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World J Gastroenterol 2008 January 7; 14(1): 90-94
World Journal of Gastroenterology ISSN 1007-9327
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RAPID COMMUNICATION

Are there tumor suppressor genes on chromosome 4p in sporadic colorectal carcinoma?

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Received: April 11, 2007 Revised: September 24, 2007

Abstract

AIM: To study the candidate tumor suppressor genes (TSG) on chromosome 4p by detecting the high frequency of loss of heterozygosity (LOH) in sporadic colorectal carcinoma in Chinese patients.

METHODS: Seven fluorescent labeled polymorphic microsatellite markers were analyzed in 83 cases of colorectal carcinoma and matched normal tissue DNA by PCR. PCR products were eletrophoresed on an ABI 377 DNA sequencer. Genescan 3.7 and Genotype 3.7 software were used for LOH scanning and analysis. The same procedure was performed by the other six microsatellite markers spanning D4S3013 locus to make further detailed deletion mapping. Comparison between LOH frequency and clinicopathological factors was performed by χ^2 test.

RESULTS: Data were collected from all informative loci. The average LOH frequency on 4p was 24.25%, and 42.3% and 35.62% on D4S405 and D4S3013 locus, respectively. Adjacent markers of D4S3013 displayed a low LOH frequency (< 30%) by detailed deletion mapping. Significant opposite difference was observed between LOH frequency and tumor diameter on D4S412 and D4S1546 locus (0% *vs* 16.67%, P = 0.041; 54.55% *vs* 11.11%, P = 0.034, respectively). On D4S403 locus, LOH was significantly associated with tumor gross pattern (11.11%, 0, 33.33%, P = 0.030). No relationship was detected on other loci compared with clinicopathological features.

CONCLUSION: By deletion mapping, two obvious high frequency LOH regions spanning D4S3013 (4p15.2) and D4S405 (4p14) locus are detected. Candidate TSG, which is involved in carcinogenesis and progression of sporadic colorectal carcinoma on chromosome 4p, may be located between D4S3017 and D4S2933 (about 1.7 cm).

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Key words: Loss of heterozygosity; Colorectal carcinoma; Chromosome 4p; Tumor suppressor gene

http://dx.doi.org/10.3748/wjg.14.90

Zheng HT, Jiang LX, Lv ZC, Li DP, Zhou CZ, Gao JJ, He L, Peng ZH. Are there tumor suppressor genes on chromosome 4p in sporadic colorectal carcinoma? *World J Gastroenterol* 2008; 14(1): 90-94

http://www.wjgnet.com/1007-9327/14/90.asp

INTRODUCTION

Colorectal cancer (CRC) constitutes the second most common neoplasm in Western countries and is the third leading cause of cancer-related death, the overall 5-year survival rate is approximately 45%[1]. Improvement in its prognosis can not be achieved without a better understanding of its etiology and tumor molecular biology. In recent years, the genetic basis of human tumors has been increasingly elucidated. As a model for both multistep and multipathway carcinogenesis, colorectal neoplastic progression provide paradigms of both oncogenes and tumor suppressor gene in epithelial tumors[2,3]. The latter changes predominate. In addition to the allelic loss on chromosome 5q, 17p and 18q, many other chromosome losses can be observed in colorectal carcinoma. Regions on chromosome 1q, 4p, 6p, 6q, 8p, 9p and 22q were lost in 25%-50% of the colorectal tumor cases studied previously[2].

Chromosome losses in colorectal tumor were first detected by cytogenesis, later, by probes of restriction fragment length polymorphisms (RFLP) and now by loss of heterozygosity (LOH) in analyzing allelic loss. The loss of tumor suppressor genes is believed to be one of the key steps to carcinogenesis of colorectal cancer[4]. The loss of one allelic at specific locus is caused by deletion mutation or loss of a chromosome from a chromosome pair[5]. When this occurs at a tumor suppressor gene locus where one of the allelics is already abnormal, it can result

in neoplastic transformation. The LOH analysis based on polymorphic microsatellite DNA has become an effective and powerful tool currently to find informative loci and candidate tumor suppressor genes[6,7]. Most investigations concentrated on defining the minimal regions of loss of specific chromosomes in various cancers in an effort to identify the putative tumor suppressor genes targeted by the loss[8].

