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# Chronic inflammation, apoptosis and (pre-)malignant lesions in the gastro-intestinal tract

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**Inflammatory conditions are characterized by activation of the transcription factor nuclear factor kappa B (NF-κB), resulting in the expression of NF-κB-regulated, inflammation-related genes, such as inducible nitric oxide synthase (iNOS) and cyclo-oxygenase-2 (COX-2). Expression of these genes contributes to the survival of cells. Indeed, exposure to pro-inflammatory cytokines in the absence of NF-κB activation leads to apoptosis.[1,2] Chronic inflammatory conditions are accompanied by constitutive activation of NF-κB and hence, to the continuous expression of pro-survival genes, as has been observed in chronic gastritis.[3] Although beneficial for the survival of cells during exposure to inflammatory stress, the continuous activation of NF-κB may also pose a risk: cells with a pro-survival phenotype may give rise to continuously proliferating cells and may thus be tumorigenic. Progression to a malignant phenotype of these cells will most likely involve additional changes in the expression of non-NF-κB regulated genes *e.g.* a shift in the balance of pro- and anti-apoptotic genes towards a more anti-apoptotic phenotype. Literature on inflammation-related genes and the apoptotic balance in pre-malignant and malignant conditions in the gastro-intestinal tract is still scarce and conflicting. In this review, we aim to give an overview of the existing literature and we will focus on inflammation- and apoptosis-related genes in the sequence of normal epithelium-inflamed epithelium-metaplasia-dysplasia-cancer in the gastrointestinal tract, in particular esophagus (Barrett's esophagus: BE), stomach (gastritis) and colon (inflammatory bowel disease: IBD).**

*Keywords:* apoptosis; COX-2; gastro-intestinal tract; iNOS; NF-κB.

## Actions of NF-κB, iNOS and COX-2

iNOS is one of three NO synthases responsible for the production of nitric oxide from L-arginine. Whereas endothelial NOS (eNOS, NOS-III) and neuronal NOS (nNOS, NOS-I) are calcium-dependent NO synthases and responsible for picomolar nitric oxide concentrations, iNOS is a calcium-independent NO synthase and responsible for NO production in the nanomolar range.[4] NO, produced by nNOS and eNOS, is necessary for physiological functions in the human body.[5,6] In contrast to the eNOS and nNOS that are active only when intracellular calcium concentrations are elevated, iNOS activity is calcium-independent. The expression of iNOS is induced by various inflammatory cytokines, in particular tumour necrosis factor alpha (TNFα), interferon-gamma and bacterial cell wall products like lipopolysaccharide (LPS).[7–9] Anti-apoptotic actions of NO include the inhibition of caspases, the proteases involved in apoptosis and elevation of cyclic nucleotides.[10] In addition, NO is able to cause DNA-damage and simultaneously inhibit DNA repair mechanisms.[11] This results in the preservation and propagation of DNA damage in proliferating cells. The COX-isoenzyme COX-2 is normally expressed at very low levels but is rapidly induced at sites of inflammation.[12,13] COX-2 predominates in inflammatory conditions and is also induced in cancer cells.[14,15] Products of COX-2 promote cell survival: the COX-2 specific inhibitor celecoxib causes regression of polyps in patients with familial adenomatous polyposis.[16] In cell lines expressing COX-2, inhibition of COX-2 sensitizes these cells to apoptotic stimuli. Therefore COX-2 expression may be a target for the chemoprotective effect of NSAIDs in pre-malignant conditions.

## Apoptosis and apoptosis-related proteins

Apoptosis is important for the removal of unnecessary, aged or damaged cells. Abnormal resistance to apoptosis entails malformations, autoimmune disease or cancer due to the persistence of unwanted cells. Apoptosis is regulated through different pathways.[17] In general, apoptosis is initiated after activation of death-receptors at the

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plasma membrane. These receptors include the TNF receptor, activated by TNF, Fas, activated by FasLigand and TRAIL- or DR-receptors, activated by TRAIL.[17] In contrast to Fas and TRAIL receptor, activation of the TNF receptor also activates a survival pathway, regulated by NF-$\kappa$B. Stimulation of death receptors will lead to the activation of initiator caspases, such as caspase-8 and caspase-10. Initiator caspases activate down-stream effector caspases such as caspase-3 that cleave essential cellular proteins leading to cell death. Activation of caspase-3 is amplified by pro-apoptotic signals released from damaged mitochondria.[18] These pro-apoptotic proteins from mitochondria include cytochrome-*c*, Diablo/Smac and HtrA2/Omi.[18] The release of these proteins into the cytoplasm causes activation of caspase-3. Therefore, the integrity of mitochondrial membranes is essential to avoid apoptosis. This is regulated to a large extent by members of the Bcl-2 family.[19] This family comprises both anti-apoptotic members (*e.g.* Bcl-2, Bcl-xl, Bfl1/A1) and pro-apoptotic members (*e.g.* Bid, Bak, Bad, Bax). Pro-apoptotic members of the Bcl-2 family contribute to the formation of pores in the outer mitochondrial membranes, facilitating leakage of pro-apoptotic proteins into the cytoplasm. Anti-apoptotic members of the Bcl-2 family antagonize the pro-apoptotic members, thus inhibiting the formation of pores. The balance between pro- and anti-apoptotic Bcl-2 family members within a cell determines its relative resistance or sensitivity to apoptosis.[19] A shift in this balance towards a more anti-apoptotic phenotype may result in transformation of a normal cell into a continuous proliferating malignant tumour cells. Some members of the Bcl-2 family, including anti-apoptotic A1/Bfl-1 and Bcl-xl are under the control of the transcription factor NF-$\kappa$B. Overexpression of Bcl-2 family members in several cancer cell types has been reported, *e.g.* hepatocellular carcinoma and leukemias.[20,21]

