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• ORIGINAL RESEARCH •

Telomere erosion is independent of microsatellite instability but related to loss of heterozygosity in gastric cancer

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Abstract

AIM **To correlate the length of the telomere to microsatellite instability (MSI) and loss of heterozygosity (LOH) of APC, MCC and DCC genes in gastric carcinomas.**

METHODS **Telomeric restriction fragment (TRF) length of gastric cancer was measured with Southern blot. LOH of APC, MCC and DCC genes, microsatellite instability (MSI) and frameshift mutation of hMSH6, TGF-βRII and BAX genes were analyzed by PCR-based methods.**

RESULTS **Sixty-eight cases of sporadic gastric carcinoma were studied for MSI using five microsatellite markers. MSI in at least one locus was detected in** 17 (25%) **of** 68 **tumors analyzed. Frameshift mutations of hMSH6, TGF-βRII and BAX were detected in** 2,6 **and 3 of gastric carcinomas respectively showing high MSI (**≥2 **loci, *n* =** 8**), but none was found in those showing low MSI (only one locus, *n*=**9**) or MSS (tumor lacking MSI or stable, *n*=**51**). Thirty-five cases, including all high MSI and low MSI, were studied for TRF. The mean TRF length was not correlated with clinicopathological parameters. No association was observed between TRF length and MSI or frameshift mutation. On the contrary, LOH at the DCC locus was related to telomere shortening (*P*<**0.01**). This tendency was also observed in APC and MCC genes, although there was no statistical significance.**

CONCLUSION **The development of gastric cancer can arise through two different genetic pathways. In high MSI gastric cancers, defective mismatch repair allows mutations to accumulate and generate the high MSI phenotype. In gastric cancers showing either low MSI or MSS, multiple deletions may represent the LOH pathway. Telomere erosion is independent of high MSI phenotype but related to the LOH pathway in gastric cancer.**

Subject headings gastric cancer; telomere restriction fragment; microsatellite instability; loss, heterozygosity

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INTRODUCTION

Eukaryotic linear chromosomes are capped by a special structure known as the telomere. In vertebrates, telomeres consist of several kilobases of tandem TTAGGG repeats bound by the related telomere-specific proteins, TRF1 and TRF2[1,2]. TRF1 regulates telomere length[3] and TRF2 maintains telomere integrity[4]. In most human cells, telomeres shorten with each cell division due to the incomplete replication of linear DNA molecules and the absence of telomere-elongating mechanisms[5]. It has been reported that telomeric repeats are often reduced in various human tumors as a consequence of many cell divisions of tumor cells, and the loss of telomeric repeats would cause additional senetic changes as a consequence of chromosomal instability.

The presence of telomeric DNA at the chromosomal termini is essential for genetic stability. Genetic instability may be classified into two different forms in which hypermutability occurs either by means of chromosomal instability or microsatellite instability (MSI)[6-10]. There is now increasing evidence that telomere shortening is involved in chromosomal instability. Indeed telomeres are thought to maintain the chromosome integrity during the cell cycle by allowing a proper segregation during cell division. Broken chromosomes that are uncapped become hotspots for different types of recombination. They may therefore undergo aberrant recombination, end-to-end fusions and bridge-fusion-breakage mechanisms. Being unprotected, the chromosomes are also susceptible to exonucleolytic degradation[11]. Telomeres would also prevent the activation of DNA-damage check-points[12]. Thus, without these essential structures, the chromosome is unstable.

MSI represents an important new form of genetic alteration characterized by widespread instability in repetitive nucleotide sequences. MSI has been found in the majority of tumors associated with hereditary non-polyposis colorectal cancer (HNPCC)[13,14] in which germ-line mutation occurs within the mismatch repair genes hMSH2, hMLH1, hPMS2 or hMSH6[15-17]. Mutations of the transforming growth factor type II receptor gene (TGF-βR II), and BAX gene are strongly correlated with MSI[18-20]. MSI is also a distinctive feature in about 10%-15% of sporadic colorectal tumors and to a varying degree in tumors of other organs, including the stomach[7,21-24]. Although alterations of telomerase activity have been reported in MSI and microsatellite stable (MSS) tumors[25,26], less clear, however, the relevance of progressive telomere shortening as a potential factor in MSI is less clear. The aim of the present study is to correlate telomere status with MSI and loss of heterozygosity (LOH) of APC, MCC and DCC genes in gastric carcinomas.

