PERGAMON

European Journal of Cancer 40 (2004) 136–141

European Journal of Cancer

www.ejconline.com

Genetic analysis of the *LKB1/STK11* gene in hepatocellular carcinomas

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Received 10 March 2003; received in revised form 6 June 2003; accepted 22 July 2003

Abstract

The tumour suppressor gene, *LKB1/STK11*, has been mapped to chromosome 19p13, a region showing frequent allelic loss in various human cancers, including hepatocellular carcinoma (HCC). Additionally, LKB1 physically associates with p53 and regulates p53-dependent apoptotic pathways. To investigate whether genetic alterations of *LKB1* could be involved in the tumorigenesis of HCC, we analysed the genetic alterations of the *LKB1* and *p53* genes in seven dysplastic nodules and 80 HCCs. We found one *LKB1* missense mutation, CCG→CTG (Pro→Leu) at codon 281 within the kinase domain. We also found allelic loss in six of 27 (22%) informative HCC cases and all of them were HBV-positive cases. In addition, we detected seven missense, one nonsense and one silent mutations (nine of 80, 11%) of *p53* in HCCs only. These results suggest that genetic alterations of the *LKB1* or *p53* genes may play an important role in tumour development or progression of a sub-set of HCCs, and may also provide alternative mechanisms to protect the HCC cell from p53-dependent apoptosis.

Keywords: *STK11/LKB1*; *p53*; Mutation; Loss of heterozygosity; Apoptosis; Hepatocellular carcinoma

1. Introduction

Primary hepatocellular carcinoma (HCC) is one of the most common causes of death from cancer in many countries of the world, especially in Asia and Africa. In Korea, it accounts for an estimated 12.2% of all malignancies, 16.4% in the male population and 6.5% in the female [1]. It is well known that infection with the hepatitis B or C virus (HBV or HCV) and ingestion of aflatoxin B1-contaminated food are important risk factors for HCC. In addition, cirrhosis resulting from heavy alcohol intake represents a major risk factor in some Western world countries. Generally, most HCCs are associated with a background of chronic liver disease, such as cirrhosis and chronic active hepatitis, suggesting a multistep process in the development of HCC [2]. However, the genetic events involved in the carcinogenesis of HCC are still unclear.

The molecular genetic approach has shown that sequential accumulations of genetic alterations such as loss or gain in the genome play a central role in the development of cancer, including the activation of oncogenes and the inactivation of tumour suppressor genes [3]. Somatic inactivation of a tumour suppressor gene is usually caused by an intragenic mutation in one allele of the gene with subsequent loss of a chromosomal region that spans the second allele. Frequent chromosomal losses in carcinomas arising from certain epithelial cells have implied that putative tumour suppressor gene(s) reside(s) on the affected chromosomal arm.

Although several chromosomal aberrations have been implicated, the significance of these changes, and the specific genes that are involved, are poorly understood. A growing body of evidence suggests that chromosome 19p, where the *LKB1* gene resides, is one of the common deletion regions and contains one or more of the genes associated with the development or progression of carcinomas in a large spectrum of tumour types, including HCC [4–6].

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doi:10.1016/S0959-8049(03)00659-2

Peutz–Jegher syndrome (PJS) is an autosomal dominant disorder characterised by mucocutaneous melanin pigmentation and intestinal hamartomatous polyposis, as well as an increased risk of cancer, including colon, stomach, breast and liver cancers [7]. The *LKB1* gene, also termed *STK11*, encoding a novel serine/threonine kinase and residing on chromosome 19p13.3 at a distance of 190 kb proximal to D19S886, was identified. Numerous germ-line mutations were detected in individuals affected by PJS [8–10]. Although the functional role of LKB1 has not been thoroughly studied, several lines of evidence suggest it may play a role in the cell cycle and apoptotic pathways [11,12]. Recently, heterozygous *LKB1* (+/−) mice have been shown to develop, not only gastrointestinal polyps, but also HCCs [13]. These data strongly suggest that inactivation of the *LKB1* gene may be one of the important novel mechanisms for HCC development.

