CHAPTER *1*

INFECTION, INFLAMMATION, AND CANCER: OVERVIEW

Hiroshi Ohshima, Noriyuki Miyoshi, and Susumu Tomono

It has been estimated that about 2 million (16.1%) of the total 12.7 million new cancer cases in 2008 were attributable to infections (1). This percentage was higher in less-developed (22.9%) than in more-developed (7.4%) countries, and varied 10-fold by region from 3.3% in Australia and New Zealand to 32.7% in sub-Saharan Africa. Four major infections with *Helicobacter pylori*, hepatitis B and C viruses, and human papillomavirus are estimated to be responsible for 1.9 million cases of gastric, liver, and cervical cancer. Cervical cancer accounts for about half of the infection-related burden of cancer in women, and in men liver and gastric cancers account for over 80%. In addition, as shown in Table 1.1, chronic infection by a variety of viruses, bacteria, or parasites and tissue inflammation such as gastritis and hepatitis, which are often caused by chronic infection, are recognized risk factors for human cancers at various sites. Furthermore, the chronic inflammation induced by chemical and physical agents such as tobacco smoke and asbestos is also associated with an increased risk of cancer. Thus, chronic bronchitis and emphysema lead to increased risks of lung cancer. Inhalation of asbestos causes chronic lung and pleural inflammation and increases the risk of mesothelioma. Gastroesophageal reflux disease and Barrett's esophagus, which are caused by abdominal obesity, gastroesophageal reflux, and cigarette smoking, induce chronic inflammation and increase the risk of esophageal adenocarcinoma. Autoimmune and inflammatory diseases of uncertain etiology are also associated with an increased risk of cancer. For example, inflammatory bowel diseases such as Crohn's disease and ulcerative colitis are associated with an increased risk of colon cancer. There is an increased risk of pancreatic cancer in chronic pancreatitis. Thus, a significant fraction of the global cancer burden is attributable to chronic infection and inflammation. It is estimated that there would be about 21% fewer cases of cancer in developing countries and 9% fewer cases in developed countries if these known infectious diseases were prevented (2).

TABLE 1.1 Infection and Inflammatory Conditions as Risk Factors for Human Cancers

Cancer site	Infection/inflammation
Breast	Inflammatory breast cancer
Cervix	Human papillomaviruses, herpes simplex virus
Esophagus	Barrett's esophagitis, gastroesophageal reflux
Gallbladder and extrahepatic biliary ducts	Stone/cholecystitis, *Salmonera typhimurium*
Kaposi's sarcoma	Human immunodeficiency viruses
Large intestine (colon/rectum)	Inflammatory bowel diseases, *Schistosomiasis japonicum*
Leukemia/lymphoma	Human T-cell leukemia virus, Epstein–Barr virus, malaria
Liver /intrahepatic biliary ducts	Hepatitis viruses B and C, cirrhosis, *Opistorchis viverrini, Clonorchis sinensis, Schistosomiasis japonicum*
Lung	Cigarette smoke, particles (asbestos, silica dust, nanomaterials, etc.)
Nasopharynx	Epstein–Barr virus
Oral cavity	Leukoplakia
Pancreas	Pancreatitis
Pleura (mesothelioma)	Asbestos
Prostate	Proliferative inflammatory atrophy
Skin	Ultraviolet radiation, sunburn, human papillomaviruses
Stomach	*Helicobacter pylori*, chronic atrophic gastritis, Epstein–Barr virus
Thyroid	Thyroiditis
Urinary bladder	Stones, bacterial infections, *Schistosomiasis haematobium*

INFECTION, INFLAMMATION, AND CANCER: POSSIBLE MECHANISMS

Although various mechanisms have been proposed for infection- and inflammation-associated carcinogenesis, at many sites carcinogenic mechanisms associated with infection and inflammation have not been fully elucidated. Both direct and indirect mechanisms may be involved in carcinogenesis associated with infection. Direct mechanisms include integration of viral DNA into the human genome, which often results in alterations of host DNA (insertion, deletion, translocation, and amplification). Products of integrated viral DNA (e.g., the X protein of hepatitis B virus and the E6 and E7 proteins of human papillomavirus) interact with tumor suppressor gene products such as pRB, p53, and Bax, inactivating these proteins in host cells (see Chapters 12 and 13). Viral products such as the E6 and E7 proteins of human papillomavirus may also immortalize infected cells (e.g., human genital keratinocytes) and interact with transcription factors of host genes (e.g., activation of c-myc by the X protein of hepatitis B virus), deregulating the cell cycle, or cell growth and death. In contrast, indirect mechanisms include inflammation-related cellular and genetic alterations and viral-infection-induced immunosuppression (e.g., human immunodeficiency virus), which can increase the risks of some types of malignancy (e.g., Kaposi's sarcoma). It is likely that both direct (integration of viral DNA into

host genome) and indirect (immunosuppression and inflammatory responses) mechanisms cooperate in various cases. This is evident because (1) many infectious agents associated with human cancer are ubiquitous and widely distributed, but only a small fraction of infected subjects develop cancer; (2) there is a long latency period between initial infection and cancer appearance; and (3) other lifestyle factors, such as smoking and dietary habits, are known to modify cancer risks associated with infection and inflammation.