MATERIAL AND METHOD

Sixty-eight cancer and corresponding normal tissues were obtained from surgically resected gastric carcinoma patients in our hospital. Each specimen was frozen immediately and

stored at -80℃ until analyzed. A 5μm section was cut from each tissue and stained with hematoxylin /eosin in order to ascertain whether the cancer cells in tissues were predominant or not. Genomic DNA was isolated by standard proteinase-K digestion and phenol-chloroform extraction protocols. Of the 68 patients with gastric cancer, 45 were men and 23 were women with an age range of 30-76 years (mean age of 56.2 years at diagnosis). None of the patients included in the present series had a family history suggestive of HNPCC and had received chemotherapy or radiation therapy.

MSI analyses included five microsatellite markers: BAT25, BAT26, BAT40, D2S123, and D5S346. PCR was performed in 15μL of reaction mixture containing 10mmol·L^{-1} Tris-HCl (pH 8.3), 50mmol·L^{-1} KCl, 1.5mmol·L^{-1} $MgCl_2$, 200μmol·L^{-1} each deoxynucleotide triphoshate, 0.5μmol·L^{-1} of each primer, 0.75unit Ampli Taq polymerase (Perkin-Elmer, Norwalk, CT, USA), and 100ng genomic DNA. The reaction was carried out in a thermal cycler at 94℃ for 1min, 55℃-62℃ for 1min, and 72℃ for 1min, for 35 cycles with an initial denaturation step of 94℃ for 5min and a final extension step of 72℃ for 10min. The PCR products were then separated on 5% polyacrylamide, 7M urea denaturing gel, and visualized by autoradiography. MSI was defined as the presence of band shift in the tumor DNA that was not present in the corresponding normal DNA. Based on the number of mutated MSI markers in each tumor, carcinomas were characterized as high MSI if they manifested instability at two or more markers, low MSI if unstable at only one marker, and MSS if they showed no instability at any markers (microsatellite stable)[27-29].

To detect frameshift mutations in coding regions, repetitive mononucleotide sequences, the (A)10 tract of TGF-βRII, the (G)8 tract of BAX and the (C)8 tract of mismatch repair gene MSH6 were amplified using published primers, respectively[30-32]. The reaction condition consisted of 35 cycles at 94℃ for 1min, 55℃ or 56℃ for 1min and 72℃ for 1min using 0.5μCi[33p] dATP into 15μL reaction mixture. DNA denaturation, electrophoresis and autoradiographic procedure were done likewise with MSI analysis, except for TGF-βRII where 8% denaturing gel was used.

Southern blot analysis to estimate telomeric restriction fragment (TRF) length was based on previously reported methods[33]. High molecular weight DNA was prepared from each sample, followed by extraction with phenol chloroform isoamyl alcohol and precipitation with 3M sodium acetate and ethanol. Genomic DNA was digested with *Hin* fI (Promega, USA)at 37℃ for 6 hours and then 10μg of digested DNA was subject to electrophoresis on 1% agarose gels. After electrophoresis, the gel was denatured in 0.5M NaOH and 1.5M NaCl for 30 minutes and neutralized in 0.5M Tris (pH 8.0) and 1.5M NaCl for 30 minutes. The DNA was then transferred onto a nitrocellulose membrane (Promega, USA) overnight. The membrane was dried at 80℃ for 1 hour and subsequently hybridized to α-^{32} P-ATP end- labeled $(TTAGGG)_4$ probe. The membranes were autoradiographed on X-ray films for 24 hours. Each lane was scanned with a densitometer and the data was used to determine the mean TRF length as previously described[34].

