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Review

Colorectal Cancer and Inflammatory Bowel Disease: Epidemiology, Risk Factors, Mechanisms of Carcinogenesis and Prevention Strategies

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Abstract. *Patients with long-standing ulcerative colitis and Crohn's disease have an increased risk of developing colorectal cancer and patients with small intestinal Crohn's disease are at increased risk of small bowel adenocarcinoma. Colorectal cancer appearing on the ground of inflammatory bowel disease is the result of a process which is believed to begin from no dysplasia progressing to indefinite dysplasia, low-grade dysplasia, high-grade dysplasia and finally to invasive adenocarcinoma, although colorectal cancer can arise without proceeding through each of these steps. Ulcerative colitis patients with total proctocolectomy and ileal pouch anal-anastomosis have a rather low risk of dysplasia in the ileal pouch, although the anal transition zone should be monitored periodically, especially if chronic pouchitis is present with associated severe villous atrophy. Concerning the risk factors predisposing to colorectal cancer in the setting of ulcerative colitis or Crohn's disease, it seems that the risk increases with longer duration and greater anatomic extent of colitis, the degree of inflammation, and the presence of primary sclerosing cholangitis and family history of colorectal cancer. Concerning the mechanisms of carcinogenesis, it is now well established that the molecular alterations responsible for sporadic colorectal cancer, namely chromosomal instability, microsatellite instability and hypermethylation, also play a role in colitis-associated colon carcinogenesis. Chemoprevention strategies include the administration of agents such as aminosalicylates, ursodeoxycholic acid, and possibly folic acid and statins, the exact role of which remains to be further elucitated.*

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Colorectal cancer (CRC) can develop on the grounds of inflammatory bowel disease (IBD), being the most common cancer among such patients. The three most important high-risk conditions for CRC are IBD and the hereditary syndromes of familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer syndrome (1).

The aim of this review is to discuss the epidemiology, risk factors and mechanisms of carcinogenesis in patients with IBD under the light of the current literature. The topics discussed are the epidemiological features of CRC in IBD, the established risk factors predisposing to colon carcinogenesis, and the molecular mechanisms involved in tumor development. Finally there is a brief discussion on the promising role of chemical agents that are currently under investigation in the prevention of CRC in patients with IBD.

Colorectal Cancer in IBD

Incidence of CRC in ulcerative colitis. The exact magnitude of the risk of cancer in ulcerative colitis (UC) is difficult to quantify due to biases and methodological errors. Early estimates of CRC incidence complicating UC often included a great proportion of patients who had more severe disease. Later population-based studies included more patients with left-sided disease or patients who had undergone colectomy and may thereby underestimate the true risk. IBD-related CRC is estimated to be responsible for less than 2% of all CRC appearing annually. In general, the risk of CRC begins to increase 8 or 10 years after the establishment of diagnosis (2-7). Depending on the study and country, the risk of developing CRC in patients with UC fluctuates between 0.9 to 8.8-fold and between 0.8 and 23-fold in patients with

pancolitis. Eaden *et al*. pooled the results of 116 studies involving almost 55,000 patients with UC (3). In this cohort of patients, 1,700 CRC were diagnosed. The probability of developing CRC 10 years after diagnosis was 2% , reaching the level of 8% after 20 years and 18% after 30 years (3).

It has been reported that in some countries, patients with UC have not been found to be at increased risk of CRC development. A Danish population-based cohort reported that the cumulative probability of CRC was 0.4% after 10 years, 1.1% after 20 years, and 3.1% at 30 years after a UC diagnosis (4). The calculated lifetime risk for development of CRC was 3.5% for UC patients compared with 3.7% for the Danish population. Nevertheless these results must be interpreted with caution. The low rate of CRC development could be related to the high rates of proctocolectomy (24% after 10 years and 32.4% after 25 years) reported in this country (4). Moreover, the systematic use of maintenance treatment with 5-ASA and the high rates of surveillance colonoscopy with proctocolectomy for dysplasia could all be important factors in reducing the incidence of CRC (5).

According to a recent analysis the risk of CRC has decreased over time in patients with UC, despite the low frequency of colectomies (6). The authors reported that the crude annual incidence rate of CRC in UC ranges from approximately 0.06% to 0.16% with a relative risk of 1.0-2.75.

The reduction in the incidence of CRC in UC patients may partly be explained by the more widespread use of maintenance therapy with 5-ASA compounds and surveillance colonoscopy (6).

Incidence of CRC in Crohn's disease. Studies on the incidence of CRC in Crohn's disease (CD) showed no statistically significant increase in cancer risk, probably due to the inclusion of all patients with CD irrespectively of the extent and duration of disease (8).

However, when patients with longstanding Crohn's colitis are considered, the risk of CRC is similar between Crohn's colitis and UC (9).

In a study from Denmark, no increase in the risk of CRC was found, either in the total group of patients or in patients with colonic CD exclusively, possibly due to maintenance treatment with 5-ASA preparations and early surgery in treatment failure (10).

In a meta-analysis of 34 studies involving 60,122 patients with CD, the relative risk for CRC was 2.59 and the relative risk for small bowel carcinoma was 28.4. In the same meta-analysis it was reported that the risk for CRC was higher in the USA and UK compared to Scandinavian countries with no evidence of temporal changes in the cancer incidence (11). It seems therefore that patients with extensive disease that has been present from a young age must be submitted to endoscopic surveillance. In another population-based study, it was found that the risk for CRC among patients with both UC and Crohn's colitis was approximately 2- to 3-fold greater than the general population and that the risk of rectal cancer was increased 2-fold in UC but not in Crohn's colitis (12). In the subgroup analysis of data patients with CD had an increased risk of colon cancer (relative risk 2.59) but not of rectal cancer (relative risk, 1.46). A significant association between the anatomic location of the diseased bowel and the risk of cancer in that segment was noticed. Patients who have only had small intestinal CD without colonic involvement are not considered to be at high risk for CRC.

Incidence of small bowel cancer in CD. The factors predisposing to small bowel carcinoma in CD patients are poorly defined, although strictured mucosa and fistulae might be involved (12-15). The risk of developing adenocarcinoma in the small intestine of patients with small bowel CD is increased, being approximately 10-12-fold greater than for the general population (16). According to other estimations, the risk is significantly increased (more than 60-fold), independently of age and gender. In a relevant study (17), one case of small bowel cancer was observed, compared with 0.3 expected cases. In another study, two cases were observed compared with 0.04 expected cases (50-fold increased occurrence) (18). In the UK, a 10-fold increased relative risk was observed (19). A Swedish study revealed a significantly increased number of small bowel cancer cases although the occurrence of CRC was not increased (20). Finally, a Canadian study also demonstrated an increased incidence of small bowel cancer (7). It must be emphasized however, that there are studies in which none of the patients developed cancer of the small intestine (10).

It could be argued that the risk for cancer in Crohn's colitis is equal to that in UC given equal extent and duration of disease (21).

