Genomic instability in ulcerative colitis : a prerequisite for cancer in the inflammatory colon ?

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Abstract

Ulcerative colitis (UC), a chronic and relapsing idiopathic inflammatory disease of the colon, although not associated with an increased mortality compared to the general population, has a substantial morbidity leading to sizable health care costs, as it carries an increased risk for development of colorectal cancer (CRC). The pathophysiology behind this carcinogenic pathway is multifactorial. This review summarizes the major pathogenetic steps from which the inflamed colonic epithelium is transformed to a dysplastic and/or cancerous one. The role of the inflammatory and immune system, the oxidative stress generated as well as the genomic stability observed in UC-associated CRC is presented so as to provide a more spherical view of the tumorigenic process and, if possible, offer new diagnostic approaches for the early detection of CRC. (Acta gastroenterol. belg., **2012**, 75, **293-299**).

Key words : ulcerative colitis, inflammation, genomic instability, oxidative stress, colorectal cancer.

Introduction

Ulcerative colitis (UC) is a chronic and relapsing idiopathic inflammatory disease of the colon, which belongs to the group of inflammatory bowel diseases (IBDs). UC is not associated with increased mortality compared to that of the general population. However, its morbidity is substantial leading to sizable health care costs (1,2). Epidemiological data suggest that the incidence and prevalence of UC vary with geographic location and ethnicity. In the USA, incidence rates range from 6.0 to 15.6 cases per 100,000 persons/years, while in Europe from 1.5 to 20.3 cases per 100,000 persons/years. As far as prevalence is concerned, figures range from 38 to 246 cases per 100,000 persons in the USA and 21 to 243 cases per 100,000 persons in Europe (1,3).

As early as 1925, physicians had recognized that patients with UC have an increased risk for the development of colorectal cancer (CRC) (4,5). Eaden *et al*. not only reported that the overall prevalence of CRC in any patient with UC is 3.7%, but, also, that that the risk of CRC for any patient with UC is estimated to be 2% after 10 years, 8% after 20 years and 18% after 30 years of disease (6). Others estimate the exponential incidence of CRC in UC to be approximately 7-10% at 20 years of disease and as high as 30% after 35 years of disease (7). In general, the risk of CRC is increased within the range of 0.5% to 1.0% per year after 8 to 10 years of disease in patients with extensive UC (1,7).

Pathophysiology of carcinogenesis in ulcerative colitis

Unlike sporadic CRC developing from a distinct adenomatous precursor outgrowing the mucosa as a polyp, cancer in UC patients arises from a focal or multifocal dysplastic mucosa (abnormalities in crypt architecture and cytological detail) in areas of inflammation (8). These mucosal areas can represent either flat lesions (the majority of cases) or raised lesions, called dysplasia-associated lesions or masses (DALMs) (9).

Any neoplastic progression in UC patients involves a stepwise progression of pathological changes that begin with an inflamed and hyperplastic epithelium that is, finally, transformed to adenocarcinoma. The histological alterations in between these two ends include, firstly, indefinite dysplasia, and, afterwards, flat dysplasia (low or high-grade). However, it remains unclear whether tumor progression can skip one or more of these steps or if one grade of dysplasia may progress to another grade (10,11).

Moreover, many questions, relating to the process of cancer formation described, need to be answered. What is known for sure involves a sequence of events (mutations in somatic cells followed by their clonal expansion) arising in an inflammatory epithelium, such as that seen in UC patients. Scientists have not revealed the exact mechanism by which these genetic abnormalities contribute to the mutator phenotype (i.e. how and when mutated cells expand into dysplasia/cancer) and how clonal succession is associated with tumor progression (i.e. if the mutation load increases as cells become increasingly dysplastic) (12-14).

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Chen *et al*., using PCR-based DNA fingerprinting techniques in three UC patients with high-grade dysplasia, revealed that crypts from UC-associated dysplasia/cancer showed alterations in 10-20% of DNA fingerprint sites, regardless of whether the crypts were dysplastic or non-dysplastic and whether the DNA came from one crypt or thousands of crypts. When single crypts with a mutational change were examined, researchers revealed that, almost, half were clonally expanded to adjacent crypts and/or to the thousands of crypts in a single biopsy. Data were also revealing when fluorescent in-situ hybridization was used to examine p53 alterations in individual crypt cells ; DNA alterations were initiated in colonic crypts and expanded to adjacent crypts through crypt fission. Their paradigm suggested that a continuous process of DNA mutations, clonal expansion through crypt fission and clonal succession can initiate the development of inflammatory-associated CRC (12).

The role of the immune and the inflammatory response

While no direct genetic cause has been found, so far, for the increased risk of CRC in patients with UC, the role of the inflammatory response and immune cells and their products has been shown to be pivotal in the initiation and progression of UC-associated CRC (15). This comes as no surprise since more and more data suggest that the chronic inflammatory environment in the colon of UC patients is responsible not only for the development of CRC but, also, for its progression (9,16). This is the rationale behind the experimental use of non-steroidal anti-inflammatory drugs (NSAIDs), like mesalamine, in the prevention of CRC (17). However, only a handful of studies have directly investigated whether the level of inflammation correlates with the risk of developing adenocarcinoma in UC patients ; disease activity has not been shown to correlate with the incidence of UC-associated CRC (11). On the other hand, the degree of active inflammation, assessed by histological criteria, has been found to be an independent risk factor for developing advanced CRC among patients with long standing UC (18,19).

The same deregulated immune response responsible in the pathogenesis of UC seems to be implicated in the formation of precancerous and cancerous cells. This response includes the loss of tolerance against luminal antigens, such as commensal bacteria residing in the intestinal lumen, due to transient breaks in the mucosal barrier and the concomitant increase of the epithelial permeability ; bacteria are allowed to infiltrate the subepithelial tissue where an inflammatory response against them is generated (20).

