Genomics & Informatics Vol. 1(2) 101-107, December 2003

Construction of Deletion Map of 16q by LOH Analysis from HCC Patients and Physical Map on 16q 23.3 - 24.1 Region

Jiyeol Chung[1], Nae Yun Choi[1], Myoung Sup Shim[1], Dong Wook Choi[2], Hyen Sam Kang[1], Chang Min Kim[3], Ung Jin Kim[4], Sun Hwa Park[5], Hyeon Kim[5] and Byeong Jae Lee[1]*

[1]School of Biological Sciences, Seoul National University, Korea, [2]Department of Surgery, Korea Cancer Center Hospital, Korea, [3]Center for Gastric Cancer, Cancer Center Hospital, National Cancer Center, Korea, [4]California Institute of Technology, U.S.A., [5]Department of Anatomy, Korea University of Medicine, Korea.

Abstract

Loss of heterozygosity (LOH) has been used to detect deleted regions of a specific chromosome in cancer cells. LOH on chromosome 16q has been reported to occur frequently in progressed hepatocellular carcinoma (HCC). Liver tissues from 37 Korean HCC patients were analyzed for LOH by using 25 polymorphic microsatellite markers distributed along 16q. Out of the 37 HCC patients studied, 21 patients (56.8%) showed LOH in various regions of 16q with at least one polymorphic marker. Puring the analysis of these 21 LOH cases, 6 patients showed interstitial LOHs in which the boundary of the LOH region was defined. With two rounds of LOH analysis, five commonly occurring interstitial LOH regions were identified; 16q21-22.1, 16q22.2 - 22.3, 16q22.3, 16q23.2 and 16q23.3 - 24.1. Among the five LOH regions the 16q23.3 - 24.1 region has been reported to be related with chromosome instability. A complete physical map, which covers the 3.2 Mb region of 16q23.3 - 24.1 (D16S402 and D16S486), was constructed to identify novel candidate tumor suppressor genes. We provide the minimally tiling path map consisting of 28 BAC clones. There was one gap between NT_10422.11 and NT_019609.9 of the human genome sequence contig (NCBI sequence build 33, April 29, 2003). This gap can be filled by sequencing the R-1425M20 clone which bridges these sequence contigs.

Keywords: chromosome 16q hepatocellular carcinoma, loss of heterozygosity, physical mapping, polymorphic marker

* Corresponding author:
E-mail imbglmg@plaza.snu.ac.kr, Tel +82-2-880-6775, Fax +82-2-872-9019
Accepted 9 November 2003

Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers, including stomach and lung cancer, in Korea (National Statistical Office, 2001). Aflatoxin B1, chronic infection of hepatitis B virus (HBV) and hepatitis C virus (HCV) are known as major risk factors for HCC (Bradley, 1999). However, the molecular mechanism of liver cell transformation has not been determined yet. The development of HCC has been reported to be a multistep process (Sugimura, 1992). Inactivation of tumor suppressor gene and activation of oncogene caused by intragenic mutations or by deletions can lead a cell to a transformed state. Several genomic regions have been reported to be involved in liver carcinogenesis. These regions are mostly located on chromosome arms 1p, 4q, 5q, 6q, 8p, 9p, 11p, 13q, 16q and 17p, indicating that dysfunctions of diverse tumor or metastasis suppressor genes located on these chromosomes are involved in the development of HCC (Boige *et al.*, 1997; Nagai *et al.*, 1997).

The studies on loss of heterozygosity (LOH) have been used for detection of chromosomal regions deleted in various types of cancer. By analyzing deleted genes in cancer patients, tumor suppressor genes, such as BRCA2 and DPC4, were identified (Hahn *et al.*, 1995; Wooster *et al.*, 1995). Therefore, detecting the regions associated with high LOH frequency is important for identifying negatively regulating genes participating in tumor growth, such as tumor suppressor genes (TSGs). High frequency of LOH has been observed on human chromosome 16q in HCC, as well as in other cancers including breast cancer (Cleton-Jansen *et al.*, 1999), prostate cancer (Carter *et al.*, 1990). and Wilm' s tumor (Maw *et al.*, 1992), indicating that one or more TSGs may exist on 16q. It would be useful to define commonly and minimally deleted regions on a specific chromosomal region of patients to search the possible TSGs. Even after the completion of the human genome project, there are still a considerable amount of gaps between certain sequence contigs. BAC contig maps of human chromosomes are still a valuable resource for positional cloning of the genes associated with diseases and, in particular, for sequencing of the gaps present on the human genome draft sequences.

