Review

NF-κB Signaling Pathway, Inflammation and Colorectal Cancer

Soly Wang[1, 2], Zhanjie Liu[1], Lunshan Wang[1] and Xiaoren Zhang[1, 3]

There is growing evidence for a connection between inflammation and tumor development, and the nuclear factor kappa B (NF-κB), a proinflammatory transcription factor, is hypothesized to promote tumorigenesis. Although the genetic evidence for the hypothesis has been lacking, recent papers have lent credence to this hypothesis. It has been reported that constitutive NF-κB activation in inflammatory bowel diseases (IBDs) increases risk of colorectal cancer (CRC) in the patients with the number of years of active disease. NF-κB activation might induce cellular transformation, mediate cellular proliferation, prevent the elimination of pre-neoplastic and fully malignant cells by up-regulating the anti-apoptosis proteins. Furthermore, NF-κB may contribute to the progression of CRC by regulating the expression of diverse target genes that are involved in cell proliferation (Cyclin D1), angiogenesis (VEGF, IL-8, COX2), and metastasis (MMP9). These findings implicate NF-κB inhibition as an important therapeutic target in CRC. However, due to lack of knowledge about the specific roles of different NF-κB subunits in different stage of carcinogenesis, and compounds to block specific subunits of NF-κB family, it will be a long time before the coming of targeting NF-κB in CRC therapy. *Cellular & Molecular Immunology*. 2009;6(5):327-334.

Key Words: NF-κB, inflammation, colorectal cancer

Introduction

Epidemiological studies revealed that chronic inflammation predisposes individuals to different cancers. The triggers of chronic inflammation that increase cancer risk include microbial infections (i.e. Helicobacter pylori for gastric cancer), autoimmune diseases (i.e. inflammatory bowel disease for colon cancer), and cryptogenic inflammatory conditions (i.e. ulcerative colitis for colorectal cancer). Accordingly, administration of non-steroidal anti-inflammatory drugs (NSAIDs) decreases the incidence of several tumors (1, 2). In recent years, though studies have revealed the connection between inflammation and carcinogenesis (3-6), the mechanistic links between the two is just beginning to emerge.

Genetic approaches have proven the role of key components of inflammation such as hematopoietic growth factors (MCSF), primary inflammatory cytokines (IL-1, TNF), IL-6, and the nuclear factor-κB (NF-κB) family members (7-9) in carcinogenesis. NF-κB has been shown to be activated in all cells, where it regulates expression of diverse target genes that promote cell proliferation, regulate immune and inflammatory response, and contribute to pathogenesis of various diseases, including cancer (10-12). This review will focus on new data that have emerged over the last couple of years implicating NF-κB signaling pathways in carcinogneneisis of colorectal cancer (CRC), the one of the most common fatal malignancies worldwide (13).

The NF-κB family and signaling pathway

NF-κB signaling pathway is a complex network that regulates a cellular pathway involved in a myriad of physiological and pathological scenarios. The detailed description of NF-κB structure, function and regulation is beyond the scope of this review (see recent review (14-16)). Briefly, five homologous subunits have been identified in mammals: RelA/p65, c-Rel, RelB, NF-κB1/p50, and NF-κB2/p52. p50 and p52 are produced by processing of their precursors NF-κB1 p105 and NF-κB2 p100, respectively. All of the NF-κB family members contain their Rel homology domain (RHD), which is required for DNA binding, homo- and heterodimerization, nuclear localization and inhibitor (IκB) binding (17, 18). NF-κB signaling

[1]Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences/Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China;

[2]Department of Pathology, Soochow University School of Medicine, Suzhou 215123, China;

[3]Correspondence to: Dr Xiaoren Zhang, Key Laboratory of Stem Cell Biology, Laboratory of Immunoregulation and Tolerance, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China. Fax: +86-21- 5492-0601, E-mail: Zhangxr68@yahoo.com

Received Sep 25, 2009. Accepted Oct 10, 2009.

pathway is regulated by IκB family. There are seven IκB family members - IκBα, IκBβ, IκBγ, IκBε, Bcl-3 and the precursor proteins p100 and p105. IκBs bind to NF-κB dimers and block their nuclear localization, thereby causing their cytoplasmic retention. Most stimuli activate NF-κB through IκB kinase-dependent (IKK-dependent) phosphorylation, polyubiquitination and subsequent proteasomal degradation of IκB proteins. The liberation of NF-κB allows translocation of heterodimer to the nucleus, where it can regulate the expression of specific genes typically involved in immune and inflammatory responses and in cell growth control (17, 19).

Many divergent stimuli activate two NF-κB pathways (29) (Figure 1). The activation events that modulate these pathways are mediated by a family of inhibitory kappa B kineses (IKKs); which consists of three core subunits, the catalytic subunits IKKα and IKKβ and several copies of a regulatory subunit IKKγ. The first, known as the classical or canonical pathway depends on IKKγ, IKKβ activation, phosphorylates mainly IκBα but also IκBβ and IκBε, which are present in the cytoplasm of unstimulated cells and undergo stimulus-induced degradation and resynthesis (20, 21). Following stimulation with the various inflammatory stimuli, including certain members of tumor necrosis factor α (TNF-α) cytokine family, IL-1, Toll-like receptor ligands (22-25), the IκB molecules are phosphorylated by the IKK complex, which leads to their degradation by the proteasome pathway (26). This pathway mostly targets p50: RelA and p50: c-Rel dimers, which translocate to the nucleus and activate the transcription of various target genes (18, 27). The activation of canonical pathway is essential for innate immunity and responsible for the inhibition of apoptosis under most conditions in response to infections or exposure to proinflammatory cytokines (28, 29). The non-canonical or alternative pathway is activated by a subset of TNF receptor superfamily members including those for BAFF, CD40