In this study, we first analyzed the LOH events on chromosome 4p using seven microsatellite markers and made further refined deletion mapping analysis spanning D4S3013 locus in 83 sporadic colorectal carcinoma cases in an attempt to identify additional candidate tumor suppressor genes involved in colorectal tumorigenesis.

MATERIALS AND METHODS

Patient sample and DNA extraction

This study was based on consecutively collected tumors in 83 patients with colorectal cancer, including 40 males and 43 females, treated at the surgical department in Shanghai First People's Hospital, China. The patients' ages ranged from 31 to 84 years with a median of 66. The cancerous tissue and adjacent normal control tissue (> 10 cm) were freshly frozen. The tissues were cut into cubes of approximately 2 mm^3 and immediately frozen in liquid nitrogen. Each patient gave his or her informed consent for the use of his or her tissue in this study. DNA was extracted using standard methods with proteinase K digestion and phenol/chloroform purification[9]. All patients were confirmed by pathology and were staged by Duke's criteria.

Microsatellite markers and PCR

Initially, 83 cases of colorectal cancer were analyzed by PCR using seven microsatellite markers (Shanghai Biology Technology Company, China) which map to chromosome 4p. DNA samples were analyzed as matched normal and tumor pairs using primers of the following microsatellite loci (hereditary location/heterozygote): pter-D4S412 (4p16.3/76)-D4S2935 (4p16.1/62)-D4S1599 (4p16.1/81)-D4S303 (4p15.33/76)-D4S3013 (4p15.2/84)-D4S391 (4p15.2/85)-D4S405 (4p14/85). The average hereditary distance was 8.65 cm[10] (Figure 1A). As the D4S3013 locus showed high LOH frequency (35.62%), six additional microsatellite markers map to chromosome 4p15 were employed to further investigate LOH. The same DNA samples were then analyzed as matched pairs for the following microsatellite markers (location/heterozygote): pter-D4S2926 (4p15.32/80)-D4S1546 (4p 15.31 /77) -D4S3017 (4p 15.31/82)-D4S2933 (4p 15.31/60)-D4S2948 (4p 15.2 /81)-D4S1551 (4p 15.2/78). The average hereditary distance was restricted within 1.03 cm[10] (Figure 1B).

LOH result analysis

A portion of each PCR product (0.5 μL) was combined with 0.1 μL Genescan 500 size standard (PE Applied Biosystems Foster City, CA, USA) and 0.9 μL formamide loading buffer. After denaturation at 96℃ for 5 min, products were eletrophoresed on 5% polyacrylamide

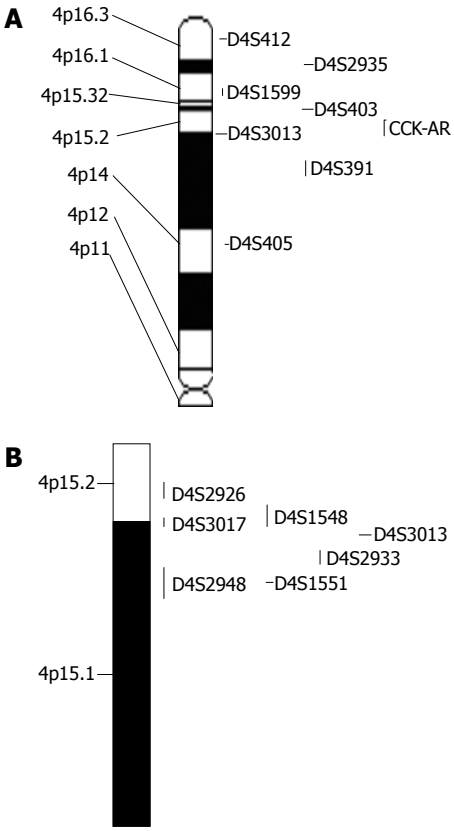

Figure 1 **A:** Microsatellite markers and candidate tumor suppressor genes on 4p (www.gdb.org); **B:** Microsatellite markers on D4S3013 (www.gdb.org).

gels using an ABI 377 DNA sequencer (PE Applied Biosystems Foster City, CA, USA) for 2.5 h. Genotype 3.7 software display individual gel lanes as electropherograms with a given size, height, and area for each detected fluorescent peak. Stringent criteria were used to score the samples. Alleles were defined as the two highest peaks within the expected size range. A ratio of T1:T2/N1:N2 of less than 0.67 or greater than 1.50 was scored as a loss of heterozygosity (Figure 2). Most amplified normal DNA produced two PCR products indicating heterozygosity. A single fragment amplified from normal DNA (homozygosity) and those PCR reactions, in which fragments were not clearly amplified, were scored as not informative. The LOH frequency of a locus is equal to the ratio of the number between allelic loss and informative cases. The average LOH frequency of chromosome 4p is the average value of each locus.