In this study, we report five commonly occurring LOH regions on human 16q from Korean HCC patients and a BAC contig map for one of the LOH regions with complete

coverage. Single gap present in the human draft sequences in this region is closed with our BAC contig sequence.

Materials and Methods

Tissue specimens

HCC and non-tumorous tissues were prepared from 37 patients who had undergone hepatectomy at the Department of Surgery, Korea Cancer Center Hospital. Tumor and non-tumor samples were kept frozen at -70℃ after removed from patients until DNA extraction.

Differentiation states of tumor samples were ranged from stages 1 to 4. There were 32 cases positive for HBV. Average tumor size was 6.2 cm. The age of patients was ranged from 24 to 67.

Microsatellite polymorphic markers

The sequences of dinucleotide polymorphic markers were obtained by searching the Genome Database (GDB: http://www.gdb.org) and from a previous report (Dogget *et al.*, 1995). The sequences of tetranucleotide repeat markers and somatic cell hybrid panels were kindly provided by Dr. Callen (Women' s and Children' s Hospital, Adelaide, Australia). Polymorphic markers retaining high heterozygosity score more than 0.7 were selected, except two markers (D16S416 and D16S518).

LOH analysis

Genomic DNA was prepared from the tissues as described by Sambrook *et al.* (2001). PCR was performed under a standard condition (Yeh *et al.*, 1996). The PCR products were labeled with α-[^{32}P]-dCTP. Amplified DNAs were subjected to 6% polyacrylamide gel electrophoresis.

The gel was dried and processed for autoradiography. Allelic loss was scored when band intensity of one allelic marker was significantly decreased (more than 50%) in tumor DNA compared to that in normal DNA.

Initial screening of high density membrane

High density filters of human D1/D2 were prepared by using Q-Bot (Genetiks) and RPCI-11 library was purchased from Children' s Hospital Okland Research Institute. Probes were prepared by amplifying gDNA with PCR and by labeling with α-[^{32}P]-dCTP. Ten to twenty probes were pooled and hybridized to each high density filter. True positive clones were selected and then subjected to deconvolution experiments to identify clones specific to individual probes (Cao *et al.*, 1999).

Fingerprinting

Each clone was digested with restriction enzyme, labeled with α-[^{32}P]-dATP and AMV-RT, subjected to polyacrylamide gel electrophoresis, processed for autoradiography and the image was then analyzed with the FPC 2.5 software (see Cao *et al.*, 1998 for detailed procedure).

Gap filling

Nine contigs were initially obtained. Gaps were filled by chromosome walking. Briefly, probes were prepared from BAC end sequences of each contig which were used to screen high density membranes. End sequences of each positive BAC clone were determined and PCR primers were designed from the BAC end sequences. All positive BAC clones obtained from each screening were used as PCR templates. The generation of PCR product was used as criteria for determining overlap. The end sequences of outermost BAC clones were used to prepare new probes for the second round screening. Chromosome walking was continued until gaps were closed. Gap closing was also determined by PCR.