p53 is the most commonly mutated gene in a variety of human cancers, including HCC [14,15]. The p53 protein has been implicated in diverse cellular processes such as cell-cycle arrest and p53-dependent apoptosis, senescence, differentiation and angiogenesis [16]. Recently, it has been reported that the N-terminal kinase domain of LKB1 is required for its interaction with p53 and the loss of LKB1 function in PJS leads to a deficiency in intestinal epithelial cell apoptosis [17]. These facts indicate that LKB1 may be one of the signal transduction molecules regulating p53-dependent apoptosis. Therefore, it is reasonable to propose that inactivation of p53 and/or LKB1 through primary structural changes, such as mutation, could be involved in the development of various human cancers or their progression, with LKB1 not only acting as a tumour suppressor but also allowing cells to become more resistant to p53-dependent apoptosis.

To determine whether genetic alterations of the *LKB1* gene could be involved in HCC development, we searched for mutations and allelic losses of the *LKB1* gene in a series of seven dysplastic nodules and 80 Korean HCCs. In addition, we performed a mutational analysis of the *p53* gene to explore the relationship between the *p53* and *LKB1* genetic status of cells in HCC development.

2. Materials and methods

2.1. Tissue samples

A total of 80 frozen HCC specimens were obtained from the Medical College, Sungkyunkwan University in Seoul, Korea. Seven dysplastic nodules from these patients were also used in this study. Informed consent was obtained from every patient. No patient had a family history. Their age ranged from 31 to 89 years, with an average age of 60 years. The male to female ratio was 66 to 14. One 6- μm section stained with haematoxylin and eosin was independently reviewed by three pathologists. The background liver showed cirrhosis in 62 (78%), chronic active hepatitis in 3 (13%), chronic persistent hepatitis in three (4%), fatty changes in 1 (1%), and non-specific changes in 4 (5%) cases, respectively.

2.2. DNA extraction

Frozen tissue samples were ground to a very fine powder in liquid nitrogen, suspended in lysis buffer, and treated with proteinase K. DNA was extracted using phenol/chloroform/isoamyl and an alcohol extraction.

2.3. SSCP and DNA sequencing

Genomic DNAs from cancer cells and corresponding non-cancerous livers were amplified with 12 sets of primers covering the entire coding region (exons 2–10) of the *LKB1* gene. These primers are described in Table 1. Numbering of the *LKB1* DNA was done using an ATG start codon according to the genomic sequence of Genbank accession No. NM000455. We also analysed the *p53* gene using five primer pairs covering the DNA binding domain of the *p53* gene [18]. Each polymerase chain reaction (PCR) was performed under standard conditions in a 10 μl reaction mixture containing 20 ng of template DNA, 0.5 μM of each primer, 0.2 μM of each deoxynucleotide triphosphate (dNTP), 1.5 mM $MgCl_2$, 0.4 unit of Taq polymerase, 0.5 μCi of