Statistical analysis

Comparisons between LOH and clinicopathological data were performed by χ^2 test. $P < 0.05$ was considered as statistically significant.

RESULTS

LOH analysis of colorectal cancer on 4p

Eighty-three colorectal cancers were analyzed for LOH at the seven marker loci spanning chromosome 4p. All loci got informative messengers. The average LOH frequency on 4p was 24.25%. Sixty-three samples (75.90%) showed

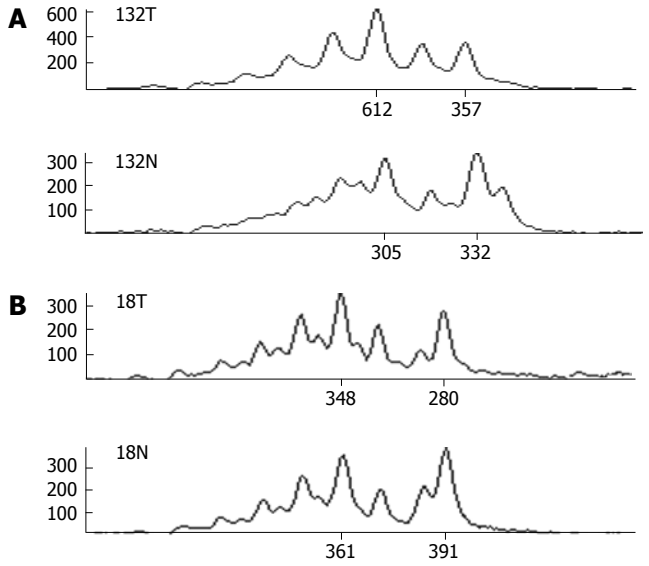

Figure 2 LOH Demonstration. **A:** Classic LOH peak: Allele ratio = (T1/T2)/(N1/N2) = (612/357)/(305/332) = 1.87 > 1.5; **B:** Heterozygosity retention: Allele ratio = (T1/T2)/(N1/N2) = (348/280)/(361/391) = 0.67 < 1.34 < 1.5. T: Tumor; N: Normal.

Table 1 LOH result and hereditary distance/location of chromo some 4p

Locus	Location	LOH cases	Normal cases	Informative rate	Distance (cM)	LOH rate (%)
D4S412	4p16.3	5	57	74.7	-	8.06
D4S2935	4p16.1	24	55	95.18	7.4	30.38
D4S1599	4p16.1	9	28	44.58	10.8	24.32
D4S403	4p15.33	3	34	44.58	5.1	8.1
D4S3013	4p15.2	26	47	86.9	7.7	35.62
D4S391	4p15.2	13	46	71.84	7.4	22.03
D4S405	4p14	22	30	62.65	13.4	42.3

at least one LOH event. Two distinct regions of frequent allelic loss at D4S3013 (4p15.2) and D4S405 (4p14) locus on chromosome were detected (Table 1). The LOH frequency was 35.62% and 42.3%, respectively. This suggested that putative tumor suppressor genes may be located near D4S3013 and D4S405 loci.

LOH deletion mapping results on 4p15 encompassing D4S3013

The chromosome region spanning D4S3013 locus on 4p15 was investigated using a saturation mapping strategy with another 6 microsatellite markers that are closely located within this region (Figure 2). To detect putative tumor suppressor genes easily, we limited average hereditary distance to 1.03 cm. Forty samples (48.19%) showed at least one LOH event. The average LOH frequency spanning D4S3013 was 24.2% (Table 2). We found that adjacent markers of D4S3013 displayed a low LOH frequency (< 30%), especially on D4S2933 locus, much less information was obtained because of more homozygosity.