## NF-$\kappa$B, iNOS and COX-2 expression in Barrett's esophagus

Barrett's esophagus (BE) is a typical pre-malignant condition of the esophagus. Normal epithelium is replaced by columnar epithelium and eventually this can evolve to adenocarcinoma.[22–24] iNOS was reported to be induced in BE and BE associated carcinoma.[25–27] COX-2 expression in BE is induced in both pre-cancerous and cancerous lesions. This could have implications for chemopreventive therapy. Although data are limited, selective inhibition of COX-2 in esophageal adenocarcinoma cells suppresses growth and induces apoptosis.[28–30] It was demonstrated that selective and non-selective COX-2 inhibitors can inhibit inflammation, COX-2 activity, and development of adenocarcinoma induced by reflux.[31] On the other hand, in a retrospective trial among patients with BE, no difference in cancer risk in BE was found in the presence or absence of COX-inhibitors of the NSAID-family.[32] Finally, one report observed no COX-2 expression in dysplastic lesions in BE.[33]

*In summary.* iNOS is induced in BE. Due to the scarcity of investigations, it is not clear what the exact consequences of iNOS in BE epithelium are with respect to survival and apoptosis. The use of iNOS inhibitors as chemopreventive intervention in BE has not been reported yet. The data on COX-2 expression and chemoprevention in BE are conflicting. No studies report on NF-$\kappa$B activation in BE.

## Apoptosis and apoptosis-related proteins in Barrett's esophagus

Apoptosis measured by counting apoptotic cells in different stages of BE was found to be increased in BE compared to normal fundus epithelium, whereas apoptosis determined by the TUNEL assay in BE was almost absent.[34,35] In BE, with or without dysplasia or carcinoma, decreased Fas expression has been reported.[36] This suggests a protective mechanism against apoptosis in BE and BE-associated adenocarcinoma. In addition to gastric acid in reflux esophagitis there is also reflux of bile in BE. Bile acids have been shown to activate Fas, inducing apoptosis in liver cells. The decreased expression of Fas may be an adaptation of epithelium against exposure to pro-apoptotic bile acids resulting in decreased sensitivity to apoptosis. Furthermore, bile acids have been shown to promote survival of cholangiocyte cell lines by activating the Epidermal Growth Factor (EGF) receptor.[37] If confirmed, this is an example of an adaptation to inflammatory stress, resulting in an anti-apoptotic phenotype and predisposing to cancer. Reports on the expression of Bax in BE demonstrated a positive association between progression to adenocarcinoma and Bax expression.[38] Increased Bax expression alone in these cells may be not pro-apoptotic. Only in response to an apoptotic trigger does Bax translocate from cytoplasm into mitochondrial membranes forming pores. In contrast, anti-apoptotic Bcl-2-family members are constitutively located in intracellular membranes including mitochondria, and therefore increased expression of these Bcl-2 members directly contribute to a more apoptosis-resistant phenotype. Reports on Bcl-2 expression in the neoplastic transformation to adenocarcinoma are scarce. Some studies showed an increased expression of Bcl-2 in neoplastic transformation, but others failed to demonstrate Bcl-2 expression at all in the epithelium of Barrett's esophagus.[39–41] In contrast, expression of the anti-apoptotic Bcl-2 family member Bcl-xl increased in the sequence towards adenocarcinoma[42] and this increase may compensate for the observed increase in Bax expression.

*In summary.* There are hardly any reports on apoptosis in BE. Distinct changes in the expression of Bcl-2 family members occur, but the consequences for the resistance against apoptosis are not clear.

## NF-κB, iNOS and COX-2 expression in pre-malignant and malignant conditions in the stomach

According to the Lauren classification,[43] gastric adenocarcinomas can be divided into those of the diffuse and those of the intestinal type. Atrophic gastritis and intestinal metaplasia can eventually result in the development of pre-malignant and malignant lesions in intestinal type cancer.[44] It is well accepted that Helicobacter pylori-associated gastritis is causally linked to both types of gastric cancer.[45–47]

Compared to normal gastric antral mucosa, NF-κB in Helicobacter pylori (Hp) gastritis is activated and translocated to the nuclei of epithelial cells and its expression correlates with the activity of gastritis.[3,48–51] NF-κB is not only activated in epithelial cells but also in endothelial cells, macrophages and B lymphocytes in the lamina propria. Several studies have demonstrated the activation of NF-κB by Helicobacter pylori in human gastric cancer cell lines and *in vivo* activation of NF-κB was demonstrated in intestinal type gastric carcinoma.[52,53] In the latter report a correlation was found between NF-κB activity and clinicopathological features of the carcinoma. iNOS is induced in the gastric epithelium of patients with Helicobacter pylori-induced gastritis[54,55] and also in epithelium of intestinal metaplasia.[56,57] Reports on the expression of iNOS in pre-malignant and malignant lesions showed an increased expression of iNOS.[58–63] Furthermore, in these studies, expression of iNOS correlates with tumour invasiveness, metastatic potential and a worse prognosis. A relationship between NF-κB activity and iNOS expression in Helicobacter pylori associated gastritis of humans has been demonstrated.[64,65] In these studies inhibition of NF-κB prevented iNOS expression and NO production. The authors suggested that iNOS inhibition was restricted to epithelial cells and did not occur in inflammatory cells of the lamina propria. Most studies report induction of COX-2 expression in Helicobacter pylori gastritis.[57,66,67] The localisation of COX-2 expression remains controversial: some studies showed COX-2 expression in both epithelial cells and lamina propria immune cells whereas other studies showed only expression in lamina propria immune cells. In addition, COX-2 expression has been demonstrated in epithelium of gastic atrophy and intestinal metaplasia and in both diffuse and intestinal type gastric adenocarcinoma,[68,69] no difference in COX-2 expression between diffuse and intestinal type gastric carcinoma was observed and not all tumour cells were positive for COX-2.[70] Inhibition of NF-κB resulted in inhibition of COX-2 expression and inhibition of proliferation of gastric cancer cells.[71]