Table 1
Primer sequence for amplifying the coding region of the *LKB1* gene

Name of primer	Nucleotide sequences	Product size (bp)
Exon 2-1 F	5′-GAACACAAGGAAGGACCGCTCACC-3′	259
Exon 2-1 R	5′-CACCTTGCCGTAAGAGCCTTCCC-3′	
Exon 2-2 F	5′-CCAAGCTCATCGGCAAGTACCTGA-3′	249
Exon 2-2 R	5′-GGGAGGGAGGAGAGAAGGAAGGAA-3′	
Exon 3 F	5′-ACGTTGGGTCGGCTGATACAC-3′	224
Exon 3 R	5′-TTCAAGGAGACGGGAAGAGGAG-3′	
Exon 4 F	5′-GTGCTCCCTGGGCCTGTGAGTG-3′	217
Exon 4 R	5′-CGGAAAGGAGCCTGCCCTGC-3′	
Exon 5 F	5′-AGGCCTCGGCCCCAGGAC-3′	234
Exon 5 R	5′-CCGTGCAGCCCTCAGGGAGT-3′	
Exon 6 F	5′-ACTCCCTGAGGGCTGCACGG-3′	215
Exon 6 R	5′-CCTCGGAGTGTGCGTGTGGTG-3′	
Exon 7 F	5′-TTCTTCCCTCCCCTCGAAATGA-3′	230
Exon 7 R	5′-CCTGACACCCCCAACCCTACAT-3′	
Exon 8 F	5′-GGTATCACCCAGGGCCTGACAACA-3′	231
Exon 8 R	5′-CGCTCAACCAGCTGCCCACAT-3′	
Exon 9 F	5′-ACAGGCGCCACTGCTTCTGGG-3′	257
Exon 9 R	5′-GAGTCAGCAGAGCCGGGCAGG-3′	
Exon 10-1 F	5′-CTGTAAGTGCGTCCCCGTGGTG-3′	205
Exon 10-1 R	5′-CGCCCTGGATTTGGTGCTCAG-3′	
Exon 10-2 F	5′-TCCCCAAGGCCGTGTGTATGAA-3′	218
Exon 10-2 R	5′-CTGTGGCATCCAGGCGTTGTC-3′	

R, reverse; F, forward; bp, base pairs.

[^{32}P]deoxycytidine triphosphate (dCTP) (Amersham, Buckinghamshire, UK), and 1 μl of 10× buffer. The reaction mixture was denatured for 12 min at 94 °C and incubated for 32 cycles (denaturing for 40 s at 94 °C, annealing for 40 s at 59–63 °C, and extending for 40 s at 72 °C). The final extension step was continued for 5 min at 72 °C. After amplification, PCR products were denatured for 5 min at 95 °C in a 1:1 dilution of sample buffer containing 98% formamide/5 mmol/l NaOH and were loaded onto a single-strand conformation polymorphism (SSCP) gel (FMC Mutation Detection Enhancement system; Intermountain Scientific, Kaysville, UT, USA) in 10% glycerol. After electrophoresis, the gels were transferred to 3-mm Whatman paper and dried, and autoradiography was performed with Kodak X-OMAT film (Eastman Kodak, Rochester, NY, USA). To detect the mutations, DNAs showing mobility shifts were cut out from the dried gel, and reamplified for 30 cycles using the same primer set. Sequencing of the PCR products was carried out using a cyclic sequencing kit (Perkin-Elmer, Foster City, CA, USA) according to the manufacturer's recommendation.

2.4. Loss of heterozygosity (LOH) analysis

HCC and the corresponding non-cancerous DNAs were amplified using the thermal cycler (MJ Reseach Institute, Watertown, MA, USA) with the microsatellite marker D19S886. Each PCR reaction was performed under standard conditions in a 10-μl reaction mixture containing 20 ng of template DNA, 0.5 μM of each primer, 0.2 μM of each deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, 0.4 unit of Taq polymerase, 0.5 μCi of [^{32}P]dCTP (Amersham, Buckinghamshire, UK), and 1 μl of 10× buffer. PCR products were then denatured and electrophoresed in a 6% polyacrylamide gel containing 7 M urea. After electrophoresis, the gels were transferred to 3 MM Whatman paper, dried, and subjected to autoradiography using Kodak-OMAT film (Eastman Kodak, Rochester, NY, USA). Complete absence of one allele in the tumour DNA sample of informative cases, as defined by direct visualisation, was considered as loss of heterozygosity (LOH).