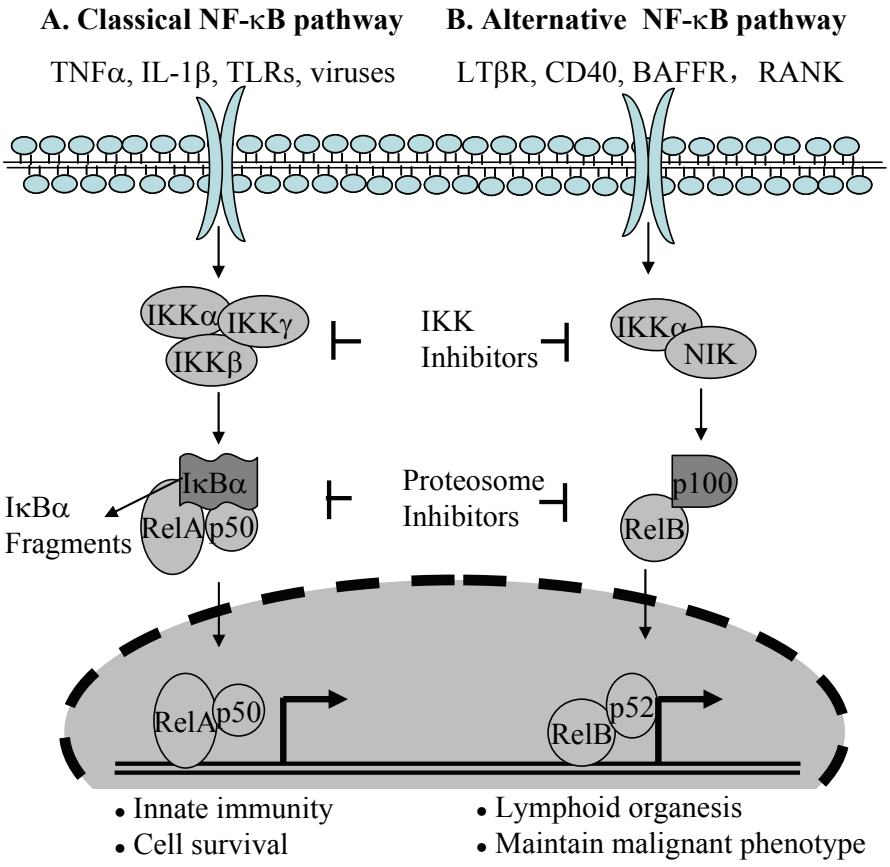

Figure 1. NF-κB signaling pathways. (A) The classical NF-κB pathway activation occurs in response to TNF-α, IL-1β, TLRs, or viruses. Intermediated kinases convey signals to the IκB complex formed by IκBα, IκBβ, IκBγ, and proteasome degradation. P105/RelA is processed to p50/RelA, which translate to the nucleus and bind promoters of genes regulating innate immunity and cell survival. (B) The alternative pathway is activated through IKKα by lymphotoxinβ receptor (LTβR), CD40 ligand (CD40L), and other TNF family members (BAFF), which activate P100/RelB for processing into p52/RelB heterodimers. The p52/RelB heterodimers then translates into the nucleus to bind the promoter of genes regulating lymphoid organesis and maintain the malignant phenotype in some cancers. Inhibitors of NF-κB pathway activation under clinical and preclinical investigation include IKK and proteasome antagonists.

ligand, receptor-activated NF-κB ligand (RANKL), and lymphotoxin β through another proteasome-dependent mechanism, dependent on IKKα and upstream kinase (NIK), thereby leading to the proteasome-dependent processing of the RelB inhibitor NF-κB2 p100, resulting in RelB-p50 nuclear translocation and DNA binding (22). The p52/RelB dimers have higher affinity for distinct κB elements (29). This pathway plays a crucial role in controlling the development and function of secondary lymphoid organs (29, 32).

The activation of both pathways relies on the inducible phosphorylation of IκB inhibitory proteins (IκBs and p100) by the IKK complex. Both pathways regulate cell survival and death (33), and now have been implicated in carcinogenesis (34, 35). In addition to the classical IKK-mediated NF-κB activation, it has been shown that certain novel pathway can activate NF-κB by IKK-independent such as UV and hydrogen peroxide (H_2O_2) induced NF-κB activation (36-38).

NF-κB links inflammation to cancer

A causal relation between inflammation and cancer has been proposed by Virchow, who hypothesized that malignant neoplasm arise at regions of chronic inflammation, reasoned that various "irritants" caused tissue injury, inflammation, and increased cell proliferation (39, 40). It has been estimated that more than 15% of all malignancies are initiated by chronic inflammatory disease (40-45). In Table 1, an outline is given of inflammation that has been associated with cancer development: skin inflammation with squamous cell carcinoma, viral hepatitis with liver cancer and inflammatory bowel disease with colorectal cancer. Although the exact mechanism of inflammation initiating neoplasm is still not completely understood, the NF-κB could well be an important player in this process since it is activated in chronic inflammation and its constitutive activation in cancers as mentioned follow.