*In summary.* NF-κB, iNOS and COX-2 are induced in Hp-gastritis, intestinal metaplasia, dysplasia and adenocarcinoma of the stomach. The localisation and degree of expression varies between studies. NF-κB activation is involved in the expression of iNOS, COX-2 and cell proliferation. Some data suggest that inhibition of NF-κB activation or NF-κB-regulated genes may sensitize gastric cancer cells to apoptosis or inhibit their proliferation.

## Apoptosis and apoptosis-related proteins in pre-malignant and malignant conditions in the stomach

Gastric intestinal metaplasia is associated with increased apoptosis compared to normal gastric mucosa.[72] Increased apoptosis, determined using the TUNEL assay, was demonstrated in intestinal type gastric carcinomas but other studies failed to confirm this finding. Since the TUNEL assay is prone to artefacts, other ways of determining apoptosis should clarify this apparent discrepancy.[73–75] In one study activated caspase-3 was not detected in gastric cancer cells nor in the gastric mucosa surrounding the gastric cancer whereas in normal gastric mucosa activated caspase 3 expression was detected. This suggests that inhibition of apoptosis, as indicated by the lack of caspase-3 activation, is involved in the transformation to gastric carcinoma.[76] In normal gastric mucosa Fas expression is hardly detectable in epithelial cells. Fas expression increases in gastric atrophy and intestinal metaplasia and is detectable in all cases with dysplasia.[77] Vollmers *et al.* reported Fas expression in the diffuse type carcinoma but not in the intestinal type carcinoma,[78] whereas we observed exactly the opposite result.[79] Another group reported high expression of Fas in gastric cancer cells and reduced Fas expression with the advancement of the carcinoma.[80] The increased Fas expression on malignant cells compared to normal gastric epithelium is difficult to explain. It remains to be determined whether the increased Fas expression really results in increased sensitivity to apoptosis. Possibly, increased Bcl-2 expression may counteract the increased Fas expression in terms of sensitivity to apoptosis: in normal gastric mucosa Bcl-2 expression is confined to only a few regenerating epithelial cells of the mucous neck region. Bcl-2 expression is increased in chronic gastritis, intestinal metaplasia and dysplasia.[81–85] Kyokane *et al.* demonstrated Bcl-2 expression in early gastric cancer of the elevated type. This elevated type probably resembles adenomatous polyps in the colon.[86] Others demonstrated Bcl-2 expression in tumour cells of both intestinal type carcinoma as well as diffuse

type gastric carcinoma, but mostly in a small percentage of the tumour cells.[87,88] However, the expression of Bcl-2 seems to be higher in intestinal type gastric cancer compared to diffuse type cancer.[75,76,89,90] Bax expression is reported in both intestinal and diffuse type carcinomas but seems to be decreased in comparison to the surrounding non-tumorous tissue, favouring an anti-apoptotic phenotype in gastric cancers.[83,91]

*In summary.* Apoptosis as determined by the expression of activated caspase-3 is reduced in gastric cancer compared to normal gastric mucosa. Reports on apoptosis using the TUNEL assay are conflicting. Expression of Fas and Bcl-2 proteins are increased in intestinal metaplasia, dysplasia and adenocarcinoma compared to the normal gastric mucosa, whereas Bax expression is reduced in gastric cancer cells.

## NF-κB, iNOS and COX-2 expression in inflammatory bowel diseases

NF-κB activity varies in inflammatory bowel diseases. NF-κB activation has been observed in macrophages in the lamina propria and in epithelial cells.[92,93] IL-10, sulphasalazine and immunosuppressive drugs have been reported to inhibit NF-κB activity in the mucosa of patients with Crohn's disease and ulcerative colitis.[94–97] iNOS is clearly expressed in epithelial cells of the inflamed gut.[98–100] The expression of COX-2 in surface epithelial cells and in lamina propria immune cells in areas of inflammation in Crohn's colitis and ulcerative colitis is strongly induced.[101,102] COX-2 overexpression has been described in sporadic colonic neoplasia and in colitis-associated neoplasia but its exact role in neoplastic transformation is not yet clear. One group reported COX-2 overexpression in ulcerative colitis associated neoplasia and in this study the increase in COX-2 expression could not be explained by inflammatory activity alone.[103] However, in this report the expression of COX-2 in adenocarcinoma in longstanding colitis was not as uniform as in the dysplastic regions.

*In summary.* Only a limited amount of data concerning NF-κB activation and COX-2 and iNOS expression in IBD-related carcinogenesis has been published. Although these proteins are induced in IBD, their role in oncogenesis is not known.

