Micronucleus analysis in patients with colorectal adenocarcinoma and colorectal polyps

Ali Karaman, Doğan Nasır Binici, Mehmet Eşref Kabalar, Züleyha Çalışkuşu

INTRODUCTION

Genomic instability plays an essential role in the development and progression of human colorectal cancer (CRC)\[1\]. Two major types of genetic instability have been described in CRC: chromosomal instability and microsatellite instability\[1\]. About 60% of CRCs develop through the chromosomal instability pathway, which is characterized by losses and gains of chromosomes (aneuploidy), as well as losses of heterozygosity\[1\].

CRC progresses through four distinct clinical stages that are described as dysplastic crypts, small benign tumors, malignant tumors invading surrounding tissues, and finally metastatic cancer. This progression involves several genetic changes such as inactivation of tumor suppressor genes and activation of oncogenes\[1\]. Mutations of the adenomatous polyposis coli (APC) gene are considered the earliest\[1\] and most prevalent genetic changes in colorectal tumorigenesis. More than 85% of colon cancers are estimated to have a somatic mutation of APC\[1\]. Furthermore, a large number of genes that trigger chromosomal instability have been identified in yeast in the past\[1\]. The underlying mechanisms leading to chromosomal instability in colorectal cancer remain to be characterized. The DNA double-strand break (DSB) is regarded as the most critical of all DNA lesions\[1\], and it has been shown that defects in the cellular response to DSBs can lead to genetic alteration, chromosomal instability, and ultimately malignant transformation\[1\].

The genome damage to the lymphocytes of peripheral blood has been widely used as a biomarker of genotoxic environmental factors, and long-term studies have demonstrated its validity and high clinical productivity\[1\]. Micronucleus (MN) is an acentric chromosome fragment or whole chromosome that is left behind during mitotic cellular division and appears in the cytoplasm of interphasic cells as a...
small additional nucleus\[^{[10]}\]. The formation of MN in dividing cells is the result of chromosome breakage due to unrepaired or mis-repaired DNA lesions, or chromosome malsegregation due to mitotic malfunction. These events may be induced by oxidative stress, exposure to clastogens or aneugens, genetic defects in cell cycle checkpoint and/or DNA repair genes, as well as deficiencies in nutrients required as co-factors in DNA metabolism and chromosome segregation machinery\[^{[11-14]}\]. All these events can cause the formation of MN through chromosomal rearrangements, altered gene expression or aneuploidy; effects associated with the chromosome instability phenotype often seen in cancer\[^{[15,16]}\].

The MN frequency test, widely accepted for \textit{in vitro} and \textit{in vivo} genotoxicity investigations, is a sensitive marker of genomic damage\[^{[17,18]}\]. The presence of an association between MN induction and cancer development is supported by a number of observations. The most substantiated include: the high frequency of MN in untreated cancer patients and in subjects affected by cancer-prone congenital diseases, e.g. Bloom syndrome or ataxia telangiectasia\[^{[15,19]}\], the presence of elevated MN frequencies in oral mucosa, used as a surrogate biomarker of cancer in clinical chemoprevention trials\[^{20}\], the correlation existing between genotoxic MN-inducing agents and carcinogenicity, e.g. ionizing and ultraviolet radiation\[^{[21,22]}\].

A major question in cancer genetics is to what extent chromosomal or genetic instability is an early event and thus a driving force of tumorigenesis\[^{[23,24]}\]. The aim of this study was to determine, by counting MN frequencies, whether chromosomal or DNA damage has an effect on the pathogenesis of early CRC.

MATERIALS AND METHODS

\textbf{Patients}

This study was conducted between May 2008 and September 2008 in the Erzurum Training and Research Hospital. Twenty-one patients with colorectal adenocarcinoma and 24 patients with colorectal polyps were studied. The study was conducted using colonoscopic specimens from subjects with the established diagnosis of colorectal polyps or colorectal adenocarcinoma in histologic analysis. Specimens were separated for each level and placed in 10% formalin solution. The pathologic specimens were reviewed independently by two pathologists.

Pathologists were blinded to the subject’s clinical history, the colonoscopic findings, and the results of the Hematoxylin-Eosin staining assay. Pathologic reading was determined for each biopsy slide with an overall pathologic diagnosis determined for each subject.