The APC, MCC and DCC genes were investigated for LOH and PCR was carried out as described[35,36]. The priming regions were located within specific tumor suppressor genes at sequence, either a restriction fragment length polymorphism (RFLP) or a variable mumber of tandem repeats-type polymorphism (VNTR)[37]. Annealing temperature, extension time and the number of amplification cycles were optimized for each primer set. After amplification, PCR products were digested with appropriate restriction enzymes (for RFLPs) or not digested (for VNTRs) and electrophoresed on 1.5% agarose gels or 8% polyacrylamide gels, which were stained with ethidium bromide and photographed under UV light. When the intensity of one allele in cancerous tissue was less than 50% of the other allele in comparison with the ratio of intensity of the allele in the corresponding normal tissue, it was judged as LOH.

One factor analysis of variance, and Chi-square test with Yates' correction were used. A P value <0.05 was considered significant.

RESUTS

Alterations of electrophoretic patterns of PCR products of five microsatellite markers, TGF-βRII, BAX, and MSH3 genes were compared between tumor and normal DNA in each patient (Figures 1,2). MSI affecting at least one locus was observed in 17 (25%) of 68 tumors, among which eight (11.8%) had high MSI and nine (13.2%) had low MIS. A comparison of MSI status with frameshift mutation is shown in Table 1. The (A)10 frameshift mutation in TGF-βRII gene was detected in 6 of 8 gastric cancers with high MSI, whereas none of the low MSI or MSS tumors showed such a mutation. The mutation in the BAX (G)8 repeat site and hMSH6 (C)8 tracts was detected in 3 of 8 and 2 of 8 gastric cancers with high MSI, respectively, and no mutation was found in tumors with low MSI and MSS.

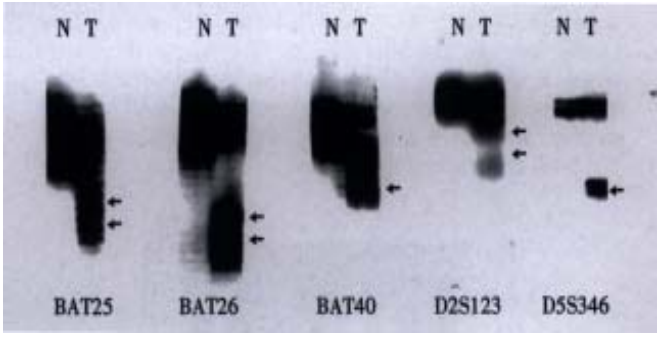

Figure 1 MSI in gastric cancer using 5 microsatellite loci (BAT-25, BAT-26, BAT40, D2S123, and D5S346). Arrows indicate variant conformers. (N: normal DNA pattern; T: tumor specimens containing variant conformers representing MIS)

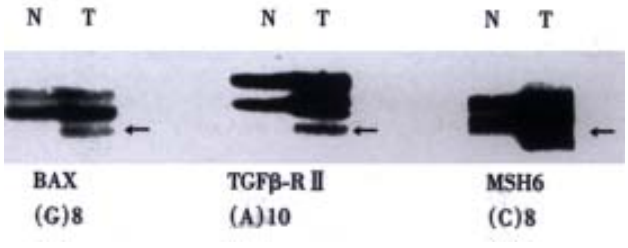

Figure 2 Frameshift mutations of hMSH6 TGF-beta RII, and BAX genes in gastric cancers. Arrows indicate conformational variants associated with frameshift mutations. (N: normal DNA; T: tumor DNA)

Table 1 Relationship between MSI status and frameshift mutation

MSI status	No. of cases	Frameshift mutations		
		MSH6	TGF β RII	BAX
MSI-H	8	2	6	3
MSI-L	9	0	0	0
MSS	51	0	0	0

Thirty-five gastric cancers, including all 8 with high MSI and 9 with low MSI, were examined for alteration in TRF length using the Southern blot technique. Since the signal of telomeric repeats is detected as a smear (Figure 3), we visually determined the peak of signal, which was confirmed using a densitometer. When the TRF length in the tumor was more than 20% shorter or longer than that in corresponding normal tissues, we classified the TRFs as shortened or elongated, as previously described[38]. Compared with the corresponding normal mucosa, 20 (57.1%) had considerable shortening (<80% of corresponding normal tissues), 12 (34.3%) had approximately the same length (between 80%-120%), and 3(8.6%) showed elongation (>120%). No correlation was found between mean TRF length and clinicopathological parameters in gastric carcinomas. However, a decrease of the mean TRF length with the age was observed except for the group aged from 50 to 59 years. The mean TRF length in the age group of 30 to 39 years was significantly longer than that in the group aged from 60 to 69 years or 70 to 79 years (P <0.05) (Table 2).