## Apoptosis and apoptosis-related proteins in inflammatory bowel diseases

In normal intestinal epithelium apoptosis is observed in the crypt and at the luminal surface.[104] Bcl-2 is expressed in the bases of crypts, whereas epithelial cells on the luminal surface express less Bcl-2.[105,106] Bax, Bcl-xl and Bak expression are confined to areas of colonic epithelial cells of the luminal surface.[106,107] There is a higher expression of Bak in the left colon compared to the right colon.[108] Fas is strongly expressed in all epithelial cells of the normal colon throughout the crypt.[109] In ulcerative colitis, apoptotic colonocytes are increased in number throughout the crypt.[110] In the same report Fas expression in the intestinal epithelium of ulcerative colitis patients was comparable to that of normal epithelial cells. Another report confirmed this.[111] In both reports Fas ligand was highly expressed compared to normal colonic epithelium. The reports on expression of apoptosis-related proteins in the epithelium of patients with ulcerative colitis and Crohn's colitis are limited. In active colitis, no change in Bcl-2 expression compared to normal colonic epithelium was observed.[105] Bcl-2 overexpression was observed in ulcerative colitis-associated neoplasia.[112] Compared to adenomas in areas involved in ulcerative colitis, Bcl-2 expression in ulcerative colitis-associated dysplastic lesions is less frequent.[113] The expression of Bcl-2 in ulcerative-colitis-associated colorectal cancer is significantly lower compared to that in sporadic colorectal cancer.[114] Another report failed to demonstrate a significant difference in Bcl-2 expression between ulcerative colitis associated neoplasia and sporadic adenocarcinomas, although this study revealed less apoptosis in the ulcerative colitis associated neoplasia compared to sporadic adenocarcinomas.[115] Bax expression is reduced in ulcerative colitis compared to normal colonic mucosa.[105] Other reports on expression of apoptosis-related proteins in inflammatory bowel disease are mainly focussed on lamina propria T cells.

*In summary.* Reports on apoptosis in inflammatory bowel diseases and associated neoplasia are limited. Conflicting data exist on the expression of Bcl-2 in colitis-associated neoplasia compared to sporadic carcinoma. Little is known about the expression in epithelium of other apoptosis-related proteins in the sequence from colitis to carcinoma.

## Perspectives

Despite the fact that advances have been made in exploring the field of pro- and anti-apoptotic proteins, little is known about the balance of these proteins in Barrett's esophagus, gastric carcinoma and in neoplastic changes of longstanding colitis. Published reports are conflicting. Thus it is difficult to draw any general conclusions. New data on the regulation of these proteins are important if one is to design new cancer therapies. There are already strong indications that inhibitors of the NF-κB-regulated gene COX-2, *e.g.* celecoxib and aspirin prevent colon cancer. Whether these findings can be extended to inhibitors of other NF-κB-regulated anti-apoptotic genes, *e.g.* iNOS, or to inhibitors of NF-κB activation itself, *e.g.* the currently used sulfasalazine[116] or

mesalamine,[117] remains to be investigated. Development of stronger chemopreventive agents and the development of novel early markers for oncogenesis in chronically inflamed mucosa may eventually lead to new chemotherapeutic strategies.

