Colonic carcinogenesis in IBD: molecular events

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Patients with ulcerative colitis (UC) and Crohn's disease (CD) are at increased risk of developing intestinal cancers via mechanisms that remain incompletely understood. Several evidences suggest a causal link between chronic inflammation and the development of cancer in the gastrointestinal tract. In fact, patients with UC are exposed to repeated episodes of inflammation that predispose to various tumorigenic events and the sequence of these events are different from those that contribute to develop a sporadic colorectal cancer. In UC carcinogenesis the early events are represented by DNA methylation that produce an inhibition of onco-suppressor genes, mutation of p53, aneuploidy and microsatellite instability. Hypermethylation of tumor suppressors and DNA mismatch repair gene promoter regions, is an epigenetic mechanism of gene silencing that contributes to tumorigenesis and might represent the first step in inflammatory carcinogenesis. P53 is frequently mutated in the early stages of UC-associated cancer, in 33-67% of patients with dysplasia and in 83-95% of UC related cancer patients. Moreover, aneuploidy is an independent risk factor for forthcoming carcinogenesis in UC. Finally, the inconsistency between the high cumulative rate of dysplasia in UC and the relatively lower incidence of invasive cancer raises the question about the mechanisms of immunosurveillance that may prevent malignant progression of neoplasm in the colon in most cases. Co-stimulatory molecule CD80 up-regulation in colonic mucosa in UC dysplasia may be one of these mechanism.

KEY WORDS: Aneuploidy, CD80, Colonic dysplasia, Colorectal cancer, Hypermethilation, Immunosurveillance, p53, Ulcerative colitis

Introduction

Patients with ulcerative colitis (UC) and Crohn's disease (CD) are at increased risk of developing intestinal cancers via mechanisms that remain incompletely understood. Several evidences suggest a causal link between chronic inflammation and the development of cancer in the gastrointestinal tract [1,2]. In fact, chronic inflammation and repeated events of inflammatory relapse in inflammatory bowel disease (IBD) expose these patients to a number of signals known to have tumorigenic effects. These signals include persistent activation of the nuclear factor-kappa B and cyclooxygenase-2/prostaglandin pathways, release of proinflammatory mediators such as tumor necrosis factor-alpha and interleukin-6, and enhanced local levels of reactive oxygen and nitrogen species. These inflammatory signals can contribute to carcinogenesis via 3 major processes: 1) by increasing oxidative stress, which promotes DNA muta-

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genesis thus contributing to tumor initiation; 2) by activating prosurvival and antiapoptotic pathways in epithelial cells, thereby contributing to tumor promotion; and 3) by creating an environment that supports sustained growth, angiogenesis, migration, and invasion of tumor cells, thus supporting tumor progression and metastasis.[3]
Neoplastic lesions in UC differ from sporadic adenomas and carcinomas in that they generally occur in younger individuals and in flat mucosa within large fields of genetic abnormalities, rather than and isolated and visible polypoid lesions [4,5,6].
Nonetheless, many of the genetic abnormalities observed in sporadic neoplasms, including alterations in the *APC*, *p53*, *bcl-2*, and K-*ras* genes, microsatellite instability, and aneuploidy, among others, are also found in UC neoplasia, albeit with different prevalence and timing in many cases[7-15].

DNA damage and genomic instability

Genomic instability includes microsatellite instability (MIN) associated with mutator phenotype, and chromosome instability (CIN) recognized by gross chromosomal abnormalities [16]. Three intracellular mechanisms are involved in DNA damage repair: nucleotide excision repair (NER), base excision repair (BER) and mismatch repair (MMR). Their alteration/inactivation can lead to mutator phenotype. The CIN pathway is typically associated with the accumulation of mutations in tumor suppressor genes and oncogenes [16]. Defects in DNA MMR and CIN pathways are responsible for a variety of hereditary cancer predisposition syndromes including hereditary non-polyposis colorectal carcinoma, Bloom syndrome, ataxia-telangiectasia, and Fanconi anaemia [16]. While there are many genetic contributors to CIN and MIN, there are also epigenetic factors that have emerged to be equally damaging the cell-cycle control. Hypermethylation of tumor suppressor and DNA MMR gene promoter regions, is an epigenetic mechanism of gene silencing that contributes to tumorigenesis. Telomere shortening has been shown to increase genetic instability and tumor formation in mice, underscoring the importance of telomere length and telomerase activity in maintaining genomic integrity [16]. Aneuploidy is an independent risk factor for forthcoming carcinogenesis in UC. A worse prognosis of patients with ulcerative colitis-associated colorectal cancer compared with those with sporadic colorectal cancer has been reported. UC-associated carcinomas presented aneuploidy at significantly higher frequency than sporadic colorectal carcinomas. Ulcerative colitis-associated colorectal cancers and aneuploid sporadic colorectal cancers share a similar prognosis, poorer than to that of diploid sporadic colorectal carcinomas. Aneuploidy proved to be the strongest independent prognostic marker for R0-resected colorectal cancer patients overall [17].