We performed MN analysis in 21 (12 females and 9 males; mean age: 57.62 ± 10.84 years) patients with CRC, in 10 (4 females and 6 males; mean age: 52.44 ± 8.36 years) patients with NP, in 14 (6 females, 8 males; mean age: 52.92 ± 9.14 years) patients with NNP and in 20 (8 females and 12 males; mean age: 50.25 ± 9.38 years) healthy controls. The patients were selected from non-smoking and nonalcoholic subjects. None of the subjects had a history of viral infection, bacterial infection or any metabolic diseases. The patients had not been treated with chemotherapy or radiotherapy during the last 4 mo. The patient and control groups were chosen for their similar habits. The hospital Ethical Committee approved the human study. All patients were analyzed prior to treatment.

\textbf{Micronucleus analysis}

For MN analysis, 2 mL of heparinized blood was drawn from each individual. Lymphocyte cultures were established by adding 0.5 mL of whole blood to 5 mL karyotyping medium (Biological Industries, Beit Haemek, Israel) with 2% phytohaemagglutinin M (PHA; Biological Industries) according to standard techniques. The culture was kept at 37°C for 72 h. Cytochalasin B (6 µg/mL, Sigma, USA) was added after 44 h of culture to block cytokinesis, allowing the identification of lymphocytes dividing in culture. Cells that had undergone the first mitosis were thus recognized as binucleated cells and were selectively screened for the presence of MN. The cells were then treated hypotonically with 0.075 mol/L KCl for 5 min at room temperature, and fixed in methanol/acetic acid (3:1). Cells were dropped onto slides and stained with 5% Giemsa in phosphate buffer (pH 6.8) for 5 min A thousand binucleated cells from each case were examined for MN by an experienced observer\[^{[25]}\].

\textbf{Statistical analysis}

The MN rates were analyzed statistically by student’s \( t \)-test. To evaluate the correlations between the age, sex, and MN rates, the coefficients of Spearman \( \rho \) correlation were calculated. A \( P \) value less than 0.05 was considered to be significant.

RESULTS

MN frequencies and clinical data obtained from the patient and control groups are shown in Table 1. According to these results, the mean MN frequency was significantly increased in CRC patients compared with controls (3.72 ± 1.34 vs 1.97 ± 0.81, \( P < 0.001 \)). Similarly, the mean MN frequency was significantly increased in NP patients compared with controls (3.58 ± 1.21 vs 1.97 ± 0.81, \( P < 0.001 \)). However, there was no difference in the mean MN frequency between CRC patients, and NP patients (\( P > 0.05 \)). Similarly, there was no difference in mean MN frequency between NNP patients and controls (2.06 ± 0.85 vs 1.97 ± 0.81, \( P > 0.05 \)). On the other hand, the mean MN frequencies did not correlate with patients’ age or sex in the CRC patients (for each, \( P > 0.05 \)). Similarly, the mean MN frequencies did not correlate with patients’ age or sex in the colon polyp patients (for each, \( P > 0.05 \)).

DISCUSSION

CRCs progress through a series of clinical and his-
Table 1 Micronucleus (MN) results of the patients with colorectal cancer and colon polyps and healthy controls (mean ± SE)

|                     | Sex (F/M) | Age (yr) | Age at diagnosis (yr) | MN/1000 BN |
|---------------------|-----------|----------|------------------------|------------|
| CRC patients (n = 21) | 12/9      | 57.62 ± 10.84 | 56.98 ± 9.45           | 3.72 ± 1.34 |
| NP patients (n = 10)   | 4/6       | 52.44 ± 8.36  | 51.68 ± 8.54           | 3.58 ± 1.21 |
| NNP patients (n = 14)  | 6/8       | 52.92 ± 9.14  | 50.48 ± 8.29           | 2.06 ± 0.85 |
| Controls (n = 20)      | 8/12      | 50.25 ± 9.38  | 51.68 ± 8.54           | 1.97 ± 0.81 |

CRC: Colorectal adenocarcinoma; NP: Neoplastic polyp; NNP: Non-neoplastic polyp.

