Accumulation of mitochondrial DNA mutation with colorectal carcinogenesis in ulcerative colitis

M Nishikawa*, 1, N Oshitani2, T Matsumoto3, T Nishigami4, T Arakawa2 and M Inoue1

1Department of Biochemistry & Molecular Pathology, Osaka City University Medical School, Osaka 545-8585, Japan; 2Department of Gastroenterology, Osaka City University Medical School, Osaka 545-8585, Japan; 3Department of Gastroenterology, Hyogo Medical School, Hyogo 663-8131, Japan; 4Department of Pathology, Hyogo Medical School, Hyogo 663-8131, Japan

We recently reported that oxidative stress elicited by chronic inflammation increases the mutation of mitochondrial DNA (mtDNA) and possibly correlates with precancerous status. Since severe oxidative stress is elicited in the colorectal mucosa of individuals with ulcerative colitis (UC), the possible occurrence of an mtDNA mutation in the inflammatory colorectal mucosa and colitic cancer was investigated. Colorectal mucosal specimens were obtained from individuals with UC with and without colitic cancer and from control subjects. The frequency of mtDNA mutations was higher in colorectal mucosal specimens from patients with UC than that from control subjects. The levels of 8-hydroxy-2'-deoxyguanosine, a DNA adduct by reactive oxygen species, were significantly higher in UC than in control. Specimens from patients with colitic cancer contained a significantly higher number of mtDNA mutations. The present observations suggest that the injury followed by the regeneration of colorectal mucosal cells associated with chronic inflammation causes accumulation of mtDNA mutations. The increased instability of genes, including those on the mtDNA, is consistent with the high and multicentric incidence of colorectal cancer in individuals with UC. Thus, analysis of mtDNA could provide a new criterion for the therapeutic evaluation, and may be useful for the prediction of risk of carcinogenesis.

British Journal of Cancer (2005) 93, 331 – 337. doi:10.1038/sj.bjc.6602664 www.bjcancer.com
Published online 14 June 2005 © 2005 Cancer Research UK

Keywords: ulcerative colitis; mitochondrial DNA; carcinogenesis; inflammation; oxidative stress

Ulcerative colitis (UC) is an idiopathic disease characterised by mucosal inflammation of the large bowel. Approximately 10 individuals per 100 000 per year are diagnosed with UC in Western countries (Russel and Stockbrugger, 1996). The number of patients with UC has also been increasing in Japan, and the incidence is now 53 per 100 000. The aetiopathogenesis of UC remains uncertain, but many factors may be involved in the initiation and propagation of the chronic inflammatory response in UC patients.

The relative risk of colorectal cancer development in UC patients is 10 times greater than in the general population (Brostrom et al, 1978; Kewenter et al, 1978; Prior et al, 1982; Ekbom et al, 1990). The risk of developing cancer, or its precursor lesion, dysplasia, increases exponentially with the duration of the disease (Lashner et al, 1989). Risk factors affecting development of colorectal cancer in UC are: greater extent of disease, duration of disease (younger onset), and severity and time course of inflammation (Collins et al, 1987; Sugita et al, 1990). The histopathogenesis of UC-associated colorectal carcinogenesis is widely believed to involve a step-wise progression from inflamed and hyperplastic epithelia, to flat dysplasia, and finally adenocarcinoma (Riddell et al, 1983). This is often contrasted with the adenoma-carcinoma sequence, thought to give rise to sporadic colon cancer. The idea that cancer derives from a multistep carcinogenesis process, entailing sequential alterations at the molecular level that may underlie tissue-level changes, has gained support from studies on many different cancers (Vogelstein et al, 1988; Sato et al, 1990; Presti et al, 1991). Similarly, 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 (Greenwald et al, 1992; Kern et al, 1994), and that of the K-ras gene is relatively less frequent (Fujimori et al, 1994) in UC-associated cancer than 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 colitic cancer (Yin et al, 1993; Brentnall et al, 1994). These distinctive molecular profiles are presumed to result from different aetiological factors and cellular environments that predispose an individual to the adenoma-carcinoma sequence or to UC-associated carcinogenesis. The mechanisms underlying these differences are yet to be elucidated.