Chromosome instability

Genomic instability in the epithelium of UC patients with dysplasia is significantly higher than the instability of UC patients without dysplasia/cancer. Genomic instability has been identified in sporadic colon cancers and adenomas, but genomic instability does not seem to be present in non-dysplastic tissues from the sporadic cases [18]. In contrast, in the epithelium of UC patients instability measured by DNA fingerprinting is widespread, precedes neoplastic transformation, and seems to be related to the extensive and chronic inflammation. These observations parallel the pathobiology of UC cancers, which differ from sporadic colon cancers, in that UC neoplasia is frequently multifocal, widespread, and may occur in flat mucosa.
What is the cause of this instability? UC colonocytes undergo high levels of genetic damage. Chronic inflammation in UC has been associated with elevated levels of reactive oxygen species and reduced oxidative defences, both of which might contribute to genetic damage [14,19-25]. Reactive oxygen species can cause DNA damage in the form of base alterations, "abasic" sites, and strand breaks and each of these types of damage could cause the genomic instability. Thus, a possible hypothesis is that in a subset of UC patients (ie, the progressors), the colonic epithelium is damaged by reactive oxygen species and this, in turn, leads to the observed genomic instability and eventually tumor progression. In support of this hypothesis, microsatellite instability in the nondysplastic mucosa of UC patients with dysplasia and cancer had previously been described [14], as well as chromosomal instability measured by *in situ* hybridization [26] and comparative genomic hybridization [27]. Moreover, it as been demonstrated that microsatellite instability can be caused by reactive oxygen species [28]. Genomic instability occurs at the same level (10%) throughout the neoplastic progression in UC. Thus, the genomic instability does not accumulate as the neoplasia progresses, but it rather occurs very early and stays at a steady level. A steady state of instability may reflect a maximal tolerated degree of genetic damage and the dynamic rate of cell death, repair, and cell turnover in the UC colon. The non progressor patients may undergo less oxidative stress in their colon than UC progressors, or perhaps they may have a lower susceptibility for genomic instability after oxidative stress because they have better protective mechanisms. For example, glutathione and glutathione *S*-transferase levels vary in UC patients and could influence the levels of oxygen-free radicals [29]. A small amount of heterogeneity was also found in the non-progressor group: 20% of the patients demonstrated increased genomic instability in negative colonic mucosa at the optimized combination of sensitivity and specificity. The percentage of patients showing instability approximates the percentage of patients with negative histology that one might expect to histologically progress

in the next 20 to 30 years [30]. If this can be confirmed by future prospective study, measurement of genomic instability could be applied to identify those UC patients at increased risk of future cancer progression. In conclusion, the finding of widespread genomic instability in UC patients with neoplasia sheds light on the underlying mechanism of UC tumorigenesis, one of a mutator phenotype; Moreover, it may provide a molecular biomarker for patients who have neoplasia or might develop it in the future, offering a useful adjunct to histological-based surveillance [31].