We compared the TRF length with MSI and frameshift mutation status. Table 3 shows the association of MSI and frameshift status to the TRF length of 35 gastric cancers. No relationship was observed between TRF length and MSI or frameshift mutation in gastric cancer.

Tissues from 35 patients were also studied for LOH of APC, MCC and DCC. In order to increase the assay sensitivity, three different sites, i.e., M2, M3 and VNTR were used for analysis of LOH at DCC genetic locus. LOH of APC, MCC and DCC were observed in 6 (27.3%) of 22, 7 (36.8%) of 19 and 18(56.3%) of 32 of informative cases, respectively (Figure 4). The relationship between LOH at various loci and the alteration in TRF length in primary tumors is shown in Table 4. LOH at the DCC locus were associated with telomere shortening (P<0.01). This tendency was also observed in APC and MCC genes, although there was no statistical significance.

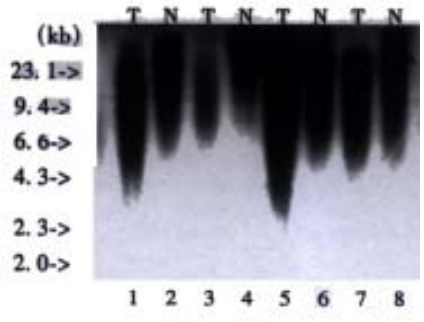

Figure 3 Southern blot analysis of telomere repeat arrays in DNA samples of patients with gastric cancer. DNA was digested with-*Hin* fI and hybridized with α-^{32} P APT end-labeled $(TTAGGG)_4$ probe.T =Tumour; N =Corresponding normal tissues.

Table 2 The relationship between the mean TRF length and clinicopathological pararmeters

Parameters	No. of cases	Mean TRF length ($\bar{x}$±s)
Gender		
Male	23	3.99±0.63
Female	12	4.32±1.00
Age(yrs)		
30-39	5	4.54±0.54
40-49	7	4.07±0.60
50-59	11	4.39±0.96
60-69	10	3.69±0.66[a]
70-79	2	3.40±0.07[a]
Size		
<5cm	12	3.80±1.20
>5cm	23	4.06±0.86
Differentiation		
Well and moderate	7	3.91±0.49
Poor	19	4.24±0.64
Mucinous	9	4.02±1.10
Clinical stage		
I and II	21	4.26±0.82
III and IV	14	3.84±0.68

[a]P<0.05 *vs* the age group of 30 to 39 years.

Table 3 Relationship between alterations of TRF length and MSI status or frameshift mutation

	No. of cases	Alterations in TRF length		
		Shortened (n=20)	Normal (n=12)	Elongated (n=3)
MSI status				
MSI-H	8	4	4	0
MSI-L	9	4	4	1
MSS	18	9	7	2
Frameshift mutation				
MSH3	35	1	1	0
TGF β RII	35	3	2	1
BAX	35	2	1	0

Table 4 Relationship between alterations of TRF length and loss of heterozygosity of APC, MCC and DCC genes

LOH	Alterations in TRF engh (LOH / Informative)		
	Shortened (n=20)	Normal (n=12)	Elongated (n=3)
APC	5 / 13	1 / 7	0 / 2
MCC	6 / 11	1 / 6	0 / 2
DCC	14 / 18[a]	3 / 11	1 / 3

[a]P<0.01.