The colonic mucosa in UC patients is infiltrated by neutrophils and macrophages (both phagocytic cells) as well as by B cells and T cells, all of which express transmembrane Toll-like receptors (TLRs), which are specific microbial recognition receptors. When an antigen, like a microbe, binds to these receptors, the nuclear factor κB (NFκB) and mitogen-activated protein kinase (MAPK) pathways are activated, promoting the inflammatory response (15). The pro-inflammatory cytokines secreted by the aforementioned inflammatory and immune cells, like tumor necrosis factor-α (TNF-α), bind to the receptor TNF-receptor (TNF-R) and promote inflammation and UC-associated CRC via upregulation of genes involved in prostaglandin synthesis to mediate tissue repair, such as cyclo-oxygenase 2 (COX-2), which in turn induces angiogenesis promoting tumor growth (21).

Oxidative stress

In parallel, this inflammatory process induces nitrosative stress and lipid peroxidation by generating an excess of radical reactive oxygen and nitrogen species (ROS and RNS, respectively), released by cells of the innate immune system, favoring tumor growth (22). Both ROS and RNS are highly reactive free radicals that can affect many metabolic processes, including those that regulate DNA, RNA, proteins, and lipids ; they are capable of causing potentially carcinogenic changes to DNA, such as single and double strand breaks and base modifications or affect regulation of genes that encode factors that prevent carcinogenesis (p53, DNA mismatch repair proteins and base excision-repair proteins) (11,20,23).

ROS produced by neutrophils and macrophages in the colonic mucosa, under the influence of pro-inflammatory cytokines, penetrate neighboring epithelial cells and causes DNA damage. ROS formation can, also, be triggered by TLR-mediated sensing of microbial antigens and through the activity of inducible nitric oxide synthase (iNOS), described below. DNA is damaged which in turn leads to telomere shortening and the induction of senescence in cells ; this mutagenic phenotype ultimately leads to dysplasia and carcinogenesis (15).

Cytokines released upregulate iNOS in macrophages and epithelial cells, resulting in increased local production of nitric oxide (NO). NO, while, on its own, has no effect on DNA, can react with oxygen and superoxide yielding highly reactive byproducts (dinitrogen trioxide and peroxynitrite, respectively), which are responsible for DNA mutations and breaks, through deamination of DNA bases (15,24,25). It is this oxidative stress (amongst other factors) that is responsible for cellular damage, through the genomic instability described in detail below, contributing to the pathogenesis of the inflamed colon and the formation of cancerous cells (Fig. 1) (26).

Genomic instability

Even though sporadic CRC and UC-associated CRC have different molecular pathophysiology due to differences in mucosal origin they share common genetic features as they are both the result of genomic instability (chromosomal instability – CIN – and microsatellite

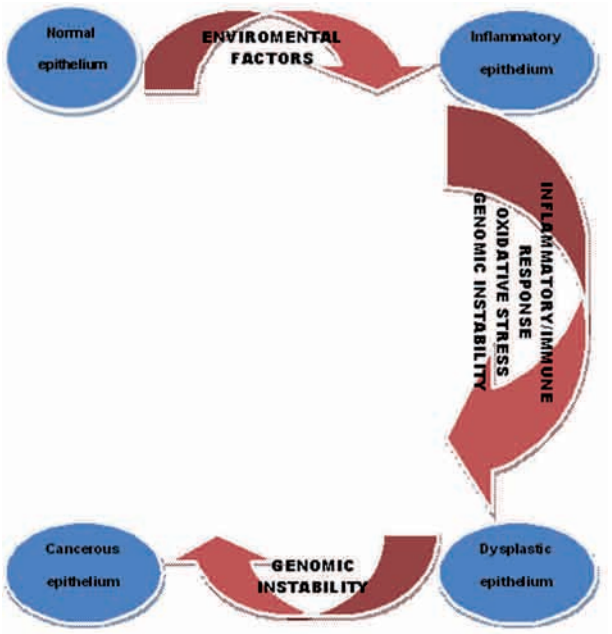

Fig. 1. — The sequence of events leading from normal to cancerous colonic epithelium along with major pathogenetic triggers.
Environmental factor can be any toxin like a bacterial or viral infection or a drug (e.g. non-steroidal anti-inflammatory drugs) ; the range of dysplasia can be wide (from indefinite to high grade).

instability – MSI –) (9,27). While both molecular mechanisms of MSI and CIN have been identified in both categories of patients and while both are responsible in the same percentage for the development of CRC (85% for CIN and 15% for MSI in both sporadic and UC-associated CRC), it is the time and the frequency with which these alterations take place that seems to be of the essence (Table 1) (27-37). Furthermore, CRC in UC patients have widespread genomic instability in their colon ; these genetic alterations have been identified long before any histologic evidence of dysplasia or cancer. On the other hand, there is no instability in the colonic mucosa of UC patients who are cancer/dysplasia-free (38-40).

Chen *et al*., using two polymerase chain reaction (PCR)-based DNA fingerprinting methods, assessed the DNA sequence variation in biopsies across the spectrum of cancerous, dysplastic, and nondysplastic mucosa in UC patients. Showing that the degree of genetic instability in nondysplastic tissue was similar to that of dysplastic/cancerous mucosa from the same patient, their data suggested that UC patients who develop dysplasia or cancer have an underlying process of genomic instability in their colonic mucosa whereas UC patients who are dysplasia-free do not (38). The presence of genetic alterations has, also been detected in the stroma of the colon of UC patients from an early stage of carcinogenesis, accompanied by a stepwise increase in the genetic instability of the colonic epithelium with progression to cancer, indicating not only that stromal genetic instability might contribute to tumorigenesis of UC-associated CRC but, also, a possible distinct carcinogenesis pathway of UC (41,42).