Microsatellite instability

Carginogenesis in UC can be associated also to microsatellite instability. Microsatellites are short repetitive sequences (1- to 5-nucleotide) of DNA that seem to be randomly distributed throughout the genome. Stability of these sequences is a good measure of the general integrity of the genome. Microsatellite instability reflects a gain or loss of repeat units in a germline microsatellite allele, indicating the clonal expansion that is a typical feature of a neoplasm. A high incidence of MSI in long standing UC with severe inflammation probably reflects genomic instability that is caused by repeated inflammatory stress. Thus, the influence of inflammation should be considered when estimating MSI in UC[32]. In fact, although the molecular mechanisms involved in the increased risk remain unclear, UC seems to be associated with microsatellite instability (MSI) [14,33]. The prevailing hypothesis of a cause for MSI in chronic inflammatory diseases is that overproduction of free radicals saturates the ability of the cell to repair DNA damage prior to replication [14,34]. Another hypothesis is that oxidative stress inactivates the human DNA mismatch repair system directly [35]. One study reported 6 of 13 mucosal samples with high MSI as having hMLH1 hypermethylation [36]. Unlike the findings in hereditary nonpolyposis colon cancer, however, other studies found little evidence for mismatch repair defects as a cause of MSI in UC [37,38]. This raises the possibility that mechanisms other than mismatch repair defects exist. An adaptive increase in the activities of 3-methyladenine DNA glycosylase (AAG) and apurinic endonuclease (APE1) in areas of UC colon undergoing active inflammation was recently observed [39]. Interestingly, this adaptive and imbalanced increase seemed associated with the MSI observed in UC. The data were consistent with a novel mechanism by which patients with chronic inflammatory diseases acquire MSI. UC patients were shown to have increased AAG and APE1 enzyme activities in epithelial areas of their colon undergoing active inflammation and UC patients with MSI seem to have the largest increase and imbalance in levels of AAG and APE1 in inflamed colonic areas. These observations indicated that the adaptive imbalanced increase in BER enzymes may have DNA-damaging effects and contribute to carcinogenesis in chronic inflammation [39]. Moreover, it was demonstrated that microsatellite instability can be caused by reactive oxygen species [40]. The non-progressor patients may be subject to less oxidative stress in their colonic mucosa than UC progressors, or perhaps they have a lower susceptibility to developing genomic instability after oxidative stress because of better protective mechanisms. For example, glutathione and glutathione *S*-transferase levels vary in UC patients and could influence the levels of oxygen-free radicals [41].

Mitochondrial DNA mutations

It is well known that reactive oxygen species (ROS) in inflammation are important inducers of both tissue injury and DNA damage [42]. Since mitochondrial DNA (mtDNA) lacks histones and related protective systems, mutations accumulate to a greater extent in this DNA subtype than in nuclear DNA [43]. The human mitochondrial genome includes a 16.5-kb circular double-stranded DNA molecule that encodes 13 polypeptides of the respiratory chain, 22 transfer RNAs, and two ribosomal RNAs required for protein synthesis. Since expression of the entire mitochondrial genome is necessary for the maintenance of mitochondrial functions, including electron transport, small changes in the mtDNA sequence can result in profound impairment of such functions, thereby enhancing generation of free radicals, which in turn accelerates the rate of DNA mutation. These highly reactive compounds can act as initiators and/or promoters, cause DNA damage, activate procarcinogens, and inactivate antioncogenes [44,45]. Therefore, the resulting injury to the mitochondria underlying chronic inflammation may contribute to the early stages of carcinogenesis. A recent study reported that oxidative stress associated with chronic hepatitis strongly enhanced the mutation of mtDNA both in cancerous and non-cancerous regions of the liver [46]. Such mutations of mtDNA are also detected in human cancers [47,48]. Interestingly, accumulation of mtDNA mutations in cancerous tissue reflected the degree of malignancy. In fact, the genetic instability in the process of colonic carcinogenesis results in the high rate of mtDNA mutation, and sequenced the colorectal mucosal mtDNA in patients with UC. The increased instability of genes in mtDNA is consistent with the high incidence of colorectal cancer in individuals with UC.

A recent study by Nishikawa et al, reported that the number of mtDNA mutations in colorectal mucosa from patients with UC is substantially higher than that previously reported with other types of cancer [49-51]. The frequency and chronological process of genetic mutations underlying sporadic cancer (adenoma-carcinoma sequence) and UC-associated carcinogenesis are different. Even though the precise mechanism for such differences