3. Results

3.1. Mutations of the LKB1 and p53 genes in HCCs

We searched for potential mutations in all nine exons of the *LKB1* gene in seven dysplastic nodules and 80 HCCs by PCR-SSCP and sequencing analysis. Direct sequencing of aberrantly migrating bands on the SSCP gel led to the identification of a mutation in one (1%) of the HCCs examined. The mutation was a missense mutation, CCG→CTG (Pro→Leu) at codon 281 (Fig. 1a) in exon 7 that encodes the LKB1 kinase domain (codons 50–337). No mutation was found in the seven dysplastic nodules. We repeated the experiment three times, including PCR-SSCP and sequencing to ensure the specificity of the results, and the data were consistent. None of the corresponding normal samples showed evidence of mutation by repeated SSCP, indicating that the mutation was a somatic mutation.

For the *p53* gene, we found seven missense, one nonsense and one silent mutation in the HCC samples alone, one at exon 4, two at exon 5, three at exon 6, and

Table 2
Genetic alterations of *LKB1* and *p53* genes in hepatocellular carcinoma (HCC)

Case no.	Age in years/gender	Hepatitis virus	Grade	Background	*LKB1*		*p53* mutation
					Mutation	LOH	
1	37/M	B	2	Cirrhosis		NI	CGA→CGG(A196A)
18	42/M	B	3	Cirrhosis		NI	CGC→CCC(A283P)
23	62/M	C	3	CPH		–	CTG→CGG(L111A)
24	58/M	None	3	Fatty change		NI	CTT→CGT(L194A)
25	60/M	B	3	Cirrhosis		NI	CGC→CTC(A175L)
27	36/F	None	1	Non-specific	CCG→CTG(P281L)	NI	
40	50/M	B	2	Cirrhosis		+	
45	45/M	B	3	Cirrhosis		NI	CGC→CAC(A158H)
64	55/M	B	2	Cirrhosis		NI	AGA→TGA(A280stop)
66	51/F	B	2	Cirrhosis		+	
68	43/M	B	3	Cirrhosis		+	
72	51/M	B	2	Cirrhosis		+	
73	57/M	B	3	Cirrhosis		+	
74	37/M	B	3	Cirrhosis		NI	CGT→TGT(A273C)
78	31/M	B	2	CAH		+	CAT→CGT(H214A)

CPH, chronic persistent hepatitis; CAH, chronic active hepatitis; NI, non-informative; –, retention of heterozygosity; +, loss of heterozygosity (LOH); M, male; F, female.

three at exon 8, respectively (Fig. 1c and d, Table 2). Clinically, all of the patients with *p53* mutations were male and 6 of them showed HBV infection and a cirrhotic background. One of them demonstrated chronic active hepatitis with HBV infection and 1 had chronic persistent hepatitis with HCV infection. The other patient revealed fatty changes and no evidence of HBV or HCV infections. The cases with *LKB1* genetic alterations did not have *p53* mutations except for case 78 (Table 2).

3.2. Allelic status

To clarify whether LOH in the *LKB1* gene is also associated with the development of human HCCs, we conducted a LOH analysis in this cohort of HCC patients using the microsatellite marker D19S886, which locates at a distance 190 kb distal to the *LKB1* gene. Patients who were heterozygous for the microsatellite marker were considered informative. 2 (29%) of 7 cases of dysplastic nodules and 27 (34%) cases of 80 HCCs were informative at this marker. We observed no LOH in the two informative dysplastic nodules. In HCCs, 6 of the 27 (22%) informative cases showed allelic loss at 19S886 (Table 2). Interestingly, all of them were HBV-positive. Statistically, there was no significant difference in the LOH frequency according to the histological grade and stage. Four of them showed Edmonson grade 2 and the rest had grade 3 (Table 2). An autoradiogram showing LOH is displayed in Fig. 1b. Although the HCC case with a *LKB1* mutation was non-informative at this marker, the SSCP gel showed both bands of the wild-type allele and aberrant bands of the mutant allele (Fig. 1a), indicating a hemizygous mutation in one allele and retention of the remaining allele. Interestingly, the patient with HBV infection and chronic active hepatitis (case 78) showed LOH at the *LKB1* locus and *p53* mutation (Table 2).