Microsatellite instability

Microsatellites, which are short repeated nucleotide sequences (as short as one or two nucleotides) are interspersed throughout the human genome (43). MSI involves the primary loss of function of genes that usually repair DNA base-pair mismatches that occur during the normal process of DNA replication in dividing cells and is characterized by the accumulation of somatic alterations in these microsatellites (due to the presence of insertions or deletions in these repetitive sequences) (44).

A study involving 57 UC patients revealed MSI in 4 of 11 cancer cases (36%), 5 of 15 dysplasia cases (33%), 5 of 11 indefinite cases (45%). No MSI was detected amongst the 20 UC patients with no dysplasia or cancer (0%) (45). While some researchers have shown that MSI+ phenotypes were present not only in UC-associated CRCs, high grade dysplasias and low grade dysplasias (67%, 67%, and 33%, respectively) but, also, in 25% of patients with inflamed UC mucosa without colonic neoplasm (46), others suggest that MSI is rare in UC-related CRC as well as non-neoplastic lesions, and, therefore, does not contribute to the progression of the colitis-dysplasia sequence (47,48).

hMLH1 is part of the mismatch repair system, a protein which, along other proteins (hMSH2, hPMS1, hPMS2, hMSH6, and hMLH3), forms specific heterodimers to coordinate DNA repair (49). Methylation of the hMLH1 promoter causes MSI in patients with sporadic CRC, via the transcriptional silencing of hMLH1 (9).

Kuismanen *et al*. investigated 46 sporadic MSI+ CRCs for changes in hMSH2 and hMLH1 protein expression. The majority of cases (78%) showed lost or reduced hMLH1 expression ; among these, 83% was associated with hMLH1 promoter hypermethylation, while the rates of loss of heterozygosity (LOH) and somatic mutation of hMLH1 were 24% and 13%, respectively. As for hMSH2, its expression was lost in 15% of CRC cased out of which, 29% showed LOH and/or somatic mutation of hMSH2 (50).

A study of 148 patients with UC/Crohn's-associated CRC, out of which the vast majority (80%) were microsatellite stable, revealed that hMLH1 promoter hypermethylation occurred frequently in the setting of MSI. hMLH1 hypermethylation was observed in 46% of patients with high levels of MSI (instability at two or more loci), in 16% of patients with low levels of MSI (instability at only one locus), and in 15% of microsatellite stable patients (no instability at any loci). Perhaps, hMLH1 promoter hypermethylation may cause MSI or contribute to its development (51). The pivotal role of

Table 1. — **Differences in time and frequency of genomic instability between sporadic and UC-associated CRC**

Sporadic CRC	UC-associated CRC
methylation of the hMLH1 promoter • very common *heterogeneous abnormalities in hMLH1, hMSH2, hMSH6, and hPMS2 repair genes* • uncommon • early event	*methylation of the hMLH1 promoter* • rare *heterogeneous abnormalities in hMLH1, hMSH2, hMSH6, and hPMS2 repair genes* • frequent • latter event
p53 loss or mutations • late event	*p53 loss or mutations* • early event
Hypermethylation of promoter CpG islands • later event • less widespread throughout the mucosa	*Hypermethylation of promoter CpG islands* • early event • more widespread throughout the mucosa
Aneuploidy • early event	*Aneuploidy* • early event
Glycosylation abnormalities (increased expression of sialosyl Tn antigen) • early event	*Glycosylation abnormalities (increased expression of sialosyl Tn antigen)* • early event
Induction of COX-2 • later event	*Induction of COX-2* • early event
k-ras gene mutations • earlier event	*k-ras gene mutations* • later event
Loss of function of tumor suppressor genes on chromosome 18q in the region of the DCC and DPC4 genes • later event	*Loss of function of tumor suppressor genes on chromosome 18q in the region of the DCC and DPC4 genes* • earlier event
c-src tyrosine kinase induction • later event	*c-src tyrosine kinase induction* • earlier event

APC : adenomatous polyposis coli ; CpG : cytosine phosphate guanine ; hMLH1 : human MutL homolog1 ; hMSH2 : human MutS homolog 2 ; hMSH6 : human MutS homolog 6 ; hPMS2 : human postmeiotic segregation increased 2 ; COX-2 : cyclooxygenase-2 ; k-ras : Kirsten rat sarcoma ; DCC : deletion in colon cancer ; DPC4 : deletion in pancreatic cancer 4 ; c-src : c-sarcoma.

hMLH1 and hMSH2 in tumorigenesis is highlighted by the fact their germline mutations are responsible for the most common inherited CRC, the hereditary nonpolyposis colon cancer (52).

Chromosomal instability

CIN, the product of abnormal segregation of chromosomes and abnormal DNA content (aneuploidy), results in loss of chromosomal material LOH at sites of a number of cancer related genes (29). This loss and gain of chromosome arms contributes to a variety of chromosome-level changes (deletions, amplifications, translocations) (53). Overall, CIN is a preneoplastic phenomenon that possibly reflects an underlying genomic instability that may predispose these patients to carcinogenesis (15). Interestingly enough, while Willenbucher *et al*. have demonstrated CIN to be also present in the non dysplastic parts of the colon of UC patients (27), Chen *et al*. have shown CIN in only a minority of UC patients (20%) without progression to neoplasia (38).

Studies in UC patients have revealed that aneuploid cell populations spread over time, occupying larger areas of the mucosa. Moreover, within a specific area of aneuploidy, subclones of aneuploid cells appear to emerge from their predecessors. It is this dysregulation of the subclones which is responsible for their expansion at the expense of the normal surrounding epithelium (54).