Populations in developing countries are susceptible to cancers caused by infectious agents. The organs and cancer associated pathogens include liver (hepatitis B virus (HBV) and hepatitis C virus (HCV)), biliary tract (*Opisthorchis viverrini*, *Clonorchis sinensis*), stomach (*Helicobacter pylori* (*H pylori*)), uterine cervix (human papillomavirus (HPV)), nasopharynx (Epstein-Barr virus (EBV)), and urinary bladder (*Schistosoma hematobium*). The proportion of cancer deaths attributable to these infectious agents has been estimated to range from 20% to 25% in developing countries and 7% to 10% in more industrialized countries (46). The first evidence implicating NF-κB activation mediated pathogen-induced carcinogenesis was identification of the REV-T viral oncogene that causes avian reticuloendothelial lymphomatosis, v-Rel, which shares a Rel transactivation domain with the mammalian homologues NF- family members (47). Hepatitis B X protein and hepatitis C 5A and core proteins have been shown to activate NF-κB and are implicated in carcinogenesis of hepatocellular carcinoma (48). In addition, the specific subtypes of human papillomavirus (HPV) infection are potentially oncogenic in the uterine cervix, vagina, vulva, anus, penis, skin, and oropharynx (49, 50). HPV genes E6 and E7, the main transforming genes of oncogenic strains, have been implicated in NF-κB activation by inactivating p53 and Rb tumor suppressor genes. Besides, some chemical and physical carcinogens implicated in initiation and/or promotion of human cancer can also activate

Table 1. Chronic inflammation and associated cancers

Chronic inflammation	Associated neoplasm
Warts	Non melanoma skin carcinoma
Osteomyelitis	Skin carcinoma in draining sinuses
Sunburned skin, skin inflammation	Melanoma, basal-cell carcinoma, squamous cell carcinoma
Gingivitis, lichen planus	Oral squamous cell carcinoma
Lichen sclerosus	Vulvar squamous cell carcinoma
Sialadenitis	Salivary gland carcinoma
Gastroesophageal reflux	Esophageal carcinoma
Chronic bronchitis	Lung carcinoma
Asbestosis, silicosis	Mesothelioma, Lung carcinoma
Gastritis	Adenocarcinoma of stomach
Hepatitis	Hepatocellular carcinoma
Ulcerative colitis, inflammatory bowel disease, Crohn's disease	Colorectal cancer
Opisthorchis, cholangitis	Cholangiosarcoma, colon carcinoma
Prostatic inflammatory atrophy	Prostate cancer
Pelvic inflammatory disease, chronic cervicitis	Ovarian carcinoma, cervical/anal carcinoma
Chronic cystitis, bladder inflammation	Bladder, liver, rectal carcinoma
Chronic cholecystitis	Gall bladder cancer
Mononucleosis	B-cell non-Hodgkin's lymphoma, Burkitt's lymphoma, Hodgkin's disease
AIDS	Non-Hodgkin's lymphoma, Kaposi's sarcoma

NF-κB. Especially, nicotine and carcinogens in tobacco, which are linked to pathogenesis of head and neck and lung malignancies, induce AKT and NF-κB, promote cell proliferation, survival, and inflammation (51, 52). Recently, talc use in women has also been implicated in promotion of ovarian cancer (53), but the mechanism needs to be further explored. Taken together, NF-κB seems to have some functions that are necessary for links inflammation with cancer.

Role of NF-κB in colorectal cancer

Chronic inflammation and colorectal cancer

Ulcerative colitis (UC) and Crohn's disease (CD) are the two major forms of inflammatory bowel diseases (IBDs). IBD primarily represents an inflammatory disorder affecting the intestine, resulting in diarrhoea, anaemia and fatigue, associated with increased risk of neoplasia experienced by patients with chronic inflammation (54). Underlying mechanisms have been the focus of a limited set of studies to date. In IBDs, long term inflammation is thought to sustain cancer initiation by the continuous release of reactive oxygen species (ROS), which are thought to ultimately cause DNA damage and the dysplastic degeneration of the repairing epithelium (55).

The inflammatory site is characterized by the release of cytokines, growth factors, proteases and ROS, which regulate the sequential recruitment of leukocytes and stimulate endothelial cells and fibroblast to divide and produce components for tissue remodeling. It has been proposed that noxious compounds released during chronic inflammation damage DNA and/or alter cell survival, results in carcinogenesis of CRC (56). NSAIDs have been shown to reduce adenoma and colon cancer development in patients with IBD and hereditary colon cancer (57, 58). In addition, COX2 inhibitors have been shown to prevent the recurrence of both adenomas and sporadic adenomatous plyps in patients with predisposition (59, 60). However, there are growing evidence that cytokines released during inflammation may contribute to cancer development, and those acting *via* the activation of NF-κB (Figure 2) (40, 61) .

The role of NF-κB in promoting carcinogenesis of colorectal cancer

In chronic inflammation, the cytokines and chemokines produced by inflammatory cells, propagate a localized inflammatory response and also enhance the survival of premalignant cells by activating NF-κB (62). Activation of the IKK/NF-κB pathway is one key survival mechanism in a variety of cancer types (63-66). Numerous studies have indicated that NF-κB can block apoptosis by regulating the anti-apoptosis proteins such as inhibitor of apoptotic proteins (IAPs) (67). Another mechanism whereby NF-κB may inhibit apoptosis is through its ability to inhibit prolonged c-Jun N-terminal kinases (JNK) activation and accumulation of reactive oxygen species (ROS) (10).