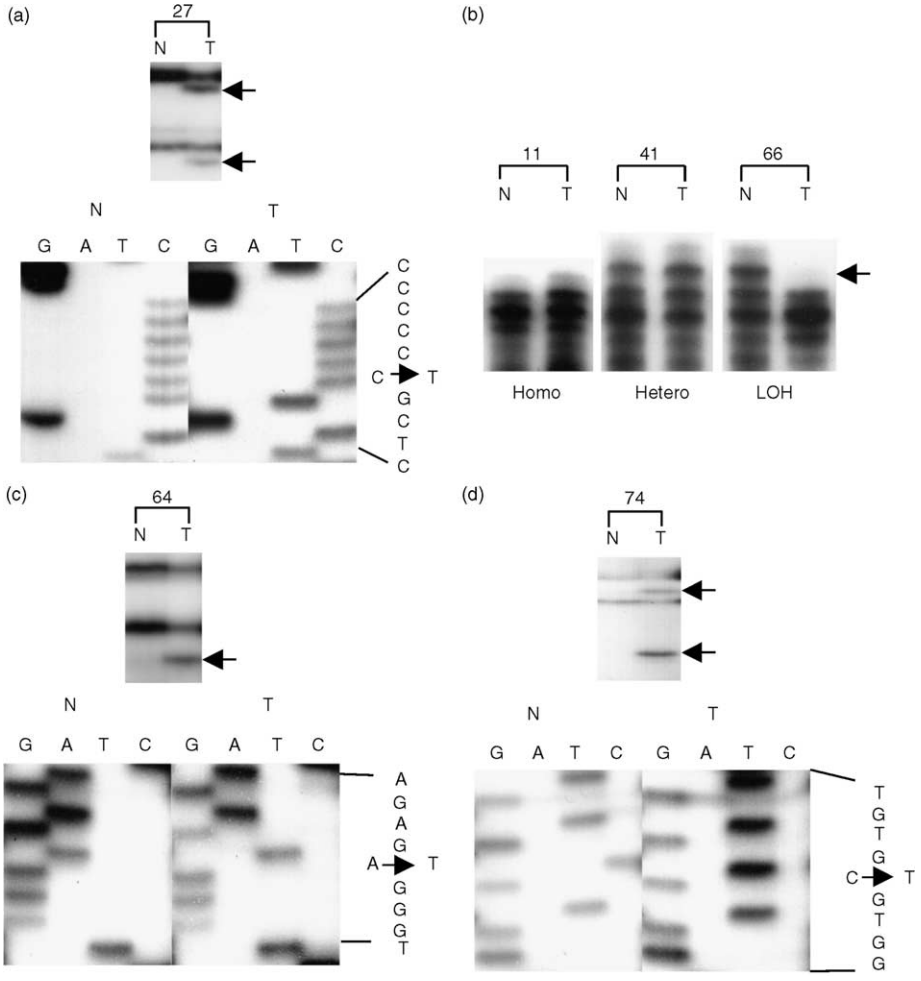

Fig. 1. Representative results showing single-strand conformation polymorphism (SSCP)-sequencing and loss of heterozygosity (LOH) analysis of the *LKB1* gene (a and b): (a) SSCP demonstrating aberrant bands (arrows) and sequencing data showing missense mutation, C to T transition at codon P281L of the *LKB1* gene in case 27, (b) LOH (arrow) at microsatellite marker D19S886, (c and d) SSCP and sequencing analysis of *p53*; a A to T transversion at codon 280 in case 64 and a C to T transition at codon 273 in case 74. Homo, homozygosity; Hetero, heterozygosity; LOH, loss of heterozygosity; N, normal DNA; T, tumour DNA.