Genomic instability in colorectal adenocarcinoma

The results of a series of genetic changes that involve activation of oncogenes and inactivations of tumor suppressor genes. In colorectal cancer, chromosomal instability is the major form of genetic instability.[27] It is generally agreed that colorectal cancers develop as a consequence of accumulation of mutations in key genes such as $K-Ras$, $Apc$, and $p53$ that are critical for regulating cell proliferation or cell cycle checkpoint control. In humans, the development of early adenomas to metastatic carcinomas takes somewhere from 20 to 40 years; it is believed that genetic instability plays a key role in accelerating the rate of mutation in cancerous cells.[20]

CRCs exhibit a defect in chromosome segregation, leading to frequent gains or losses of chromosomes (> 10 per chromosome per generation).[28] Chromosome instability has been detected in the smallest adenoma, suggesting that chromosomal instability may occur at very early stages of colorectal carcinogenesis.[29] Extensive research during the past has led to the identification of genes that play a major role in the development of colorectal cancer. For example, mutations or deletions of the adenomatous polyposis coli ($Apc$) gene, encoding a 310-kDa cytoplasmic protein, are commonly found in inherited familial adenomatous polyposis patients and in sporadic colorectal cancers.[32,33] Such mutations appear to be an early event during colorectal tumorigenesis.[4]

The most commonly affected gene in sporadic colon cancer with defective DNA mismatch repair (MMR) is $hMLH1$, with the primary mechanism of gene inactivation being hypermethylation of the promoter.[34] These tumors account for approximately 15% of sporadic colon cancers. The majority of sporadic colon cancers (85%), however, are proficient in DNA MMR but show another form of genomic instability at the gross chromosomal level, which has been called chromosomal instability. Such chromosomal instability represents the end result of a number of processes, including mutations in mitotic checkpoint genes, microtubule spindle defects, and telomere dysfunction.[35]

Two types of genetic instability have been identified, with chromosomal instability predominating.[31,30] The molecular basis for chromosomal instability is just beginning to be explored.[37] A large number of gene alterations can give rise to chromosomal instability in Saccharomyces cerevisiae.[5,38] These genes include those involved in chromosome condensation, sister-chromatid cohesion, kinetochore structure and function, and microtubule formation and dynamics as well as checkpoints that monitor the progress of the cell cycle. To date, the only genes implicated in aneuploidy in human tumor cells are those of the latter class. Heterozygous mutations in the mitotic spindle checkpoint gene $\alpha$BUB1 were detected in a small portion of colorectal tumors with the chromosomal instability[39]. The identification of aneuploidy at early stages of tumor formation in MYH- and $Apc$-mutant polyps is interesting also in view of previous reports showing that loss of $Apc$ function in primary mouse cell lines results in chromosomal instability due to a kinetochore attachment defect at mitosis[36]. It is generally accepted that $Apc$'s main tumor suppressing activity resides in its capacity to bind and regulate Wnt/$\beta$-catenin signal transduction.[40] However, additional $Apc$ functions in cytoskeletal organization, mitotic spindle assembly, cell migration, and apoptosis may play important roles in tumor progression and malignant transformation.[41,42]

It has been demonstrated that chromosomes display nonrandom changes in cancer cells. These include structural rearrangements, e.g. deletions, amplifications or translocations that arise from breaks in DNA, as well as alterations in the number of intact chromosomes, known as whole-chromosome missegregations, originating from errors in cell division (mitosis). As a result of the accumulation of such processes, chromosomal instability is thought to play a key role in tumor development[40].

In the present study, we investigated whether cytogenetic abnormalities participate in the pathogenesis of early CRC. Cytogenetic endpoints are sensitive biomarkers that are widely accepted to evaluate chromosome damage.[43,44] MN assay provides a measure of both chromosome breakage and chromosome loss or nondisjunction in clastogenic and aneugenic events, respectively.[11,13]

MN assay is a sensitive indicator of exogenously or endogenously caused genetic damage and MN frequency has become an important end point in genotoxicity testing both in vivo and in vitro.[37,48] Elevated levels of MN are indicative of defects in DNA repair and chromosome segregation which could result in generation of daughter cells with altered gene dosage, or deregulation of gene expression that could lead to the evolution of the chromosome instability phenotype often seen in cancer.[10,11,15,21]. These considerations give
mechanistic support to a possible causal association between MN frequency and the risk of cancer. A recently published cohort study linking the frequency of micronuclei in lymphocytes of healthy subjects to the risk of cancer reported stomach cancer among the sites most specifically associated with micronuclei frequency\textsuperscript{49}. Similar findings have also been reported for preneoplastic lesions of colon\textsuperscript{45}, esophagus\textsuperscript{46} and cervix\textsuperscript{47}. In particular, the higher risks noted for stomach and intestinal cancers, are in agreement with the literature, which emphasizes the role of chromosome rearrangements in the early stages of these tumours\textsuperscript{47,48}.