NF-κB proteins were detected in the epithelial cells and macrophages from patients with UC, CD and unspecific colitis thus providing evidence for constitutive NF-κB activation (68-70), and these patients have been associated with an increased risk of developing colitis-associated cancer (54). The proinflammatory stimuli such as bacterial products *via* Toll-like receptors or cytokines (e.g. TNF-α and IL-1α) induce NF-κB activation in different cell types and the block of the NF-κB signaling has been shown to suppress experimental colitis (68). On the other hand, activated NF-κB translocates into the nucleus inducing the expression of cytokines such as TNF-α and IL-6, and chemokines, all contributing to the inflammation-related tissue damage. This elevated IKK/NF-κB activity may lead to aberrant upregulation of certain tumorigenic, adhesion proteins, chemokines, and inhibitors of apoptosis that promote cell survival (63, 64). Therefore, NF-κB may contribute to the development of colitis associated colorectal cancer by sustaining the ongoing inflammatory process in the gut mucosa. Inactivation of the IκB kinase/NF-κB pathway at the site of inflammation has been shown to attenuate the formation of inflammation-associated tumors (7). The experimental data obtained in animal model of colitis associated colorectal cancer, inhibition of interleukin 6 (IL-6) trans-signaling dramatically reduced the number and size of the colon cancer (71). The IL-6 is a multifunctional NF-κB-regulated cytokines that acts on epithelial and immune cells, and regulate preneoplastic growth during CRC tumorigenesis (43).

Activation of NF-κB in epithelial cells is directly involved in the promotion and progression steps of colitis associated colorectal cancer. To investigate the role of NF-κB in CRC development, conditional knockout mice lacking of the expression of IKKβ in the epithelial cells in AOM/DSS colitis associated CRC model was used by Greten et al. (72), who demonstrated that the number of colon tumors reduced (but not tumor size), although inflammation was not reduced comparing wild type with knockout mice. They also showed that the decrease in tumor incidence was accompanied by increased epithelial apoptosis. These data indicated that NF-κB activation is involved in the promotion phase of CRC development.

The role of NF-κB in facilitating colorectal cancer maintenance and invasion

NF-κB has been shown to regulate many genes differently expressed and implicated in cell proliferation, tumorigenesis, and metastasis in cancer. Cyclin D1 and cMyc, two NF-κB target genes have an important role in cell growth and proliferation (73, 74), and key angiogenesis factors such as vascular endothelial growth factor (VEGF) and interleukin-8 (64), are directly or indirectly enhanced by NF-κB activation. Cancer growth inhibition *via* NF-κB inhibition has been shown in colon (75), and the similar results has been shown in other several flavors of human neoplasia such as lung (76) and breast (77). To investigate the role of NF-κB in inflammation-induced tumor growth, one study of experimental murine colon cancer metastasis model revealed

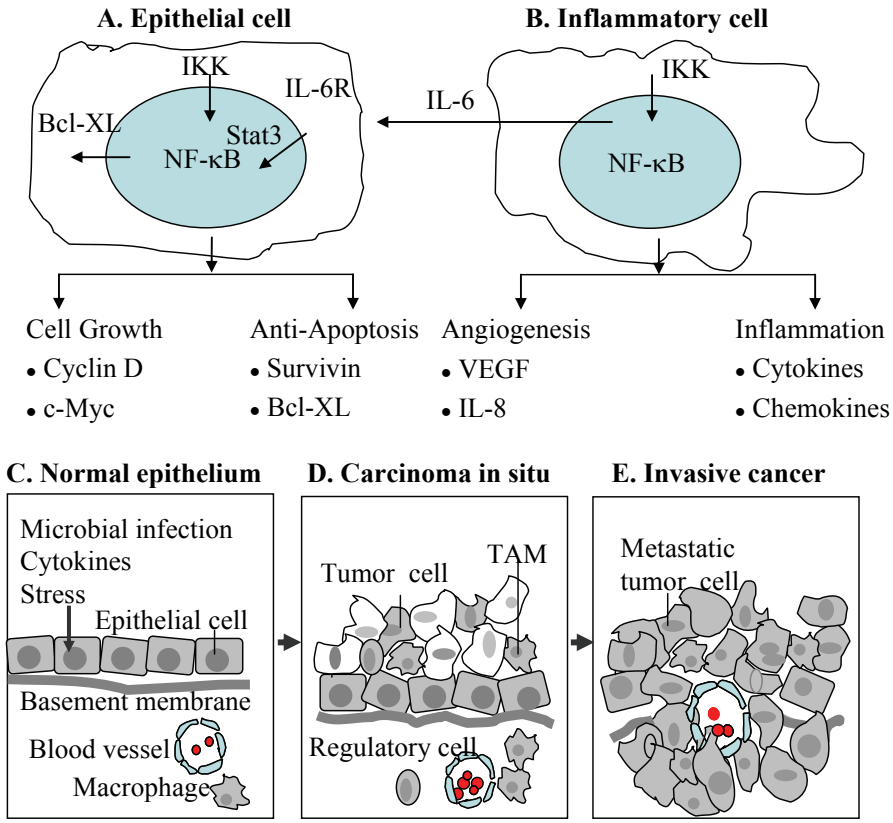

Figure 2. Inflammation and progression of CRC. NF-κB promotes the development of CRC by acting in two different cells. (A) NF-κB activation in intestine epithelial cells (IEC) results in proliferation of IEC by upregulating survival gene such as CyclinD and Bcl-XL. (B) Activation of NF-κB in inflammatory cells also contributes to CRC development by inducing expression of angiogenic factors, chemokines and epithelial cell growth factors, such as IL-6. (C) Normal epithelium. Chronic inflammation in the IEC resulted from microbial infection, cytokines, and stress, can lead to gene mutation of IEC. (D) Carcinoma in situ. Activation of NF-κB induced production of chemokines and cytokines, which attract tumor associated macrophages (TAM) and regulatory T cells. The chemkines receptor can be induced on initialted cells, and be necessary for tumor cell proliferation and invasion. (E) Invasive cancer. The chemokines and cytokines mediated signaling promotes expression of genes associated with invasion and metastasis.