Patients with ileoanal pouch anastomosis for ulcerative colitis. Patients with UC who have undergone proctocolectomy with ileal pouch anal anastomosis have a very small risk of dysplasia in the mucosa of the pouch itself. The risk is probably higher in patients with chronic pouchitis and associated severe villous atrophy (22), although opposite findings have been published (23). In a study involving 160 patients with an average surveillance time of 8.4 years, it was noticed that in 1,800 pouch-years of surveillance, only one patient developed low-grade dysplasia in the pouch (24). The risk of cancer is greater in the anal transitional mucosa between the pouch and the anal canal, particularly if a cuff of rectal mucosa has been left, and if the indications for the ileoanal pouch anastomosis were rectal dysplasia or cancer (25). However, opposite results have been published. In a relevant study ileal-pouch mucosal dysplasia was found to be uncommon, occurring in only 1 of 138 patients (26). Villous atrophy and colonic metaplasia were not observed.

It is believed that a program of periodic endoscopy with biopsies could be of value in patients with chronic pouchitis and severe villous atrophy or patients in whom the primary indication for ileoanal pouch anastomosis was dysplasia or cancer.

Risk Factors Associated with Increased CRC in UC

Inflammation. Inflammation is an important risk factor for the development of CRC, and severity of inflammation has been directly linked to CRC risk (27). The epidemiological data clearly support this assumption. It is generally accepted that there is a strong link between endoscopic or histologic score of inflammation and CRC or dysplasia. Increased risk has also been linked to postinflammatory polyps and strictures, while macroscopically normal colon is not associated with neoplastic risk (28). In a recent study, a significant association between inflammation and progression to advanced neoplasia was demonstrated (29). The hypothesis that inflammation predisposes cancer development is further supported by the fact that CRC risk increases with longer duration of colitis, greater extent of colitis, the concomitant presence of other inflammatory manifestations such as primary sclerosing cholangitis, and the fact that certain drugs used to treat inflammation may prevent the development of CRC.

Concerning the mechanisms of carcinogenesis, it has been hypothesized that inflammation results in neoplastic transformation by enhancing epithelial cell turnover in the colonic mucosa. Mucosal biopsies from patients with UC demonstrate higher rates of mitosis and apoptosis, especially in areas of active inflammation. On the other hand mutagenic assault and sustained DNA damage appear to drive the whole process. Several inflammation-associated genes such as *cyclooxygenase-2, nitric oxide synthase-2* and the *interferon-inducible gene 1–8U* are increased in inflamed mucosa and remain elevated in colonic neoplasms (30). Toll-like receptor4 (TLR4) signalling is critical for colon carcinogenesis in chronic colitis. A recent study revealed that TLR4 is overexpressed in human and murine inflammation-associated colorectal neoplasia and that TLR4-deficient mice were protected from colon carcinogenesis. TLR4 activation appears to promote the development of colitis-associated CRC by enhancement of Cox-2 expression and increased EGFR signalling (31). Altered expression of intestinal epithelial tight junction proteins might contribute to neoplastic progression.

It has been reported that claudin-1 and claudin-2 expression is elevated in active IBD. Claudin-1 and claudin-2 expression correlate positively with inflammatory activity. Beta-catenin is also activated in IBD. Beta-catenin transcriptional activity is elevated in chronic injury and this may contribute to increased claudin-1 and claudin-2 expression. Thus increased claudin-1 and -2 expression may be involved at the early stages of transformation in IBD-associated neoplasia (33).

Oxidative stress. Oxidative stress and oxidative cellular damage are important features of UC. The activities of phagocytic leukocytes are increased in UC patients, resulting in enhanced generation of pro-oxidant molecules. Oxidative stress in inflamed tissue can pave the way for malignant tumors (34) and nitric oxide may contribute to the pathogenesis of CRC (35). Enhanced formation of 8-nitroguanine, representative of nitrative damage to nucleobases, has been detected in manyinflammatory conditions including IBD.

Cytokines. It is now becoming clear that cytokines and growth factors released during inflammation may influence the carcinogenesis process. Interleukin-6 and -23, which play key roles in the induction and maintenance of gut inflammation in IBD, have been recently shown to influence the development and growth of colitis-associated CRC (36, 37).

Nuclear factor-kappaB (NF-κB) regulates the expression of various cytokines and modulates the inflammatory processes in IBD (38). Actually, several lines of evidence suggest that activation of NF-κB may cause cancer. It has been observed that NF-κB genes can be oncogenes, and that NF-κB controls apoptosis, cell-cycle progression and proliferation, and cell differentiation (39).

Tumor necrosis factor-alpha (TNF-α) promotes cancer development through induction of gene mutations. It has been shown that TNF-alpha treatment in cultured cells resulted in increased gene mutations, gene amplification, micronuclei formation, and chromosomal instability (40). TNF-alpha can cause DNA damage through reactive oxygen species and could lead to increased malignant transformation of mouse embryo fibroblasts.

Duration of UC. Duration of UC is among the most important risk factors for CRC development. Retrospective data show a 5.4% CRC incidence rate among patients with pancolitis. In a cohort of patients with IBD for whom the median time from diagnosis of IBD to CRC was 17 years, 21% of the tumors developed before 10 years of disease (41). This observation must be taken into account when planning a surveillance programm for patients with UC.

Extent of UC. Extent of colitis is an independent risk factor for the development of CRC. The more colonic surface that is involved with colitis the greater the CRC risk. Patients with proctitis had a 70% higher risk than that expected in the general population. A meta-analysis showed that the prevalence of CRC among patients with extensive UC was 5.4% (42), although there are no unified criteria concerning "extent of colitis" as different studies have used different

radiologic, endoscopic and histological criteria. It is suggested that histological detection of extent of colitis is probably the most important element that must be taken into account when estimating the risk of CRC development.

Other factors. Other factors that have shown to contribute to CRC development include family history of CRC, smoking, and the presence of pseudopolyps, primary sclerosing cholangitis and backwash ileitis (43-45).

A family history of CRC increases the risk of CRC by at least two-fold as compared to patients with UC without positive family history for CRC. The positive family history of CRC remained independently associated with CRC risk even after controlling for variables such as primary sclerosing cholangitis, surveillance colonoscopy, presence of pseudopolyps, mesalazine therapy and use of NSAIDs (46, 47).

Primary sclerosing cholangitis (PSC) appearing on the ground of UC increases the risk of CRC by 4.8-fold compared with patients with UC without PSC. Patients with PSC-IBD are at especially high risk for CRC and dysplasia. Therefore, patients with PSC-IBD should be enrolled in colonoscopic surveillance program regardless of UC duration (48, 49).

Other risk factors such as *backwash ileitis* (50) and young age at diagnosis have been implicated although their exact role remains to be determined. For young age at diagnosis, a meta-analysis suggested that the overall annual incidence rate of 0.6% in studies restricted to pediatric patients was only numerically higher than that calculated for adults (0.3%) (3). Some authors suggest that younger age at UC onset is an independent risk factor.

Smoking reduces the risk of CRC in UC by 50% but increases the risk of CRC in CD 4-fold, perhaps reflecting the opposite effect smoking has on inflammation in each disease (42).

Pseudopolyps increase the risk of CRC in UC by 2.5-fold perhaps either as a marker of more severe inflammation in the past, or because they may obscure the sensitivity of surveillance colonoscopy (43).

