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Apoptosis in (pre-) malignant lesions in the gastro-intestinal tract

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

2004

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Woude, C. J. V. D. (2004). Apoptosis in (pre-) malignant lesions in the gastro-intestinal tract. Groningen: s.n.

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CHAPTER 2

CHRONIC INFLAMMATION, APOPTOSIS AND (PRE-)MALIGNANT LESIONS IN THE GASTRO-INTESTINAL TRACT

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Adapted from Apoptosis 2004;9:123-130.

1. NF- κ 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 adenocarcinoma¹⁻³.

iNOS was reported to be induced in BE and BE associated carcinoma⁴⁻⁶. 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 apoptosis⁷⁻⁹. It was demonstrated that selective and non-selective COX-2 inhibitors can inhibit inflammation, COX-2 activity, and development of adenocarcinoma induced by reflux¹⁰. 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-family¹¹. Finally, one report observed no COX-2 expression in dysplastic lesions in BE¹².

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- κ B activation in BE.

2. 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^{13,14}. In BE, with or without dysplasia or carcinoma, decreased Fas expression has been reported¹⁵. 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) receptor¹⁶. 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 expression¹⁷. Increased Bax expression alone in these cells may not be pro-apoptotic per se. 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 esophagus¹⁸⁻²⁰. In contrast, expression of the anti-apoptotic Bcl-2 family member Bcl-xl increased in the sequence towards adenocarcinoma²¹ 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.

3. NF-κB, iNOS AND COX-2 EXPRESSION IN PRE-MALIGNANT AND MALIGNANT CONDITIONS IN THE STOMACH

According to the Lauren classification²², 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 cancer²³. It is well accepted that *Helicobacter pylori*-associated gastritis is causally linked to both types of gastric cancer²⁴⁻²⁶.

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^{3,27-30}. 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^{31,32}. In the latter report a correlation

was found between NF- κ B activity and clinicopathological features of the carcinoma. iNOS is induced in the gastric epithelium of patients with *Helicobacter pylori*-induced gastritis^{33,34} and also in epithelium of intestinal metaplasia^{35,36}. Reports on the expression of iNOS in pre-malignant and malignant lesions showed an increased expression of iNOS³⁷⁻⁴². Furthermore, in these studies, expression of iNOS correlates with tumor invasiveness, metastatic potential and a worse prognosis. A relationship between NF- κ B activity and iNOS expression in *Helicobacter pylori* associated gastritis of humans has been demonstrated^{43,44}. In these studies inhibition of NF- κ B 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^{45,46,57}. 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 gastric atrophy and intestinal metaplasia and in both diffuse and intestinal type gastric adenocarcinoma^{47,48}, no difference in COX-2 expression between diffuse and intestinal type gastric carcinoma was observed and not all tumor cells were positive for COX-2⁴⁹. Inhibition of NF- κ B resulted in inhibition of COX-2 expression and inhibition of proliferation of gastric cancer cells⁵⁰.

In summary: NF- κ B, 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- κ B activation is involved in the expression of iNOS, COX-2 and cell proliferation. Some data suggest that inhibition of NF- κ B activation or NF- κ B-regulated genes may sensitize gastric cancer cells to apoptosis or inhibit their proliferation.

4. 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 mucosa⁵¹. 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 discrepancy⁵²⁻⁵⁴. 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 carcinoma⁵⁵. 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 dysplasia⁵⁶. Vollmers et al reported Fas expression in the diffuse type carcinoma but not in the intestinal type carcinoma⁵⁷, whereas we observed exactly the opposite result⁵⁸. Another group reported high expression of Fas in gastric cancer cells and reduced Fas expression with the advancement of the carcinoma⁵⁹. 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 dysplasia⁶⁰⁻⁶⁴. Kyokane et al demonstrated Bcl-2 expression in early gastric cancer of the elevated type. This elevated type probably resembles adenomatous polyps in the colon⁶⁵. Others demonstrated Bcl-2 expression in tumor cells of both intestinal type carcinoma as well as diffuse type gastric carcinoma, but mostly in a small percentage of the tumor cells^{66,67}. However, the expression of Bcl-2 seems to be higher in intestinal type gastric cancer compared to diffuse type cancer^{68,69,75,76}. 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^{70,83}.

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.

5. NF- κ B, iNOS AND COX-2 EXPRESSION IN INFLAMMATORY BOWEL DISEASES

NF- κ B activity varies in inflammatory bowel diseases (IBD). NF- κ B activation has been observed in macrophages in the lamina propria and in epithelial cells^{71,72}. IL-10, sulphasalazine and immunosuppressive drugs have been reported to inhibit NF- κ B activity in the mucosa of patients with Crohn's disease and ulcerative colitis⁷³⁻⁷⁶. iNOS is clearly expressed in epithelial cells of the inflamed gut⁷⁷⁻⁷⁹. 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^{80,81}. 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 alone⁸². 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- κ B 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.

6. APOPTOSIS AND APOPTOSIS-RELATED PROTEINS IN INFLAMMATORY BOWEL DISEASES

In normal intestinal epithelium apoptosis is observed in the crypt and at the luminal surface⁸³. Bcl-2 is expressed in the bases of crypts, whereas epithelial cells on the luminal surface express less Bcl-2^{84,85}. Bax, Bcl-xl and Bak expression are confined to areas of colonic epithelial cells of the luminal surface^{86,106}. There is a higher expression of Bak in the left colon compared to the right colon⁸⁷. Fas is strongly expressed in all epithelial cells of the normal colon throughout the crypt⁸⁸. In ulcerative colitis, apoptotic colonocytes are increased in number throughout the crypt⁸⁹. 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 this⁹⁰. 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 observed⁹⁶. Bcl-2 overexpression was observed in ulcerative colitis-associated neoplasia⁹¹. Compared to adenomas in areas involved in ulcerative colitis, Bcl-2 expression in ulcerative colitis-associated dysplastic lesions is less frequent⁹². The expression of Bcl-2 in ulcerative-colitis-associated colorectal cancer is significantly lower compared to that in sporadic colorectal cancer⁹³. 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 adenocarcinomas⁹⁴. Bax expression is reduced in ulcerative colitis compared to normal colonic mucosa⁹⁵. Other reports on expression of apoptosis-related proteins in IBD are mainly focussed on lamina propria T cells.

In summary: Reports on apoptosis in IBD 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.

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