that lipopolysaccharide (LPS)-induced metastatic growth response depends on both TNF-α production by host hematopoietic cells and NF-κB activation in tumor cells. Inhibition of NF-κB in both colon and mammary carcinoma cells converts the LPS-induced growth response to LPS-induced tumor regression (78). This study confirmed that NF-κB-induced inflammation is directly linked to growth stimulation of malignant cells. A further mechanism whereby NF-κB might be involved is through binding to the promoter of Cyclin D1 and thus stimulating gene transcription (79).

Formation of new blood vessels is essential for tumor progression, and has been shown to be dependent on growth factors (e.g., TNF, VEGF) and chemokines (e.g., monocyte chemoattractant protein-1, IL-8), which are upregulated by NF-κB signaling (80, 81). Cyclooxygenase 2 (COX2), a protein involved in inflammation, is increased in more aggressive forms of colorectal cancer, and is known to promote angiogenesis. It can be induced by NF-κB (82). SC-514, an inhibitor of IKKβ, blocks the TNF-α-induced activation of NF-κB as well as the expression of matrix metalloprotease-9 (MMP-9), migration and invasion of murine colon adenocarcinoma cells (83).

To investigate the role of NF-κB activation in tumorigenesis in a mouse model of CAC, the AOM/DSS model, tumor development was investigated in mice lacking IKK-β expression restricted to myeloid cells or intestinal epithelial cells. Accordingly, these mice exhibited decline in tumor load, most of tumors presented in these mice were smaller in size compared to tumors in WT mice (72), which indicates that the inflammation-related release of cytokines might target epithelium thus promoting the expansion of transformed cells in an NF-κB dependent manner, the expression of most of cytokines and growth factors characterizing the inflammatory process and involved in tumor progression relies on NF-κB activation in immune cells (84). Taken together, NF-κB seems to have a variety of functions that are required for CRC promotion and CRC

maintenance.

NF-κB inhibition: strategy for colorectal cancer therapy

The pivotal role of the NF-κB signaling pathway in apoptosis, tumor promotion and tumor maintenance, strongly suggests that NF-κB inhibitors would be useful in cancer therapy, and much efforts is currently invested in developing NF-κB inhibitors in cancers (10, 14, 85, 86). Several agents have been implemented to inhibit IKK/NF-κB activity, because of the relevance of this process in cancer and those related with inflammation, such as curcumin, ginseng extract, resveratrol, green tea extract have anti-inflammatory and anti-proliferative propertiese (87). Proteosome inhibitors regulates the degradation of IκB and hence inhibits NFκB, and a few publications have documented their efficacy in triggering apoptosis in cancer cell lines in combination with either chemotherapeiutic drugs or death-inducing cytokines (88-90). Sulfasalazine and methotrexate are widely used for treatment of acute states of IBD by inhibiting NF-κB activation (91, 92), thus, may also decrease the risk of developing cancer. NSAIDs and aspirin reduce the risk of gastric cancer in human and the incidence of colorectal cancer in animal models (93, 94).

Concluding remarks

Colorectal cancer represents a life-threatening complication of inflammatory bowel diseases, and NF-κB was natural suspects in providing a mechanistic link between inflammation and carcinogenesis. The molecular mechanisms underling this process have only recently started to be clarified with biochemical and genetic studies (95). The results of Pikarsky et al. (8) , Greten et al. (72) , and Luo et al. (78) identified the NF-κB signaling pathway could be regarded as a rational target for cancer therapy, and there is significant enthusiasm from cell culture experiments and animal models for the use of NF-κB inhibitors as a new anti-cancer therapy in recent years (14, 96, 97). However, several questions remain. First, the biggest question is whether this type of approach will be of general applicability. For example, IKKβ inhibitors more specifically inhibit IκB phosphorylation and degradation, and subsequent classical NF-κB signaling pathway that is initially implicated in colorectal cancer (10, 98), but only partly inhibit NF-κB activation, and do not appear functional in neck and head cancers (99). Therefore, the specific roles of different NF-κB subunits in different cancer types, especially in biological behavior (such as survival, proliferation, angiogenesis, differentiation, and metastasis) of malignancies, need to be explored further. The connection between inflammation and cancer is now generally accepted, but the question is that whether anti-inflammation is the best target for cancer therapy. An ideal NF-κB inhibitor should prevent NF-κB activation without any apparent effects on other signaling pathway and should avoid long-term immunosuppression.

Acknowledgements

This study was supported in part by a grant from the Jiangsu Provincial Natural Science Foundation (No. 2006540), Suchow University Young Natural Science Foundation and Key Project of Chinese Academy of Science (KSCX1-YW-22-04).