Clinical characteristics of CRC arising on the ground of UC and CD (colitis associated CRC). Colitis-associated CRC arising in patients with IBD has several distinguishing clinical features compared with sporadic CRC. It usually affects individuals at a younger age than the general population and demonstrates a more proximal distribution in the colon. There is also a higher rate of two or more synchronous primary CRCs. Colitis-associated CRC progresses to invasive adenocarcinoma from flat and nonpolypoid dysplasia more frequently than sporadic CRC. An *hMSH2* mutation could be more frequent in UC patients who developed high-grade dysplasia and cancer than in those who did not. Colitis-associated CRC more often have a higher proportion of mucinous and signet ring cell histology and there is background of chronic inflammation in colitis (42).

It has been suggested that telomere shortening is implicated in cancer and aging and might link these two biologic events. An elegant study revealed that colonocyte telomeres shorten with age almost twice as rapidly in UC patients as in normal controls and that this shortening occurs within approximately 8 years of disease duration (51). Gamma-H2AX intensity is higher in colonocytes of UC patients than in controls and is not dependent on age or telomere length. Shortening of telomeres might explain the increased and earlier risk of CRC in UC.

Concerning CD, it seems that it worsens the prognosis of CRC particularly in cases with regional spread. Patients with CRC developing on the ground of CD are usually younger although the stage of CRC does not differ.

Dysplasia in UC

The incidence of dysplasia in UC is difficult to determine and varies significantly in different studies. In a relevant study the cumulative probability of developing dysplasia or CRC was 7.7% at 20 years and 15.8% at 30 years (52). In a meta-analysis the cancer incidence was 14 of 1,000 person-years' duration and the incidence of any advanced lesion was 30 of 1,000 person-years' duration. When low-grade dysplasia is detected on surveillance there is a 9-fold risk of developing cancer and 12-fold risk of developing any advanced lesion (53). Among patients with low-grade dysplasia who undergo immediate colectomy, 19% will already harbor concurrent CRC or high-grade dysplasia and 29%-54% will develop advanced neoplasia over the next 5 years (54, 55). High-grade dysplasia carries a 43% risk of synchronous malignancy (56).

Dysplasia arising on the grounds of UC is usually patchy and it may precede the development of carcinoma. Flat dysplasia is detected microscopically in random biopsies from "normal" mucosa. Its detection therefore depends on adequate sampling, or chromoendoscopy which highlights suspicious lesions thus permitting targeted biopsies.

The management of patients with dysplasia and IBD depends on the degree of dysplasia. A patient with multifocal flat low-grade dysplasia or repetitive flat low-grade dysplasia should be encouraged to undergo prophylactic proctocolectomy.

Disease;associated lesion or mass (DALM). DALM is an important endoscopic finding in patients with UC. A 40% possibility for the lesion to be a carcinoma has been reported and the possibility increases if the dysplasia is of high degree. DALM is an indication for colectomy irrespective of the grade of dysplasia in preoperative biopsies. However, not all types of polypoid dysplasia in patients with IBD carry the same significance. Some polyps may be adenomatous polyps unrelated to colitis and can be managed by endoscopic polypectomy (57).

Molecular pathogenesis of sporadic CRC. Sporadic CRC arises as a result of genomic instability. The two main types of genomic instability contributing to colon carcinogenesis are chromosomal instability (CIN) and microsatellite instability (MSI), accounting for 85 and 15% of sporadic CRC, respectively. The importance of the global genomic status (MSI status and CIN status) and epigenomic status [CpG island methylator phenotype (CIMP) status] in CRC seems to be very high because it could characterize the clinical, pathological and biological characteristics of CRC (58).

CIN results in abnormal segregation of chromosomes and aneuploidy. As a result, loss of chromosomal material often occurs, such as *APC* and *p53*. Loss of *APC* function is an early event in sporadic CRC pathogenesis. The great majority of all sporadic CRC show loss of *APC* function, usually through protein truncation or allelic loss. Among the 15% of colon carcinomas that retain wild-type *APC*, point mutations have been found in β-catenin that change one of the four serine/threonine residues in the *N*-terminus. These mutations thus render β-catenin refractory to phosphorylation by glycogen synthase kinase-3b, increasing free β-catenin levels. CIN is associated with a worse prognosis in CRC, and should be evaluated as a prognostic marker, together with MSI status, in all clinical trials (59).

Once a sporadic adenoma forms, induction of *k-ras* oncogene and loss of function of tumor suppressor genes on chromosome 18q in the region of the deletion in colon cancer (*DCC*) and in pancreatic cancer (*DPC4*) genes occur. Loss of *p53* gene function occurs late and is believed to be the defining event that drives the adenoma to carcinoma.

The MSI pathway involves the primary loss of function of genes (*hMSH2, hMLH1, hPMS1, hPMS2, hMSH6, hMLH3*) that repair DNA base-pair mismatches. DNA mismatch repair deficiency results in strong mutator phenotype and MSI. MSI is characterized by length alterations within simple repeated sequences, microsatellites. Cancers with MSI exhibit many differences in genotype and phenotype relative to cancers without MSI, irrespective of their hereditary or sporadic origins (60).

Epigenetic alterations can also contribute to altered gene expression in colon carcinogenesis. The CpG island methylator phenotype occurs when cytosines in the promoter region of genes become extensively methylated. A number of human cancer genes that contain hypermethylation of promoter CpG islands have been identified including *hMLH*, and *E-cadherin* (61).

Molecular pathogenesis of colitis-associated CRC. Whereas adenomatous polyps are considered to be the major precursor of sporadic CRC, Colitis-associated CRC involves the development of epithelial dysplasia. The major carcinogenic pathways that lead to sporadic CRC, namely CIN, MIN, and hypermethylation, also occur in colitis-associated CRC (62, 63). The neoplastic transformation in IBD is thought to be similar to the adenoma-carcinoma sequence in sporadic CRC. However, unlike sporadic CRC in colitic mucosa, it is not unusual for dysplasia or cancer to be multifocal.

CIN and MSI in colitis-associated CRC appeared with the same frequency (85% CIN, 15% MSI) as in sporadic CRC (64-66). Distinguishing features of colitis-associated CRC, however, are differences in the timing and frequency of these alterations. For example, *APC* loss of function, considered to be a very common early event in SCC, is much less frequent and usually occurs late in the colitis-associated dysplasia-carcinoma sequence. Conversely, *p53* mutations in sporadic CRC usually occur late in the adenoma-carcinoma sequence, whereas in patients with colitis, *p53* mutations occur early and are often detected in mucosa that is non-dysplastic or indefinite for dysplasia. The carcinogenesis process in UC-associated CRC is associated with the MSI pathway through *TGFβRII* mutation by a dysfunction of the mismatch repair system (67).

CIN is the most frequent form of genomic instability in colitis-associated cancers. Monosomies and polysomies are frequently conserved between non-dysplastic and dysplastic epithelium, and between dysplasia and cancer. Several studies revealed an increasing frequency of chromosome losses or gains from non-dysplastic epithelia to dysplasia and carcinoma. No chromosomal anomalies were detected in UC patients at low risk for carcinoma development. CIN is an early event in the progression to colitis-associated CRC, and may contribute to widespread aneuploidy and eventually dysplasia.