PRODUCTION OF INFLAMMATORY MEDIATORS AND REACTIVE OXIDANTS

Inflammation is the normal physiological response to tissue injury. The cellular and tissue responses to injury can increase the blood supply and enhance vascular permeability and migration of white blood cells to damaged sites. Granulocytes, monocytes, and lymphocytes are recruited to the injured area and concomitantly produce soluble mediators such as acute-phase proteins, eicosanoids, interleukins, and cytokines. Cytokines can be divided into pro-inflammatory [interleukin (IL)-1, IL-6, IL-15, IL-17, and IL-23 and tumor necrosis factor (TNF)-α] and anti-inflammatory [IL-4, IL-10, IL-13, transforming growth factor (TGF)-β, and interferon (IFN)-α]. Pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 play an important role in inflammation and cancer development. These cytokines activate various transcription factors, such as nuclear factor (NF)-κB and signal transducer and activator of transcription (STAT) 3, which promote cell growth, suppress apoptotic cell death, and stimulate production of growth factors, cytokines, and a variety of oxidant-generating enzymes. Furthermore, these pro-inflammatory cytokines also activate multiple oncogenic pathways, such as the mitogen-activated protein kinase (MAPK) cascade, an important signaling pathway involved in various processes of carcinogenesis, such as cell proliferation and migration, and angiogenesis. Similarly, infection and inflammation activate multiple oncogenic pathways, such as the PI3K/AKT/GSK3β/STAT3 and β-catenin pathways (3) and induce aberrant expression of activation-induced cytidine deaminase (AID) (see Chapter 9)—all of which are important in promoting carcinogenesis. Upon activation, AID can deaminate cytosine to produce uracil in DNA and thus can induce C:G-to-T:A transition and other types of mutations. The enzyme cyclooxygenase-2, which plays pivotal roles in the progression of a variety of cancers through prostaglandin synthesis, can be induced by NF-κB and can be activated by excess nitric oxide (NO), produced by inducible NO synthase (iNOS) in inflamed tissues.

NF-κB activation by pro-inflammatory cytokines stimulates various inflammatory cells (e.g., macrophages, neutrophils, basophils, eosinophils) to produce potent reactive oxygen species (ROSs) and reactive nitrogen species (RNSs), primarily to attack and destroy invading microorganisms and foreign bodies. However, if foreign agents are not eliminated rapidly, inflammation becomes chronic, which often causes extensive tissue damage, due to continuous production of excessive active proteolytic enzymes, inflammatory mediators, and potent oxidants.

During infection or inflammation, various oxidant-generating enzymes are activated, including NADPH oxidase and xanthine oxidase, which produce a superoxide anion, iNOS, which produces excess NO, and peroxidases such as myeloperoxidase and eosinophil peroxidase, which generate hypochlorous acid (HOCl) and hypobromous acid (HOBr), respectively. Furthermore, these oxidants can react with lipids to generate lipid peroxidation products, which may react further with nucleobases in DNA and RNA and amino acid residues in proteins to form adducts (see Chapters 5 and 6).

DNA AND PROTEIN DAMAGE BY ROSs AND RNSs

The oxidants can cause oxidative damage to nucleobases and sugar moieties of DNA and RNA. Many different products resulting from oxidative DNA damage have been identified. The best studied nucleobase modification includes 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), thymidine glycol, and 5-hydroxymethyl-2′-deoxyuridine. Recent advances in mass spectrometric analysis of DNA damage present in inflamed tissues have allowed us to detect numerous DNA adducts in human tissues (see Chapter 6). Especially significant increases in nucleobase modifications induced by inflammatory oxidants have been observed, including 5-chlorocytosine (halogenation damage by HOCl) in both DNA and RNA, and increased deamination product hypoxanthine in DNA in the colon of *Helicobacter hepaticus*–infected mice, an animal model for inflammatory bowel diseases and colon carcinogenesis (4). RNSs such as dinitrogen trioxide (N_2O_3), formed by oxidation of NO, can deaminate nucleobases (e.g., adenine to hypoxanthine, guanine to xanthine and cytosine to uracil, 5-methylcytosine to thymine). NO also reacts with superoxide anion to form a highly reactive nitrating and oxidizing species, peroxynitrite anion ($ONOO^-$), which can damage DNA and RNA to induce DNA strand breaks and form oxidation and nitration products of nucleobases such as 8-oxodG and 8-nitroguanine (see Chapter 4). Thus, oxidative and nitrative damage can lead to point and frameshift mutations, to single- and double-stranded breaks, and to chromosome abnormalities.