## References


1. Schoemaker MH, Ros JE, Homan M, *et al.* Cytokine regulation of pro- and anti-apoptotic genes in rat hepatocytes: NF-kappaB-regulated inhibitor of apoptosis protein 2 (cIAP2) prevents apoptosis. *J Hepatol* 2002; **36**: 742–750.
2. Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM. Suppression of TNF-alpha-induced apoptosis by NF-kappaB. *Science* 1996; **274**: 787–789.
3. Van den Brink GR, ten Kate FJ, Ponsioen CY, *et al.* Expression and activation of NF-$\kappa$B in the antrum of the human stomach. *J Immunol* 2000; **164**: 3353–3359.
4. Michel T, Xie QW, Nathan C. Molecular biological analysis of nitric oxide synthases. In: Feelisch M and Stamler JS, eds. *Methods in Nitric Oxide Research*. UK: John Wiley and Sons, 1995: 161–175.
5. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; **327**: 524–526.
6. Kobzik L, Reid MB, Bredt DS, Stamler JS. Nitric oxide in skeletal muscle. *Nature* 1994; **372**: 546–548.
7. Kilbourn RG, Belloni P. Endothelial cell production of nitrogen oxides in response to interferon gamma in combination with tumour necrosis factor, interleukin-1 or endotoxin. *J Natl Cancer Inst* 1990; **82**: 772–776.
8. Farrell AJ, Blake DR. Nitric oxide. *Ann Rheum Dis* 1996; **55**: 7–20.
9. Vane JR, Mitchell JA, Appleton I, *et al.* Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. *Proc Natl Acad Sci* 1994; **91**: 2046–2050.
10. Kim PKM, Zamora R, Petrosko P, Billiar TR. The regulatory role of nitric oxide in apoptosis. *Int Immunopharmacol* 2001; **1**: 1421–1441.
11. Jaiswal M, LaRusso NF, Gores GJ. Nitric oxide in gastrointestinal epithelial cell carcinogenesis: Linking inflammation to oncogenesis. *Am J Physiol* 2001; **281**: G626–G634.
12. Jouzeau JY, Terlain B, Abid A, *et al.* Cyclo-oxygenase isoenzymes. *Drugs* 1997; **53(4)**: 564–582.
13. Frölich JC. A classification of NSAIDs according to the relative inhibition of cyclooxygenase isoenzymes. *TiPS* 1997; **18**: 30–34.
14. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994; **107**: 1183–1188.
15. Ristimäki A, Honkanen N, Jänkälä H, Sipponen P, Härkönen M. Expression of cyclooxygenase-2 in human gastric carcinoma. *Cancer Res* 1997; **57**: 1276–1280.
16. Steinbach G, Lynch PM, Phillips RK, *et al.* The effect of celecoxib, a cyclooxygenase inhibitor, in familial adenomatous polyposis. *N Engl J Med* 2000; **29**: 2946–2952.
17. Reed JC. Mechanisms of apoptosis. *Am J Pathol* 2000; **157**: 1415–1430.
18. Van Gurp M, Festjens N, van Loo G, Saelens X, Vandenabeele P. Mitochondrial intermembrane proteins in cell death. *Biochem Biophys Res Comm* 2003; **304**: 487–497.
19. Gross A, McDonnell JM, Korsmeyer SJ. Bcl-2 family members and the mitochondria in apoptosis. *Genes Development* 1999; **13**: 1899–1911.
20. Watanabe J, Kushihata F, Honda K, Mominoki K, Matsuda S, Kobayashi N. Bcl-xl overexpression in hepatocellular carcinoma. *Int J Oncol* 2002; **21**: 515–519.
21. Montserrat E. Chronic lymphoproliferative disorders. *Curr Opin Oncol* 1997; **9**: 34–41.
22. Levine DS, Haggit RC, Irvine S, *et al.* Natural history of high-grade dysplasia in Barrett's esophagus. *Gastroenterology* 1996; **110**: A550.
23. Hameeteman W, Tytgat GNJ, Houthoff HJ, van den Tweel JG. Barrett's oesophagus: Development of dysplasia and adenocarcinoma. *Gastroenterolgy* 1989; **96**: 1249–1256.
24. Champion G, Richter JE, Vaezi MF. Duodenogastro-esophageal reflux: Relationship to pH and importance in Barrett's esophagus. *Gastroenterology* 1994; **107**: 747–754.
25. Wilson KT, Fu S, Ramanujam KS, Meltzer SJ. Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's Esophagus and associated adenocarcinomas. *Cancer Res* 1998; **58**: 2929–2934.
26. Goldstein SR, Yang GY, Chen X, Curtis SK, Yang CS. Studies of iron deposits, inducible nitric oxide synthase and nitrotyrosine in a rat model for esophageal adenocarcinoma. *Carcinogenesis* 1998; **19**: 1445–1449.
27. Forrester K, Ambs S, Lupold SE, *et al.* Nitric oxide-induced p53 accumulation and regulation of inducible nitric oxide synthase expression by wild-type p53. *Proc Natl Acad Sci USA* 1996; **93**: 2442–2447.
28. Souza RF, Shewmake K, Beer DG, Cryer B, Spechler SJ. Selective inhibition of cyclooxygenase-2 suppresses growth and induces apoptosis in human esophageal adenocarcinoma cells. *Cancer Res* 2000; **60**: 5767–5772.
29. Shirvani VN, Ouatu-Lascar R, Kaur BS, Omary MB, Triadafilopoulos G. Cyclooxygenase 2 expression in Barrett's esophagus and adenocarcinoma: *Ex vivo* induction by bile salts and acid exposure. *Gastroenterology* 2000; **118(3)**: 487–496.
30. Morris CD, Armstrong GR, Bigley G, Green H, Attwood SEA. Cyclooxygenase-2 expression in the Barrett's metaplasia-dysplasia-adenocarcinoma sequence. *Am J Gastroenterol* 2001; **96**: 990–996.
31. Buttar NJ, Wang KK, Leontovich O, *et al.* Chemoprevention of esophageal adenocarcinoma by COX-2 inhibitors in an animal model of Barrett's Esophagus. *Gastroenterology* 2002; **122**: 1101–1112.
32. Tsibouris P, Hendricksce MT, Zissis M, Isaacs PE. NSAIDs do not protect patients with Barrett Oesophagus from adenocarcinoma development. *Gastroenterology* 2001; **120**: A3022.
33. Garewal HS, Ramsey L, Sampliner RE, Payne C, Bernstein H, Bernstein C. Post-ablation biomarker abnormalities in Barrett's Esophagus (BE): Are we increasing the cancer risk? *Gastroenterology* 2001; **120**: A79.
34. Halm U, Tannapfel A, Breitung B, Breidert M, Wittemkind CW, Moss. Apoptosis and cell proliferation in the metaplasia-dysplasia-carcinoma-sequence of Barrett's esophagus. *Hepatogastroenterology* 2000; **47**: 962–966.
35. Katada N, Hindler RA, Smyrk TC, *et al.* Apoptosis is inhibited early in the dysplasia-carcinoma sequence of Barrett esophagus. *Arch Surg* 1997; **132**: 728–733.
36. Younes M, Lechago J, Ertan A, Finnie D, Younes A. Decreased expression of Fas (CD95/APO1) associated with goblet cell metaplasia in Barrett's esophagus. *Hum Pathol* 2000; **31(4)**: 434–438.
37. Werneburg NW, Yoon JH, Higuchi H, Gores GJ. Bile acids activate EGF receptor via a TGF-alpha-dependent mechanism