Results

Polymorphic marker analysis

Forty polymorphic markers with high heterozygosity score (higher than 0.70 according to GDB) were initially selected. Some markers, however, having lower heterozygosity score than 0.7 were selected to keep marker intervals approximately 3 Mb. The markers consist of thirty one dinucleotide and nine tetranucleotide repeat polymorphic markers. To validate these markers, selected markers were subjected to PCR-based analysis. As a result, 21 dinucleotide polymorphic markers and 4 tetranucleotide repeat markers were finally selected to be useful for LOH analysis on HCC cases. The heterozygosity scores of each marker in the GDB database were compared to those obtained from the LOH study of Korean HCC cases. It should be noted that some markers showed higher dissimilarity in heterozygosity score in Korean population compared to those in the database (see Table 1). This discrepancy in marker informativeness may be due to ethnic difference (Uthoff *et al.*, 2002). Although heterozygosity score information of tetranucleotide repeat markers could not be obtained from GDB database, we chose them because of marker intervals. The heterozygosity scores of the tetranucleotide polymorphic markers ranged from 0.4 to 0.71 in 37 Koreans. Tetranucleotide repeat markers showed single specific bands. On the other hand, dinucleotide repeat markers showed multiple bands ranging from 4 to 6 (Fig. 1). Multiple bands from dinucleotide repeat markers might be occurred due to polymerase slippage.

Table 1. Frequency of LOH and heterozygosity percentage at 16q of HCC patients.

	Chromosomal location	LOH frequency %	Heterozygosity score in Korean	Heterozygosity score in GDB
D16S411	16q11.1	46.2	0.743	0.7943
D16S416	16q11.2	38.5	0.371	0.4258
D16S2623*	16q12.1	47.8	0.657	-
D16S415	16q12.2	62.5	0.229	0.7361
D16S419	16q13	52.4	0.6	0.7653
D16S503	16q21	50	0.514	0.81
D16S767*	16q22.1	55.6	0.514	-
D16S485	16q22.2	60	0.571	0.8
D16S512	16q22.2	54.5	0.629	0.764
D16S515	16q22.2	65.5	0.829	0.801
D16S518	16q22.3	36	0.714	0.597
D16S3098	16q23.1	36	0.714	0.733
D16S505	16q23.2	60	0.714	0.7647
D16S422	16q 23.3	68.4	0.543	0.7993
D16S402	16q 23.3	54.2	0.686	0.8682
D16S2625*	16q24.1	52.4	0.6	-
D16S539*	16q24.1	56	0.714	-
D16S486	16q24.1	50	0.4	0.8571

* Tetranucleotide repeat markers (heterozygosity score could not be obtained from GDB)

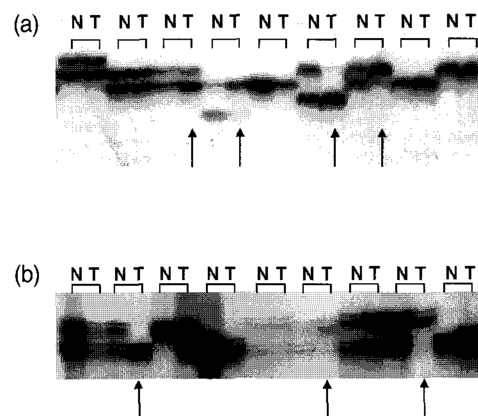

Fig. 1. Patterns of LOH observed with tetranucleotide repeat marker (panel A) and dinucleotide repeat marker (panel B). N and T designate DNA sample isolated from non-tumor and tumor tissues, respectively. Arrows indicate distinct patterns of LOH.

LOH analysis and deletion mapping on human chromosome 16q

LOH mapping was carried out in two steps in this study. Overall LOH profile on 16q was examined in the first step. Markers that have long intervals (3 Mb in average) were chosen for the first step LOH analysis, and the rough LOH regions were determined. In the second step, additional markers were used to reduce the length of each LOH region.

Eighteen markers, showing 3 Mb of interval size in average, were used in the first step of LOH analysis. Twenty one out of 37 HCC patients showed LOH with at least one polymorphic marker, however, 16 patients showed no LOH at all. Fifteen out of the 21 LOH-showing patients were observed to have extensive deletions throughout the entire long arm of chromosome 16, while the other six patients showed interstitial LOHs on the 16q. The position of each marker was determined according to the sequence contig of human genomic sequence database and Généthon linkage map provided by NCBI. Four common LOH regions were observed in the first step LOH analysis. The first region was located between D16S503 and D16S512 marker (10.48 Mb in length). The second region was located between D16S512 and D16S3098 (7.38 Mb). The third region was located between D16S505 and D16S402 (1.62 Mb). The fourth region was located between D16S402 and D16S486 (3.2 Mb).