Our study, which showed increased MN frequencies in the lymphocytes of CRC and colon polyp patients, could support these observations, as the induction of changes in DNA that lead to mutations plays a role in carcinogenicity. Establishment of inherited susceptibility factors is important in recognizing individuals at a higher risk of developing CRC, so that they may benefit from early detection and prevention programs. Many investigators have demonstrated genomic instability and abnormalities in patients with CRC\textsuperscript{19-21}. Further, experimental evidence shows that early colorectal adenomas have allelic imbalance\textsuperscript{22}. bCDC4 mutations have been shown to occur early in colorectal tumorigenesis\textsuperscript{19,23}. An association between MN and cancer has been reported\textsuperscript{19}. The causes of this association may be structural chromosomal aberrations and aneuploidy\textsuperscript{19}. The presence of an association between the frequency of micronuclei in lymphocytes and cancer risk has been suggested\textsuperscript{11,12,13}. Our findings of a high level of MN frequency in patients with CRC or NP seem to support this association. Thus, MN assay may be performed in lymphocytes as an indicator of genomic instability relevant to colorectal tumorigenesis.

In conclusion, our results indicate that the increased MN frequency in lymphocytes of patients with CRC and NP may reflect genomic instability or deficiency of DNA repair capacity. Further, these results suggest increased chromosome/DNA instabilities may be associated with the pathogenesis of early CRC.

### Applications

MN analysis has come into use as a sensitive means of monitoring DNA damage. MN analysis may be used as a marker to estimate the risk of CRC.

### Terminology

Micronucleus (MN): MN is an acentric chromosome fragment or whole chromosome that is left behind during mitotic cellular division and appears in the cytoplasm of interphase cells as a small additional nucleus.

### Peer review

This study indicated genetic impairment and genetic instability may play an important role in CRC. Further, MN frequency is a promising biomarker for assessing the risk of neoplastic progression in colorectal adenocarcinoma.

### REFERENCES

1. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 1998; 396: 643-649
2. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61: 759-767
3. The Genetic Basis for Human Cancer. 1st ed. In: Vogelstein B, Kinzler KW (eds). Toronto: McGraw Hill, 1998
4. Powell SM, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN, Vogelstein B, Kinzler KW. APC mutations occur early during colorectal tumorigenesis. Nature 1992; 359: 235-237
5. Kolodner RD, Putnam CD, Myung K. Maintenance of genome stability in Saccharomyces cerevisiae. Science 2002; 297: 552-557
6. van Gent DC, Hoeijmakers JH, Kanaar R. Chromosomal stability and the DNA double-stranded break connection. Nat Rev Genet 2001; 2: 196-206
7. Zhou BB, Elledge SJ. The DNA damage response: putting checkpoints in perspective. Nature 2000; 408: 433-439
8. Mills KD, Ferguson DO, Alt FW. The role of DNA breaks in genomic instability and tumorigenesis. Immunol Res 2003; 194: 77-95
9. Hagmar L, Stromberg U, Bonassi S, Hansteen IL, Knudsen LE, Lindholm C, Norppa H. Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts. Cancer Res 2004; 64: 2258-2263
10. Kirsch-Volders M, Sofuni T, Aardema M, Albertini S, Eastmond D, Fenech M, Ishidate M Jr, Kirchner S, Lorge E, Morita T, Norppa H, Surrallés J, Vanhauwaert A, Wakata A. Report from the in vitro micronucleus assay working group. Mutat Res 2003; 540: 153-163
11. Fenech M, Holland N, Chang WP, Zeiger E, Bonassi S. The HUMAN MicroNucleus Project--An international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. Mutat Res 1999; 428: 271-283
12. Umegaki K, Fenech M. Cytokinesis-block micronucleus assay in WIL2-NS cells: a sensitive system to detect chromosomal damage induced by reactive oxygen species and activated human neutrophils. Mutagenesis 2000; 15: 261-269
13. Mateuca R, Lombaert N, Aka PV, Decordier I, Kirsch-Volders M. Chromosomal changes: induction, detection methods and applicability in human biomonitoring. Biochimie 2006; 88: 1515-1531
14. Fenech M, Baghurst P, Luderer W, Turner J, Record S, Ceppi M, Bonassi S. Low intake of calcium, folate, nicotinic acid, vitamin E, retinol, beta-carotene and high intake of pantothenic acid, biotin and riboflavin are significantly associated with increased genome instability—results from a dietary intake and micronucleus index survey in South Australia. Carcinogenesis 2005; 26: 991-999
15. Fenech M. Chromosomal biomarkers of genom instability relevant to cancer. Drug Discov Today 2002; 7: 1128-1137
16. Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E. HUMAN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus

### COMMENTS

**Background**

It is known there is an increased micronucleus (MN) frequency rate in neoplastic disease. Colorectal adenocarcinoma (CRC) is a common cause of cancer-related deaths worldwide, despite improved diagnostic and therapeutic implications. Hence, early diagnosis has critical importance. The aim of this study was to determine, by counting MN frequencies, whether chromosomal or DNA damage has an effect on the pathogenesis of early CRC.