Aust *et al*. reported losses on chromosomes 18q, 8p, 17p, and gains on 8q, 20q, and 13q on patients with CRC, either UC-related or sporadic. However, differences in the frequency and timing of specific alterations were, also, observed. For instance, chromosome 5q was lost in nearly half of UC-related CRC in contrast with approximately ¼ of sporadic CRC (56% and 26%, respectively), while 18q loss was associated with stage progression only in sporadic cancers. In contrast, alterations of chromosome 8 were associated with stage progression in UC-related, but not in sporadic CRC (55).

Loss of adenomatous polyposis coli (APC) function, usually through protein truncation or allelic loss is evident in as much as 85% of all CRCs. The APC gene, considered the "gatekeeper" of the colon, is located on chromosome 5q21-q22 (29). Loss of APC function results in elevated β-catenin concentration (a subunit of the cadherin protein complex), which could be the initiating event in intestinal tumorigenesis, through a process not yet fully understood (30-32). In patients with mucosa of the colon negative or indefinite for dysplasia, mutations in APC are rarely, if ever, detected as the vast majority of patients with low-grade dysplasia or cancer have no mutations in APC. Allelic deletion of APC occurs in less than one third of UC-associated CRCs (11,56).

The loss of p53 function is thought to play a crucial role in the pathogenesis of UC-associated CRC. Burmer *et al*. demonstrated in UC patients that loss of a p53

allele was found in 85% of biopsy specimens classified histologically as carcinoma, 63% with high grade dysplasia, and 33% with low grade dysplasia. LOH for p53 was also found in 9% of biopsy specimens indefinite for dysplasia (57). Other researchers, through the use of single-strand conformation polymorphism analysis, DNA sequencing, and LOH studies, revealed that point mutations affecting p53 in dysplastic and cancerous UC lesions were detected in 26 lesions from 20 patients. Missense, nonsense, and tandem mutations were also detected (58).

Interestingly enough, p53 mutations appear to be an early genetic event that precedes p53 LOH. Data revealing that mutations, but not LOH, were found in non dysplastic or only indefinite for dysplasia UC mucosa adjacent to dysplastic areas suggest that p53 mutations precede aneuploidy in the process of carcinogenesis (59). The finding that in UC patients, higher p53 codon 247 and 248 mutation frequencies were observed in the inflamed regions when compared with non inflamed regions of their colon, suggesting that these mutations maybe the result of the inflammatory environment (60).

The 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) gene as well as induction of the k-ras oncogene, has also been observed in UC-associated CRC (29,61,62). Data, also, suggest that activation of the src proto-oncogene, which encode cytoplasmic, membrane-associated protein tyrosine kinases, is an early event in the genesis of UC-associated CRC (63,64). Expression of the mucin-associated carbohydrate antigen sialosyl-Tn (glycosylation abnormalities) seems to correlate with malignant transformation in UC patients as it is expressed in aneuploid, diploid, and nondysplastic mucosa areas (36,65,66).

DNA hypermethylation - epigenetic alterations

Methylation of CpG islands in several genes (have, also, been suggested to precede dysplasia in UC patients (35,67,68). A recent study amongst 19 patients with CRC arising within UC revealed that the CpG island methylator phenotype was observed in 5% of UC-associated CRCs (69). A number of human cancer genes that contain hypermethylation of promoter CpG islands have been identified like p14 (ARF), p16 (INK4a), and E-cadherin (CDH1) (70-74).

Conclusions

UC, a chronic inflammatory disease and the result of an altered host response to intestinal flora under the effect of an environmental insult (bacterial or viral infection, drugs), has substantial morbidity since UC patients are at an increased risk for developing CRC (1). However, CRC can develop in patients without any histological evidence of UC. What is known for sure is that the carcinogenesis pathway, involving the transformation of the inflammatory colonic epithelium to a dysplastic and/or cancerous one, is multifactorial (75).

Carcinogenesis is promoted under the influence of an inflammatory colonic epithelium. This inflammatory environment though is not enough ; otherwise all UC patients would develop CRC. Individual components of the innate and adaptive immune response have, also, been implicated in carcinogenesis. Free radicals, like ROS and NOS, produced by inflammatory cells, can affect regulation of genes that encode factors that prevent carcinogenesis and induce DNA damage. This genomic instability is one of the defining genetic fingerprints of UC-associated CRC. CIN, MSI and DNA hypermethylation are evident in the majority of UC patients who develop dysplasia and/or cancer and are present in the inflamed colonic mucosa long ago before any histopathologic evidence of dysplastic and/or cancerous cells. However, no direct genetic cause has been found for the increased risk of CRC in UC patients (15).

Up to now, screening of UC patients with endoscopy for the development of any dysplastic lesion (polypoid, flat, localized, or multifocal) is the only armament available to the treating physician. The endoscopist must bear in mind that dysplasia in UC can be found at distant sites from the cancer itself or before the cancer develops and is difficult to recognize on colonoscopy, as it often arises from flat, normal-appearing mucosa. Therefore, the standard histological examination of random intestinal biopsy samples might be inefficient as a method of cancer surveillance (15). Researchers have, even, proposed surveillance strategies so as to reduce the risk of CRC in UC patients according to the histopathology of the biopsy samples ; proctocolectomy is indicated in the case of dysplasia of any grade found in an endoscopically non-resectable polyp and high-grade dysplasia found in flat mucosa. In the case of a solitary low- or high-grade dysplasia found in a discrete adenoma-like polyp, polypectomy and accelerated surveillance are warranted (28,29,76). However, current surveillance protocols seem to lack efficacy (77). New imaging modalities like chromoendoscopy, digital chromoendoscopy, high-definition endoscopy and confocal laser endomicroscopy may increase the diagnostic yield of endoscopy and reduce the risk of death from UC-associated CRC (78-80).