To reduce the size of each LOH region, we further performed LOH analysis by using 7 additional polymorphic markers located inside of each LOH region. Four markers (D16S3019, D16S397, D16S496 and D16S3066) were used to reduce the length of the first region. Because D16S3019, D16S397 and D16S496 showed LOHs or homozygosities in the six interstitial LOH cases, these markers did not contribute to reduce the length of the first LOH region. D16S3066 was located inside of the first LOH region and 0.73 Mb apart from D16S516. This marker did not show LOH in two patients. The first region, therefore, could be reduced by 0.37 Mb. D16S266, D16S3090 and D16S516 markers were used to reduce the second LOH region.

D16S3090 showed retention of LOH. Both D16S266 and D16S516 showed no LOH in three cases. D16S266 was located approximately in the middle of the second LOH region, and the markers neighboring on both right and left side of D16S266 showed LOH. Therefore, the second LOH region could be divided into two LOH regions (LOH region 2 and 3). The location of D16S516 was 2.63 Mb apart inside from D16S3098, and it showed no LOH. The length of LOH region 3 was, therefore, reduced to 2.63 Mb. No additional markers were found in the third and fourth LOH regions in the first step analysis. The size of these regions, therefore, could not be reduced.

By combining the first and second step of LOH analysis, five LOH regions were obtained. Region 1 was located at 16q21-22.1 between markers D16S503 and D16S3066, region 2 at 16q22.2 - 22.3 between D16S512 and D16S266, region 3 at 16q22.3 between D16S266 and D16S516, region 4 at 16q23.2 between D16S505 and D16S402, and region 5 at 16q23.3 - 24.1 between

D16S402 and D16S486. The lengths of LOH region 1 to 5 were 9.7, 2.6, 2.4, 1.6 and 3.2 Mb, respectively. The LOH analysis data and position of markers were summarized as an integrated map in Fig. 2.

Relationship between clinicopathological data and LOH

Relationship between the LOH occurrence and clinicopathological parameters was analyzed. No significant difference was observed in gender, age and status of hepatitis viruses between LOH patients and non-LOH patients. Big difference was, however, observed in average tumor size of LOH patients compared to those of non-LOH patients. The average size of tumor in LOH patients was approximately 2 cm larger than those in non-LOH patients (data not shown). The average α-fetoprotein level of the patients showing LOH was 35.7 μg/ml. This value is much higher than that of the patients showing non-LOH (488 ng/ml in average). However, there is still no evidence at the molecular level to explain the significance of this relationship.

Physical mapping of the LOH region 5

Our final goal is to isolate novel gene(s) which is(are) related with HCC. FRA16D, located in the region 5, was reported to be related to chromosome instability (Doggett *et al*, 1995). No TSGs have been reported yet in this region. We, therefore, decided to generate a physical map between D16S402 and D15S486 (16q23.3 - 16q24.1) by using BAC clones. Human BAC library A was screened by a PCR-based method as previously described (Kim *et al*., 1996). Nine positive BACs were obtained by using 10 STSs, the positions of which were determined with the previous YAC-based physical map of chromosome 16 (Doggett *et al*., 1995). The nine BACs were digested with *Not*I enzyme, subjected to preparative pulsed field gel electrophoresis, the inserts isolated from the gel and the isolated inserts used as probes for the screening of human D1 and D2 library. Seventy eight BACs were obtained from high density membrane screening. The false positive clones were removed by Southern hybridization. Thirteen and thirty one clones were identified as real positives from library D1 and library D2, respectively. Six contigs were obtained by fingerprinting the positive BACs from A, D1, and D2 library.