The frequency of MIN in colitis-associated CRC fluctuates between 8 and 21% while in dysplastic regions its frequency fluctuates between 13 to 19%. It must be emphasized that MIN could also be detected in inflamed and regenerative epithelia. The relatively high frequency of MIN in non-dysplastic, inflamed epithelia suggests that MIN may be associated with chronic inflammation and oxidative stress.

Methylation is an important mechanism contributing to the genetic alterations in colitis-associated CRC. Methylation of CpG islands in several genes seems to precede dysplasia and is more widespread throughout the mucosa of UC patients (68).

Aneuploidy in UC. Usually measured by flow-cytometry on fresh biopsies, aneuploidy occurs in approximately 33% of patients with long-standing UC. Aneuploidy correlates directly to dysplasia; 20-50% of dysplastic lesions and 50-90% of cancers demonstrate aneuploidy (69). Aneuploidy although seems to be a useful marker for developing neoplastic lesions, may not be universally present and may not be necessary for progression to cancer.

Regions of aneuploidy in the large bowel of UC patients are frequently associated with dysplasia, and precede the appearance of histological changes. Aneuploidy is more frequent in patients with disease duration of more than 10

years, but aneuploidy has also been detected in colon samples of patients not though to be at risk for developing colorectal neoplasia (70).

Despite the usefulness of flow-cytometry in assessing patients with IBD, it is not universally applied in the follow-up of patients with long-standing UC, probably because the method is not available everywhere.

Tumor suppressor gene alteration in colitis-associated CRC and dysplasia. A genetic and epigenetic model, involving both the activation of oncogenes such as ras and the inactivation of tumor suppressor genes such as *APC* and *p53* in the development of the majority of CRC has been proposed (71).

The frequency and timing of occurrence of these genetic alterations differ remarkably in sporadic CRC and colitis-associated CRC.

p53 oncogene. *p53* protein accumulation, which is associated with *p53* mutation as well as wild-type *p53* overexpression, is frequently detected in UC dysplasia and carcinoma (69). *p53* alterations have also been detected in non-dysplastic, regenerative epithelium and precede the development of UC-associated dysplasia and carcinoma. *p53* allelic loss was observed in nearly 70% of CRC and 45% of dysplastic lesions. Almost 70% of colitis-associated CRC and 20% of dysplastic lesions analyzed contained *p53* mutations. The percentage of *p53* mutation-containing samples is increasing with the morphological progression to carcinoma.

Rb tumor suppressor gene. The tumor suppressor gene *Rb* is often mutated or lost in epithelial tumors. *Rb* loss of heterozygosity (LOH) was detected in 25% of UC patients with carcinoma, DALM, or dysplasia. Overall, *Rb* LOH has been observed in 30% of colitis-associated CRC and 20% of dysplasias studied. (72). Studies of the *p16* locus (9p21) showed a high rate of *p16* loss in dysplasias as well as in inflamed epithelia and adjacent normal epithelia. Methylation of the *p16* promoter has been observed in 75% of dysplastic or cancerous lesions. Alterations of *p16* may be important early markers of carcinogenic progression in UC patients.

APC protooncogene. Mutant APC proteins have been detected in 17% of UC-associated dysplasia- or carcinoma-bearing patients (73). Nearly 30% of dysplastic lesions and 59% of cancers exhibited *APC* LOH. In contrast to sporadic CRC carcinogenesis, *APC* alteration is a relatively late event in the dysplasia sequence and occurs in a subset of UC-associated colorectal carcinomas (73).

Deleted in colon cancer tumor suppressor gene (DCC). Losses at chromosome 18q are relatively rare events during UC-associated carcinogenesis (74). LOH of 18q, the site of the putative DCC was observed in 12% of cancers and 33% of the dysplasia lesions, and was not detected in non-dysplastic, inflamed epithelia.

K-ras oncogene. Studies indicated a lower but significant frequency of *K-ras* mutation (75). Overall, *K-ras* mutation was detected in 24% of UC-associated lesions. *K-ras* mutation seems to play a significant role in the later stages of UC-associated carcinogenesis.

Mismatch repair genes. Alterations in mismatch repair genes may contribute to the MIN^+ subset of UC-associated carcinomas (76).

CARD2/NOD2 may be a genetic factor that predispose to sporadic CRC. The type A (estrogen receptor) occurs as a function of age and is found on normal colon and CRC. Type G however is cancer associated and leads to silencing of genes such as *hMLH1, p16* and *p14*. Fujii *et al*. using methylation specific PCR found methylation of the *OR* gene in 77% of non-neoplastic epithelium in UC with neoplasia, but only in 24% without neoplasia (77). Analysis of *OR* gene methylation could be a marker for identifying patients at risk for developing CRC.

In summary, genetic instability in the stroma, especially regarding tumor suppressor gene markers, may play an important role in early-phase, UC-associated tumorigenesis (78). To date, only four molecular markers named aneuploidy, p53, MSI, and mucin-associated sial-Tn antigen, have been evaluated. However, there is potential for molecular diagnostics to enhance the management of patients with long-standing IBD (63). Olliner *et al*. described 699 genes exhibiting altered expression with dysplasia development, by using microarrays in UC patients without dysplasia, UC with DALM, and UC with adenocarcinoma (79), thus emphasizing the difficulties in applying these alterations in everyday clinical practice.

Normal bacteria flora in colitis-associated CRC. Although the mechanisms of bacteria-induced carcinogenesis remain unclear, it is well established that the normal bacterial flora is a prerequisite for the development of inflammation and inflammation-related CRC and that bacterial flora potentiates tumor formation independently of inflammation (80).

Specific bacterial infection promotes colonic tumor formation in genetically susceptible mice. Unusual bacterial infection might be associated with CRC (80). The capacity of bacteria flora to cause inflammation and cancer depends on the activity of many constituents of the flora rather than on a single species. This capacity might be enhanced or reduced as a result of significant changes in the species diversity and abundance within the flora.

However, no human counterparts of the critical defects artificially created in rodents have been identified. It is plausible that the defects or polymorphisms of human genes

could in collaboration with a normal bacterial flora lead to cancer in a patient over a period of decades.

Chemoprevention of colitis-associated CRC. Chemoprevention refers to the use of natural or synthetic chemical agents to reverse, suppress or delay the process of carcinogenesis. Generally, data on chemoprevention on patients with long-standind UC are not clearly definitive, referring to either retrospective case-control or cohort studies.

The agents studied so far, are analyzed below.

Aspirin and NSAIDs. Aspirin and other NSAIDs markedly reduce the incidence of and mortality from sporadic CRC. Since many patients with IBD take NSAIDs in the form of 5-ASA it would be possible that aminosalicylates might also be protective the available data suggest that this may be so (43).

Folic acid. In the setting of sporadic CRC, low folate intake has been associated with an increased risk for developing CRC and colon adenomas. Concerning the role of folic acid on colon carcinogenesis in patients with UC a beneficial effect albeit insignificant has consistently been demonstrated in all studies. It is of interest that folic acid also seems to exert also a protective effect on patients with UC and concomitant primary sclerosing cholangitis.