References

1. Flossmann E, Rothwell PM. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet. 2007;369:1603-1613.
2. Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. N Engl J Med. 2007;356:2131-2142.
3. Mantovani A. Cancer: inflammation by remote control. Nature. 2005;435:752-753.
4. Baniyash M. Chronic inflammation, immunosuppression and cancer: new insights and outlook. Semin Cancer Biol. 2006; 16:80-88.
5. Hagemann T, Balkwill F, Lawrence T. Inflammation and cancer: a double-edged sword. Cancer Cell. 2007;12:300-301.
6. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454:436-444.
7. Greten FR, Eckmann L, Greten TF, et al. IKKβ links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell. 2004;118:285-296.
8. Pikarsky E, Porat RM, Stein I, et al. NF-κB functions as a tumour promoter in inflammation-associated cancer. Nature. 2004;431:461-466.
9. Karin M. Nuclear factor-κB in cancer development and progression. Nature. 2006;441:431-436.
10. Luo JL, Kamata H, Karin M. IKK/NF-κB signaling: balancing life and death--a new approach to cancer therapy. J Clin Invest. 2005;115:2625-2632.
11. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-κB activity. Annu Rev Immunol. 2000;18: 621-663.
12. Hayden MS, Ghosh S. Signaling to NF-κB. Genes Dev. 2004;18:2195-2224.
13. Weir HK, Thun MJ, Hankey BF, et al. Annual report to the nation on the status of cancer, 1975-2000, featuring the uses of surveillance data for cancer prevention and control. J Natl Cancer Inst. 2003;95:1276-1299.
14. Baud V, Karin M. Is NF-κB a good target for cancer therapy? Hopes and pitfalls. Nat Rev Drug Discov. 2009;8:33-40.
15. Lee CH, Jeon YT, Kim SH, Song YS. NF-κB as a potential molecular target for cancer therapy. Biofactors. 2007;29:19-35.
16. Ghosh S, Hayden MS. New regulators of NF-κB in inflammation. Nat Rev Immunol. 2008;8:837-848.
17. Baldwin AS, Jr. The NF-κB and IκB proteins: new discoveries and insights. Annu Rev Immunol. 1996;14:649-683.
18. Ghosh S, Karin M. Missing pieces in the NF-κB puzzle. Cell. 2002;109 Suppl:S81-96.
19. Ghosh S, May MJ, Kopp EB. NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol. 1998;16:225-260.