in human cholangiocyte cell lines. *Am J Physiol* 2003; **285**: G31–G36.
38. Woodward TA, Klingler PD, Genko PV, Wolfe JT. Barrett's Esophagus, apoptosis and cell cycle regulation: Correlation of p53 with Bax, Bcl-2 and p21 protein expression. *Anticancer Res* 2000; **20**: 2427–2432.
39. Chatelain D, Flejou JF. High-grade dysplasia and superficial adenocarcinoma in Barrett's esophagus: Histological mapping and expression of p53, p21 and Bcl-2 oncoproteins. *Virchows Arch* 2003; **442**: 18–24.
40. Goldblum JR, Rice TW. Bcl-2 protein expression in the Barrett's metaplasia-dysplasia-carcinoma sequence. *Modern Pathol* 1995; **8**: 866–869.
41. Bhargava P, Eisen GM, Holterman BS, *et al.* Endoscopic mapping and surrogate markers for better surveillance in Barrett esophagus. *Am J Clin Pathol* 2000; **114**: 552–563.
42. van der Woude CJ, Jansen PL, Tiebosch AT, *et al.* Expression of apoptosis-related proteins in Barrett's metaplasia-dysplasia-carcinoma sequence: A switch to a more resistant phenotype. *Hum Pathol* 2002 ; **33**: 686–692.
43. Lauren P. The two histological main types of gastric cancer: Diffuse and so-called intestinal type carcinoma. *Acta Parthol Microbiol Scand* 1965; **64**: 331–349.
44. Jass JR. Role of intestinal metaplasia in the histogenesis of gastric carcinoma. *J Clin Pathol* 1980; **33**: 801–810.
45. Huang JQ, Sridhar S, Chen Y, *et al.* Meta-analysis of the relationship between Helicobacter pylori seropositivity and gastric cancer. *Gastroenterology* 1998; **114**: 1169–1179.
46. Eslick GD, Lim LLY, Byles JE, *et al.* Association of Helicobacter pylori infection with gastric carcinoma: A meta-analysis. *Am J Gastroenterol* 1999; **94**: 2373–2379.
47. Danesh J. Helicobacter pylori infection and gastric cancer: Systematic review of the epidemiological studies. *Aliment Pharmacol Ther* 1999; **13**: 851–856.
48. Isomoto H, Mizuta Y, Miyazaki M, *et al.* Implication of NF-$\kappa$B in Helicobacter pylori-associated gastritis. *Am J Gastroenterol* 200; **95**: 2768–2776.
49. Keates S, Hitti YS, Upton M, *et al.* Helicobacter pylori infection activates NF-$\kappa$B in gastric epithelial cells. *Gastroenterology* 1997; **113**: 1099–1109.
50. Sharma SA, Tummuru MKR, Blaser MJ, Kerr LD. Activation of IL-8 gene expression by Helicobacter pylori is regulated by transcription factor nuclear factor-kB in gastric epithelial cells. *J Immunol* 1998; **160**: 2401–2407.
51. Isomoto H, Miyazaki M, Mizuta Y, *et al.* Expression of nuclear factor–kB in Helicobacter pylori-infected gastric mucosa detected with southwestern histochemistry. *Scand J Gastroenterol* 2000; **35**: 247–254.
52. Aihara M, Tsuchimoto D, Takizawa H, *et al.* Mechanisms involved in Helicobacter pylori-induced interleukin-8 production by a gastric cancer cell line, MKN45. *Infect Immun* 1997; **65**: 3218–3224.
53. Sasaki N, Morisaki T, Hashizume K, *et al.* Nuclear factor-kB p65 (relA) transcription factor is constitutively activated in human gastric carcinoma tissue. *Clin Canc Res* 2001; 7: 4136–4142.
54. Wilson KT, Ramujam KS, Mobley HLT, Masselman RF, James SP, Meltzer SJ. Helicobacter pylori stimulates inducible nitric oxide synthase expression and activity in murine macrophage cell line. *Gastroenterology* 1996; **111**: 1524–1533.
55. Rachmilewitz D, Karmeli F, Eliakim R, *et al.* Enhanced gastric nitric oxide synthase activity in duodenal ulcer patients. *Gut* 1994; **35**: 1394–1397.
56. Tatemichi M, Ogura T, Nagata H, Esumi H. Enhanced expression of inducible nitric oxide synthase in chronic gastritis with intestinal metaplasia. *J Clin Gastroenterol* 1998; **27**: 240–245.
57. Fu S, Ramanujam KS, Wong A, *et al.* Increased expression and cellular localization of inducible nitric oxide synthase and cyclooxygenase 2 in Helicobacter pylori gastritis. *Gastroenterology* 1999; **116**: 1319–1329.
58. Pignatelli B, Bancel B, Esteve J, *et al.* Inducible nitric oxide synthase, anti-oxidant enzymes and Helicobacter pylori infection in gastritis and gastric precancerous lesions in humans. *Eur J Cancer Prev* 1998; **7**: 439–447.
59. Goto T, Haruma K, Kitadai Y, *et al.* Enhanced expression of nitric oxide synthase and nitrotyrosine in gastric mucosa of gastric cancer patients. *Clin Canc Res* 1999; **5**: 1411–1415.
60. Rajnakova A, Moochhala SM, Goh PMY, Ngoi SS. Expression of nitric oxide synthase, cyclooxygenase, and p53 in different stages of human gastric adenocarcinoma. *Cancer Lett* 2001; **172**: 177–185.
61. Doi C, Noguchi Y, Marat D, *et al.* Expression of nitric oxide synthase in gastric cancer. *Cancer Lett* 1999; **144**: 161–167.
62. Koh E, Noh SH, Lee YD, *et al.* Differential expression of nitric oxide synthase in human stomach cancer. *Cancer Lett* 1999; **146**: 173–180.
63. Lishi H, Tatsuta M, Baba M, Yamamoto R, Uehara H, Nakaizumi A. Inhibition of experimental gastric carcinogenesis, induced by N-methyl-N'-nitrosoguanidine in rats, by sodium nitroprusside, a nitric oxide generator. *Eur J Cancer* 1998; **34**: 554–557.
64. Zhang XJ, Ruiz B, Correa P, Miller MJS. Cellular dissociation of NF-$\kappa$B and inducible nitric oxide synthase in helicobacter pylori infection. *Free Radical Biol Med* 2002; **29**: 730–735.
65. Lim JW, Kim H, Kim KH. NF-$\kappa$B, inducible nitric oxide synthase and apoptosis by Helicobacter pylori infection. *Free Rad Biol Med* 2001; **31**: 355–366.
66. Mccarthy CJ, Crofford LJ, Greenson J, Scheiman JM. Cyclooxygenase expression in gastric antral mucosa before and after eradication of helicobacter pylori infection. *Am J Gastroenterol* 1999; **94**: 1218–1223.
67. Jackson LM, Wu KC, Mahida YR, Jenkins D, Hawkey CJ. Cyclooxygenase (COX) 1 and 2 in normal, inflamed, and ulcerated human gasrtric mucosa. *Gut* 2000; **47**: 762–770.
68. Sung JJY, Leung WK, Go MYY, *et al.* Cyclooxygenase-2 expression in Helicobacter pylori-associated premalignant and malignant gastric lesions. *Am J Pathol* 2000; **157**: 729–735.
69. Lim HY, Joo HJ, Choi JH, *et al.* Increased expression of cyclooxygenase-2 protein in human gastric carcinoma. *Clin Cancer Res* 2000; **6**: 519–525.
70. Ristimaki A, Honkanen N, Jankala H. Expression of cyclooxygenase-2 in human gastric mucosa. *Cancer* 1997; **57**: 1276–1280.
71. Lim JW, Kim H, Kim KH. Nuclear factor–kB regulates cyclooxygenase-2 expression and cell proliferation in human gastric cancer cells. *Lab Invest* 2001; **82**: 349–360.
72. Van Grieken NC, Meijer GA, zur Hausen A, Meuwissen SG, Baak J, Kuipers EJ. Increased apoptosis in gastric mucosa adjacent to intestinal metaplasia. *J Clin Pathol* 2003; **56**: 358–361.
73. Shinohara T, Ohsima K, Murayama H, Kikuchi M, Yamashita Y, Shirakusa T. Apoptosis and proliferation in gastric carcinoma: The association with histological type. *Histopathology* 1996; **29**: 123–129.
74. Ikeguchi M, Cai J, Yamane N, Maeta M, Kaibara N. Clinical significance of spontaneous apoptosis in advanced gastric adenocarcinoma. *Cancer* 1999; **85**: 2329–2335.
75. Hoshi T, Sasano H, Kato K, *et al.* Immunohistochemistry of caspase-3/CPP32 in human stomach and its correlation