4. Discussion

PJS patients have a dramatically increased incidence of cancer in a wide variety of tissues, including the colon, breast, stomach, uterine cervix, lung, and liver [7]. Since Hemminki and colleagues identified the *LKB1* gene, germline mutations in the *LKB1* gene have been reported to account for 50–70% of PJS patients [10,19,20]. These results indicate that the *LKB1* acts as a tumour suppressor gene and that the development of hamartomas and various types of cancers in PJS patients may be the result of inactivation of the *LKB1* gene. Recently, Nakau and colleagues have reported that all LKB1 (+/−) mice had nodular foci in the liver after 61 weeks and that nine of 12 nodules in male mice showed HCC tumours with marked vascularisation [13]. In addition, a growing body of evidence suggests that inactivation of LKB1 may be responsible for the development of HCC and that genetic alteration of *LKB1* gene may be also associated with the development of sporadic forms of HCC.

In the present study, we found one missense mutation of the *LKB1* gene in the 80 HCCs examined. The mutation revealed a C→T transition type mutation at codon 281 in exon 7, which lies within the mutational hot spot corresponding to codons 279–281, and this nucleotide transition would change an amino acid from proline to a leucine (Fig. 1a and Table 2). Interestingly, the patient was a HBV- and HCV-negative female and the background liver showed non-specific changes, suggesting that there may be an additional mechanism in the carcinogenesis process. Thus, it is likely that somatic mutation of the *LKB1* gene may occur as an early event and be involved in the acceleration of carcinogenesis in HCC. Of particular note, an identical mutation has been detected in ovarian cancer [21]. Furthermore, our results may underestimate the prevalence of *LKB1* somatic mutations in HCC, as the sensitivity rate of the SSCP analysis for the detection of single base substitution is estimated to be 80% [22]. However, most reports have found little evidence of somatic mutational inactivation of *LKB1* [23–26].

Deletions of chromosome 19p have been reported in many types of human malignancy, including HCC, which raises the possibility of the presence of tumour suppressor gene in this region [4–6]. In this study, we found LOH in 6 (22%) of 27 informative cases of HCCs, and all of the cases were HBV-positive (Table 2). Interestingly, a similar low frequency of LOH was also observed in sporadic colon, testicular, ovary and breast tumours [21,23,27]. However, Nakau and colleagues have reported that LKB (+/−) mice develop small nodular foci of HCC with continuous proliferation [13]. Several reasons may account for this discrepancy. First, we cannot completely rule out the possibility of an epigenetic inactivation of the *LKB1* gene through DNA methylation in the development of human HCCs, like colorectal and breast carcinomas [28]. Infection with HBV or HCV, ingestion of aflatoxin B1-contaminated food, and heavy alcohol intake are to be important risk factors for human HCC. The influence of a susceptibility gene on the human HCC risk may depend on environmental and lifestyle factors. Another explanation is the possibility that inactivation of another candidate tumour suppressor gene(s) on chromosome 19p might be associated with the development of human HCC.

Despite the fact that epidemiological evidence indicates that HBV is a major risk factor for the development of HCC [29], the exact mechanism of this association has not been elucidated. The site of cellular DNA at which HBV integrates frequently undergoes rearrangement [30], resulting in translocation [31], and deletion [31,32]. Interestingly, all cases with LOH detected in this study were HBV-positive. It is reasonable that the process of carcinogenesis itself may result from the inappropriate survival of DNA-damaged cells that should otherwise have been removed by apoptosis. The p53 protein is a key protein to integrate signals emanating from a wide range of cellular stresses and allows the cell to respond to these insults by activating a set of genes whose products facilitate adaptive and protective activities, which include apoptosis and cell-cycle arrest [33]. Furthermore, the kinase domain of LKB1 is required for its interaction with p53 and loss of LKB1 function in PJS leads to a deficiency in intestinal epithelial cell apoptosis [17]. Hence, mutations in *LKB1* may remove the selection pressure on p53 and allow cancer to develop by evading the apoptotic checkpoint regulated by p53 in a variety of cell types. In this study, we found nine somatic mutations of the *p53* gene in HCC cases only. These results suggest that inactivation of *LKB1* or *p53* caused by alterations of the primary structure by mutation or deletion may be one of the possible mechanisms preventing p53-dependent apoptosis.