It is well known that reactive oxygen species (ROS) in inflammation are important inducers of both tissue injury and DNA damage (Beckman and Ames, 1997). Since mitochondrial DNA (mtDNA) lacks histones and related protective systems,
mutations accumulate to a greater extent in it than in nuclear DNA (Croteau and Bohr, 1997). The human mitochondrial genome comprises 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 anti-oncogenes (Trush and Kensing, 1991; Feig et al, 1994). Therefore, the resulting injury to the mitochondria underlying chronic inflammation may contribute to the early stages of carcinogenesis. We recently reported that oxidative stress associated with chronic hepatitis strongly enhanced the mutation of mtDNA both in cancerous and noncancerous regions of the liver (Nishikawa et al, 2001). Such mutations of mtDNA are also detected in human cancers (Polyak et al, 1998; Fliss et al, 2000). Interestingly, accumulation of mtDNA mutations in cancerous tissue reflected the degree of malignancy. We therefore hypothesised that the genetic instability in the process of carcinogenesis results in the high rate of mtDNA mutation, and sequenced the colorectal mucosal mtDNA in individuals with UC. The increased instability of genes in mtDNA is consistent with the high incidence of colorectal cancer in individuals with UC.

METHODS

Tissue specimens

The present study was performed in accordance with the Helsinki Declaration of 1975 (1983 revision) and was approved by the ethics committee of the Osaka City University Medical School. The entire sequence was analysed from two controls (Control 1: 62-year-old male; Control 2: 33-year-old male), and four patients with UC (UC-Case 1: 37-year-old male with active colitis; UC-Case 2: 72-year-old female with inactive colitis; UC-Case 3: 58-year-old female with active colitis; and UC-Case 4: 48-year-old male with active colitis complicated with dysplasia-associated lesion or mass, DALM). The particular sequence of the displacement loop (D-loop) was studied in 19 patients with UC without cancer (15 men and four women, mean age 44.8 years, range 26–58), seven patients with UC-associated cancer (three men and four women, mean age 42.6 years, range 33–66), and nine controls (six men and three women, mean age 43.2 years, range 26–66). Samples of mucosal tissue in inflammatory lesions were obtained either by biopsy or as surgical specimens taken from controls suspected of having lower intestinal disease, or from normal mucosa of patients with colorectal cancer or adenoma, and patients with UC who underwent colonoscopy or surgery. The procedure conformed to the approved Institutional Review Board guidelines. Mucosal specimens included 19 samples from 19 UC patients without cancer, four noncancerous and seven cancerous samples from seven patients with colitic cancer, and nine samples from nine control subjects without UC.

Polymerase chain reaction amplification

Fresh specimens were frozen, microdissected with a cryostat, and digested with proteinase K (0.1 mg ml⁻¹) in the presence of 1% SDS. DNA was extracted using phenol–chloroform, followed by ethanol precipitation as described previously (Nishikawa et al, 2001). Each DNA sample (50 ng) was subjected to amplification by polymerase chain reaction (PCR) with overlapping sets of primers to screen the entire mitochondrial genome. Polymerase chain reaction (an initial incubation at 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min) was performed in a final volume of 50 µl with a GeneAmp PCR system 9600 (Perkin-Elmer, Oak Brook, IL, USA). Histopathological diagnosis was confirmed by conventional haematoxylin and eosin staining under light microscopy.

The generation of large PCR products excluded the possibility of nuclear pseudogenes complicating the analysis (Parfait et al, 1998). Furthermore, the primers were selected to avoid such complication by analysis with cell lines devoid of mtDNA.

