance renal lipid peroxidation and H₂O₂ generation with concomitant reduction in glutathione content, antioxidant enzymes in kidneys. So, Fe-NTA was used to induce oxidative DNA damage in a study. Since 8-hydroxyguanine (8-OH-Gua) is one of the major markers of oxidative DNA damage, the formation of 8-OH-Gua in nuclear DNA leads to GC→TA transversions, which are involved in carcinogenesis. 8-OH-Gua and 8-OH-Gua repair activities were determined in rat kidneys after intra-peritoneal injection of Fe-NTA to identify the carcinogenicity of oxygen radical from Fe-NTA. The quantities of 8-OH-Gua in DNA were determined by HPLC, whereas 8-OH-Gua repair activities were determined with an endonuclease assay, using a 22-mer double strand DNA containing 8-OH-Gua at a specific position as the substrate.

Under mild oxidative stress, cells can control a variety of adaptation mechanisms to deal with oxidative stress. The first adaptation to ROS stress is increasing of the redox-buffering system. Another important cellular adaptation mechanism is incremental expression of antioxidant enzymes (i.e., SOD, catalase and peroxidase). These mechanisms provide more sustainable protection to cells against ROS stress (Pelicano et al., 2004). Thus, under decreasing cellular antioxidant enzymes (i.e., SOD), the risk of DNA damage increases. Siomek et al. (2010) studied oxidative DNA damage in Cu, Zn-superoxide dismutase deficient mice. The level of oxidative damage increased in only some organs (viz., the liver and kidney) but not in urinary excretions (Siomek et al., 2010).

6. Conclusion

The relationship between inflammation and cancer has been studied extensively. During

inflammation, inflammatory cells are recruited to inflammatory sites, leading to a respiratory burst from higher uptake of oxygen to release various pro-inflammatory cytokines as well as ROS—important mediators linking inflammation and cancer. Under physiological conditions, there is constant endogenous production of ROS, which act as signaling molecules for metabolism, cell cycle and intercellular transduction pathways. The homeostasis of ROS in living organisms is a balance between ROS production and elimination by the antioxidant system. Oxidative stress occurs when ROS exceeds its elimination. In chronic inflammation, more ROS are generated than the antioxidant system can neutralize, leading to oxidative stress. The effect of oxidative stress on living organism—such as DNA damage—modify cancer-related proteins and regulate transcription (i.e., nuclear factor κ B: NF- κ B), signal transducer activator of transcription 3 (STAT3), which may lead to an increase or a decrease carcinogenesis, depending on the level of oxidative stress (Figure 5).

Mild to moderate oxidative stress leads to cancer development, whereas high oxidative stress can induce cell death. Current approaches to cancer treatment use this principle to kill cancer via ROS-generating agents such as radiotherapy and some types of chemotherapy. ROS have a multifaceted role at the cellular level by acting both as cytotoxic agents as well as signal transducers involved in physiological cell proliferation and death. ROS thus have an interesting role in cancer, not only inducing carcinogenesis but also in its treatment. Since ROS are associated with carcinogenesis, chemopreventive agents such as antioxidants may be useful in this type of patient by arresting cancer development at the initiation stage. The tightly regulated redox balance involved with the complex network of physiological and pathological pathways is important for consideration in the development of cancer therapy.

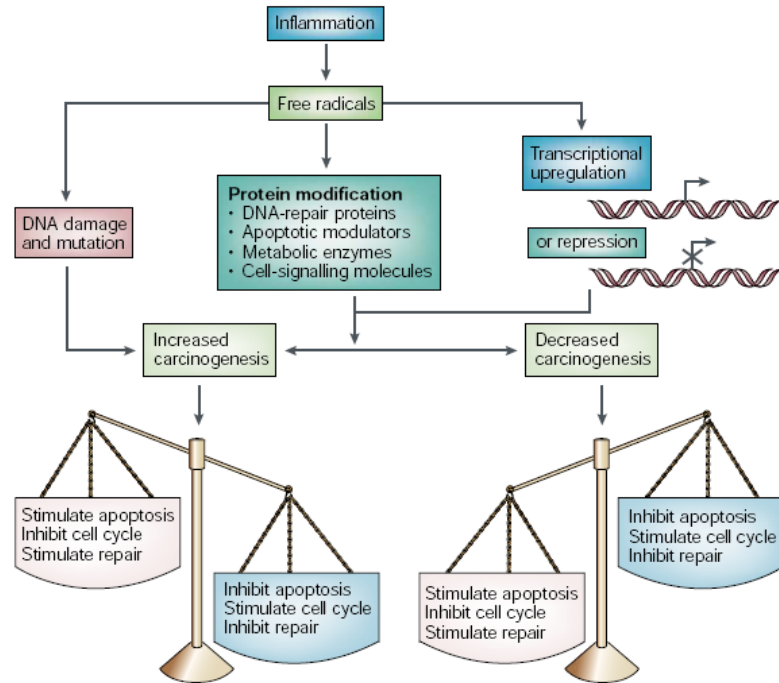


Figure 5 Chronic inflammation and production of free radicals regulate multiple cellular processes (Hussain et al., 2003)

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