Chromosome walking was carried out to fill the gaps located among those six contigs. BACs located at the terminal regions of each contig were subjected to

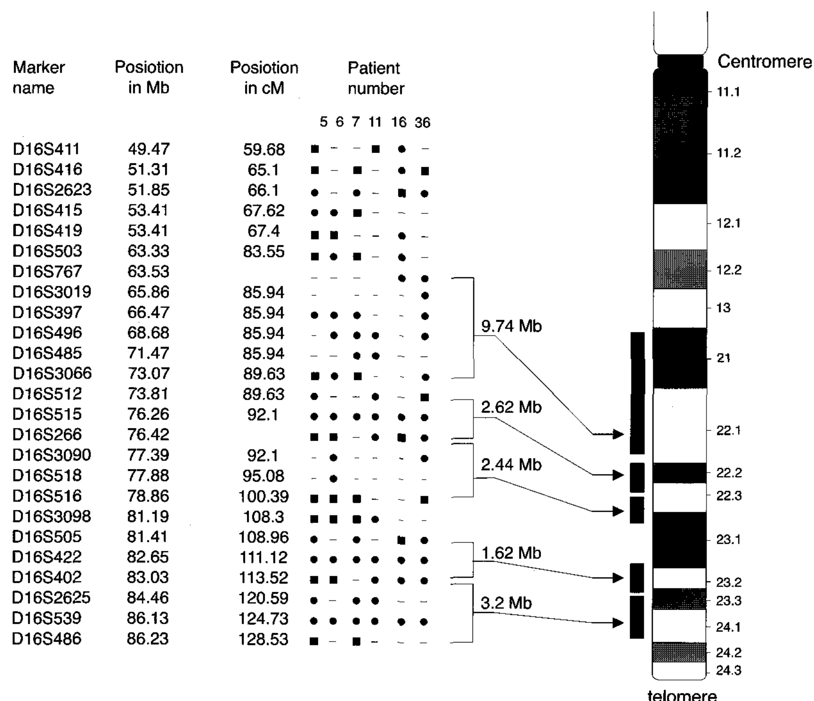

Fig. 2. Summary of LOH analysis. Closed circles, closed squares, and dashes designate patients showing LOH, retention of heterozygosity and non-informative homozygosity, respectively. The bars represent the maximum size and location of each LOH region determined on human genomic sequence data.

sequencing. BAC end sequences were used to generate new STS markers. D1 and D2 library screening was carried out with the probes corresponding to these BAC end sequence markers. By repeating BAC end sequencing and chromosome walking, four gaps were filled. There was still one gap left between the first five contigs and the sixth contig with D1 and D2 library, but this gap was filled by the RPCI-11 library screening.

By using the end-sequenced BACs as starting points and also by adding minimally overlapping BACs to each other, minimal tiling path was constructed, spanning between D16S402 and D16S486 in 1.5X coverage (Fig. 3). Mega BLAST search was performed to find the location of each STS marker, including the BAC end sequences, on the human genomic sequence database in NCBI. One gap existed on the sequence contig of human genome sequence at NCBI (Sequence Build 33, April 29, 2003). This gap was bridged by a R-1425M20 clone. We identified 89 STS markers and 128 BAC clones in these regions (data is available upon request).

Discussion

In this study, we constructed a deletion map of 16q by using LOH analysis and constructing a physical map on one of the LOH regions. It is important to define the commonly deleted region in human cancers to isolate gene(s) related to growth inhibition. Five interstitial LOH regions were located on 16q. It has been reported that the LOH region 1 contains genes related to metastasis of cancer cell (Nam *et al*., 2002). Interestingly, 16q contains 8 cadherin gene clusters.