Sequence analysis

Aberrant PCR products were purified with a QIAquick PCR purification kit (Qiagen, Chatsworth, CA, USA) and sequenced with an Applied Biosystems DNA sequencer (Perkin-Elmer, Oak Brook, IL, USA) and a Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA). The entire sequence of mtDNA was examined, by using the 17 sets of specific primers (Polyak et al, 1998; Fliss et al, 2000; Nishikawa et al, 2001), for two control (Control 1, Control 2) subjects and three individuals with UC (UC-Case 1, UC-Case 2, UC-Case 3). The entire sequence was also analysed for samples from noninflammatory, inflammatory and dysplastic regions in UC-Case 4. The sequence of D-loop (nucleotide position 100–600) was examined with the specific primers 5’-TACCCCTATTAAACCTACGOGGA-3’ (sense) and 5’-TCACTGGAACGGGATGCTTGCG-3’ (antisense) in nine control subjects, 19 individuals with UC without cancer, and seven colitic cancers. All mutations were confirmed by repeated analysis of DNA extracted from the specimens.

Long PCR analysis to detect large deletions in mtDNA

DNA samples (500 ng) were subjected to amplification by a long PCR using an LA PCR Kit (TaKaRa Biomedicals, Kusatsu, Japan) with primer 1 (5’-GGGACATAAGGGTGTCGGGC-3’) and primer 2 (5’-GGGACATAGGGTGTCGGGCCTC-3’). When any deletions were present, products smaller than 16.5-kbp would be generated by this primer set. The amplification profile involved an initial incubation at 94°C for 1 min followed by 14 cycles of 98°C for 20 s, 68°C for 20 min, 20 cycles of 98°C for 20 s, 68°C for 20 min, 15 s, and 72°C for 1 min in a final volume of 50 µl with a GeneAmp PCR system 9600 (Perkin-Elmer, Oak Brook, IL, USA). The resulting products were electrophoresed and stained with ethidium bromide.

Measurement of 8-hydroxy-2'-deoxyguanosine levels in the mucosal specimens

DNA extracted from mucosal specimens of nine control subjects and 10 individuals with UC was suspended in 10 mM Tris-HCl and 0.1 mM EDTA (pH 8.0). In all, 5 µl of 200 mM sodium acetate buffer (pH 4.8) and 5 µg nuclease P1 (USB, Cleveland, OH, USA) were added to 45 µl DNA samples. After purging with a nitrogen steam, the mixtures were incubated at 37°C for 1 h to digest the DNA to nucleotides. Then, 5 µl of 500 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, and 0.6 units Escherichia coli alkaline phosphatase (Toyobo, Tokyo, Japan) were added to the samples. After purging with a nitrogen steam, the mixtures were incubated at 37°C for 1 h to hydrolyse the nucleotides to nucleosides. The nucleoside samples were used for the determination of 8-hydroxy-2'-deoxyguanosine (8-OHdG) by competitive ELISA kit (8-OHdG Check; Japan Institute for the Control of Aging, Tokyo, Japan).

Statistical analysis

Statistical analyses of the results were made with Student’s t-test. Spearmann’s correlation coefficient and regression analysis was
Mitochondrial DNA mutation and colorectal carcinogenesis
M Nishikawa et al

RESULTS
The entire mitochondrial genomes of four UC specimens and two control specimens were amplified by PCR and sequenced manually. In UC-Case 4, the entire mitochondrial genomes from three different specimens, noninflamed, inflamed, and DALM, were analysed. The nuclear positions and changes of mtDNA mutations found in Control 1, Control 2, UC-Case 1, UC-Case 2, and UC-Case 3 are indicated in Table 1. When compared with the mtDNA sequence stored in GenBank (accession no. #J01415) and MITOMAP (http://www.mitomap.org/), the mtDNA sequence obtained from the control specimens was found to contain 10