is not known, increased ROS generation in the UC intestine [52,53] is thought to be a major cause of DNA damage in the inflammatory process [54]. Thus, the high incidence of mtDNA mutation in the colorectal mucosa of UC patients indicates that mutation of nuclear DNA is also enhanced in the epithelial cells in the colon of UC patients during long-lasting inflammation [49]. The observation that most mtDNA mutations found in UC patients were homoplasmic in nature indicates that the mutated mtDNA had become dominant in the colorectal mucosa of UC individuals. Mitochondrial DNA harboring certain types of mutations might result in the generation of abnormal proteins, increasing the leakage of electrons from the electron transport chain [49]. The amounts of endogenously produced free radicals might thus be increased in cells with mutant mtDNA [49] The resulting increase in oxidative stress could enhance the mutation of mtDNA and probably nuclear DNA, thereby promoting the early stage of carcinogenesis, in tissues with chronic inflammation. Given the clonal nature and large number of mtDNA copies, mutation of the mitochondrial genome in the colorectal mucosa of UC individuals is indicative of genomic instability that enhances carcinogenesis [49]. The high incidence of mtDNA mutation in colorectal tissues of individuals with UC suggests that the rate of DNA mutation is enhanced in their mucosal cells by oxidative stress caused by chronic inflammation and, hence, malignant transformation occurs more easily than in normal subjects [49].

DNA hypermethylation in UC carcinogeneisis

An other mechanism involved in the UC associated carcinogeneisis is the hypermethylation of Death-Associated Protein Kinase (DAPK) promoter in patients with long-standing UC. DAPK is a pro-apoptotic ptrotein implied in various apoptotic system. In these patients Kuester et al. found that DAPK is overexpressed in inflamed mucosa, suggesting a protective role of this molecule. Thus, inactivation of DAPK by promoter hypermethylation might be crucial for accumulation of DNA damage in inflamed mucosa of UC, and might therefore contribute to the initiation of the neoplastic process and development of UC-associated carcinoma [55]. Increased expression of DNA methyltransferase (DNMT)-1 in non-neoplastic epithelium may precede or be a relatively early event in UC-associated tumorigenesis, and may help predict the risk of colorectal neoplasia in UC [56]. Garrety-Park et al evaluated the methylation status of 10 genes (p16, p14, runt-related transcript factor-3 (RUNX3), cyclooxygenase-2 (COX-2), E-cadherin, methylated-in-tumor-1 (MINT1), MINT31, HPP1, estrogen receptor, SLC5A8) in UC-CRC tumors and non-neoplastic sections from both UC-CRC cases and UC controls [57]. RUNX3, MINT1, and COX-2 resulted to be hypermethylated in presence of CRC in patients with UC, thus these genes could be used as biomarkers for colorectal dysplasia [57].

Oncogenes involvement on inflammatory colonic carcinogenesis: NF-kB and COX-2 pathways

In inflammatory process, the activation of the transcription factor nuclear factor kB (NF-kB) induces the expression of many genes involved in cell survival, including antiapoptotic genes [58,59]. NF-kB regulated proteins inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) in the gastritis-metaplasia-gastric cancer sequence and in the metaplasia-dysplasia-adenocarcinoma sequence in Barrett's oesophagus were extensively studied [60]. Nitric oxide, produced by iNOS, was shown to inhibit apoptosis by inhibiting caspase activity, although chronic exposure to high concentrations of nitric oxide can also promote apoptosis [61]. Van der Wounde et al. found that expression of iNOS was increased in UC-associated dysplasia, whereas iNOS expression was absent in UC-associated carcinoma [62].

COX-2 is an inducible cyclooxygenase whose production is stimulated by interleukin-1, tumor necrosis factor, and many other mediators [63,64]. COX-2 is thought to play a role in the reparative process after mucosal injury in the gastrointestinal tract [63,64]. A number of studies suggest that COX-2 plays a role in sporadic colorectal neoplasia, based on its over expression in colonic adenomas and carcinomas, as shown by both immunohistochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR) [65-67]. Agoff et al. examined COX-2 expression at the protein and mRNA levels on numerous spatially mapped mucosal samples in total colectomy specimens from UC patients who had developed dysplasia or carcinoma. They demonstrated that COX-2 over expression in UC-associated neoplasia occurs early, beginning in mucosa that is diploid and negative for dysplasia, and in mucosa that is non inflammed and that COX-2 protein over expression detected by immunohistochemistry in >60 mapped mucosal samples occurs early in UC-associated neoplastic progression [15].