In conclusion, we detected somatic mutations of the *LKB1* and *p53* genes in HCC. We suspect that mutations of the *LKB1* or *p53* genes may provide the HCC cell with protection from p53-dependent apoptosis and also may play a role in tumour progression in a sub-set of HCCs. Additional studies are needed to verify these initial observations and the functional analysis of mutations identified in this study will certainly broaden our understanding of the pathogenesis of human HCC.

Acknowledgements

This work was supported by the Medical Research Center (R13-2002-005-01004-0) from the Korea Science and Engineering Foundation.

References

1. Suh CI, Suh KA, Park SH, Chang HJ, Ko JW, Ahn DH. Annual report of the central cancer registry in Korea—1998. *J Korean Cancer Assoc* 2000, **32**, 827–834.
2. Sakamoto M, Hirohashi S, Shimosato Y. Early stages of multistep hepatocarcinogenesis: adenomatous hyperplasia and early hepatocellular carcinoma. *Hum Pathol* 1991, **22**, 172–178.
3. Kumar V, Cotran RS, Robbins SL. Neoplasia. In *Robbins Basic Pathology,* 7th ed. Philadelphia, Saunders, 2003, 165–210.
4. Okabe H, Ikai I, Matsuo K, *et al*. Comprehensive allelotype study of hepatocellular carcinoma: potential differences in pathways to hepatocellular carcinoma between hepatitis B virus-positive and -negative tumors. *Hepatology* 2000, **31**, 1073–1079.
5. Qin LX, Tang ZY, Ye SL, *et al*. Chromosome 8p deletion is associated with metastasis of human hepatocellular carcinoma when high and low metastatic models are compared. *J Cancer Res Clin Oncol* 2001, **127**, 482–488.
6. Guan XY, Fang Y, Sham JST, *et al*. Recurrent chromosome alterations in hepatocellular carcinoma detected by comparative genomic hybridisation. *Genes Chromosomes Cancer* 2000, **29**, 110–116.
7. Aaltonen LA, Peutz-Jeghers syndrome. In Vogelstein B, Kinzler KW, eds. *The Genetic Basis of Human Cancer*, 2nd ed. 337–341. New York, McGraw-Hill
8. Hemminki A, Tomlinson IP, Markie D, *et al*. Localization of a susceptibility locus for Peutz-Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis. *Nat Genet* 1997, **15**, 87–90.
9. Amos CI, Bali D, Thil TJ, *et al*. Fine mapping of a genetic locus for Peutz-Jeghers syndrome on chromosome 19p. *Cancer Res* 1997, **57**, 3653–3656.
10. Hemminki A, Markie D, Tomlinson P, *et al*. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature* 1998, **391**, 184–187.
11. Tiainen M, Ylikorkala A, Makela TP. Growth suppression by lkb1 is mediated by a G_1 cell cycle arrest. *Proc Natl Acad Sci USA* 1999, **96**, 9248–9251.
12. Marignani PA, Kanai F, Carpenter CL. LKB1 associates with Brg1 and is necessary for Brg-1 induced growth arrest. *J Biol Chem* 2001, **276**, 32415–32418.
13. Nakau M, Miyoshi H, Seldin MF, Imamura M, Oshima M, Taketo MM. Hepatocellular carcinoma caused by loss of heterozygosity in Lkb1 gene knockout mice. *Cancer Res* 2002, **62**, 4549–4553.
14. Sugo H, Takamori S, Kojima K, Beppu T, Futagawa S. The significance of p53 mutations as an indicator of the biological behavior of recurrent hepatocellular carcinomas. *Surg Today* 1999, **29**, 849–855.