Table 1  mtDNA changes detected in the entire sequence of Control 1, 2 and UC-Case 1–3

| Control 1 | Control 2 | UC-Case 1 | UC-Case 2 | UC-Case 3 |
|-----------|-----------|-----------|-----------|-----------|
| Position  | Gene      | Change    | Position  | Gene      | Change    | Position  | Gene      | Change    | Position  | Gene      | Change    |
| 248       | D-loop    | del A     | 741       | 12S rRNA  | A→G       | 191       | D-loop    | del A     | 500       | D-loop    | del C     |
| 2472      | 16SrRNA   | del A     | 2687      | 16SrRNA   | del C     | 200       | D-loop    | A→C       | 514       | D-loop    | del CA    |
| 3969      | ND1       | C→T       | 6479      | COI       | ins A     | 490       | D-loop    | A→C       | 751       | 12S rRNA  | A→G       |
| 4769      | ND2       | A→G       | 8639      | ATPase6   | T→C       | 1000      | 12S rRNA  | A→G       | 2686      | 16SrRNA   | G→C       |
| 6392      | COI       | C→T       | 8850      | ATPase6   | A→G       | 1438      | 12S rRNA  | A→G       | 4386      | Q         | T→C       |
| 9053      | ATPase6   | G→A       | 8860      | ATPase6   | A→G       | 1468      | 12S rRNA  | C→G       | 4769      | ND2       | A→G       |
| 10 309     | ND3       | T→A       | 11 720     | ND4       | G→A       | 3010      | 12S rRNA  | G→A       | 4958      | ND2       | A→G       |
| 10 590     | ND4L      | T→G       | 15 326     | Cyt.b     | G→A       | 3285      | L         | T→G       | 5895      | COI       | ins C     |
| 11 947     | ND4       | A→C       | 3338      | ND1       | C→T       | 4662      | ND2       | A→G       | 7337      | COI       | G→A       |
| 11 953     | ND4       | A→C       | 4768      | ND2       | T→G       | 4868      | ND2       | C→T       | 8861      | ATPase6   | C→G       |
|           |           |           | 5178      | ND2       | C→T       | 5942      | COI       | A→G       | 11 084    | ND4       | A→G       |
|           |           |           | 9860      | COIII     | T→G       | 9883      | COIII     | T→G       | 11 771    | ND4       | A→G       |
|           |           |           | 11 853     | ND4       | T→G       | 11 247     | ND4       | C→G       | 12 417    | ND5       | C→T       |
|           |           |           | 11 457     | ND4       | C→G       | 11 712     | ND4       | C→G       | 13 386    | ND5       | C→T       |
|           |           |           | 11 719     | ND4       | G→A       | 11 956     | ND4       | C→T       | 14 364    | ND6       | G→A       |
|           |           |           | 14 570     | ND6       | C→T       | 14 675     | D-loop    | C→T       | 15 043    | Cyt.b     | G→A       |
|           |           |           | 16 245     | D-loop    | C→T       | 16 029     | D-loop    | T→C       | 16 184    | D-loop    | C→T       |

ND = NADH (reduced nicotinamide adenine dinucleotide) dehydrogenase; CO = cytochrome c oxidase; ATPase, ATP synthase; Cyt.b, cytochrome b; F, V, L, I, Q, M, W, A, N, C, Y, D, K, G, R, H, E, T, and P = tRNAs for phenylalanine, valine, leucine, isoleucine, glutamine, methionine, tryptophan, alanine, asparagine, cysteine, tyrosine, serine, aspartate, lysine, glycine, arginine, histidine, glutamate, threonine, and proline, respectively. ins = insertion; del = deletion.