Besides any surveillance protocol, the use of anti-inflammatory medications as an attempt to suppress the degree of active inflammation and, hence, lower the risk for UC-associated CRC, has produced conflicting results. Studies so far have not established that any anti-inflammatory agent used to treat UC has any chemopreventive effects against cancer (11). Finally, the role for prophylactic proctocolectomy in high risk UC patients for CRC (like those with with primary sclerosing cholangitis) is still controversial (81).

Understanding the genetics behind the transformation of the inflamed colonic epithelium into a dysplastic

and/or cancerous may offer the treating physician new molecular screening approaches. Aneuploidy, mutations in p53 and k-ras, increased expression of sialyl Tn antigen may provide robust diagnostic information (29). DNA double-strand breaks (DSBs) and their products like the variant histone H2A.x, which rapidly becomes phosphorylated to form γ-H2A.x at the site of the DSB, 8-hydroxydeoxyguanosine (8□OHdG), formed when the DNA base deoxyguanosine is oxidatively modified by ROS, α□methylacyl-CoA-racemase (AMACR), an enzyme that catalyzes the racemization of α□methyl-branched carboxylic coenzyme A thioesters, could serve as specific biomarkers of oxidative DNA damage and, therefore, preneoplasia (15). As scientists gain more and more insight on the tumorigenesis pathway and the genomic instability that accompanies it, new biologic markers (with high sensitivity and specificity) could be the next best thing in UC-associated CRC detection.