ROSs and RNSs react with proteins to modify amino acid residues by oxidation, nitrosation, nitration, and halogenation. During aging, oxidative stress, and some pathological conditions, modified forms of proteins accumulate, resulting in alterations of protein structure and function. Tyrosine residues in proteins are modified by various RNSs to form 3-nitrotyrosine and react with ROSs such as HOCl and HOBr to form 3-chloro- or 3-bromotyrosines. Thiols, metals, and radical residues in protein are also modified by ROSs and RNSs. For example, the p53 tumor suppressor protein may be inactivated with excess RNSs through formation of disulfide bonds via S-nitrosation (5) and/or nitration of tyrosine residues (6). Similarly, various DNA repair enzymes such as 8-oxoguanine DNA glycosylase 1 (7) and O^6-methylguanine transferase (8) are inactivated by ROSs and RNSs, which may increase further mutations induced by ROSs and RNSs. Caspases and other pro-apoptotic enzymes have been reported to be inhibited by ROSs and RNSs, resulting in prevention of apoptotic cell death (9).

In contrast, NO and/or other reactive species are capable of activating the proto-oncogene *c-Ha-ras* product p21 protein via S-nitrosation (10). The activated

p21 protein leads to an escape of transformed cells from cell-cycle control, rendering them independent to stimulation by growth factors, giving them almost unlimited proliferative capacity. ROSs and RNSs can also activate other enzymes, such as telomerase, by whose action cells acquire replicative potential (11), and metalloproteases, which facilitate invasion by cancer cells surrounding tissues (12).

INFLAMMATION, EPIGENETIC MODIFICATION, AND microRNA

Epigenetic modifications are DNA-associated modifications that are inherited upon somatic cell replication, which include DNA methylation and histone modifications (13,14). Cytosine methylation in promoter CpG islands is associated with gene silencing. In cancer cells, the presence of regional hypermethylation (aberrant DNA methylation) and global hypomethylation has been reported. In particular, many tumor suppressor genes that have promoter CpG islands [e.g., *CDKN2A*, mutL homolog (*MLH1*), and cadherin-1] are hypermethylated and thus silenced permanently. It has been hypothesized that inflammatory signals mainly from macrophages, such as IL-1β and IL-6 and oxidative stress, possibly produced by iNOS, probably recruit a complex with DNA methyltrasferase (DNMT) 1 and histone methyltransferase (EZH2) to promoter CpG islands, which aberrantly methylate DNA at scattered CpG sites within a CpG island (14). If a promoter CpG island of a tumor suppressor gene is hypermethylated and silenced, such methylation promotes carcinogenesis (13).

Similarly, microRNAs (miRNAs) play an important role in inflammation and cancer (see Chapter 10). miRNAs are small, noncoding RNAs that regulate the translation of specific genes by base pairing with target RNAs. Inflammation signals lead to altered miRNAs expression, which may contribute to inflammation and cancer. During inflammation, miRNA expression in epithelial cells can be altered through various mechanisms, such as activations of NF-κB and/or activator protein-1 or stimulation with cytokines. For example, increased levels of *miR-21*, which targets a number of tumor suppressor genes, such as programmed cell death 4, are found in several chronic inflammatory diseases (e.g., ulcerative colitis), and various cancers and inflammatory stimuli can increase the expression of *miR-21* (15). In contrast, the expression of *miR-7*, which targets epidermal growth factor receptor (*Egfr*) and other signaling pathways and thus acts as a tumor suppressor, is inhibited by activated macrophages in *Helicobacter*-infected gastritis (16). Upon activation with a variety of inflammatory stimuli, the aberrant expression of many different miRNAs (e.g., *let-7*, *miR-9*, *miR-98*, *miR-214*) has been shown to occur (15).

CONCLUDING REMARKS

Inflammation facilitates the initiation of normal cells and their growth and progression to malignancy. Possible mechanisms include production of pro-inflammatory cytokines and oxidants, such as ROSs and RNSs, activation of signaling and oncogenic pathways associated with inflammation and carcinogenesis, and inactivation of

TABLE 1.2   Roles of Infection and Inflammation in Various Stages of Carcinogenesis

DNA damage or mutation by ROSs and RNSs	
Inhibition of antioxidant enzymes	
Inhibition of DNA repair enzymes	
Inhibition of apoptosis	(Evading apoptosis)[a]
Inactivation of tumor-suppressor functions	(Insensitivity to antigrowth signals)
Activation of oncogenic pathways	(Self-sufficiency in growth signals)
Production of growth factors	(Self-sufficiency in growth signals)
Telomerase activation	(Limitless replicative potential)
Increased vascular permeability	
Angiogenesis induction	(Sustained angiogenesis)
Metalloproteinase activation	(Tissue invasion)
Subversion of host immune system	