Among these, E-cadherin, cadherin 11, cadherin 16, cadherin 3 and cadherin 5 are located on LOH region 1. It is possible that the LOH region 1 is broader than the other LOH regions because the cadherin gene cluster is widely spread throughout the entire region 1. D16S515 marker in LOH region 2 was reported to show 23.3% of LOH frequency in Taiwanese (Chou *et al*., 1998). However, this marker showed 65.5% of LOH frequency in Koreans. This difference in LOH frequency was possibly originated from the cause of HCC development or treatment of HCC patients. It has been reported that LOH was frequently observed in the LOH region 3 from HCC patients. In addition, the CTRB locus inside of the LOH region 3 was also reported to be a commonly deleted region in HCC (Tsuda *et al*., 1990; Yakicier *et al*., 2001). Chromosome 16q23.1-24.1, including the LOH regions 4 and 5, was

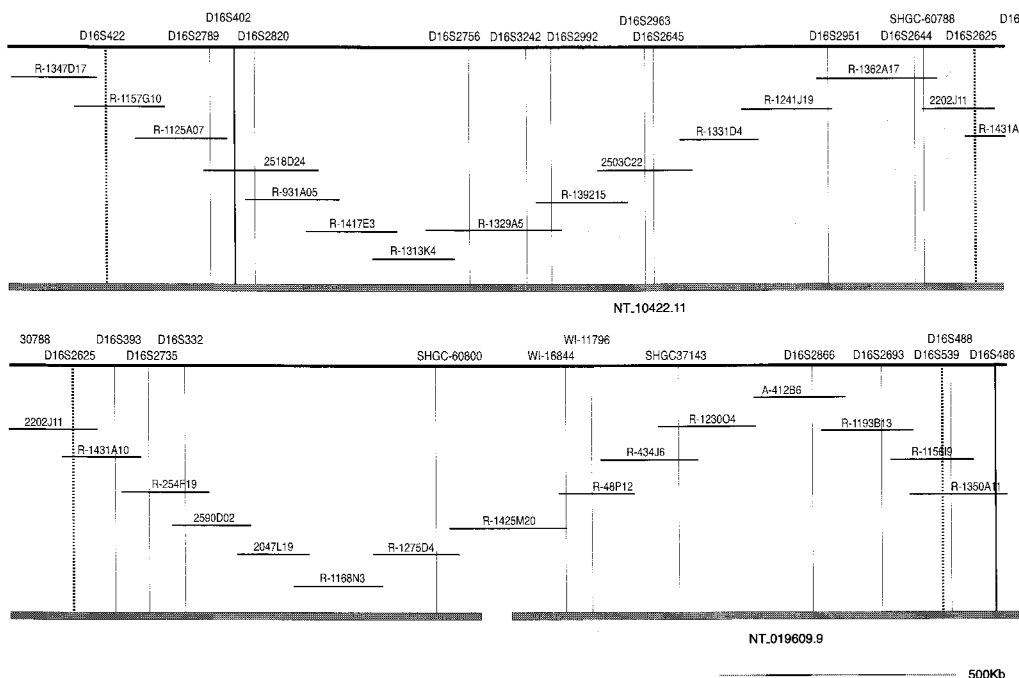

Fig. 3. Schematic drawing of physical map in minimal tiling path between D16S402 and D16S486 (16q23.3 - 24.1). This map is oriented from centromere (left) to telomere (right). Vertical lines designate STS markers, dotted vertical lines designate polymorphic markers showing LOH, and bold vertical lines designate polymorphic markers showing non-LOH. Black horizontal lines designate BAC clones. Slashed horizontal lines designate sequence contigs. Scale bar (500 Kb) was shown below the map.

reported to be a consistently deleted region among the patients analyzed by using comparative genomic hybridization (CGH) studies in HCC (Balsara *et al.*, 2001). High frequency of LOH in this CGH region from breast and prostate cancer was reported (Gunnarsson *et al.*, 2000; Matsuyama *et al.*, 2003).

Although the human genome sequencing was announced to be finished, there are still many sequence-gaps in the human genome sequence. We found one sequence-gap in LOH region 5 located at 16q23.3 - 24.1. Based on complete physical map, gap between NT_10422.11 and NT_019609.9 was bridged by the R-1425M20 BAC clone. Sequencing of R-1425M20 could fill the gap between those sequence contigs. Thirty-three genes were predicted to be located in LOH region 5 according to the NCBI map viewer (Build 34 Version 1). Among these, one down regulated gene (OKL38) in liver cancer was identified by the virtual northern provided by National Cancer Institute' s Cancer Genome Anatomy Project (http://cgap.nci.nih.gov). The ratio of OKL38 expression level in HCC to normal liver was 0/6. We are currently examining the expression level of the OKL38 gene in HCC patients. Because LOH analysis does not give information if the LOH regions are located in *cis*-position or *trans*-position on sister chromatids, it is necessary to carry out haplotype analysis to determine the phase of deletions on chromosome.