The mechanism of action is possibly related to maintainance of the normal DNA methylation process and the steady-state levels of DNA precursors (81). It has been reported that in IBD patients with normal homocysteinemia, the increase in carcinogenic risk is negligible. Conversely, in patients with hyperhomocysteinemia, folate deficiency may be associated with increased colorectal carcinogenesis in IBD patients (82). Since folic acid is quite safe and inexpensive, the administration of this compound should be considered for CRC risk reduction in patients with longstanding IBD.

Ursodeoxycholic acid (UDCA). In animal models of colon carcinogenesis, UDCA inhibits carcinogenesis. This effect could be due to reduction of colonic concentration of the secondary bile acid deoxycholic acid (83). UDCA also has antioxidant effect. A study performed on UC patients with concomitant primary sclerosing cholangitis demonstrated that UDCA use was associated with decreased prevalence of colonic dysplasia (84). UDCA use was associated with a significant protection against the development of dysplasia and cancer. However, it is not clear whether UDCA can prevent neoplastic progression in UC patients without primary sclerosing cholangitis (85,86). In UC patients with primary sclerosing cholangitis, UDCA did not reduce the risk of developing cancer or dysplasia (87). However, UDCA may reduce mortality. UDCA may prevent further progression of manifest low-grade dysplasia in colorectal IBD. It seems that prolonged treatment or an increased dose may be needed to fully exploit the chemopreventive properties of this compound.

Immunomodulators. There are insufficient data regarding the chemopreventive role of immunomodulators in CRC development in IBD patients. There is no recommendation on whether patients who require immunomodulator therapy should continue their 5-ASA therapies. It seems that treatment with 6-mercaptopurine is not chemopreventive (88). No reduction in the risk of dysplasia or CRC could be anticipated by the use of immunosuppressants. In UC patients with no initial history of dysplasia, 6MP/AZA use appears to have little or no effect on the rate of neoplastic transformation in the colon.

Recent observations identified TNF-α as a crucial mediator of the initiation and progression of colitis-associated CRC. In an experimental study (89), the authors noticed that treating wild-type mice with azoxymethane and dextran sulfate sodium resulted in increase of TNF-α expression and the number of infiltrating leukocytes expressing the receptor p55 (TNF-Rp55) in the lamina propria and submucosal regions of the colon. Consequently, multiple colonic tumors developed. Mice lacking TNF-Rp55 and treated with azoxymethane and dextran sulfate sodium showed reduced mucosal damage, reduced infiltration of macrophages and neutrophils, and attenuated subsequent tumor formation. Furthermore, administration of etanercept, a TNF-α antagonist, to wild-type mice after treatment with azoxymethane and dextran sulfate sodium markedly reduced the number and size of tumors and reduced colonic infiltration by neutrophils and macrophages. This suggests that targeting TNF-α may be useful in treating CRC in patients with UC. This assumption, however, contrasts with the fear of gastroenterologists concerning the development of lymphomas and other solid tumors in patients with IBD receiving biological agents for a long period of time.

Calcium. Limited data concerning the influence of calcium on the CRC development in patients with UC exist. No conclusions can be drawn from the data. Although there are indications suggesting that calcium supplementation might prevent the formation of colorectal adenomatous polyps, this does not constitute sufficient evidence to recommend the general use of calcium supplements to prevent CRC (90).

Statins. Again, there are limited data concerning the role of statins on CRC development. In a population-based case-control study of patients who had diagnosis of CRC in northern Israel between 1998 and 2004, statin therapy was associated with a modest reduction in CRC in the non-IBD population, but a substantial 94% risk reduction in patients with IBD was observed in a subset analysis of a small number of patients (91).

There are however, experimental studies describing encouraging results. Treatment with fluvastatin in mice with UC resulted in a reduction of colitis and carcinogenesis, shown by inhibition of the decrease in colorectal length and the incidence of colorectal dysplasia, with a reduction in anti-8-hydroxy-2'-deoxyguanosine antibody (a biological marker of *in vivo* oxidative DNA damage)-positive cells of the colorectal mucosa and the activity of the DNA-synthesizing enzyme thymidine kinase in colorectal tissues (92).

In an experimental colitis-associated CRC model, simvastatin significantly reduced tumor development by induction of apoptosis and suppression of angiogenesis. In the xenograft model, tumors from animals treated with simvastatin had smaller volumes, larger necrotic areas, lower expression of VEGF and higher apoptotic scores (93). All the available experimental evidence suggests that statins could be a potential chemopreventive and therapeutic agent for colitis-associated CRC. However, further clinical studies for a long period of time are needed in order to clarify the role of statins in the prevention of CRC in man.

Mesalamine. Most of the available studies support the use of mesalazine for the prevention of UC-related CRC. A dose of at least 1.2 g/d is most likely to be effective (94). Concerning the mode of action of 5-ASA, the drug seems to decrease the inflammation, block the transcription of NF κB by up-regulating or stabilizing its natural inhibitor IκB, and induces apoptosis. Moreover, it seems to have antiproliferative effects on human colon cancer cell lines, it has a significant antioxidant effect and causes reduction of aberrant crypt foci (43).

Corticosteroids. There are a small number of studies on corticosteroid usage, some of which have found a beneficial effect, on occasions the difference being statistically significant. However, due to serious adverse effects, corticosteroids should not be prescribed for this indication (43). No relevant studies are available for budesonide.

Conclusion

IBD clearly predisposes to CRC development although the risk differs in different parts of the world. Cancer follows the sequence of no dysplasia, indefinite dysplasia, low-grade dysplasia, high-grade dysplasia and carcinoma. Low-grade dysplasia can progress to CRC without the intermediate stage of high-grade dysplasia. Similar to sporadic CRC, colitis-associated CRC is a consequence of sequential episodes of somatic genetic mutation and clonal expansion. In IBD, neoplastic lesions arise within areas of the mucosa that have been involved with colonic inflammation. However, in some countries, including Greece, the incidence of CRC in patients with IBD appears to be relatively low. A balance between cell proliferation and apoptosis may partly explain this epidemiological feature. Knowledge of the mechanisms of carcinogenesis could identify patients at high risk for development of CRC. In the near future, some chemopreventive agents could play a role in reducing the incidence of CRC in IBD patients. The future looks promising with respect to new developments in the management of cancer risk in IBD.

References

1 Forbes GM: Colorectal cancer screening tests: pros and cons, and for whom? Expert Rev Gastroenterol Hepatol *2*: 197-205, 2008.

2 Ekbom A, Helmick C, Zack M and Adami HO: Ulcerative colitis and colorectal cancer. A population-based study. N Engl J Med *323*: 1228-1233, 1990.

3 Eaden JA, Abrams KR and Mayberry JF: The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut *48*: 526-535, 2001.

4 Winther KV, Jess T, Langholz E, Munkholm P and Binder V: Long-term risk of cancer in ulcerative colitis: a population-based cohort study from Copenhagen County. Clin Gastroenterol Hepatol *2*: 1088-1095, 2004.