Two potential mechanisms may explain the relationship between COX-2 over expression and neoplastic progression in UC: it may increase malondialdehyde levels or it may up-regulate *bcl-2*. The first hypothesis contends that increased COX-2 activity, in part brought about by the normal physiological response to injury and inflammation, may accelerate genetic damage through increased production of malondialdehyde, a mutagenic by-product of COX-mediated prostaglandin synthesis and lipid peroxidation[68,69]. This malondialdehyde production would be in addition to that produced by the constitutive activity of COX-1, which is thought to be important in sporadic colorectal neoplasia [63]. In support of this hypoth-

esis, elevated levels of malondialdehyde have been detected both in sporadic colon cancer and in inflammatory bowel disease [70-73]. After the initiation of neoplasia, COX-2 may promote tumor progression by increasing expression of *bcl-2* [74,75]. *Bcl-2* generates resistance to apoptosis, and *bcl-2* up-regulation has also been demonstrated in UC-associated neoplasia. Moreover, overexpression of *bcl-2* is reversible by both nonspecific COX inhibitors [77] and by highly selective COX-2 inhibitors [77].

The overexpression of COX-2 in colorectal adenomas and adenocarcinomas suggests that treatment of individuals without colorectal neoplasms with selective COX-2 inhibitors might lower their risk of developing a cancer. Promising preliminary studies in rodents treated with selective COX-2 inhibitors showed suppression of neoplastic development with minimal toxic side effects [77-79]. Specifically, the prevalence of gastrointestinal side effects, such as ulceration and bleeding, are lower in animals treated with selective COX-2 inhibitors than those given nonspecific cyclooxygenase inhibitors, such as aspirin and other NSAIDs [80,81].

Other oncogenes

Dysplasias and carcinomas associated with UC tend to develop as multiple and superficially extended lesions called DALM (dysplasia-associated lesion or mass) [82-85]. DALMs appear to be frequent in areas of more active inflammation. Therefore, a chronic inflammation –dysplasia- carcinoma sequence has been proposed [86]. Moreover, UC-associated cancer is presumed to arise from an accumulation of genetic alterations in tumor suppressor genes, oncogenes, and genes encoding DNA repair proteins, as well as an overall loss of genomic stability. Comparisons of the molecular alteration profiles of sporadic and UC-associated colorectal cancers have indicated distinct differences. The timing and frequency of the molecular genetic alterations in UC-associated cancers appear to be unique. Mutation of the adenomatous polyposis coli (*APC*) gene is less frequent [87], and that of the *K-ras* gene is relatively less frequent [88]. In UC-associated cancer than in sporadic adenoma and cancer. In contrast, *p53* is frequently mutated in the early stages of UC-associated cancer, 33–67% in dysplasia and 83–95% in UC related cancer [89,90]. These distinctive molecular profiles are presumed to result from different aethiological factors and cellular environments that predispose an individual to the adenoma-carcinoma sequence or to UC-associated carcinogenesis [86].

The mechanisms underlying these differences are yet to be elucidated [86]. In fact, despite knowledge about the use of different genetic markers in detecting colitis associated neoplasia, the pathogenesis of colon cancer in chronic UC is still poorly understood, but there are indications that the pathogenesis is different from that of sporadic colon cancer, namely: (1) dysplasia in UC is preceded by a long history of chronic inflammation and can be seen at sites distant from the cancer, whereas dysplasia in sporadic colon cancer is usually associated with a discrete polyp without inflammation. (2) Mutations in the *ras* protooncogene are present in 40–60% of sporadic colon cancers and are probably an early event; in contrast, in cancer associated with UC, these mutations are less frequently seen and are probably a late event [91-93]. (3) Loss of heterozygosity (LOH) of the *p53* gene and *src* activation occur in UC non-dysplastic epithelium, UC associated dysplasia, and in UC associated carcinoma, whereas there is an absence of LOH of *p53* in regions with negative, indefinite, or low grade dysplastic histology [94]. (4) LOH at the *APC* loci in UC was noted in dysplasia with associated carcinoma, but LOH of *APC* was not present either in cases of non-dysplastic epithelium or in high grade dysplasia alone. Conversely, LOH of APC is present in 20% of colonic adenomas [95,96].