Figure 1  Location of mtDNA mutations in specimens of noninflammatory, inflammatory, and DALM from a patient with UC (UC-Case 3). Arrows indicate the positions of mutations in mtDNA. Abbreviations: ND, NADH (reduced nicotinamide adenine dinucleotide) dehydrogenase; CO, cytochrome c oxidase; ATPase, ATP synthase; Cyt.b, cytochrome b; F, V, L, I, Q, M, W, A, N, C, Y, D, K, G, R, H, E, T, and P, tRNAs for phenylalanine, valine, leucine, isoleucine, glutamine, methionine, tryptophan, alanine, asparagine, cysteine, tyrosine, serine, aspartate, lysine, glycine, arginine, histidine, glutamate, threonine, and proline, respectively.
and eight mutations. This fact is consistent with a previous observation that mtDNA in colorectal mucosa contains much changes per individual (Polyak et al., 1998). The three UC specimens contained 25 (UC-Case 1), 25 (UC-Case 2), and 28 (UC-Case 3) nucleotide changes. Consistent with previous observations (Polyak et al., 1998; Fliss et al., 2000; Nishikawa et al., 2001), most of the mtDNA mutations found in the present study were homoplasmic.

Total mtDNA sequences could be compared in three different samples in a patient with UC (UC-Case 4). The mtDNA sequence obtained from the noninflammatory region (near/around the inflammatory region) contained 41 mutations. Surprisingly, the inflammatory and DALM specimens contained one and five further mutations, respectively, in addition to 41 mutations found in the noninflamed region in this patient (Figure 1).

To determine any large deletion profile in mtDNA, DNA samples from Control 1, Control 2, and UC-Case 1, UC-Case 2, UC-Case 3 and UC-Case 4 were subjected to a long PCR analysis (Figure 2). However, no deletion was found in these six samples. Furthermore, all of DNA samples used in the present study were analysed with the same results. As shown in Table 1 and Figure 1, the frequency of mtDNA mutations was relatively high in the D-loop. We then compared the position and the number of mutations in the D-loop region (between nucleotides 100 and 600) among nine controls, 19 patients with UC without cancer, and seven patients with colitic cancer (Table 2: position; Figure 3: number). For control specimens, the median number of mutations in the D-loop was 0.67. The corresponding values were 2.74 for specimens of UC without

Table 2  mtDNA sequence changes (nucleotide position 100–600) in control, UC without cancer, and UC with cancer

| Control | UC without cancer | UC with cancer |
|---------|------------------|----------------|
| Sample number | Position | Sample number | Position | Sample number | Position | Sample number | Position |
| 1 | 248, 548 | 1 | 248, 302, 482 | 1 | 199, 248, 302, 514, 527 | 1 | 194, 248, 302, 320, 514, 527 |
| 2 | 302 | 2 | 499 | 2 | 194, 248, 527 | 2 | 199, 248, 302, 514, 527 |
| 3 | 514 | 3 | 302, 482 | 3 | 191, 195, 199, 248, 302, 320, 514, 527 | 3 | 248, 302, 320, 514 |
| 4 | — | 4 | 199, 248, 302, 320, 514, 527 | 4 | 194, 248, 302, 320, 514, 527 | 4 | 191, 195, 199, 248, 302, 317, 320, 456, 514, 527 |
| 5 | — | 5 | 302, 482 | 5 | 199, 248, 302, 514, 527 | 5 | 194, 248, 302, 514, 527 |
| 6 | — | 6 | 248, 302, 320, 514 | 6 | 194, 248, 302, 514, 527 | 6 | 194, 248, 302, 320, 514, 527 |
| 7 | 302 | 7 | — | 7 | 194, 248, 302, 320, 514, 527 | 7 | 194, 248, 302, 320, 514, 527 |
| 8 | 302 | 8 | 514 | 8 | — | 8 | — |
| 9 | — | 9 | 248, 302 | 9 | 199, 248, 302, 514, 527 | 9 | 199, 248, 302, 514, 527 |
| 10 | 199, 248, 302, 514, 527 | 10 | 199, 248, 302, 514, 527 | 10 | 199, 248, 302, 514, 527 | 10 | 199, 248, 302, 514, 527 |
| 11 | 514 | 11 | 514 | 11 | 514 | 11 | 514 |
| 12 | 302, 482, 514 | 12 | 302, 482, 514 | 12 | 302, 482, 514 | 12 | 302, 482, 514 |
| 13 | 302, 499 | 13 | 302, 499 | 13 | 302, 499 | 13 | 302, 499 |
| 14 | 194, 199, 302, 527 | 14 | 194, 199, 302, 527 | 14 | 194, 199, 302, 527 | 14 | 194, 199, 302, 527 |
| 15 | 199, 248, 302, 514, 527 | 15 | 199, 248, 302, 514, 527 | 15 | 199, 248, 302, 514, 527 | 15 | 199, 248, 302, 514, 527 |
| 16 | — | 16 | — | 16 | — | 16 | — |
| 17 | 199, 248, 320, 514 | 17 | 199, 248, 320, 514 | 17 | 199, 248, 320, 514 | 17 | 199, 248, 320, 514 |
| 18 | 194, 248, 527 | 18 | 194, 248, 527 | 18 | 194, 248, 527 | 18 | 194, 248, 527 |
| 19 | 248, 302, 482, 514 | 19 | 248, 302, 482, 514 | 19 | 248, 302, 482, 514 | 19 | 248, 302, 482, 514 |