5 Langholz E, Munkholm P, Davidsen M and Binder V: Colorectal cancer risk and mortality in patients with ulcerative colitis. Gastroenterology *103*: 1444-1451, 1992.

6 Lakatos PL and Lakatos L: Risk for colorectal cancer in ulcerative colitis: Changes, causes and management strategies World J Gastroenterol *14*: 3937-3947, 2008.

7 Bernstein CN, Blanchard JF, Kliewer E and Wajda A: Cancer risk in patients with inflammatory bowel disease: a population-based study. Cancer *91*: 854-862, 2001.

8 Mellemkjaer L, Johansen C, Gridley G, Linet MS, Kjaer SK and Olsen JH: Crohn's disease and cancer risk (Denmark). Cancer Causes Control *11*: 145-150, 2000

9 Sachar DB: Cancer in Crohn's disease: dispelling the myths. Gut *35*: 1507-1508, 1994.

10 Jess T, Winther KV, Munkholm P, Langholz E and Binder V: Intestinal and extra-intestinal cancer in Crohn's disease: follow-up of a population-based cohort in Copenhagen County, Denmark. Aliment Pharmacol Ther *19*: 287-93, 2004.

11 Canavan C, Abrams KR and Mayberry J: Meta-analysis: colorectal and small bowel cancer risk in patients with Crohn's disease. Aliment Pharmacol Ther *23*: 1097-1104, 2006.

12 von Roon AC, Reese G, Teare J, Constantinides V, Darzi AW and Tekkis PP: The risk of cancer in patients with Crohn's disease. Dis Colon Rectum *50*: 839-55, 2007.

13 Sjodahl RI, Myrelid P and Soderholm JD: Anal and rectal cancer in Crohn's disease. Colorectal Dis *5*: 490-495, 2003.

14 Ky A, Sohn N, Weinstein MA and Korelitz BI: Carcinoma arising in anorectal fistulas of Crohn's disease. Dis Colon Rectum *41*: 992-996, 1998.

15 Connell WR, Sheffield JP, Kamm MA, Ritchie JK, Hawley PR and Lennard-Jones JE: Lower gastrointestinal malignancy in Crohn's disease. Gut *35*: 347-352, 1994.

16 Solem CA, Harmsen WS, Zinsmeister AR and Loftus EV Jr: Small intestinal adenocarcinoma in Crohn's disease: a case-control study. Inflamm Bowel Dis *10*: 32-35, 2004.

17 Ekbom A, Helmick C, Zack M and Adami HO: Extracolonic malignancies in inflammatory bowel disease. Cancer *67*: 2015-2019, 1991.
18 Munkholm P, Langholz E, Davidsen M and Binder V: Intestinal cancer risk and mortality in patients with Crohn's disease. Gastroenterology *105*: 1716-1723, 1993.
19 Fielding JF, Prior P, Waterhouse JA and Cooke WT: Malignancy in Crohn's disease. Scand J Gastroenterol *7*: 3-7, 1972.
20 Persson PG, Karlén P, Bernell O, Leijonmarck CE, Broström O, Ahlbom A and Hellers G: Crohn's disease and cancer: a population-based cohort study. Gastroenterology *107*: 1675-1679, 1994.
21 Friedman S: Cancer in Crohn's disease. Gastroenterol Clin North Am *35*: 621-639, 2006.
22 Gullberg K, Ståhlberg D, Liljeqvist L, Tribukait B, Reinholt FP, Veress B and Löfberg R: Neoplastic transformation of the pelvic pouch mucosa in patients with ulcerative colitis. Gastroenterology *112*: 1487-1492, 1997.
23 Thompson-Fawcett MW, Marcus V, Redston M, Cohen Z and McLeod RS: Risk of dysplasia in long-term ileal pouches and pouches with chronic pouchitis. Gastroenterology *121*: 275-281, 2001.
24 Herline AJ, Meisinger LL, Rusin LC, Roberts PL, Murray JJ, Coller JA, Marcello PW and Schoetz DJ: Is routine pouch surveillance for dysplasia indicated for ileoanal pouches? Dis Colon Rectum *46*: 156-159, 2003.
25 O'Riordain MG, Fazio VW, Lavery IC, Remzi F, Fabbri N, Meneu J, Goldblum J and Petras RE: Incidence and natural history of dysplasia of the anal transitional zone after ileal pouch-anal anastomosis: results of a five-year to ten-year follow-up. Dis Colon Rectum *43*: 1660-1665, 2000.
26 Nilubol N, Scherl E, Bub DS, Gorfine SR, Marion J, Harris MT, Kornbluth A, Lichtiger S, Rubin P, George J, Chapman M, Harpaz N, Present D and Bauer JJ: Mucosal dysplasia in ileal pelvic pouches after restorative proctocolectomy. Dis Colon Rectum *50*: 825-831, 2007.
27 Itzkowitz SH and Yio X: Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J Physiol Gastrointest Liver Physiol *287*: G7-17, 2004.
28 Rutter M, Saunders B, Wilkinson K, Rumbles S, Schofield G, Kamm M, Williams C, Price A, Talbot I and Forbes A: Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. Gastroenterology *126*: 451-459, 2004.
29 Gupta RB, Harpaz N, Itzkowitz S, Hossain S, Matula S, Kornbluth A, Bodian C and Ullman T: Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. Gastroenterology *133*: 1099-1105, 2007.
30 Clevers H: Colon cancer–understanding how NSAIDs work. N Engl J Med *354*: 761-763, 2006.
31 Fukata M, Chen A, Vamadevan AS, Cohen J, Breglio K, Krishnareddy S, Hsu D, Xu R, Harpaz N, Dannenberg AJ, Subbaramaiah K, Cooper HS, Itzkowitz SH and Abreu MT: Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. Gastroenterology *133*: 1869-1881, 2007.
32 Zeissig S, Bürgel N, Günzel D, Richter J, Mankertz J, Wahnschaffe U, Kroesen AJ, Zeitz M, Fromm M and Schulzke JD: Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. Gut *56*: 61-72, 2007
33 Weber CR, Nalle SC, Tretiakova M, Rubin DT and Turner JR: Claudin-1 and claudin-2 expression is elevated in inflammatory bowel disease and may contribute to early neoplastic transformation. Lab Invest *88*: 1110-1120, 2008
34 Roessner A, Kuester D, Malfertheiner P and Schneider-Stock R: Oxidative stress in ulcerative colitis-associated carcinogenesis. Pathol Res Pract *204*: 511-524, 2008.
35 Sawa T and Ohshima H: Nitrative DNA damage in inflammation and its possible role in carcinogenesis. Nitric Oxide *14*: 91-100, 2006.
36 Fantini MC and Pallone F: Cytokines: from gut inflammation to colorectal cancer. Curr Drug Targets *9*: 375-380, 2008.
37 Atreya R and Neurath MF: Involvement of IL-6 in the pathogenesis of inflammatory bowel disease and colon cancer. Clin Rev Allergy Immunol *28*: 187-196, 2005.
38 Schottelius AJ and Dinter H: Cytokines, NF-κB, microenvironment, intestinal inflammation and cancer. Cancer Treat Res *130*: 67-87, 2006.
39 Tang W, Wang W, Zhang Y, Liu S, Liu Y and Zheng D: Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced chemokine release in both TRAIL-resistant and TRAIL-sensitive cells *via* nuclear factor kappa B. FEBS J *276*: 581-593, 2009.
40 Yan B, Wang H, Rabbani ZN, Zhao Y, Li W, Yuan Y, Li F, Dewhirst MW and Li CY: Tumor necrosis factor-alpha is a potent endogenous mutagen that promotes cellular transformation. Cancer Res *66*: 11565-11570, 2006.
41 Brackmann S, Andersen SN, Aamodt G, Langmark F, Clausen OP, Aadland E, Fausa O, Rydning A and Vatn MH: Relationship between clinical parameters and the colitis-colorectal cancer interval in a cohort of patients with colorectal cancer in inflammatory bowel disease. Scand J Gastroenterol *44*: 46-55, 2009.
42 Xie J and Itzkowitz SH: Cancer in inflammatory bowel disease World J Gastroenterol *14*: 378-389, 2008.
43 Velayos FS, Loftus EV Jr, Jess T, Harmsen WS, Bida J, Zinsmeister AR, Tremaine WJ and Sandborn WJ: Predictive and protective factors associated with colorectal cancer in ulcerative colitis: a case-control study. Gastroenterology *130*: 1941-1949, 2006.
44 Siegel CA and Sands BE: Risk factors for colorectal cancer in Crohn's colitis: a case-control study. Inflamm Bowel Dis *12*: 491-496, 2006.
45 Pinczowski D, Ekbom A, Baron J, Yuen J and Adami HO: Risk factors for colorectal cancer in patients with ulcerative colitis: a case-control study. Gastroenterology *107*: 117-120, 1994.
46 Nuako KW, Ahlquist DA, Mahoney DW, Schaid DJ, Siems DM and Lindor NM: Familial predisposition for colorectal cancer in chronic ulcerative colitis: a case-control study. Gastroenterology *115*: 1079-1083, 1998.
47 Askling J, Dickman PW, Karlén P, Broström O, Lapidus A, Löfberg R and Ekbom A: Family history as a risk factor for colorectal cancer in inflammatory bowel disease. Gastroenterology *120*: 1356-1362, 2001.
48 Soetikno RM, Lin OS, Heidenreich PA, Young HS and Blackstone MO: Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis: a meta-analysis. Gastrointest Endosc *56*: 48-54, 2002.
49 Broome U and Bergquist A: Primary sclerosing cholangitis, inflammatory bowel disease, and colon cancer. Semin Liver Dis *26*: 31-41, 2006.