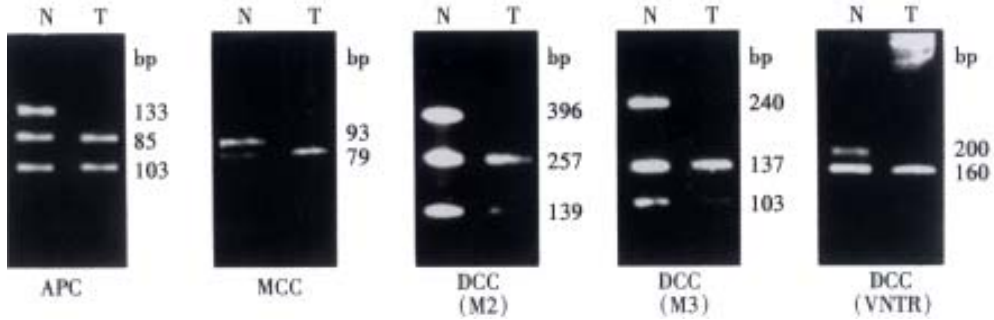

Figure 4 Representative LOH analysis of APC, MCC and DCC genes. (APC: Rsa1 RFLP in APC exon 11, loss of 133bp allele is seen in tumor DNA, MCC: A 14bp insertion / deletion polymorphism in MCC exon 10 gives rise to a 93 or 79 allele. Loss of the 93bp allele is seen in the tumor. DCC: Losses of 396bp, 240bp and 200bp alleles are seen at M2, M3 and VNTR polymorphic sequences in the tumor DNA)

DISCUSSION

In this study, 17(25%) of 68 sporadic gastric cancers had MSI in at least one locus. This finding is similar to previous studies[23,39]. There is now evidence that MSI cancer comprises distinctive high MSI and low MSI categories[40,41]. High MSI cancers are distinguished clinicopathologically and in their spectrum of genetic alterations from cancers showing low MSI and MSS cancers[40,41]. Our previous studies indicated that high MSI gastric cancers often show lower frequency of LOH of APC, MCC and DCC genes than low MSI and MSS cancers[41]. In our series of 68 gastric cancers, 8 were classified as high MSI, 9 as low MSI and 51 as MSS. Frameshift mutations of hMSH6, TGF-β RII and BAX were detected in gastric carcinomas with high MSI, but no mutation of these genes was found in those showing low MSI or MSS. These results indicate that these genes are mutational targets in high MSI tumor cells and support the notion that high MIS tumors identify an alterative pathway of tumorigenesis that has been proposed by Vogelstein and co-workers[42].

In the present study, 35 gastric carcinomas were examined for TRF length using the Southern blot. Telomere shortening was detected in 57.1% of the gastric carcinomas, indicating that telomere erosion may play a role in the gastric carcinoma development. It has been reported that telomere shortening was associated with a short survival and disease recurrent in prostate cancer[43]. In the current study, subdivision of the tumors according to telomere length did not reveal an obvious relationship between the shortening of telomere and tumor size, depth of invasion, node metastasis or clinical stages, indicating a limited role of the telomere shortening in predicting prognosis of gastric carcinomas. This finding is in agreement with the recently published data on renal cell carcinoma and colorectal carcinomas[44,45].

TRF reduction was demonstrated in various human malignant tumors[38,45-47]. In contrast to these results, it was also found that telomeres in some tumors are similar to, even elongated, as compared with normal tissues[36,48]. In this study, the mean TRF length was reduced in 20 of 35 tumors, similar in 12 cases and elongated in 3 cases, as compared with the corresponding normal mucosa. The discrepancy in TRF length may be related for several reasons. For tumor samples with TRF of normal length, there are at least three possibilities: ① The majority of cells in such tumors have not experienced many cell divisions nor experienced critical shortening of telomeric repeats; ② In most tumor cells, telomerase already activated, elongated the once shortened telomeric repeats back to normal length, as observed in HPV-immortalized epithelial cells *in vitro*[49]; and ③ The amount of tumor cells also affects TRF analysis. Therefore, with the predominance of normal cells in tumor specimens, telomere loss may be underestimated[48]. We found that the TRF length in tumors was progressively reduced with age, suggesting that patients' age should be considered in evaluating the TRF lengths of tumor. Finally, end-to-end chromosome fusion observed in some tumors could lead to telomere elongation[50].