Relationship between clinicopathological features and LOH on 4p

On D4S412 locus, none LOH was detected in patients with

Table 2 LOH result and hereditary distance/location of detailed deletion mapping spanning D4S3013

Locus	Location	LOH cases	Normal cases	Informative rate	Distance (cM)	LOH rate (%)
D4S2926	4p15.32	7	38	54.22	-	15.56
D4S1546	4p15.31	9	38	56.63	1.6	19.15
D4S3017	4p15.31	13	46	71.08	0.5	22.03
D4S3013	4p15.2	26	47	87.95	1.2	35.62
D4S2933	4p15.31	2	14	19.28	0.5	12.5
D4S2948	4p15.2	15	49	77.11	1.9	23.44
D4S1551	4p15.2	12	64	91.57	0.5	21.05

tumor larger than 5 cm in diameter (0/27), while in patients with tumor less than 5 cm in diameter, LOH frequency was 14.29% (5/35, $P = 0.041$). On the contrary, on D4S1546 locus, LOH frequency was 35.29% (6/17) in the former; and only 10% (3/30) in the latter locus ($P = 0.030$). Notably, on D4S403 locus, LOH was significantly associated with tumor gross pattern. In tumor of the massive, ulcerative and encroaching pattern, the LOH frequency was 10%, 0%, 33.33%, respectively ($P = 0.030$). No significant relationship was found between clinicopathological features and LOH on other loci (data not shown).

DISCUSSION

Inactivation of tumor suppressor genes appears to be one of the genetic mechanisms involved in the development of colorectal cancer[11,12]. Deletion of tumor suppressor genes occur frequently in human malignances. Such events can be detected using markers from the region of genome that include a tumor suppressor gene. Allelic deletions detected as LOH have been proved useful for mapping regions of DNA that contains tumor suppressor genes, i.e., LOH at specific chromosomal regions strongly suggests the existence of tumor suppressor genes at the relevant segment.

A great deal of evidence supported the presence of tumor suppressor genes in the short arm of chromosome 4. These include the reversion of the immortal phenotype by chromosome 4 transfer[13] and the frequent occurrence of losses in or near the 4p14-4p16 region in bladder cancer[14]. LOH has been observed at distal 4p in sporadic neuroblastoma with an incidence raging from 20% to 29%[15,16]. Using array comparative genomic hybridization, Hurst *et al*[17] reported the loss frequency of 4p to be 52% in bladder cancer. More importantly, Shirapurkar *et al*[18] observed the loss frequency of > 50% at 4p15.1-4p15.3 in malignant mesothelioma and lung carcinoma. LOH on 4p was 21% and > 30% in differentiated adenocarcinoma of stomach as well[19,20]. Head and neck squamous cell carcinoma, invasive cervical cancer and acinic cell carcinoma also showed a high allelic loss frequency[21-23].

In colorectal tumors, previous alletic typing[24], cytogenetic[25-27] and comparative genomic hybridization[28] studies have reported moderate losses(0%-30%) of chromosome 4. These data have not raised special interest in this chromosome as a candidate to harbor a tumor suppressor gene, therefore, colorectal cancer investigations

have not included a detailed analysis of loss in this chromosome. Choi *et al*[29] reported a LOH frequency of 24%-30% at just several loci on chromosome 4 in colorectal cancer. Later, Arribas *et al*[30,31], used AP-PCR method and suggested chromosome 4p14-4p16 may contain tumor suppressor gene, because LOH frequency on D4S2397 was as high as 35%. These reports indicate that 4p14-4p16 region displayed frequent loss in a couple of cancers, so 4p14-4p16 region is of important value for TSG screening.

D4S3013 locus region, 4p15.2, was concordant with several reports in other tumors before[14,16,20,21]. In this study, we investigated the LOH on 4p in 83 sporadic cases of colorectal cancer. The results showed putative tumor suppressor gene may harbor adjacent to D4S405 and D4S3013 locus. We made further detailed deletion mapping spanning D4S3013 locus, and found that the surrounding markers of D4S3013 displayed a low LOH frequency (< 30%). Therefore, we speculate that the candidate TSG may be located between D4S3017 and D4S2933, about 1.7 cm in hereditary distance.