50 Heuschen UA, Hinz U, Allemeyer EH, Stern J, Lucas M, Autschbach F, Herfarth C and Heuschen G: Backwash ileitis is strongly associated with colorectal carcinoma in ulcerative colitis. Gastroenterology *120*: 841-847, 2001.

51 Risques RA, Lai LA, Brentnall TA, Li L, Feng Z, Gallaher J, Mandelson MT, Potter JD, Bronner MP and Rabinovitch PS: Ulcerative colitis is a disease of accelerated colon aging: evidence from telomere attrition and DNA damage. Gastroenterology *135*: 410-418, 2008.

52 Lennard-Jones JE, Melville DM, Morson BC, Ritchie JK and Williams CB: Precancer and cancer in extensive ulcerative colitis: findings among 401 patients over 22 years. Gut *31*: 800-806, 1990.

53 Thomas T, Abrams KA, Robinson RJ and Mayberry JF: Meta-analysis: Cancer risk of low-grade dysplasia in chronic ulcerative colitis. Aliment Pharmacol Ther *25*: 657-668, 2007.

54 Ullman T, Croog V, Harpaz N, Sachar D and Itzkowitz S: Progression of flat low-grade dysplasia to advanced neoplasia in patients with ulcerative colitis. Gastroenterology *125*: 1311-1319, 2003.

55 Odze RD, Farraye FA, Hecht JL and Hornick JL: Long-term follow-up after polypectomy treatment for adenoma-like dysplastic lesions in ulcerative colitis. Clin Gastroenterol Hepatol *2*: 534-541, 2004.

56 Kornfeld D, Ekbom A and Ihre T: Is there an excess risk for colorectal cancer in patients with ulcerative colitis and concomitant primary sclerosing cholangitis? A population based study. Gut *41*: 522-525, 1997.

57 Torres C, Antonioli D and Odze RD: Polypoid dysplasia and adenomas in inflammatory bowel disease: a clinical, pathologic, and follow-up study of 89 polyps from 59 patients. Am J Surg Pathol *22*: 275-284, 1998.

58 Ogino S and Goel A: Molecular classification and correlates in colorectal cancer. J Mol Diagn *10*: 13-27, 2008.

59 Walther A, Houlston R, and Tomlinson I. Association between chromosomal instability and prognosis in colorectal cancer: a meta-analysis. Gut *57*: 941-950, 2008.

60 Imai K and Yamamoto H: Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. Carcinogenesis *29*: 673-680, 2008.

61 Fukushige S, Kondo E and Horii A: Methyl-CpG targeted recruitment of p300 reactivates tumor suppressor genes in human cancer cells. Biochem Biophys Res Commun *379*: 1021-1026, 2009

62 Itzkowitz S: Colon carcinogenesis in inflammatory bowel disease: applying molecular genetics to clinical practice. J Clin Gastroenterol *36*: S70-74, 2003.

63 Itzkowitz SH: Molecular biology of dysplasia and cancer in inflammatory bowel disease. Gastroenterol Clin North Am *35*: 553-571, 2006.

64 Schulmann K, Mori Y, Croog V, Yin J, Olaru A, Sterian A, Sato F, Wang S, Xu Y, Deacu E, Berki AT, Hamilton JP, Kan T, Abraham JM, Schmiegel W, Harpaz N and Meltzer SJ: Molecular phenotype of inflammatory bowel disease-associated neoplasms with microsatellite instability. Gastroenterology *129*: 74-85, 2005.

65 Tahara T, Inoue N, Hisamatsu T, Kashiwagi K, Takaishi H, Kanai T, Watanabe M, Ishii H and Hibi T: Clinical significance of microsatellite instability in the inflamed mucosa for the prediction of colonic neoplasms in patients with ulcerative colitis. J Gastroenterol Hepatol *20*: 710-715, 2005.

66 van Dieren JM, Wink JC, Vissers KJ, van Marion R, Hoogmans MM, Dinjens WN, Schouten WR, Tanke HJ, Szuhai K, Kuipers EJ, van der Woude CJ and van Dekken H: Chromosomal and microsatellite instability of adenocarcinomas and dysplastic lesions (DALM) in ulcerative colitis. Diagn Mol Pathol *15*: 216-222, 2006.

67 Fujiwara I, Yashiro M, Kubo N, Maeda K and Hirakawa K: Ulcerative colitis-associated colorectal cancer is frequently associated with the microsatellite instability pathway. Dis Colon Rectum *51*: 1387-1394, 2008.