References

1. OSTERMAN M.T., LICHTENSTEIN G.R. Ulcerative colitis. *In* : FELDMAN M., FRIEDMAN L.S., BRANDT L.J. (eds). Sleisenger and Fordtran's gastrointestinal and liver disease. Volume 2. 9th ed. Philadelphia : Saunders, Elsevier, 2010 : 1975-2014.
2. WILKS S. Lectures on pathological anatomy. London : Ongman, Brown, Green, Longman, & Roberts, 1859.
3. LOFTUS E.V. Jr. Clinical epidemiology of inflammatory bowel disease : Incidence, prevalence, and environmental influences. *Gastroenterology*, 2004, **126** : 1504-17.
4. CROHN B.B., ROSENBERG H. The sigmoidoscopic picture of chronic ulcerative colitis. *Am. J. Med. Sci*., 1925, **170** : 220-8.
5. SMITH R.A., VON ESCHENBACH A.C., WENDER R., LEVIN B., BYERS T., ROTHENBERGER D. *et al.* ; ACS Prostate Cancer Advisory Committee, ACS Colorectal Cancer Advisory Committee, ACS Endometrial Cancer Advisory Committee. American Cancer Society guidelines for the early detection of cancer : update of early detection guidelines for prostate, colorectal, and endometrial cancers. Also : update 2001 – testing for early lung cancer detection. *CA. Cancer. J. Clin*., 2001, **51** : 38-75 ; quiz 77-80.
6. EADEN J. Review article : colorectal carcinoma and inflammatory bowel disease. *Aliment. Pharmacol. Ther.*, 2004, **20** : 24-30.
7. LEWIS J.D., DEREN J.J., LICHTENSTEIN G.R. Cancer risk in patients with inflammatory bowel disease. *Gastroenterol. Clin. North. Am*., 1999, **28** : 459-77.
8. KULAYLAT M.N, DAYTON M.T. Ulcerative colitis and cancer. *J. Surg. Oncol*., 2010, **101** : 706-12.
9. VIENNOT S., DELEPORTE A., MOUSSATA D., NANCEY S., FLOURIÉ B., REIMUND J.M. Colon cancer in inflammatory bowel disease : recent trends, questions and answers. *Gastroenterol. Clin. Biol*., 2009, **33** : S190-201.
10. TRIANTAFILLIDIS J.K., NASIOULAS G., KOSMIDIS P.A. Colorectal cancer and inflammatory bowel disease : epidemiology, risk factors, mechanisms of carcinogenesis and prevention strategies. *Anticancer. Res*., 2009, **29** : 2727-37.
11. ULLMAN T.A, ITZKOWITZ S.H. Intestinal inflammation and cancer. *Gastroenterology*, 2011, **140** : 1807-16.
12. CHEN R., RABINOVITCH P.S., CRISPIN D.A., EMOND M.J., BRONNER M.P., BRENTNALL T.A. The initiation of colon cancer in a chronic inflammatory setting. *Carcinogenesis*., 2005, **26** : 1513-9.
13. LOEB L.A. Mutator phenotype may be required for multistage carcinogenesis. *Cancer. Res*., 1991, **51** : 3075-9.
14. KIM K., SHIBATA D. Methylation reveals a niche : stem cell succession in human colon crypts. *Oncogene*., 2002, **21** : 5441-9.
15. THORSTEINSDOTTIR S., GUDJONSSON T., NIELSEN O.H., VAINER B., SEIDELIN J.B. Pathogenesis and biomarkers of carcinogenesis in ulcerative colitis. *Nat. Rev. Gastroenterol. Hepatol*., 2011, **8** : 395-404.
16. RISQUES R.A., RABINOVITCH P.S., BRENTNALL T.A. Cancer surveillance in inflammatory bowel disease : new molecular approaches. *Curr. Opin. Gastroenterol*., 2006, **22** : 382-90.
17. SLATTERY M.L., SAMOWITZ W., HOFFMAN M., MA K.N., LEVIN T.R., NEUHAUSEN S. Aspirin, NSAIDs, and colorectal cancer : possible involvement in an insulin-related pathway. *Cancer. Epidemiol. Biomarkers. Prev*., 2004, **13** : 538-45.
18. RUTTER M., SAUNDERS B., WILKINSON K., RUMBLES S., SCHOFIELD G., KAMM M. *et al*. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology*, 2004, **126** : 451-9.
19. GUPTA R.B., HARPAZ N., ITZKOWITZ S., HOSSAIN S., MATULA S., KORNBLUTH A. *et al*. Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis : a cohort study. *Gastroenterology*, 2007, **133** : 1099-105.
20. WESTBROOK A.M., SZAKMARY A., SCHIESTL R.H. Mechanisms of intestinal inflammation and development of associated cancers : lessons learned from mouse models. *Mutat. Res*., 2010, **705** : 40-59.
21. COUSSENS L.M., RAYMOND W.W., BERGERS G., LAIG-WEBSTER M., BEHRENDTSEN O., WERB Z. *et al*. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes. Dev*., 1999, **13** : 1382-97.
22. CASORELLI I., PANNELLINI T., DE LUCA G., DEGAN P., CHIERA F., IAVARONE I. *et al*. The Mutyh base excision repair gene influences the inflammatory response in a mouse model of ulcerative colitis. *PLoS. One*, 2010, **5** : e12070.
23. ROESSNER A., KUESTER D., MALFERTHEINER P., SCHNEIDER-STOCK R. Oxidative stress inulcerative colitis-associated carcinogenesis. *Pathol. Res. Pract*., 2008, **204** : 511-24.
24. CAULFIELD J.L., WISHNOK J.S., TANNENBAUM S.R. Nitric oxide-induced deamination of cytosine and guanine in deoxynucleosides and oligonucleotides. *J. Biol. Chem*., 1998, **273** : 12689-95.
25. NEDOSPASOV A.A. Is N2O3 the main nitrosating intermediate in aerated nitric oxide (NO) solutions in vivo ? If so, where, when, and which one ? *J. Biochem. Mol. Toxicol*., 2002, **16** : 109-20.
26. ITZKOWITZ S.H., YIO X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease : the role of inflammation. *Am. J. Physiol. Gastrointest. Liver. Physiol*., 2004, **287** : G7-17.
27. WILLENBUCHER R.F., AUST D.E., CHANG C.G., ZELMAN S.J., FERRELL L.D., MOORE D.H. 2ND *et al*. Genomic instability is an early event during the progression pathway of ulcerative-colitis-related neoplasia. *Am. J. Pathol*., 1999, **154** : 1825-30.
28. ITZKOWITZ S.H., HARPAZ N. Diagnosis and management of dysplasia in patients with inflammatory bowel diseases. *Gastroenterology*, 2004, **126** : 1634-48.
29. XIE J., ITZKOWITZ S.H. Cancer in inflammatory bowel disease. *World J. Gastroenterol*., 2008, **14** : 378-89.
30. REDSTON M.S., PAPADOPOULOS N., CALDAS C., KINZLER K.W., KERN S.E. Common occurrence of APC and K-ras gene mutations in the spectrum of colitis-associated neoplasias. *Gastroenterology*, 1995, 108 : 383-92.
31. TARMIN L., YIN J., HARPAZ N., KOZAM M., NOORDZIJ J., ANTONIO L.B. *et al*. Adenomatous polyposis coli gene mutations in ulcerative colitis-associated dysplasias and cancers versus sporadic colon neoplasms. *Cancer. Res*., 1995, **55** : 2035-8.
32. AUST D.E., TERDIMAN J.P., WILLENBUCHER R.F., CHANG C.G., MOLINARO-CLARK A., BARETTON G.B. *et al*. The APC/beta-catenin pathway in ulcerative colitis-related colorectal carcinomas : a mutational analysis. *Cancer*, 2002, **94** : 1421-7.
33. MORIN P.J., SPARKS A.B., KORINEK V., BARKER N., CLEVERS H., VOGELSTEIN B. *et al*. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science*, 1997, **275** : 1787-90.
34. SVRCEK M., EL-BCHIRI J., CHALASTANIS A., CAPEL E., DUMONT S., BUHARD O. *et al*. Specific clinical and biological features characterize inflammatory bowel disease associated colorectal cancers showing microsatellite instability. *J. Clin. Oncol*., 2007, **25** : 4231-8.
35. ISSA J.P., AHUJA N., TOYOTA M., BRONNER M.P., BRENTNALL T.A. Accelerated age-related CpG island methylation in ulcerative colitis. *Cancer. Res*., 2001, **61** : 3573-7.
36. ITZKOWITZ S.H., YOUNG E., DUBOIS D., HARPAZ N., BODIAN C., CHEN A. *et al*. Sialosyl-Tn antigen is prevalent and precedes dysplasia in ulcerative colitis : a retrospective case-control study. *Gastroenterology*, 1996, **110** : 694-704.
37. RAPOZO D.C., GRINMANN A.B., CARVALHO A.T., DE SOUZA H.S., SOARES-LIMA S.C., DE ALMEIDA SIMÃO T. *et al*. Analysis of mutations in TP53, APC, K-ras, and DCC genes in the non-dysplastic mucosa of patients with inflammatory bowel disease. *Int. J. Colorectal. Dis*., 2009, **24** : 1141-8.
38. CHEN R., RABINOVITCH P.S., CRISPIN D.A., EMOND M.J., KOPROWICZ K.M., BRONNER M.P. *et al*. DNA fingerprinting