It has been reported that telomerase activity and microsatellite instability are independent events in colorectal carcinogenesis[25]. To our knowledge, this is the first report on the relationship between MSI and telomere length. We did not find any correlation between telomere erosion and MSI, suggesting that the MSI pathway is independent of telomere erosion.

It has been found that chromosomal instability is correlated with telomere erosion and inactivation of G2 checkpoint function in human fibroblasts[12]. Alterations in the TRF length were often associated with LOH of the p53 gene and Rb gene, but less often with mutation of k-ras and p53 genes in lung cancer[38]. We found that LOH at the DCC locus was associated with telomere shortening. Although not statistically significant, LOH at the APC and MCC genetic loci tends to occur in telomere shortened gastric cancers, indicating that telomere erosion may be involved in the LOH pathway.

In summary, our data indicate that telomere shortening may play a role in gastric carcinogenesis. There are at least two distinct genetic instabilities in gastric tumorigenesis: one is the chromosomal instability (or suppressor pathway) and the other is microsatellite instability (or MSI pathway). The former, may include tumors with low MIS as well as MSS and accumulation of loss of tumor suppressor genes such as p53, Rb, APC, MCC and DCC plays an important role in their carcinogenesis; whereas the latter consists of a small subset of gastric cancer with high MSI, defective repair of mismatched bases results in an increased mutation rate at the nucleotide level, and consequent widespread MSI. Telomere shortening may be involved in the LOH pathway but independent of the MSI pathway. Our analysis of TRFs should further provide some clues to the molecular mechanisms underlying the profound genomic instability in the MSI and LOH pathway for gastric carcinoma.

REFERENCES

1 Chong L, van Steensel B, Broccoli D, Erdjument-Bromage H, Hanish J, Tempst P, de Lange T. A human telomeric protein. *Science*, 1995;270:1663-1667
2 Broccoli D, Smogorzewska A, Chong L, de Lange T. Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2. *Nat Gent*, 1997;17:231-235
3 van Steensel B, de Lange T. Control of telomere length by the human telomeric protein TRF1. *Nature*, 1997;385:740-743
4 van Steensel B, Smogorzewska A, de Lange T. TRF2 protects human telomeres from end-to-end fusions. *Cell*, 1998;92:401-413
5 Greider CW. Telomere length regulation. *Annu Rev Biochem*, 1996;65:337-365
6 Lengauer C, Kinzler KW, Vogelstein B. Genetic instability in colorectal cancers. *Nature*, 1997;386:623-627
7 Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science*, 1993;260:816-819
8 Fearon ER, Vogelstein BA. A genetic model for colorectal tumorigenesis. *Cell*, 1990;61:759-767
9 Tsushimi T, Noshima S, Oga A, Esato K, Sasaki K. DNA amplification and chromosomal translocations are accompanied by chromosomal instability: analysis of seven human colon cancer cell lines by comparative genomic hybridization and spectral karyotyping. *Cancer Genet Cytogenet*, 2001;126:34-38
10 Loukola A, Eklin K, Laiho P, Salovaara R, Kristo P, Jarvinen H, Mecklin JP, Launonen V, Aaltonen LA. Microsatellite marker analysis in screening for hereditary nonpolyposis colorectal cancer (HNPCC). *Cancer Res*, 2001;61:4545-4549
11 Sandell LL, Zakian VA. Loss of a yeast telomere: arrest recovery, and chromosome loss. *Cell*, 1993;75:729-739
12 Filatov L, Golubovskaya V, Hurt JC, Byrd LL, Phillips JM, Kaufmann WK. Chromosomal instability is correlated with telomere erosion and inactivation of G2 checkpoint function in human fibroblasts expressing human papillomavirus type 16 E6 oncoprotein. *Oncogene*, 1998;16:1825-1838
13 Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Powell SM, Jen J, Hamilton SR. Clues to the pathogenesis of familial colorectal cancer. *Science*, 1993; 260:812-816
14 Wu BP, Zhang YL, Zhou DY, Gao CF, Lai ZS. Microsatellite instability, MMR gene expression and proliferation kinetics in colorectal cancer with famillial predisposition. *World J Gastroenterol*, 2000;6:902-905
15 Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A.

Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature*, 1994;368:258-261
16 Papadopoulos N, Nicolaides NC, Liu B, Parsons R, Lengauer C, Palombo F, D'Arrigo A, Markowitz S, Willson JK, Kinzler KW. Mutations of GTBP in genetically unstable cells. *Science*, 1995; 268:1915-1917
17 Wang Q, Lasset C, Desseigne F, Saurin JC, Maugard C, Navarro C, Ruano E, Descos L, Trillet-Lenoir V, Bosset JF, Puisieux A. Prevalence of germline mutations of hMLH1, hMSH2, Hpms1, hPMS2, and hMSH6 genes in 75 French kindreds with nonpolyposis colorectal cancer. *Hum Genet*, 1999;105:79-85
18 Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B. Inactivation of the type II TGF-Beta receptor in colon cancer cells with microsatellite instability. *Science*, 1995;268:1336-1338
19 Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, Perucho M. Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. *Science*, 1997;275:967-969
20 Souza RF, Appel R, Yin J, Wang S, Smolinski KN, Abraham JM, Zou TT, Shi YQ, Lei J, Cottrell J, Cymes K, Biden K, Simms L, Leggett B, Lynch PM, Frazier M, Powell SM, Harpaz N, Sugimura H, Young J, Meltzer SJ. Microsatellite instability in the insulin -like growth factor II receptor gene in gastrointestinal tumors. *Nat Genet*, 1996;14:255-257
21 Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutation in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature*, 1993;363: 558-561
22 Jass JR, Do KA, Simms LA, Iino H, Wynter C, Pillay SP, Searle J, Radford-Smith G, Young J, Leggett B. Morphology of sporadic colorectal cancer with DNA replication errors. *Gut*, 1998; 42:673-679
23 Chung YJ, Park SW, Song JM, Lee KY, Seo EJ, Choi SW, Rhyu MG. Evidence of genetic progression in human gastric carcinomas with microsatellite instability. *Oncogene*, 1997;15:1719-1726
24 Potocnik U, Glavac D, Golouh R, Ravnik-Glavac M. Causes of microsatellite instability in colorectal tumors: implications for hereditary non-polyposis colorectal cancer screening. *Cancer Genet Cytogenet*, 2001;126:85-96
25 Unate H, Ikeguchi M, Kaibara N, Okamura D, Nishihara S, Katoh M, Oshimura M. Telomerase activity and microsatellite instability in colorectal cancer and adenoma. *Int J Oncol*, 1998; 13:1223-1228
26 Linn JF, Lango M, Halachmi S, Schoenberg MP, Sidransky D. Microsatellite analysis and telomerase activity in archived tissue and urine samples of bladder cancer patients. *Int J Cancer*, 1997;74:625-629
27 Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: Development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res*, 1998;58:5248-5257
28 Fang DC, Zhou XD, Luo YH, Wang DX, Lu R, Yang SM, Liu WW. Microsatellite instability and loss of heterozygosity of suppressor gene in gastric cancer. *Shijie Huaren Xiaohua Zazhi*, 1999;7:479-481
29 Sood AK, Holmes R, Hendrix MJ, Buller RE. Application of the National Cancer Institute international criteria for determination of microsatellite instability in ovarian cancer. *Cancer Res*, 2001;61:4371-4374
30 Togo G, Toda N, Kanai F, Kato N, Shiratori Y, Kishi K, Imazeki F, Makuuchi M, Omata M. A transforming growth factor beta type II receptor gene mutation common in sporadic cecum cancer with microsatellite instability. *Cancer Res*, 1996;56:5620-5623
31 Yagi OK, Akiyama Y, Nomizu T, Iwama T, Endo M, Yuasa Y. Proapototic gene BAX is frequently mutated in hereditary nonpolyposis colorectal cancers but not in adenomas. *Gastroenterology*, 1998;114:268-274