We found several loci were significantly associated with clinicopathological features. On D4S412 locus, no LOH was detected in patients with tumor larger than 5 cm in diameter, while in patients with tumor less than 5 cm in diameter, the LOH frequency was 14.29% ($P = 0.041$). On the contrary, on D4S1546 locus, the LOH frequency showed opposite phenomenon. On D4S403 locus, LOH was significantly associated with tumor gross pattern. Similarly, Arribas *et al*[31] found solely at the D4S2397 locus was indicative of a shorter disease-free survival ($P = 0.027$). Choi *et al*[29] found 4p loss was significantly associated with early onset of colorectal cancer. The effect of 4p loss on the early-onset disease is unlikely to be the result of tumor aggressiveness, because 4p loss was not found to be correlated with cancer-related death. Nishizuka *et al*[20] found 4p LOH had an essentially similar frequency in early and advanced differentiated adenocarcinoma. The differential behavior of LOH at different markers suggested that distinct mechanisms and/or selection presses participate in the mutational event that affect this chromosomal region during the tumorigenic process.

Regarding allelic loss at 4p, cholecystokinin type A receptor (CCK-AR) gene maps near D4S2397[32,33] (Figure 1). Recent reports have suggested that cholecystokinin receptor may function as a tumor suppressor gene[34,35].

In summary, we investigated LOH on 4p in sporadic colorectal carcinoma in Chinese patients and detected two high deletion regions encompassing D4S3013 (4p15.2) and D4S405 (4p14). Candidate TSG, involved in sporadic colorectal carcinoma on chromosome 4p, may be located between D4S3017 and D4S2933 (about 1.7 cm). Further related gene screening and functional studies may contribute to the identification of the tumor suppressor gene in these regions.

COMMENTS

Background

Cancer arises from the accumulation of inherited polymorphism (i.e. SNPs) and mutation and/or sporadic somatic polymorphism (i.e. non-germline polymorphism) in cell cycle, DNA repair, and growth signaling genes. Neoplastic progression is generally characterized by the accumulation of multiple somatic-cell genetic alterations as the tumor progresses to advanced stages. The classic mechanism of tumor suppressor gene inactivation is described by two-hit modes in which one allele is mutated (or promoter hypermethylation or a small intragenic deletion) and the other allele is lost through a number of possible mechanisms, resulting in loss of heterozygosity at multiple loci. Loss of heterozygosity is the most common molecular genetic alteration observed in human cancers. In the model of colorectal tumorigenesis, mutational inactivation of tumor suppressor genes predominates.

Research frontiers

Most genome-wide scans for loss of heterozygosity (LOH) have been conducted at low resolution with a relatively small number of polymorphic markers. For example, an average of 120 microsatellites have been used to determine the allelotype of multiple different human neoplasms in a series of studies since 1995, and the highest density microsatellite allelotype was about 280 polymorphic markers before the year 2000. SNPs are the most common form of sequence variation in human genome, occurring approximately every 1200 base pairs (bps). High density mapping of genetic losses reveals potential tumor suppressor loci and might be useful for clinical classification of individual tumors. SNP array has been introduced recently for genome-wide screening of chromosome imbalance. Higher density SNP array can effectively detect small regions of chromosomal changes and provide more information regarding the boundaries of loss regions.

Innovations and breakthroughs

A great deal of evidence supported the presence of tumor suppressor genes in the short arm of chromosome 4. Much less studies have been reported in colorectal cancer. Previous allelotyping analysis of cancer by many groups was used with a relatively low density of markers. By deletion dense markers mapping, we detected two obvious high frequency LOH regions spanning D4S3013 and D4S405 locus in colorectal cancer. Candidate TSG, might be located between D4S3017 and D4S2933 (about 1.7 cm).

Applications

We used this method to detect some major allelic loss regions in genome-wide scans of LOH in patients with colorectal cancer.

Terminology

LOH is caused by a variety of genetic mechanisms, including physical deletion of chromosome non-disjunction and mitotic non-disjunction followed by republication of the remaining chromosomes, mitotic recombination and gene conversion. The mechanisms of LOH are remarkably chromosome-specific. Some chromosomes display a complete loss. However, more than half of the losses are associated with a only partial loss of a chromosome rather than a whole chromosome. LOH is also a common form of allelic imbalance and the detection of LOH has been used to identify genomic regions that harbor tumor suppressor genes and to characterize different tumor types, pathological stages and progression.