In a recent study of van der Woude et al observed that *Bcl-Bxl* expression was absent in chronic UC, but was clearly positive in tumour cells in the inflammatory carcinogenesis. [62] Moreover, they noted interesting differences in the expression of *Fas* and *Bcl-xl* between tumour cells in UC associated carcinoma and tumour cells in sporadic carcinoma. *Fas* was strongly expressed in most UC associated dysplasia and tumour cells, whereas it was weakly expressed in tumour cells of the sporadic carcinomas. Furthermore, *Bcl-xl* was clearly expressed in chronic UC tumour cells, but showed only weak staining in sporadic colon cancer cells. Because high grade dysplasia is an indication for colectomy, whereas sporadic adenomas may be removed endoscopically, even if they occur in an area histologically involved with colitis, the differential expression of *Fas* and *Bcl-xl* could be used to distinguish chronic UC associated premalignant lesions from sporadic adenomas, and thus prevent unnecessary colectomy. The different expression patterns of proapoptotic and anti-apoptotic proteins did not result in differences in apoptosis. Activated caspase 3, used as a marker of apoptosis, was weakly present in both chronic UC associated colon cancer and sporadic colon cancer, and may be the result of increased apoptosis in the presence of increased (tumour) cell proliferation [62]. Finally, network analysis discovered that *Sp1* and *c-myc* proteins may play roles in UC early and late stages of neoplastic progression, respectively. Two over-expressed proteins in the non-dysplastic tissue of UC progressors, CPS1 and S100P, were further confirmed by IHC analysis [97]. Last but not least, telomerase and ILK activation occurs during the later stages of carcinoma progression, whereas upregulation of survivin, c-MYB, and Tcf-4 is a feature of the early stage of development of neoplasia, and thus, they might serve as early indicators for UC-associated colorectal carcinogenesis [98].

The importance of IL6/p-STAT3 in patients with inflammation-induced CRC was demonstrated. Moreover, SOCS3 may be involved in UC pathogenesis and the absence of SOCS3 seems critical for CRC progression [99]. Oncogenic Smad3 signaling, altered by chronic inflammation and eventually somatic mutations, promotes UC-associated neoplastic progression by upregulating growth-related protein [100].

Immunosurveillance in inflammatory colonic carcinogenesis

The cumulative risk of colon cancer is approximately 8% twenty years after the initial diagnosis and rises to 18% at 30 years. [101,102] Adenocarcinoma of the colon develops from a dysplastic precursor lesion. In UC patients the pre-malignant histological change is broadly referred to as dysplasia, rather than adenoma, since very often the dysplasia is not polypoid [103]. Even if recent data assessed that at least 25% of UC patients may be diagnosed with low grade dysplasia in a 10 year follow up period, some studies, such as the one by Lynch et al in 1993, suggested that low grade dysplasia will develop in all UC patients if they are followed for a sufficient length of time [104,105]. The inconsistency between the high cumulative rate of dysplasia and the relatively lower incidence of cancer raises the question about the mechanisms of surveillance that may prevent malignant progression of neoplasm in the colon in most cases.

Tumour cell escape from immunosurveillance is a well known mechanism that enables unrestrained neoplastic cell growth and metastatic spread. This immune escape is thought to be facilitated both by defence mechanism of tumour cells and by an impaired function of the immune system [106,107].

Both CD4 and CD8 T lymphocytes are the main cells responsible for anti tumour immunity [108,109]. The effective activation of naive T lymphocytes requires engagement of the T cell receptor (TCR) with the major histocompatibility complex (MHC)-antigen-complex in the presence of co-stimulation molecules which ligate the antigen-presenting cell (APC) to the T cell [110-112]. The presentation of MHC-antigen-complex in the absence of co-stimulatory signal induces T-cell anergy [113]. Such co-stimulatory signals are provided by the interaction of CD80 or CD86 on APC surface with their receptors expressed by T-cells [114,115]. CD80 or CD86 binding to CD28 induces tyrosine phosporylation of several substrates and enhances the T cell activation promoted by the MHC-TCR interaction [116]. Increase in CD4/CD8 ratio was observed in sentinel lymph nodes draining dysplastic epithelium compared to normal mucosa. The increase in CD4(+) T cells in relation to CD8(+) T cells correlated with the degree of dysplasia reflected by a significant increase in the ratio against low-grade dysplasia compared to indefinite dysplastic lesions. The T-cell response was specific to antigens from dysplastic epithelial lining as seen in proliferation assays. The observation suggests an important surveillance role for the immune system against premalignant intestinal lesions in patients with long-standing ulcerative colitis [117].