20. Senftleben U, Li ZW, Baud V, Karin M. IKKβ is essential for protecting T cells from TNFα-induced apoptosis. Immunity. 2001;14:217-230.
21. Karin M, Lin A. NF-κB at the crossroads of life and death. Nat Immunol. 2002;3:221-227.
22. Xiao G, Harhaj EW, Sun SC. NF-κB-inducing kinase regulates the processing of NF-κB2 p100. Mol Cell. 2001;7:401-409.
23. Desai A, Konda VR, Darland G, et al. META060 inhibits multiple kinases in the NF-κB pathway and suppresses LPS - mediated inflammation *in vitro* and *ex vivo*. Inflamm Res. 2009; 58:229-234.
24. Pindolia KR, Noth CJ, Xu YX, Janakiraman N, Chapman RA, Gautam SC. IL-4 upregulates IL-1-induced chemokine gene expression in bone marrow stromal cells by enhancing NF-kB activation. Hematopathol Mol Hematol. 1996;10:171-185.
25. Senftleben U, Cao Y, Xiao G, et al. Activation by IKKα of a second, evolutionary conserved, NF-κB signaling pathway. Science. 2001;293:1495-1499.
26. Hayden MS, Ghosh S. Shared principles in NF-κB signaling. Cell. 2008;132:344-362.
27. Li ZW, Chu W, Hu Y, et al. The IKKβ subunit of IκB kinase (IKK) is essential for nuclear factor κB activation and prevention of apoptosis. J Exp Med. 1999;189:1839-1845.
28. Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol. 2001;11:372-377.
29. Bonizzi G, Karin M. The two NF-κB activation pathways and their role in innate and adaptive immunity. Trends Immunol. 2004;25:280-288.
30. Derudder E, Dejardin E, Pritchard LL, Green DR, Korner M, Baud V. RelB/p50 dimers are differentially regulated by tumor necrosis factor-α and lymphotoxin-β receptor activation: critical roles for p100. J Biol Chem. 2003;278:23278-23284.
31. Coope HJ, Atkinson PG, Huhse B, et al. CD40 regulates the processing of NF-κB2 p100 to p52. EMBO J. 2002;21:5375-5385.
32. Dejardin E. The alternative NF-κB pathway from biochemistry to biology: pitfalls and promises for future drug development. Biochem Pharmacol. 2006;72:1161-1179.
33. Senftleben U, Karin M. The IKK/NF-κB pathway. Crit Care Med. 2002;30:S18-S26.
34. Keats JJ, Fonseca R, Chesi M, et al. Promiscuous mutations activate the noncanonical NF-κB pathway in multiple myeloma. Cancer Cell. 2007;12:131-144.
35. Annunziata CM, Davis RE, Demchenko Y, et al. Frequent engagement of the classical and alternative NF-κB pathways by diverse genetic abnormalities in multiple myeloma. Cancer Cell. 2007;12:115-130.
36. Kato T, Jr, Delhase M, Hoffmann A, Karin M. CK2 is a C-terminal IκB kinase responsible for NF-κB activation during the UV response. Mol Cell. 2003;12:829-839.
37. Algul H, Tando Y, Beil M, et al. Different modes of NF-κB/Rel activation in pancreatic lobules. Am J Physiol Gastrointest Liver Physiol. 2002;283:G270-281.
38. Byun MS, Jeon KI, Choi JW, Shim JY, Jue DM. Dual effect of oxidative stress on NF-κB activation in HeLa cells. Exp Mol Med. 2002;34:332-339.
39. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357:539-545.
40. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002; 420:860-867.
41. Lawrence T, Willoughby DA, Gilroy DW. Anti-inflammatory lipid mediators and insights into the resolution of inflammation. Nat Rev Immunol. 2002;2:787-795.
42. Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. Nat Rev Immunol. 2004;4:641-648.
43. Grivennikov S, Karin E, Terzic J, et al. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell. 2009;15: 103-113.
44. Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. CA Cancer J Clin. 2006;56:69-83.
45. Schottenfeld D, Beebe-Dimmer JL. Advances in cancer epidemiology: understanding causal mechanisms and the evidence for implementing interventions. Annu Rev Public Health. 2005;26:37-60.
46. Pisani P, Parkin DM, Munoz N, Ferlay J. Cancer and infection: estimates of the attributable fraction in 1990. Cancer Epidemiol Biomarkers Prev. 1997;6:387-400.
47. Hiscott J, Kwon H, Genin P. Hostile takeovers: viral appropriation of the NF-κB pathway. J Clin Invest. 2001;107: 143-151.
48. Chan CF, Yau TO, Jin DY, Wong CM, Fan ST, Ng IO. Evaluation of nuclear factor-κB, urokinase-type plasminogen activator, and HBx and their clinicopathological significance in hepatocellular carcinoma. Clin Cancer Res. 2004;10:4140-4149.
49. Herrero R, Castellsague X, Pawlita M, et al. Human papillomavirus and oral cancer: the international agency for research on cancer multicenter study. J Natl Cancer Inst. 2003;95:1772-1783.
50. IAfRoC (IARC). Monographs on the evaluation of carcinogenic risk to humans: human papillomaviruses. Lyon, France: IARC Publications; 2005.
51. Aggarwal BB, Shishodia S, Sandur SK, Pandey MK, Sethi G. Inflammation and cancer: how hot is the link? Biochem Pharmacol. 2006;72:1605-1621.
52. Tsurutani J, Castillo SS, Brognard J, et al. Tobacco components stimulate Akt-dependent proliferation and NFκB-dependent survival in lung cancer cells. Carcinogenesis. 2005;26:1182-1195.
53. Wu AH, Pearce CL, Tseng CC, Templeman C, Pike MC. Markers of inflammation and risk of ovarian cancer in Los Angeles County. Int J Cancer. 2009;124:1409-1415.
54. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut. 2001;48: 526-535.
55. Seril DN, Liao J, Yang GY, Yang CS. Oxidative stress and ulcerative colitis-associated carcinogenesis: studies in humans and animal models. Carcinogenesis. 2003;24:353-362.
56. Meira LB, Bugni JM, Green SL, et al. DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice. J Clin Invest. 2008;118:2516-2525.
57. Sangha S, Yao M, Wolfe MM. Non-steroidal anti-inflammatory drugs and colorectal cancer prevention. Postgrad Med J. 2005;81:223-227.
58. Hoffmeister M, Chang-Claude J, Brenner H. Do older adults using NSAIDs have a reduced risk of colorectal cancer? Drugs Aging. 2006;23:513-523.
59. Bertagnolli MM, Eagle CJ, Zauber AG, et al. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med. 2006;355:873-884.
60. Steinbach G, Lynch PM, Phillips RK, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med. 2000;342:1946-1952.
61. Karin M, Lawrence T, Nizet V. Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. Cell. 2006;124:823-835.

62. Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest. 2007;117: 1175-1183.
63. Karin M, Cao Y, Greten FR, Li ZW. NF-κB in cancer: from innocent bystander to major culprit. Nat Rev Cancer. 2002;2: 301-310.
64. Richmond A. NF-κB, chemokine gene transcription and tumour growth. Nat Rev Immunol. 2002;2:664-674.
65. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57-70.
66. Schulze-Bergkamen H, Krammer PH. Apoptosis in cancer-implications for therapy. Semin Oncol. 2004;31:90-119.
67. Kucharczak J, Simmons MJ, Fan Y, Gelinas C. To be, or not to be: NF-κB is the answer-role of Rel/NF-κB in the regulation of apoptosis. Oncogene. 2003;22:8961-8982.
68. Neurath MF, Pettersson S, Meyer zum Buschenfelde KH, Strober W. Local administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF-κB abrogates established experimental colitis in mice. Nat Med. 1996;2:998-1004.
69. Rogler G, Brand K, Vogl D, et al. Nuclear factor κB is activated in macrophages and epithelial cells of inflamed intestinal mucosa. Gastroenterology. 1998;115:357-369.
70. Ardite E, Panes J, Miranda M, et al. Effects of steroid treatment on activation of nuclear factor κB in patients with inflammatory bowel disease. Br J Pharmacol. 1998;124:431-433.
71. Becker C, Fantini MC, Schramm C, et al. TGF-β suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. Immunity. 2004;21:491-501.
72. Greten FR, Arkan MC, Bollrath J, et al. NF-κB is a negative regulator of IL-1β secretion as revealed by genetic and pharmacological inhibition of IKKβ. Cell. 2007;130:918-931.
73. Guttridge DC, Albanese C, Reuther JY, Pestell RG, Baldwin AS, Jr. NF-κB controls cell growth and differentiation through transcriptional regulation of cyclin D1. Mol Cell Biol. 1999;19: 5785-5799.
74. Chen C, Edelstein LC, Gelinas C. The Rel/NF-κB family directly activates expression of the apoptosis inhibitor Bcl-x(L). Mol Cell Biol. 2000;20:2687-2695.
75. Fernandez-Majada V, Aguilera C, Villanueva A, et al. Nuclear IKK activity leads to dysregulated notch-dependent gene expression in colorectal cancer. Proc Natl Acad Sci U S A. 2007; 104:276-281.
76. Tew GW, Lorimer EL, Berg TJ, Zhi H, Li R, Williams CL. SmgGDS regulates cell proliferation, migration, and NF-κB transcriptional activity in non-small cell lung carcinoma. J Biol Chem. 2008;283:963-976.
77. Rahman KM, Ali S, Aboukameel A, Sarkar SH, et al. Inactivation of NF-κB by 3,3'-diindolylmethane contributes to increased apoptosis induced by chemotherapeutic agent in breast cancer cells. Mol Cancer Ther. 2007;6:2757-2765.
78. Luo JL, Maeda S, Hsu LC, Yagita H, Karin M. Inhibition of NF-κB in cancer cells converts inflammation-induced tumor growth mediated by TNFα to TRAIL-mediated tumor regression. Cancer Cell. 2004;6:297-305.
79. Baldwin AS. Control of oncogenesis and cancer therapy resistance by the transcription factor NF-κB. J Clin Invest. 2001;107:241-246.
80. Loch T, Michalski B, Mazurek U, Graniczka M. (Vascular endothelial growth factor (VEGF) and its role in neoplastic processes). Postepy Hig Med Dosw. 2001;55:257-274.
81. Huang S, DeGuzman A, Bucana CD, Fidler IJ. Nuclear factor-κB activity correlates with growth, angiogenesis, and metastasis of human melanoma cells in nude mice. Clin Cancer Res. 2000;6:2573-2581.
82. Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell. 1998;93:705-716.
83. Choo MK, Sakurai H, Kim DH, Saiki I. A ginseng saponin metabolite suppresses tumor necrosis factor-α-promoted metastasis by suppressing nuclear factor-κB signaling in murine colon cancer cells. Oncol Rep. 2008;19:595-600.
84. Hayden MS, West AP, Ghosh S. NF-κB and the immune response. Oncogene. 2006;25:6758-6780.
85. Monks NR, Biswas DK, Pardee AB. Blocking anti-apoptosis as a strategy for cancer chemotherapy: NF-κB as a target. J Cell Biochem. 2004;92:646-650.
86. Ravi R, Bedi A. NF-κB in cancer - a friend turned foe. Drug Resist Updat. 2004;7:53-67.
87. Bharti AC, Aggarwal BB. Chemopreventive agents induce suppression of nuclear factor-κB leading to chemosensitization. Ann N Y Acad Sci. 2002;973:392-395.
88. Frelin C, Imbert V, Griessinger E, Loubat A, Dreano M, Peyron JF. AS602868, a pharmacological inhibitor of IKK2, reveals the apoptotic potential of TNF-α in Jurkat leukemic cells. Oncogene. 2003;22:8187-8194.
89. Frelin C, Imbert V, Griessinger E, et al. Targeting NF-κB activation *via* pharmacologic inhibition of IKK2-induced apoptosis of human acute myeloid leukemia cells. Blood. 2005;105:804-811.
90. Ziegelbauer K, Gantner F, Lukacs NW, et al. A selective novel low-molecular-weight inhibitor of IκB kinase-β (IKK-β) prevents pulmonary inflammation and shows broad anti-inflammatory activity. Br J Pharmacol. 2005;145:178-192.
91. Weber CK, Liptay S, Wirth T, Adler G, Schmid RM. Suppression of NF-κB activity by sulfasalazine is mediated by direct inhibition of IκB kinases α and β. Gastroenterology. 2000;119:1209-1218.
92. Majumdar S, Aggarwal BB. Methotrexate suppresses NF-κB activation through inhibition of IκBα phosphorylation and degradation. J Immunol. 2001;167:2911-2920.
93. Farrow DC, Vaughan TL, Hansten PD, et al. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev. 1998;7:97-102.
94. Zaridze D, Borisova E, Maximovitch D, Chkhikvadze V. Aspirin protects against gastric cancer: results of a case-control study from Moscow, Russia. Int J Cancer. 1999;82:473-476.
95. Naugler WE, Karin M. NF-κB and cancer-identifying targets and mechanisms. Curr Opin Genet Dev. 2008;18:19-26.
96. Katsman A, Umezawa K, Bonavida B. Chemosensitization and immunosensitization of resistant cancer cells to apoptosis and inhibition of metastasis by the specific NF-κB inhibitor DHMEQ. Curr Pharm Des. 2009;15:792-808.
97. Salminen A, Lehtonen M, Suuronen T, Kaarniranta K, Huuskonen J. Terpenoids: natural inhibitors of NF-κB signaling with anti-inflammatory and anticancer potential. Cell Mol Life Sci. 2008;65:2979-2999.
98. Karin M, Yamamoto Y, Wang QM. The IKK NF-κB system: a treasure trove for drug development. Nat Rev Drug Discov. 2004;3:17-26.
99. Yu M, Yeh J, Van Waes C. Protein kinase casein kinase 2 mediates inhibitor-κB kinase and aberrant nuclear factor-κB activation by serum factor(s) in head and neck squamous carcinoma cells. Cancer Res. 2006;66:6722-6731.