32 Yin J, Kong D, Wang S, Zou TT, Souza RF, Smolinski KN, Lynch PM, Hamilton SR, Sugimura H, Powell SM, Young J, Abraham JM, Meltzer SJ. Mutation of hMSH3 and hMSH6 mismatch repair genes in genetically unstable human colorectal and gastric carcinomas. *Human Mutat*, 1997;10:474-478
33 Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *EMBO J*, 1992;11:1921-1929
34 Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature*, 1990;345:458-460
35 Boynton RF, Blount PL, Yin J, Brown VL, Huang Y, Tong Y, McDaniel T, Newkirk C, Resau JH, Raskind WH. Loss of heterozygosity involving the APC and MCC genetic loci occurs in the majority of human esophageal cancers. *Proc Natl Acad Sci USA*, 1992;89:3385-3388
36 Fang DC, Jass JR, Wang DX. Loss of heterozygosity and loss of expression of the DCC gene in gastric cancer. *J Clin Pathol*, 1998;51:593-596
37 Nakamura Y, Leppert M, O'Connell P, Wolff R, Holm T, Culver M, Martin C, Fujimoto E, Hoff M, Kumlin E. Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science*, 1987;235:1616-1622
38 Hiyama K, Ishioka S, Shirotani Y, Inai K, Hiyama E, Murakami I, Isobe T, Inamizu T, Yamakido M. Alterations in telomeric repeat length in lung cancer are associated with loss of heterozygosity in p53 and Rb. *Oncogene*, 1995;10:937-944
39 Kim HS, Woo DK, Bae SI, Kim YI, Kim WH. Microsatellite instability in the adenomacarcinoma sequence of the stomach. *Lab Invest*, 2000;80:57-64
40 Jass JR, Biden KG, Cummings MC, Simms LA, Walsh M, Schoch E, Meltzer SJ, Wright C, Searle J, Young J, Leggett BA. Characterization of a subtype of colorectal cancer combining features of the suppressor and mild mutator pathways. *J Clin Pathol*, 1999;52:455-460
41 Fang DC, Jass JR, Wang DX, Zhou XD, Luo YH, Young J. Infrequent loss of heterozygosity of APC / MCC and DCC genes in gastric cancer showing DNA microsatellite instability. *J Clin Pathol*, 1999;52:504-508
42 Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal tumor development. *N Engl J Med*, 1988;319:525-532
43 Donaldson L, Fordyce C, Gilliland F, Smith A, Feddersen R, Joste N, Moyzis R, Griffith J. Association between outcome and telomere DNA content in prostate cancer. *J Urol*, 1999;162: 1788-1792
44 Dahse R, Fiedler W, Ernst G, Kosmehl H, Schlichter A, Schubert J, Claussen U. Changes in telomere lengths in renal cell carcinomas. *Cell Mol Biol*, 1996;42:477-485
45 Takagi S, Kinouchi Y, Hiwatashi N, Chida M, Nagashima F, Takahashi S, Negoro K, Shimosegawa T, Toyota T. Telomere shortening and the clinicopathologic characteristics of human colorectal carcinomas. *Cancer*, 1999;86:1431-1436
46 Paz-y-Mino C, Perez JC, Davalos V, Sanchez ME, Leone PE. Telomeric associations in cigarette smokers exposed to low levels of X-rays. *Mutat Res*, 2001;490:77-80
47 Wong KK, Chang S, Weiler SR, Ganesan S, Chaudhuri J, Zhu C, Artandi SE, Rudolph KL, Gottlieb GJ, Chin L, Alt FW, DePinho RA. Telomere dysfunction impairs DNA repair and enhances sensitivity to ionizing radiation. *Nat Genet*, 2000;26:85-88
48 Engelhardt M, Albanell J, Drullinsky P, Han W, Guillem J, Scher HI, Reuter V, Moore MA. Relative contribution of normal and neoplastic cells determines telomerase activity and telomere length in primary cancers of the prostate, colon, and sarcoma. *Clin Cancer Res*, 1997;3:1849-1857
49 Klingelhutz AJ, Barber SA, Smith PP, Dyer K, McDougall JK. Restoration of telomeres in human papillomavirus- immortalized human anogenital epithelial cells. *Mol Cell Biol*, 1994;14:961-969
50 Lindsey J, McGill NI, Lindsey LA, Green DK, Cooke HJ. *In vivo* loss of telomeric repeats with age in human. *Mutat Res*, 1991;256:45-48

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