Figure 2  Long PCR analysis to detect large deletions in mtDNA. DNA samples from Control 1 (lane 1), 2 (lane 2) and UC-Case 1–4 (lane 3–6, respectively) were subjected to long PCR analysis as described in the text. M, molecular marker.
DISCUSSION

Our data show that the number of mtDNA mutations in colorectal mucosa from patients with UC tissue is substantially higher than that previously reported with other types of cancer (Polyak et al., 1998; Fliss et al., 2000). 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 (Oshitani et al., 1993; Tomobuchi et al., 2001) is thought to be a major cause of DNA damage in the inflammatory process (Seril et al., 2003), as suggested in Table 3. 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 colorectal epithelial cells of UC patients during long-lasting inflammation. 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. The amounts of endogenously produced free radicals might thus be increased in cells with mutant mtDNA. 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.

Each colorectal mucosal cell contains hundreds of mitochondria and each mitochondrion contains ~10 genomes (Wallace, 1992). The mitochondria with mtDNA mutations in colorectal mucosal cells proliferate selectively when such cells are fused with normal cells (Poljak et al, 1998), possibly because certain mutant mtDNA molecules exhibit a replicative advantage. The D-loop region of mtDNA is important for both replication and expression of the mitochondrial genome because it contains the leading-strand origin and promotes replication and transcription, respectively (Ta Brennan O, Lofberg R, Nordenvall B, Ost A, Hellers G (1978) The risk of colorectal cancer in ulcerative colitis. An epidemiologic study. Scand J Gastroenterol 3: 331 – 337