abnormalities can distinguish ulcerative colitis patients with dysplasia and cancer from those who are dysplasia/cancer-free. *Am. J. Pathol*., 2003, **162** : 665-72.
39. RABINOVITCH P.S., DZIADON S., BRENTNALL T.A., EMOND M.J., CRISPIN D.A., HAGGITT R.C. *et al*. Pancolonic chromosomal instability precedes dysplasia and cancer in ulcerative colitis. *Cancer Res*., 1999, **59** : 5148-53.
40. WILLENBUCHER R.F., ZELMAN S.J., FERRELL L.D., MOORE D.H. 2ND, WALDMAN F.M. Chromosomal alterations in ulcerative colitis related neoplastic progression. *Gastroenterology*, 1997, **113** : 791-801.
41. ISHIGURO K., YOSHIDA T., YAGISHITA H., NUMATA Y., OKAYASU T. Epithelial and stromal genetic instability contributes to genesis of colorectal adenomas. *Gut*, 2006, **55** : 695-702.
42. MATSUMOTO N., YOSHIDA T., OKAYASU I. High epithelial and stromal genetic instability of chromosome 17 in ulcerative colitis-associated carcinogenesis. *Cancer Res*., 2003, **63** : 6158-61.
43. SUZUKI H., HARPAZ N., TARMIN L., YIN J., JIANG H.Y., BELL J.D. *et al*. Microsatellite instability in ulcerative colitis-associated colorectal dysplasias and cancers. *Cancer Res*., 1994, **54** : 4841-4.
44. THIBODEAU S.N., BREN G., SCHAID D. Microsatellite instability in cancer of the proximal colon. *Science*, 1993, **260** : 816-19.
45. FUJIWARA I., YASHIRO M., KUBO N., MAEDA K., HIRAKAWA K. Ulcerative colitis-associated colorectal cancer is frequently associated with the microsatellite instability pathway. *Dis. Colon. Rectum*, 2008, **51** : 1387-94.
46. TAHARA T., INOUE N., HISAMATSU T., KASHIWAGI K., TAKAISHI H., KANAI T. *et al*. Clinical significance of microsatellite instability in the inflamed mucosa for the prediction of colonic neoplasms in patients with ulcerative colitis. *J. Gastroenterol. Hepatol*., 2005, **20** : 710-5.
47. LØVIG T., ANDERSEN S.N., CLAUSEN O.P., ROGNUM T.O. Microsatellite instability in long-standing ulcerative colitis. *Scand. J. Gastroenterol*., 2007, **42** : 586-91.
48. TAKAHASHI S., KOJIMA Y., KINOUCHI Y., NEGORO K., TAKAGI S., AIHARA H. *et al*. Microsatellite instability and loss of heterozygosity in the nondysplastic colonic epithelium of ulcerative colitis. *J. Gastroenterol*., 2003, **38** : 734-9.
49. KOLODNER R.D., MARSISCHKY G.T. Eukaryotic DNA mismatch repair. *Curr. Opin. Genet. Dev*., 1999, **9** : 89-96.
50. KUISMANEN S.A., HOLMBERG M.T., SALOVAARA R., DE LA CHAPELLE A., PELTOMÄKI P. Genetic and epigenetic modification of MLH1 accounts for a major share of microsatellite-unstable colorectal cancers. *Am. J. Pathol*., 2000, **156** : 1773-9.
51. FLEISHER A.S., ESTELLER M., HARPAZ N., LEYTIN A., RASHID A., XU Y. *et al*. Microsatellite instability in inflammatory bowel disease-associated neoplastic lesions is associated with hypermethylation and diminished expression of the DNA mismatch repair gene, hMLH1. *Cancer. Res*., 2000, 60 : 4864-8.
52. LYNCH P.M. The hMSH2 and hMLH1 genes in hereditary nonpolyposis colorectal cancer. *Surg. Oncol. Clin. N. Am*., 2009, **18** : 611-24.
53. LENGAUER C., KINZLER K.W., VOGELSTEIN B. Genetic instability in colorectal cancers. *Nature*, 1997, **386** : 623-7.
54. RUBIN C.E., HAGGITT R.C., BURMER G.C., BRENTNALL T.A., STEVENS A.C., LEVINE D.S. *et al*. DNA aneuploidy in colonic biopsies predicts future development of dysplasia in ulcerative colitis. *Gastroenterology*, 1992, **103** : 1611-20.
55. AUST D.E., WILLENBUCHER R.F., TERDIMAN J.P., FERRELL L.D., CHANG C.G., MOORE D.H. 2ND. *et al*. Chromosomal alterations in ulcerative colitis-related and sporadic colorectal cancers by comparative genomic hybridization. *Hum. Pathol*., 2000, **31** : 109-14.
56. UMETANI N., SASAKI S., WATANABE T., SHINOZAKI M., MATSUDA K., ISHIGAMI H. *et al*. Genetic alterations in ulcerative colitis-associated neoplasia focusing on APC, K-ras gene and microsatellite instability. *Jpn. J. Cancer. Res*., 1999, **90** : 1081-7.
57. BURMER G.C., RABINOVITCH P.S., HAGGITT R.C., CRISPIN D.A., BRENTNALL T.A., KOLLI V.R. *et al*. Neoplastic progression in ulcerative colitis : histology, DNA content, and loss of a p53 allele. *Gastroenterology*, 1992, **103** : 1602-10.
58. YIN J., HARPAZ N., TONG Y., HUANG Y., LAURIN J., GREENWALD B.D. *et al*. p53 point mutations in dysplastic and cancerous ulcerative colitis lesions. *Gastroenterology*, 1993, **104** : 1633-39.
59. BRENTNALL T.A., CRISPIN D.A., RABINOVITCH P.S., HAGGITT R.C., RUBIN C.E., STEVENS A.C. *et al*. Mutations in the p53 gene : an early marker of neoplastic progression in ulcerative colitis. *Gastroenterology*, 1994, **107** : 369-78.