Peer review

This is a report that describes the LOH events in sporadic colorectal cancer in Chinese patients, further studies must benefit from this paper. The data presented is clear and concise in the text.

REFERENCES

1 **Westra JL**, Plukker JT, Buys CH, Hofstra RM. Genetic alterations in locally advanced stage II/III colon cancer: a search for prognostic markers. *Clin Colorectal Cancer* 2004; **4**: 252-259
2 **Fearon ER**, Volgelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767
3 **Hardy RG**, Meltzer SJ, Jankowski JA. ABC of colorectal cancer. Molecular basis for risk factors. *BMJ* 2000; **321**: 886-889
4 **Kataoka M**, Okabayashi T, Johira H, Nakatani S, Nakashima A, Takeda A, Nishizaki M, Orita K, Tanaka N. Aberration of p53 and DCC in gastric and colorectal cancer. *Oncol Rep* 2000; **7**: 99-103
5 **Lengauer C**, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. *Nature* 1998; **396**: 643-649

6 **Baker SJ**, Fearon ER, Nigro JM, Hamilton SR, Preisinger AC, Jessup JM, vanTuinen P, Ledbetter DH, Barker DF, Nakamura Y, White R, Vogelstein B. Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science* 1989; **244**: 217-221

7 **Kinzler KW**, Nilbert MC, Vogelstein B, Bryan TM, Levy DB, Smith KJ, Preisinger AC, Hamilton SR, Hedge P, Markham A. Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. *Science* 1991; **251**: 1366-1370

8 **Thiagalingam S**, Laken S, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B, Lengauer C. Mechanisms underlying losses of heterozygosity in human colorectal cancers. *Proc Natl Acad Sci USA* 2001; **98**: 2698-2702

9 **Xu SF**, Peng ZH, Li DP, Qiu GQ, Zhang F. Refinement of heterozygosity loss on chromosome 5p15 in sporadic colorectal cancer. *World J Gastroenterol* 2003; **9**: 1713-1718

10 **Dib C**, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996; **380**: 152-154

11 **Kataoka M**, Okabayashi T, Johira H, Nakatani S, Nakashima A, Takeda A, Nishizaki M, Orita K, Tanaka N. Aberration of p53 and DCC in gastric and colorectal cancer. *Oncol Rep* 2000; **7**: 99-103

12 **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

13 **Ning Y**, Weber JL, Killary AM, Ledbetter DH, Smith JR, Pereira-Smith OM. Genetic analysis of indefinite division in human cells: evidence for a cell senescence-related gene(s) on human chromosome 4. *Proc Natl Acad Sci USA* 1991; **88**: 5635-5639

14 **Polascik TJ**, Cairns P, Chang WY, Schoenberg MP, Sidransky D. Distinct regions of allelic loss on chromosome 4 in human primary bladder carcinoma. *Cancer Res* 1995; **55**: 5396-5399

15 **Caron H**, van Sluis P, Buschman R, Pereira do Tanque R, Maes P, Beks L, de Kraker J, Voute PA, Vergnaud G, Westerveld A, Slater R, Versteeg R. Allelic loss of the short arm of chromosome 4 in neuroblastoma suggests a novel tumour suppressor gene locus. *Hum Genet* 1996; **97**: 834-837

16 **Luttikhuis ME**, Powell JE, Rees SA, Genus T, Chughtai S, Ramani P, Mann JR, McConville CM. Neuroblastomas with chromosome 11q loss and single copy MYCN comprise a biologically distinct group of tumours with adverse prognosis. *Br J Cancer* 2001; **85**: 531-537

17 **Hurst CD**, Fiegler H, Carr P, Williams S, Carter NP, Knowles MA. High-resolution analysis of genomic copy number alterations in bladder cancer by microarray-based comparative genomic hybridization. *Oncogene* 2004; **23**: 2250-2263

18 **Shivapurkar N**, Virmani AK, Wistuba II, Milchgrub S, Mackay B, Minna JD, Gazdar AF. Deletions of chromosome 4 at multiple sites are frequent in malignant mesothelioma and small cell lung carcinoma. *Clin Cancer Res* 1999; **5**: 17-23