**Research frontiers**

The MN frequency test, widely accepted for in vitro and in vivo genotoxicity investigations, is a sensitive marker of genomic damage. Therefore, in this study, we aimed to determine, by assessing MN rates, whether genetic impairment and DNA damage have an effect on the pathogenesis of CRC.

**Innovations and breakthroughs**

Our results suggest increased genomic instability may be associated with the pathogenesis of early CRC. The identification of increased MN frequency rate in patients with colorectal lesions may be helpful in the early diagnosis of CRC.
 assay using isolated human lymphocyte cultures. Mutat Res 2003; 534: 65-75
17 Fenech M. The in vitro micronucleus technique. Mutat Res 2000; 455: 81-95
18 Miller B, Pötter-Locher F, Seelbach A, Stopper H, Utesch D, Madle S. Evaluation of the in vitro micronucleus test as an alternative to the in vitro chromosomal aberration assay: position of the GUM Working Group on the in vitro micronucleus test. Gesellschaft für Umwelt-Mutationsforschung. Mutat Res 1998; 410: 81-116
19 Fenech M, Holland N, Chang WP, Zeiger E, Bonassi S. The HUman MicroNucleus Project—An international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. Mutat Res 1999; 428: 271-283
20 Van Schooten FJ, Besaratinia A, De Flora S, D’Agostini F, Izzotti A, Camarino A, Balm AJ, Dallinga JW, Bast A, Haenen GR, Van’t Veer L, Baas P, Sakai H, Van Zandwijk N. Effects of oral administration of N-acetyl-L-cysteine: a multi-biomarker study in smokers. Cancer Epidemiol Biomarkers Prev 2002; 11: 167-175
21 Chang WP, Hwang BF, Wang D, Wang JD. Cytogenetic effect of chronic low-dose, low-dose-rate gamma-radiation in residents of irradiated buildings. Lancet 1997; 350: 330-333
22 Bettega D, Calzolari P, Doneda L, Belloni F, Tallone L, Redpath JL. Differential effectiveness of solar UVB subcomponents in causing cell death, oncogenic transformation and micronucleus induction in human hybrid cells. Int J Radiat Biol 2003; 79: 211-216
23 Nowak MA, Komarova NL, Sengupta A, Jallepalli PV, Shih IeM, Vogelstein B, Lengauer C. The role of chromosomal instability in tumour initiation. Proc Natl Acad Sci USA 2002; 99: 16226-16231
24 Michor F, Iwasa Y, Komarova NL, Nowak MA. Local regulation of homeostasis favors chromosomal instability. Curr Biol 2003; 13: 581-584
25 Fenech M, Morley AA. Measurement of micronuclei in lymphocytes. Mutat Res 1985; 147: 29-36
26 Cruz-Bustillo Claren D. Molecular genetics of colorectal cancer. Rev Esp Enferm Dig 2004; 96: 48-59
27 Chung DC. The genetic basis of colorectal cancer: insights into critical pathways of tumorigenesis. Gastroenterology 2000; 119: 854-865
28 Lengauer C, Kinzler KW, Vogelstein B. Genetic instability in colorectal cancers. Nature 1997; 386: 623-627
29 Rajagopalan H, Nowak MA, Vogelstein B, Lengauer C. The significance of unstable chromosomes in colorectal cancer. Nat Rev Cancer 2003; 3: 695-701
30 Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertson M, Juslin G, Stevens J, Spirio L, Robertson M. Identification and characterization of the familial adenomatous polyposis coli gene. Cell 1991; 66: 589-600
31 Kinzler KW, Nilbert MC, Su LK, Vogelstein B, Bryan TM, Levy DB, Smith KJ, Preisanger AC, Hedge P, McKeanne D. Identification of FAP locus genes from chromosome 5q21. Science 1991; 253: 661-665
32 Bodmer W, Bishop T, Karran P. Genetic steps in colorectal cancer. Nat Genet 1994; 6: 217-219