68 Maeda O, Ando T, Watanabe O, Ishiguro K, Ohmiya N, Niwa Y and Goto H: DNA hypermethylation in colorectal neoplasms and inflammatory bowel disease: a mini review. Inflammopharmacology *14*: 204-206, 2006.

69 Rosman-Urbach M, Niv Y, Birk Y, Smirnoff P, Zusman I, Morgenstern S and Schwartz B: A high degree of aneuploidy, loss of *p53* gene, and low soluble p53 protein serum levels are detected in ulcerative colitis patients. Dis Colon Rectum *47*: 304-313, 2004.

70 Holzmann K, Klump B, Borchard F, Gregor M and Porschen R: Flow cytometric and histologic evaluation in a large cohort of patients with ulcerative colitis: correlation with clinical characteristics and impact on surveillance. Dis Colon Rectum *44*: 1446-1455, 2001.

71 Jass JR: Colorectal cancer: a multipathway disease. Crit Rev Oncog *12*: 273-287, 2006.

72 Greenwald BD, Harpaz N, Yin J, Huang Y, Tong Y, Brown VL, McDaniel T, Newkirk C, Resau JH and Meltzer SJ: Loss of heterozygosity affecting the *p53, Rb,* and *mcc/apc* tumor suppressor gene loci in dysplastic and cancerous ulcerative colitis. Cancer Res *52*: 741-745, 1992.

73 Umetani N, Sasaki S, Watanabe T, Shinozaki M, Matsuda K, Ishigami H, Ueda E and Muto T: Genetic alterations in ulcerative colitis-associated neoplasia focusing on *APC, K-ras* gene and microsatellite instability. Jpn J Cancer Res *90*: 1081-1087, 1999.

74 Mikami T, Mitomi H, Hara A, Yanagisawa N, Yoshida T, Tsuruta O and Okayasu I: Decreased expression of CD44, alpha-catenin, and deleted colon carcinoma and altered expression of beta-catenin in ulcerative colitis-associated dysplasia and carcinoma, as compared with sporadic colon neoplasms. Cancer *89*: 733-740, 2000.

75 Andersen SN, Lovig T, Clausen OP, Bakka A, Fausa O and Rognum TO: Villous, hypermucinous mucosa in long standing ulcerative colitis shows high frequency of *K-ras* mutations. Gut *45*: 686-692, 1999.

76 Fujiwara I, Yashiro M, Kubo N, Maeda K and Hirakawa K: Ulcerative colitis-associated colorectal cancer is frequently associated with the microsatellite instability pathway. Dis Colon Rectum *51*: 1387-94, 2008.

77 Fujii S, Tominaga K, Kitajima K, Takeda J, Kusaka T, Fujita M, Ichikawa K, Tomita S, Ohkura Y, Ono Y, Imura J, Chiba T and Fujimori T: Methylation of the *oestrogen receptor* gene in non-neoplastic epithelium as a marker of colorectal neoplasia risk in longstanding and extensive ulcerative colitis. Gut *54*: 1287-1292, 2005.

78 Yagishita H, Yoshida T, Ishiguro K, Numata Y and Okayasu I: Epithelial and stromal genetic instability linked to tumor suppressor genes in ulcerative colitis-associated tumorigenesis. Scand J Gastroenterol *43*: 559-566, 2008.

79 Colliver DW, Crawford NP, Eichenberger MR, Zacharius W, Petras RE, Stromberg AJ and Galandiuk S: Molecular profiling of ulcerative colitis-associated neoplastic progression. Exp Mol Pathol *80*: 1-10, 2006.
80 Yang L and Pei Z: Bacteria, inflammation, and colon cancer. World J Gastroenterol *12*: 6741-6746, 2006.
81 Biasco G and Di Marco MC: Folate and prevention of colorectal cancer in ulcerative colitis. Eur J Cancer Prev *14*: 395-398, 2005.
82 Phelip JM, Ducros V, Faucheron JL, Flourie B and Roblin X: Association of hyperhomocysteinemia and folate deficiency with colon tumors in patients with inflammatory bowel disease. Inflamm Bowel Dis *14*: 242-248, 2008.
83 Kohno H, Suzuki R, Yasui Y, Miyamoto S, Wakabayashi K and Tanaka T: Ursodeoxycholic acid *versus* sulfasalazine in colitis-related colon carcinogenesis in mice. Clin Cancer Res *13*: 2519-2525, 2007.
84 Tung BY, Emond MJ, Haggitt RC, Bronner MP, Kimmey MB, Kowdley KV and Brentnall TA: Ursodiol use is associated with lower prevalence of colonic neoplasia in patients with ulcerative colitis and primary sclerosing cholangitis. Ann Intern Med *134*: 89-95, 2001.
85 Pardi DS, Loftus EV Jr, Kremers WK, Keach J and Lindor KD: Ursodeoxycholic acid as a chemopreventive agent in patients with ulcerative colitis and primary sclerosing cholangitis. Gastroenterology *124*: 889-893, 2003.
86 Wolf JM, Rybicki LA and Lashner BA: The impact of ursodeoxycholic acid on cancer, dysplasia and mortality in ulcerative colitis patients with primary sclerosing cholangitis. Aliment Pharmacol Ther *22*: 783-788, 2005.
87 Sjöqvist U, Tribukait B, Ost A, Einarsson C, Oxelmark L and Löfberg R: Ursodeoxycholic acid treatment in IBD-patients with colorectal dysplasia and/or DNA-aneuploidy: a prospective, double-blind, randomized controlled pilot study. Anticancer Res *24(5B)*: 3121-3127, 2004.
88 Matula S, Croog V, Itzkowitz S, Harpaz N, Bodian C, Hossain S and Ullman T: Chemoprevention of colorectal neoplasia in ulcerative colitis: the effect of 6-mercaptopurine. Clin Gastroenterol Hepatol *3*: 1015-1021, 2005.
89 Popivanova BK, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, Oshima M, Fujii C and Mukaida N: Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. J Clin Invest *118*: 560-570, 2008.
90 Weingarten MA, Zalmanovici A and Yaphe J: Dietary calcium supplementation for preventing colorectal cancer and adenomatous polyps. Cochrane Database Syst Rev CD003548, 2008.
91 Poynter JN, Gruber SB, Higgins PD, Almog R, Bonner JD, and Rennert HS, Low M, Greenson JK and Rennert G: Statins and the risk of colorectal cancer. N Engl J Med *352*: 2184-2192, 2005.
92 Suzuki S, Tajima T, Sassa S, Kudo H, Okayasu I and Sakamoto S: Preventive effect of fluvastatin on ulcerative colitis-associated carcinogenesis in mice. Anticancer Res *26(6B)*: 4223-4228, 2006.
93 Cho SJ, Kim JS, Kim JM, Lee JY, Jung HC and Song IS: Simvastatin induces apoptosis in human colon cancer cells and in tumor xenografts, and attenuates colitis-associated colon cancer in mice. Int J Cancer *123*: 951-957, 2008.
94 Munkholm P, Loftus EV Jr, Reinacher-Schick A, Kornbluth A, Mittmann U and Esendal B: Prevention of colorectal cancer in inflammatory bowel disease: value of screening and 5-amino-salicylates. Digestion *73*: 11-19, 2006.

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