19 **Tamura G**, Sakata K, Nishizuka S, Maesawa C, Suzuki Y, Terashima M, Eda Y, Satodate R. Allelotype of adenoma and differentiated adenocarcinoma of the stomach. *J Pathol* 1996; **180**: 371-377

20 **Nishizuka S**, Tamura G, Terashima M, Satodate R. Loss of heterozygosity during the development and progression of differentiated adenocarcinoma of the stomach. *J Pathol* 1998; **185**: 38-43

21 **Pershouse MA**, El-Naggar AK, Hurr K, Lin H, Yung WK, Steck PA. Deletion mapping of chromosome 4 in head and neck squamous cell carcinoma. *Oncogene* 1997; **14**: 369-373

22 **Sherwood JB**, Shivapurkar N, Lin WM, Ashfaq R, Miller DS, Gazdar AF, Muller CY. Chromosome 4 deletions are frequent in invasive cervical cancer and differ between histologic variants. *Gynecol Oncol* 2000; **79**: 90-96

23 **el-Naggar AK**, Abdul-Karim FW, Hurr K, Callender D, Luna MA, Batsakis JG. Genetic alterations in acinic cell carcinoma of the parotid gland determined by microsatellite analysis. *Cancer Genet Cytogenet* 1998; **102**: 19-24

24 **Vogelstein B**, Fearon ER, Kern SE, Hamilton SR, Preisinger AC, Nakamura Y, White R. Allelotype of colorectal carcinomas. *Science* 1989; **244**: 207-211

25 **Muleris M**, Salmon RJ, Dutrillaux B. Cytogenetics of colorectal adenocarcinomas. *Cancer Genet Cytogenet* 1990; **46**: 143-156

26 **Bardi G**, Sukhikh T, Pandis N, Fenger C, Kronborg O, Heim S. Karyotypic characterization of colorectal adenocarcinomas. *Genes Chromosomes Cancer* 1995; **12**: 97-109

27 **Thorstensen L**, Qvist H, Nesland JM, Giercksky KE, Lothe RA. Allelotype profiles of local recurrences and distant metastases from colorectal-cancer patients. *Int J Cancer* 1996; **69**: 452-456

28 **Ried T**, Knutzen R, Steinbeck R, Blegen H, Schrock E, Heselmeyer K, du Manoir S, Auer G. Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumors. *Genes Chromosomes Cancer* 1996; **15**: 234-245

29 **Choi SW**, Lee KJ, Bae YA, Min KO, Kwon MS, Kim KM, Rhyu MG. Genetic classification of colorectal cancer based on chromosomal loss and microsatellite instability predicts survival. *Clin Cancer Res* 2002; **8**: 2311-2322

30 **Arribas R**, Risques RA, Gonzalez-Garcia I, Masramon L, Aiza G, Ribas M, Capella G, Peinado MA. Tracking recurrent quantitative genomic alterations in colorectal cancer: allelic losses in chromosome 4 correlate with tumor aggressiveness. *Lab Invest* 1999; **79**: 111-122

31 **Arribas R**, Ribas M, Risques RA, Masramon L, Tortola S, Marcuello E, Aiza G, Miro R, Capella G, Peinado MA. Prospective assessment of allelic losses at 4p14-16 in colorectal cancer: two mutational patterns and a locus associated with poorer survival. *Clin Cancer Res* 1999; **5**: 3454-3459

32 **Huppi K**, Siwarski D, Pisegna JR, Wank S. Chromosomal localization of the gastric and brain receptors for cholecystokinin (CCKAR and CCKBR) in human and mouse. *Genomics* 1995; **25**: 727-729

33 **Inoue H**, Iannotti CA, Welling CM, Veile R, Donis-Keller H, Permutt MA. Human cholecystokinin type A receptor gene: cytogenetic localization, physical mapping, and identification of two missense variants in patients with obesity and non-insulin-dependent diabetes mellitus (NIDDM). *Genomics* 1997; **42**: 331-335

34 **Detjen K**, Fenrich MC, Logsdon CD. Transfected cholecystokinin receptors mediate growth inhibitory effects on human pancreatic cancer cell lines. *Gastroenterology* 1997; **112**: 952-959

35 **Reubi JC**, Schaer JC, Waser B. Cholecystokinin(CCK)-A and CCK-B/gastrin receptors in human tumors. *Cancer Res* 1997; **57**: 1377-1386

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