Acknowledgments

The authors thank David Callen for providing information of polymorphic markers and providing hybrid panel. We also thank S.S. Kim for his kind reading the manuscript and suggestions. This work was supported by the National Research and Development Program and Genomic Program and New Frontier (MOST, Korea) to B.J. Lee. J.Chung was supported by BK21- life science program.

References

Balsara, B.R., Pei, J., De Rienz,o A., Simon, D., Tosolini, A., Lu, Y.Y., Shen, F.M., Fan, X., Lin, W.Y., Buetow, K.H., London, W.T., and Testa, J.R. (2001). Human hepatocellular carcinoma is characterized by a highly consistent pattern of genomic imbalances, including frequent loss of 16q23.1-24.1. *Genes Chromosomes Cancer* 30, 245-253.

Boige, V., Laurent-Puig, P., Fouchet, P., Flejou, J.F., Monges, G., Bedossa, P., Bioulac-Sage, P., Capron, F., Schmitz, A., Olschwang, S., and Thomas, G. (1997). Concerted nonsyntenic allelic losses in hyperploid hepatocellular carcinoma as determined by a high-resolution allelotype. *Cancer Res.* 57, 1986-1990.

Bradley, D.W. (1999). Hepatitis viruses: their role in human cancer. *Proc. Assoc. Am. Physicians* 111, 588-593.

Cao, Y., Kangm H.L., Xu, X., Wang, M., Dho, S.H., Huh, J.R., Lee, B.J., Kalush, F., Bocskai, D., Ding, Y., Tesmer, J.G., Lee, J., Moon, E., Jurecic, V., Baldini, A., Weier, H.U., Doggett, N.A., Simon, M.I., Adams, M.D., and Kim, U.J. (1999). A 12-Mb complete coverage BAC contig map in human chromosome 16p13.1-p11.2. *Genome Res.* 9, 763-774.

Carter, B.S., Ewing, C.M., Ward, W.S., Treiger, B.F., Aalders, T.W., Schalken, J.A., Epstein, J.I., and Isaacs, W.B. (1990). Allelic loss of chromosomes 16q and 10q in human prostate cancer. *Proc. Natl. Acad. Sci. USA* 87, 8751-8755.

Chou, Y.H., Chung, K.C., Jeng, L.B., Chen, T.C., and Liaw, Y.F. (1998). Frequent allelic loss on chromosomes 4q and 16q associated with human hepatocellular carcinoma in Taiwan. *Cancer Lett.* 123, 1-6.

Cleton-Jansen, A.M., Moerland, E.W., Pronk, J.C., van Berkel, C.G., Apostolou, S., Crawford, J., Savoia, A., Auerbach, A.D., Mathew, C.G., Callen, D.F., and Cornelisse, C.J. (1999). Mutation analysis of the Fanconi anaemia A gene in breast tumours with loss of heterozygosity at 16q24.3. *Br. J. Cancer* 79, 1049-1052.

Doggett, N.A., Goodwin, L.A., Tesmer, J.G., Meincke, L.J., Bruce, D.C., Clark, L.M., Altherr, M.R., Ford, A.A., Chi, H.C., Marrone, B.L., Longmire, J.L., Lane, S.A., Whitmore, S.A. *et al.*, (1995). An integrated physical map of human chromosome 16. *Nature* 377 (6547 Suppl), 335-365.

Gunnarsson, C., Olsson, B.M., and Stal, O.; Southeast Sweden Breast Cancer Group. (2001). Abnormal expression of 17beta-hydroxysteroid dehydrogenases in breast cancer predicts late recurrence. *Cancer Res.* 61, 8448-8451.