Potentially immunogenic proteins such as the products of oncogenes or oncosuppressor mutated proteins are expressed by colorectal cancer cells but they are not rejected by the immune system. In addition, APC infiltrating colorectal carcinoma that express MHC class II, do not express CD80 or CD86 [118]. In vitro CD80 and CD86 expression by human carcinoma cell lines, up regulated by IFN?, was attributed to the early stage of tumourigenesis when they were selected [119]. As a matter of fact, the role of co-stimulatory molecules in immune response to tumour growth was already suggested in 1995 by Antonia et al who showed that surface CD80 expression can be induced by an oncogenic insult and its down-regulation at a later stage in the carcinogenesis process may lead to immunosurveillance escaping [120]. In previous studies our group demonstrated that there is a significant, specific CD80 over expression in the colon mucosa of patients with UC and dysplasia and a down-regulation at later stages in the carcinogenetic process, while in the non- inflammatory carcinogenesis pathway CD80 is significantly lower at all stages [121,122].

Conclusions

Patients with UC are exposed to repeated episodes of inflammation that predispose to various tumorigenic events and the sequence of these events are different from those that contribute to develop a sporadic colorectal cancer. In fact, as reported in Fig. 1, in UC the early events are represented by DNA methylation that produce an inhibition of onco-suppressor genes, mutation of p53, aneuploidy and microsatellite instability. Hypermethylation of tumor suppressors and DNA mismatch repair gene promoter regions, is an epigenetic mechanism of gene silencing that contributes to tumorigenesis and might represent the first step in inflammatory carcinogenesis. *P53* is frequently mutated in the early stages of UC-associated cancer, in 33–67% of patients with dysplasia and in 83–95% of UC related cancer patients. Moreover, aneuploidy is an independent risk factor for forthcoming carcinogenesis in UC. Finally, the inconsistency between the high cumulative rate of dysplasia in UC and the relatively lower incidence of invasive cancer raises the question about the mechanisms of immunosurveillance that may prevent malignant progression of neoplasm in the colon in most cases. Co-stimulatory molecule CD80 up-regulation in colonic mucosa in UC dysplasia may be one of these mechanism.

Riassunto

I pazienti affetti da rettocolite ulcerosa o morbo di Crohn presentano un rischio aumentato di sviluppare un cancro dell'intestino attraverso meccanismi ancora non completamente chiariti. Molte evidenze suggeriscono un collegamento causale tra l'infiammazione cronica e lo sviluppo del cancro del tratto gstrointestinale. Infatti, i pazienti con rettocolite ulceroso sono esposti a ripetuti episodi infiammatori che predispongono a vari eventi tumorigenici e la sequenza di tali eventi è diversa da quella che contribuisce allo sviluppo del cancro colorettale su base non infiammatoria. Nella carcinogenesi in colite ulcerosa gli eventi precosi sono rappresentati dalla mutilazione del DNA che produce un'inibizione dei geni oncosoppressori di p53, aneuploidia e instabilità dei microsatelliti. L'ipermetilazione degli oncosoppressori e dei geni riparatori del mismatch è un meccanismo epigenetico di silenziamento genetico che contribuisce alla tumorigenesi e potrebbe rappresentare il primo step della carcinogenesi associata alla colite.
La proteina p53 è frequentemente mutata negli stadi precoci della carcinogenesi associata alla colite nel 33-67% dei pazienti con displasia e nel 83-95% dei pazienti con colite e cancro. Inoltre l'aneuploidia è un fattore di rischio indipendente per il successivo sviluppo di cancro in colite. Infine, la discrepanza tra l'alto tasso cumulativo di displasia in colite e la relativamente più bassa incidenza di cancro invasivo solleva la questione sui meccanismi di imunosorveglianza che possono impedire la progressione maligna nella maggior parte dei casi. L'iperespressione della molecola di costimolazione CD80 nella mucosa del colon dei pazienti con colite e displasia potrebbe essere uno di questi meccanismi.

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