[a]Six hallmark capabilities necessary for carcinogenesis proposed by Hanahan and Weinberg (17,18) are shown in parentheses.

tumor-suppressor genes and their products, through genetic alterations and epigenetic mechanisms such as aberrant DNA methylation, aberrant expression of miRNAs, and post-translational modifications of gene products. Hanahan and Weinberg (17,18) recently proposed six major characteristics (self-sufficiency in growth signals, insensitivity to antigrowth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis) that are required for a normal cell to become a tumor cell. These pathways could be disrupted by genetic alterations in genes involved in carcinogenesis (oncogenes and tumor suppressor genes) or by epigenetic processes (e.g., gene methylation; miRNAs; post-translational modifications of proteins, including histones; DNA repair enzymes), and modification of the gene expression pattern. Diverse pro-inflammatory cytokines, ROSs, and RNSs are generated in inflamed tissues and can cause genetic and epigenetic changes, affecting the six major characteristics noted above (Table 1.2).

Better understanding of the molecular mechanisms by which chronic infection and inflammation increases cancer risks will lead to the development of new strategies for cancer prevention at various sites (19). In the following chapters, cancers associated with infection and inflammation are reviewed comprehensively, and possibilities for cancer prevention by modulating inflammatory processes are discussed.

REFERENCES

1. de Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, et al. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol 2012;13:607–615.

2. Pisani P, Parkin DM, Muñoz N, Ferlay J. Cancer and infection: estimates of the attributable fraction in 1990. Cancer Epidemiol Biomark Prev 1997;6:387–400.

3. Ding SZ, Goldberg JB, Hatakeyama M. *Helicobacter pylori* infection, oncogenic pathways and epigenetic mechanisms in gastric carcinogenesis. Future Oncol. 2010;6:851–862.

4. Mangerich A, Knutson CG, Parry NM, Muthupalani S, Ye W, Prestwich E, et al. Infection-induced colitis in mice causes dynamic and tissue-specific changes in stress response and DNA damage leading to colon cancer. Proc Natl Acad Sci USA 2012;109:E1820–E1829.

5. Calmels S, Hainaut P, Ohshima H. Nitric oxide induces conformational and functional modifications of wild-type p53 tumor suppressor protein. Cancer Res 1997;57:3365–3369.

6. Chazotte-Aubert L, Hainaut P, Ohshima H. Nitric oxide nitrates tyrosine residues of tumor-suppressor p53 protein in MCF-7 cells. Biochem Biophys Res Commun 2000;267:609–613.

7. Jaiswal M, LaRusso NF, Nishioka N, Nakabeppu Y, Gores GJ. Human Ogg1, a protein involved in the repair of 8-oxoguanine, is inhibited by nitric oxide. Cancer Res 2001;61:6388–6393.

8. Liu L, Xu-Welliver M, Kanugula S, Pegg AE. Inactivation and degradation of *O*(6)-alkylguanine-DNA alkyltransferase after reaction with nitric oxide. Cancer Res 2002;62:3037–3043.

9. Cauwels A, Brouckaert P. Survival of TNF toxicity: dependence on caspases and NO. Arch Biochem Biophys 2007;462:132–139.

10. Lander HM, Hajjar DP, Hempstead BL, Mirza UA, Chait BT, Campbell S, et al. A molecular redox switch on p21(ras): structural basis for the nitric oxide–p21(ras) interaction. J Biol Chem 1997;272:4323–4326.

11. Vasa M, Breitschopf K, Zeiher AM, Dimmeler S. Nitric oxide activates telomerase and delays endothelial cell senescence. Circ Res 2000;87:540–542.

12. Kar S, Subbaram S, Carrico PM, Melendez JA. Redox-control of matrix metalloproteinase-1: a critical link between free radicals, matrix remodeling and degenerative disease. Respir Physiol Neurobiol 2010;174:299–306.

13. Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007;128:683–692.

14. Chiba T, Marusawa H, Ushijima T. Inflammation-associated cancer development in digestive organs: mechanisms and roles for genetic and epigenetic modulation. Gastroenterology 2012;143:550–563.

15. Schetter AJ, Heegaard NH, Harris CC. Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. Carcinogenesis 2010;31:37–49.

16. Kong D, Piao YS, Yamashita S, Oshima H, Oguma K, Fushida S et al. Inflammation-induced repression of tumor suppressor miR-7 in gastric tumor cells. Oncogene 2012;31:3949–3960.

17. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.

18. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–674.

19. Coussens LM, Zitvogel L, Palucka AK. Neutralizing tumor-promoting chronic inflammation: A magic bullet? Science 2013;339:286–291.