Hahn, S.A., Schutte, M., Hoque, A.T., Moskaluk, C.A., da Costa, L.T., Rozenblum, E., Weinstein, C.L., Fischer, A., Yeo, C.J., Hruban, R.H., and Kern, S.E. (1996). DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science* 271, 350-353.

Kim, U.J., Birren, B., Slepak, T., Mancino, V., Boysen, C., Kang, H.L., Simon, M.I., and Shizuya, H. (1996). Constuction and characterization of a human bacterial artificial chromosome library. *Genomics* 34, 213 - 218.

Matsuyama, H., Pan, Y., Yoshihiro, S., Kudren, D., Naito, K., Bergerheim, U.S., and Ekman, P. (2003). Clinical significance of chromosome 8p, 10q, and 16q deletions in prostate cancer. *Prostate* 54, 103-111.

Maw, M.A., Grundy, P.E., Millow, L.J., Eccles, M.R., Dunn, R.S., Smith, P.J., Feinberg, A.P., Law, D.J., Paterson, M.C., Telzerow, P.E., *et al.* (1992). A third Wilms' tumor locus on chromosome 16q. *Cancer Res.* 52, 3094-1098.

Nagai, H., Pineau, P., Tiollais, P., Buendia, M.A., and Dejean, A. (1997). Comprehensive allelotyping of human hepatocellular carcinoma. *Oncogene* 14, 2927-2933.

National Statistical Office, Republic of Korea. (2001). Annual report on the cause of death statistics (based on vital registration).

Nam, J.S., Ino, Y., Sakamoto, M., and Hirohashi, S. (2002). Ras farnesylation inhibitor FTI-277 restores the E-cadherin/catenin cell adhesion system in human cancer cells and reduces cancer metastasis, *Jpn. J. Cancer Res.* 93, 1020-1028.

Sambrook J., Fritsch, T., and Maniatis, T. (2001). *Molecular cloning: A Laboratory Manual*, 3rd edition (Cold Spring Harbor Laboratory Press).

Sugimura T. (1992). Multistep carcinogenesis: a 1992 perspective. *Science* 258, 603-607.

Tsuda, H., Zhang, W.D., Shimosato, Y., Yokota, J., Terada, M., Sugimura, T., Miyamura, T., and Hirohashi, S. (1990). Allele loss on chromosome 16 associated with progression of human hepatocellular carcinoma. *Proc. Natl. Acad. Sci. U S A* 87, 6791-6794.

Uthoff, S.M., Crawford, N.P., Eichenberger, M.R., Hamilton, C.J., Petras, R.E., Martin, E.R., and Galandiuk, S. (2002). Association of ulcerative colitis with the inflammatory bowel disease susceptibility locus IBD2 in non-Jewish Caucasians and evidence of genetic heterogeneity among racial and ethnic populations with Crohn disease. *Am. J. Med. Genet.* 113, 242-249.

Wooster, R., Bignell, G., Lancaster, J., Swift, S., Seal, S., Mangion, J., Collins, N., Gregory, S., Gumbs, C., and Micklem, G. (1995). Identification of the breast cancer susceptibility gene BRCA2. *Nature* 378, 789-792.

Wu, C., Zhu, S., Simpson, S., and de Jong, P.J. (1996). DOP-vector PCR: a method for rapid isolation and sequencing of insert termini from PAC clones. *Nucleic Acids Res.* 24, 2614-2615.

Yakicier, M.C., Legoix, P., Vaury, C., Gressin, L., Tubacher, E., Capron, F., Bayer, J., Degott, C., Balabaud, C., and Zucman-Rossi, J. (2001). Identification of homozygous deletions at chromosome 16q23 in aflatoxin B1 exposed hepatocellular carcinoma. *Oncogene* 20, 5232-5238.

Yeh, S.H., Chen, P.J., Lai, M.Y., and Chen D.S. (1996). Allelic loss on chromosomes 4q and 16q in hepatocellular carcinoma: association with elevated alpha-fetoprotein production. *Gastroenterology* 110, 184-192.