with cell proliferation and apoptosis. *Anticancer Res* 1998; **18**: 4347–4354.
76. Kania J, Konturek SJ, Marlicz K, Hahn EG, Konturek PC. Expression of survivin and caspase-3 in gastric cancer. *Dig Dis Sci* 2003; **48**: 266–271.
77. Li H, Liu N, Guo L, Li JW, Liu J. Frequent expression of soluble Fas and Fas ligand in Chinese stomach cancer and its preneoplastic lesions. *Int J Mol Med* 2000 ; **5**: 473–476.
78. Vollmers HP, Dämmrich J, Hensel F, *et al.* Differential expression of apoptosis receptors on diffuse and intestinal type stomach carcinoma. *Cancer* 1997; **79**: 433–440.
79. van der Woude CJ, Kleibeuker JH, Tiebosch AT, *et al.* Diffuse and intestinal type gastric carcinomas differ in their expression of apoptosis related proteins. *J Clin Pathol* 2003; **56**: 699–702.
80. Ohno S, Tachibana M, Shibakita M, *et al.* Prognostic significance of Fas and Fas ligand system-associated apoptosis in gastric cancer. *Ann Surg Oncol* 2000; **7**: 750–757.
81. Lauwers GY, Scott GV, Hendricks J. Immunohistochemical evidence of abberant bcl-2 protein expression in gastric epithelial dysplasia. *Cancer* 1994; **73**: 2900–2904.
82. Cho JH, Kim WH. Altered topographic expression of p21WAF1/CIP1/SDI1, bcl-2 and p53 during gastric carcinogenesis. *Pathol Res Prast* 1998; **194**: 309–317.
83. Nakamura T, Nomura S, Skai T, *et al.* Expression of Bcl-2 oncoprotein in gastrointestinal and uterine carcinomas and their premalignant lesions. *Hum Pathol* 1997; **28**: 309–315.
84. Clarke NR, Safatle-Ribeiro AV, Ribeiro U, Sakai P, Reynolds JC. Bcl-2 expression in gastric remnant mucosa and gastric cancer 15 or more years after partial gastrectomy. *Mod Pathol* 1997; **10**: 1021–1027.
85. Saegusa M, Takano Y, Okayasu I. Bcl-2 expression and its association with cell kinetics in human gastric carcinomas and intestinal metaplasia. *J Cancer Res Clin Oncol* 1995; **121**: 357–363.
86. Kyokane K, Ito M, Sato Y, Ina K, Ando T, Kusugami K. Expression of Bcl-2 and p53 correlates with the morphology of gastric neoplasia. *J Pathol* 1998; **184**: 382–389.
87. Inada T, Kikuyama S, Ichikawa A, Igarashi S, Ogata Y. Bcl-2 expression as a prognostic factor of survival of gastric carcinoma. *Anticancer Res* 1998; **18**: 2003–2010.
88. Nakata B, Muguruma K, Hirakawa K, *et al.* Predictive value of Bcl-2 and Bax protein expression for chemotherapeutic effect in gastric cancer. *Oncology* 1998; **55**: 543–547.
89. Klein RF, Vollmers HP, Mller-Hermelink HK. Different expression of Bcl-2 in diffuse and intestinal-type stomach carcinomas. *Oncol Rep* 1996; **3**: 1–4.
90. Krajewska M, Fenoglio-Preiser CM, Krajewski S, *et al.* Immunohistochemical analysis of Bcl-2 family proteins in adenocarcinomas of the stomach. *Am J Pathol* 1996; **149**: 1449–1457.
91. Konturek PC, Konturek SJ, Sulekova Z, *et al.* Expression of hepatocyte growth factor, transforming growth factor alpha, apoptosis related proteins Bax and Bcl-2 and gastrin in human gastric cancer. *Aliment Pharmacol Ther* 2001; **15**: 989–999.
92. Schreiber S, Nikolaus S, Hampe J. Activation of nuclear factor kappa B in inflammatory bowel diseases. *Gut* 1998; **42**: 477–484.
93. Rogler G, Brand K, Vogl D, *et al.* Nuclear factor kappa B is activated in macrophages and epithelial cells of inflamed intestinal mucosa. *Gastroenterology* 1998; **115**: 357–369.
94. Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. *Nature* 1998; **396**: 77–80.
95. Egan LJ, Mays DC, Huntoon CJ, *et al.* Inhibition of interleukin-1-stimulated NF-kappaB RelA/p65 phosphorylation by mesalamine is accompanied by decreased transcriptional activity. *J Biol Chem* 1999; **274**: 26448–26453.
96. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS. Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science* 1995; **270**: 283–286.
97. Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF-kappa B pathway in the treatment of inflammation and cancer. *J Clin Invest* 2001; **107**: 135–142.
98. Singer II, Kawka DW, Scott S, *et al.* Expression of inducible nitric oxide synthase and nitrotyrosine in colonic epithelium in inflammatory bowel disease. *Gastroenterology* 1996; **111**: 871–885.
99. Iwashita E, Miyahara T, Hino K, Tokunaga T, Wakisaka H, Sawazaki Y. High nitric oxide synthase activity in endothelial cells in ulcerative colitis. *J Gastroenterol* 1995; **30**: 551–554.
100. Dijkstra G, Moshage H, van Dullemen HM, *et al.* Expression of nitirc oxide synthases and formation of nitrotyrosine and reactive oxygen species in inflammatory bowel disease. *J Pathol* 1998; **186**: 416–421.
101. Singer II, Kawka DW, Schloemann S, Tessner T, Riehl T, Stenson WF. Cyclooxygenase 2 is induced in colonic epithelial cells in inflammatory bowel disease. *Gastroenterology* 1998; **115**: 297–306.
102. Hendel J, Nielsen OH. Expression of cyclooxygenase-2 mRNA in active inflammatory bowel disease. *Am J Gastroenterol* 1997; **92**: 1170–1173.
103. Agoff SN, Brentnall TA, Crispin DA, *et al.* The role of cyclooxygenase 2 in ulcerative colitis-associated neoplasia. *Am J Pathol* 2000; **157**: 737–745.
104. Ciccocioppo R, Di Sabatino A, Gasbarrini G. Apoptosis and gastrointestinal tract. *Ital J Gastroenterol Hepatol* 1999; **116**: 177–184.
105. Limura M, Nakamura T, Shinozaki S, *et al.* Bax is downregulated in inflamed colonic mucosa of ulcerative colitis. *Gut* 2000; **47**: 228–235.
106. Ogura E, Senzaki H, Yamamoto D, *et al.* Prognostic significance of Bcl-2, Bcl-xl/s, Bax and Bak expressions in colorectal carcinomas. *Oncol Rep* 1999; **6**: 365–369.
107. Moss SF, Agarwal B, Arber N. Increased intestinal Bak expression results in apoptosis. *Biochem Biophys Res Commun* 1996; **223**: 199–203.
108. Liu LU, Holt PR, Krivosheyev V, Moss SF. Human right and left colon differ in epithelial cell apoptosis and in expression of Bak, a pro-apoptotic Bcl-2 homologue. *Gut* 1999; **45**: 45–50.
109. Möller P, Koretz K, Leithäuser F, *et al.* Expression of APO-1 (CD95), a member of the NGF/TNF receptor superfamily, in normal and neoplastic colon epithelium. *Int J Cancer* 1994; **57**: 371–377.
110. Sträter J, Wellisch I, Riedl S, *et al.* CD95 (APO-1/Fas)-mediated apoptosis in colon epithelial cells: A possible role in ulcerative colitis. *Gastroenterology* 1997; **113**: 160–167.
111. Ueyama H, Kiyohara T, Sawada N, *et al.* High Fas ligand expression on lymphocytes in lesions of ulcerative colitis. *Gut* 1998; **43**: 48–55.
112. Bronner MP, Culin C, Reed JC, Furth EE. The bcl-2 proto-oncogene and the gastrointestinal epithelial tumor progression model. *Am J Pathol* 1995; **146**: 20–26.
113. Mueller E, Vieth M, Stolte M, Mueller J. The differentiation of true adenomas from colitis-associated dysplasia in

ulcerative colitis: A comparative immunohistochemical study. *Hum Pathol* 1999; **30**: 898–905.
114. Ilyas M, Tomlinson IPM, Hanby AM, Yao T, Bodmer WF, Talbot IC. Bcl-2 expression in colorectal tumors. Evidence of different pathways in sporadic and ulcerative-colitis-associated carcinomas. *Am J Pathol* 1996; **149**: 1719–1726.
115. Mikami T, Yoshida T, Akino F, Motoori T, Yajima M, Okayasa I. Apoptosis regulation differs between ulcerative colitis-associated and sporadic colonic tumors. Association with survivin and bcl-2. *Am J Clin Pathol* 2003; **119**: 723–730.
116. Wahl C, Liptay S, Adler G, Schmid RM. Sulfasalazine: A potent and specific inhibitor of NF-kappa B. *J Clin Invest* 1998; **101**: 1163–1174.
117. Kaiser GC, Yan F, Polk DB. Mesalamine blocks TNF growth inhibition and NF-kappa B activation in mouse colonocytes. *Gastroenterology* 1999; **116**: 602–609.