15. Lee SN, Park CK, Sung CO, *et al*. Correlation of mutation and immunohistochemistry of p53 in hepatocellular carcinomas in Korean people. *J Korean Med Sci* 2002, **17**, 801–805.
16. El-Deiry WS. Regulation of p53 downstream genes. *Semin Cancer Biol* 1998, **8**, 345–357.
17. Karuman P, Gozani O, Odze RD, *et al*. The Peutz-Jegher gene product LKB1 is a mediator of p53-dependent cell death. *Mol Cell* 2001, **7**, 1307–1319.
18. Park WS, Lee HK, Lee JY, *et al*. p53 mutations in solar keratoses. *Hum Pathol* 1996, **27**, 1180–1184.
19. Ylikorkala A, Avizienyte E, Tomlinson IP, *et al*. Mutations and impaired function of LKB1 in familial and non-familial Peutz-Jeghers syndrome and a sporadic testicular cancer. *Hum Mol Genet* 1999, **8**, 45–51.
20. Nakagawa H, Koyama K, Miyoshi Y, *et al*. Nine novel germline mutations of STK11 in ten families with Peutz-Jeghers syndrome. *Hum Genet* 1998, **103**, 168–172.
21. Nishioka Y, Kobayashi K, Sagae S, *et al*. Mutational analysis of STK11 gene in ovarian carcinomas. *Jpn J Cancer Res* 1999, **90**, 629–632.
22. Sheffield VC, Beck JS, Kwitek AE, Sandstrom DW, Stone EM. The sensitivity of single-strand conformation polymorphism anlaysis for the detection of single base substitutions. *Genomics* 1993, **6**, 325–332.
23. Avizienyte E, Roth S, Loukola A, *et al*. Somatic mutations in LKB1 are rare in sporadic colorectal and testicular tumors. *Cancer Res* 1998, **58**, 2087–2090.
24. Park WS, Moon YW, Yang YM, *et al*. Mutations of the STK11 gene in sporadic gastric carcinoma. *Int J Oncol* 1998, **13**, 601–604.
25. Wang ZJ, Taylor F, Churchman M, Norbury G, Tomlinson I. Genetic pathways of colorectal carcinogenesis rarely involve the PTEN and LKB1 genes outside the inherited hamartoma syndromes. *Am J Pathol* 1998, **153**, 363–366.
26. Avizienyte E, Loukola A, Roth S, *et al*. LKB1 somatic mutations in sporadic tumors. *Am J Pathol* 1999, **154**, 677–681.
27. Bignell GR, Barfoot R, Seal S, Collins N, Warren W, Stratton MR. Low frequency of somatic mutations in the LKB1/Peutz-Jeghers syndrome gene in sporadic breast cancer. *Cancer Res* 1998, **58**, 1384–1386.
28. Esteller M, Avizienyte E, Corn PG, *et al*. Epigenetic inactivation of LKB1 in primary tumors associated with the Peutz-Jeghers syndrome. *Oncogene* 2000, **19**, 164–168.
29. Robbins WS. Molecular events in the pathogenesis of hepadnavirus associated hepatocellular carcinoma. *Annu Rev Med* 1994, **45**, 297–323.
30. Ogata N, Tokino T, Kamimura T, Asakura H. A comparison of the molecular structure of integrated hepatitis B virus genomes in hepatocellular carcinoma cells and hepatocytes derived from the same patient. *Hepatology* 1990, **11**, 1017–1023.
31. Hino O, Shows TB, Rogler CE. Hepatitis B virus integration site in hepatocellular carcinoma at chromosome 17:18 translocation. *Proc Natl Acad Sc USA* 1986, **83**, 8338–8342.
32. Nakamura T, Tokino T, Nagaya T, Matsubara K. Microdeletion associated with the integration process of hepatitis B virus DNA. *Nucleic Acids Res* 1988, **16**, 4865–4873.
33. Wyllie AH, Bellamy CO, Bubb VJ, *et al*. Apoptosis and carcinogenesis. *Br J Cancer (Suppl)* 1999, **80**, 34–37.