ACKNOWLEDGEMENTS

This work was supported in part by grants from the Ministry of Education, Science, Sports, and Culture and the Special Coordination funds for Science and Technology from the Science and Technology Agency, as well as by the National Institute of Health of Japan.
the p53, Rb, and mcc/apc tumor suppressor gene loci in dysplastic and cancerous ulcerative colitis. Cancer Res 52: 741 – 745
Kern SE, Redston M, Seymour AB, Caldas C, Powell SM, Kornacki S, Kinzler KW (1994) Molecular genetic profiles of colitis-associated neoplasms. Gastroenterology 107: 420 – 428
Kewenter J, Ahlman H, Hulten L (1978) Cancer risk in extensive ulcerative colitis. Ann Surg 188: 824 – 828
Lashner BA, Silverstein MD, Hanauer SB (1989) Hazard rates for dysplasia and cancer in ulcerative colitis. Results from a surveillance program. Dig Dis Sci 34: 1536 – 1541
Nishikawa M, Nishiguchi S, Shiomi S, Tamori S, Koh N, Takeda T, Kubo S, Hirohashi K, Kinoshita H, Sato E, Inoue M (2001) Somatic mutation of mitochondrial DNA in cancerous and noncancerous liver tissue in individuals with hepatocellular carcinoma. Cancer Res 61: 1843 – 1845
Oshitani N, Kitano A, Okabe H, Nakamura S, Matsumoto T, Kobayashi K (1993) Location of superoxide anion generation in human colonic mucosa obtained by biopsy. Gut 34: 936 – 938
Parfait B, Rustin P, Munnich A, Rotig A (1998) Co-amplification of nuclear pseudogenes and assessment of heteroplasmy of mitochondrial DNA mutations. Biochem Biophys Res Commun 247: 57 – 59
Polyak K, Li Y, Zhu H, Lengauer C, Willson JK, Markowitz SD, Trush MA, Kinzler KW, Vogelstein B (1998) Somatic mutations of the mitochondrial genome in human colorectal tumors. Nat Genet 20: 291 – 293
Presti J, Reuter VE, Galan T, Fair WR, Cordon-Cardo C (1991) Molecular genetic alterations in superficial and locally advanced human bladder cancer. Cancer Res 51: 5405 – 5409
Prior P, Gyde SN, Macartney JG, Thompson H, Waterhouse JA, Allan RN (1982) Cancer morbidity in ulcerative colitis. Gut 23: 490 – 497
Riddell RH, Goldman H, Ransohoff DF, Appelman HD, Fenoglio CM, Haggitt RC, Ahren C, Correa P, Hamilton SR, Morson BC (1983) Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. Hum Pathol 14: 931 – 966
Russel MG, Stockbrugger RW (1996) Epidemiology of inflammatory bowel disease: an update. Scand J Gastroenterol 31: 417 – 427
Sato T, Tanigami A, Yamakawa K, Akiyama F, Kasumi F, Sakamoto G, Nakamura Y (1990) Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. Cancer Res 50: 7184 – 7189
Seril DN, Lian J, Tang TY, Yang CS (2003) Oxidative stress and ulcerative colitis-associated carcinogenesis: studies in humans and animal models. Carcinogenesis 24: 353 – 362
Sugita A, Sachar DB, Bodian C, Ribeiro MB, Aufses JH, Greenstein AJ (1990) Colorectal cancer in ulcerative colitis. Influence of anatomical extent and age at onset on colitis-cancer interval. Gut 32: 167 – 169
Taam JW (1999) The mitochondrial genome: structure, transcription, translation and replication. Biochim Biophys Acta 1410: 103 – 123
Taylor RW, Barron MJ, Borthwick GM, Gospel A, Chinnery PF, Samuels DC, Taylor GA, Plusa SM, Needham SJ, Greaves LC, Kirkwood TB, Turnbull DM (2003) Mitochondrial DNA mutations in human colon crypt stem cells. J Clin Invest 112: 1351 – 1360
Tomobuchi M, Oshitani N, Matsumoto T, Kitano A, Seki S, Tetsuo A (2001) In situ generation of nitric oxide by myenteric neurons but not by mononuclear cells of the human colon. Clin Exp Pharmacol Physiol 28: 13 – 18
Trush MA, Kessler TW (1991) An overview of the relationship between oxidative stress and chemical carcinogenesis. Free Radic Biol Med 10: 201 – 209
Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AMM, Bos JL (1988) Genetic alterations during colorectal-tumor development. N Engl J Med 319: 525 – 532
Wallace DC (1992) Diseases of the mitochondrial DNA. Annu Rev Biochem 61: 1175 – 1212
Yamamoto H, Tanaka M, Katayama M, Obayashi T, Nimura Y, Ozawa T (1992) Significant existence of deleted mitochondrial DNA in cirrhotic liver surrounding hepatic tumor. Biochim Biophys Res Commun 182: 913 – 920
Yin J, Harpaz N, Tong Y, Huang Y, Laurin J, Greenwald BD, Hontanosas M, Newkirk C, Meltzer SJ (1993) p53 point mutations in dysplastic and cancerous ulcerative colitis. Gastroenterology 104: 1633 – 1639