33 Bodmer WF, Bailey CJ, Bodmer J, Bussey HJ, Ellis A, Gorman P, Luebello FC, Murday VA, Rider SH, Scambler P. Localization of the gene for familial adenomatous polyposis on chromosome 5. Nature 1987; 328: 614-616
34 Cunningham JM, Christensen ER, Tester DJ, Kim CY, Roche PC, Burgart LJ, Thibodeau SN. Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. Cancer Res 1998; 58: 3455-3460
35 Grady WM. Genomic instability and colon cancer. Cancer Metastasis Rev 2004; 23: 11-27
36 Sen S. Aneuploidy and cancer. Curr Opin Oncol 2000; 12: 82-88
37 Maser RS, DePinho RA. Connecting chromosomes, crisis, and cancer. Science 2002; 297: 565-569
38 Nasmyth K. Segregating sister genomes: the molecular biology of chromosome separation. Science 2002; 297: 559-565
39 Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. Mutations of mitotic checkpoint genes in human cancers. Nature 1998; 392: 300-303
40 Fodde R, Smits R, Clevers H. APC, signal transduction and genetic instability in colorectal cancer. Nat Rev Cancer 2001; 1: 55-67
41 Fodde R. The multiple functions of tumour suppressors: it’s all in APC. Nat Cell Biol 2003; 5: 190-192
42 Kirsch-Volders M. Towards a validation of the micronucleus test. Mutat Res Rev 1997; 392: 1-4
43 Norppa H. Cytogenetic biomarkers. IARC Sci Publ 2004; 179-205
44 Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N, Kirsch-Volders M, Zeiger E, Ban S, Barale R, Bigatti MP, Bolognesi C, Cebulska-Wasilewska A, Fabianova E, Fucic A, Hagner L, Joksic G, Martelli A, Migliore L, Mirkova E, Scarfi MR, Zijno A, Norppa H, Fenech M. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. Carcinogenesis 2007; 28: 625-631
45 Cardoso J, Molenaar L, de Menezes RX, Nowak MA, Rosenberg C, Moslein G, Sampson J, Moreau H, Boer JM, Fodde R. Chromosomal instability in MYH- and APC-mutant adenomatous polyps. Cancer Res 2006; 66: 2514-2519
46 Doak SH, Jenkins GJ, Parry EM, D’Souza FR, Griffiths AP, Toffazzal N, Shah V, Baxter JN, Parry JM. Chromosome 4 hyperploidy represents an early genetic aberration in premalignant Barrett’s oesophagus. Gut 2003; 52: 623-628
47 Olaarkers AJ, Sotelo R, Solozza-Luna G, Gonsebatt ME, Guzman P, Mohar A, Eastmond DA. Tetraploidy and chromosomal instability are early events during cervical carcinogenesis. Carcinogenesis 2006; 27: 337-343
48 Stevenius Y, Gorunova L, Jonson T, Larsson N, Hoglund M, Mandahl N, Mertens F, Miileman F, Gisselsson D. Structural and numerical chromosome changes in colon cancer develop through telomere-mediated anaphase bridges, not through mitotic multipolarity. Proc Natl Acad Sci USA 2005; 102: 5541-5546
49 Sieber OM, Heimann K, Gorman P, Lamllum H, Crabtree M, Simpson CA, Davies D, Neale K, Hodgson SV, Roylance RL, Phillips RK, Bodmer WF, Tomlinson IP. Analysis of chromosomal instability in human colorectal adenomas with two mutational hits at APC. Proc Natl Acad Sci USA 2002; 99: 16910-16915
50 Little MP, Wright EG. A stochastic carcinogenesis model incorporating genomic instability fitted to colon cancer data. Math Biosci 2003; 183: 111-134
51 Komarova NL, Lengauer C, Vogelstein B, Nowak MA. Dynamics of genetic instability in sporadic and familial colorectal cancer. Cancer Biol Ther 2002; 1: 685-692
52 Rajagopalan H, Jallepalli PV, Rago C, Velculescu VE, Kinzler KW, Vogelstein B, Lengauer C. Inactivation of hCDC4 can cause chromosomal instability. Nature 2004; 428: 77-81
53 Shih IM, Zhou W, Goodman SN, Lengauer C, Kinzler KW, Vogelstein B. Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. Cancer Res 2001; 61: 818-822

S-Editor Cheng JX L-Editor Logan S E-Editor Yin DH