60. HUSSAIN S.P., AMSTAD P., RAJA K., AMBS S., NAGASHIMA M., BENNETT W.P. *et al*. Increased p53 mutation load in noncancerous colon tissue from ulcerative colitis : a cancer-prone chronic inflammatory disease. *Cancer Res*., 2000, **60** : 3333-7.
61. BORCHERS R., HEINZLMANN M., ZAHN R., WITTER K., MARTIN K., LOESCHKE K. *et al*. K-ras mutations in sera of patients with colorectal neoplasias and long-standing inflammatory bowel disease. *Scand. J. Gastroenterol*., 2002, **37** : 715-8.
62. LYDA M.H., NOFFSINGER A., BELLI J., FENOGLIO-PREISER C.M. Microsatellite instability and K-ras mutations in patients with ulcerative colitis. *Hum. Pathol*., 2000, **31** : 665-71.
63. CARTWRIGHT C.A., COAD C.A., EGBERT B.M. Elevated c-Src tyrosine kinase activity in premalignant epithelia of ulcerative colitis. *J. Clin. Invest*., 1994, **93** : 509-15.
64. RUSTGI A.K. c-src tyrosine kinase activity : a marker of dysplasia in ulcerative colitis. *Gastroenterology*, 1994, **107** : 1565-6.
65. ITZKOWITZ S.H., MARSHALL A., KORNBLUTH A., HARPAZ N., MC HUGH J.B., AHNEN D. *et al*. Sialosyl-Tn antigen : initial report of a new marker of malignant progression in long-standing ulcerative colitis. *Gastroenterology*, 1995, **109** : 490-7.
66. KARLÉN P., YOUNG E., BROSTRÖM O., LÖFBERG R., TRIBUKAIT B., OST K. *et al*. Sialyl-Tn antigen as a marker of colon cancer risk in ulcerative colitis : relation to dysplasia and DNA aneuploidy. *Gastroenterology*, 1998, **115** : 1395-404.
67. KOIZUMI K., ALONSO S., MIYAKI Y., OKADA S., OGURA H., SHIIYA N. *et al*. Array-based identification of common DNA methylation alterations in ulcerative colitis. *Int. J. Oncol*., 2012, **40** : 983-94.
68. TAHARA T., SHIBATA T., NAKAMURA M., OKUBO M., YAMASHITA H., YOSHIOKA D. *et al*. Association between polymorphisms in the XRCC1 and GST genes, and CpG island methylation status in colonic mucosa in ulcerative colitis. *Virchows. Arch*., 2011, **458** : 205-11.
69. SANCHEZ J.A., DEJULIUS K.L., BRONNER M., CHURCH J.M., KALADY M.F. Relative role of methylator and tumor suppressor pathways in ulcerative colitis-associated colon cancer. *Inflamm. Bowel. Dis*., 2011, **17** : 1966-70.
70. GARRITY-PARK M.M., LOFTUS E.V. JR., SANDBORN W.J., BRYANT S.C., SMYRK T.C. Methylation status of genes in non-neoplastic mucosa from patients with ulcerative colitis-associated colorectal cancer. *Am. J. Gastroenterol*., 2010, **105** : 1610-9.
71. MORIYAMA T., MATSUMOTO T., NAKAMURA S., JO Y., MIBU R., YAO T. *et al*. Hypermethylation of p14 (ARF) may be predictive of colitic cancer in patients with ulcerative colitis. *Dis. Colon Rectum*, 2007, **50** : 1384-92.
72. SATO F., HARPAZ N., SHIBATA D., XU Y., YIN J., MORI Y. *et al*. Hypermethylation of the p14(ARF) gene in ulcerative colitis-associated colorectal carcinogenesis. *Cancer Res*., 2002, **62** : 1148-51.
73. HSIEH C.J., KLUMP B., HOLZMANN K., BORCHARD F., GREGOR M., PORSCHEN R. Hypermethylation of the p16INK4a promoter in colectomy specimens of patients with long-standing and extensive ulcerative colitis. *Cancer Res*., 1998, **58** : 3942-5.
74. WHEELER J.M., KIM H.C., EFSTATHIOU J.A., ILYAS M., MORTENSEN N.J., BODMER W.F. Hypermethylation of the promoter region of the E-cadherin gene (CDH1) in sporadic and ulcerative colitis associated colorectal cancer. *Gut*, 2001, **48** : 367-71.
75. JAWAD N., DIREKZE N., LEEDHAM S.J. Inflammatory bowel disease and colon cancer. Recent. Results. *Cancer Res*., 2011, **185** : 99-115.
76. BAUMGART D.C. Endoscopic surveillance in Crohn's disease and ulcerative colitis : who needs what and when ? *Dig. Dis*., 2011, **29** : S32-5.
77. SCHMIDT C., BIELECKI C., FELBER J., STALLMACH A. Surveillance strategies in inflammatory bowel disease. *Minerva. Gastroenterol. Dietol*., 2010, **56** : 189-201.
78. KIESSLICH R. Chromoendoscopy : what is its true value for ulcerative colitis surveillance ? *Dig. Dis*., 2010, **28** : 445-51.
79. ULLMAN T.A. Should chromoendoscopy be the standard of care in ulcerative colitis dysplasia surveillance ? *Gastroenterol. Hepatol. (NY)*, 2010, **6** : 616-20.
80. HLAVATY T., HUORKA M., KOLLER T., ZITA P., KRESANOVA E., RYCHLY B. *et al*. Colorectal cancer screening in patients with ulcerative and Crohn's colitis with use of colonoscopy, chromoendoscopy and confocal endomicroscopy. *Eur. J. Gastroenterol. Hepatol*., 2011, **23** : 680-9.
81. MATTAR M.C., LOUGH D., PISHVAIAN M.J., CHARABATY A. Current management of inflammatory bowel disease and colorectal cancer. *Gastrointest. Cancer. Res*., 2011, **4** : 53-61.