Establishment and characterisation of six human biliary tract cancer cell lines

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Human cell lines established from biliary tract cancers are rare, and only five have been reported previously. We report the characterisation of six new six biliary tract cancer cell lines (designated SNU-245, SNU-308, SNU-478, SNU-869, SNU-1079 and SNU-1196) established from primary tumour samples of Korean patients. The cell lines were isolated from two extrahepatic bile duct cancers (one adenocarcinoma of common bile duct, one hilar bile duct cancer), two adenocarcinomas of ampulla of Vater, one intrahepatic bile duct cancer (cholangiocarcinoma), and one adenocarcinoma of the gall bladder. The cell phenotypes, including the histopathology of the primary tumours and in vitro growth characteristics, were determined. We also performed molecular characterisation, including DNA fingerprinting analysis and abnormalities of K-ras, p15, p16, p53, hMLH1, hMSH2, DPC4, β-catenin, E-cadherin, hOGG1, STK11, and TGF-βRII genes by PCR – SSCP and sequencing analysis. In addition, we compared the genetic alterations in tumour cell lines and their corresponding tumour tissues. All lines grew as adherent cells. Population doubling times varied from 48 – 72 h. The culture success rate was 20% (six out of 30 attempts). All cell lines showed (i) relatively high viability; (ii) absence of mycoplasma or bacteria contamination; and (iii) genetic heterogeneity by DNA fingerprinting analysis. Among the lines, three lines had p53 mutations; and homozygous deletions in both p16 and p15 genes were found three and three lines, respectively; one line had a heterozygous missense mutation in hMLH1; E-cadherin gene was hypermethylated in two lines. Since the establishment of biliary tract cancer cell lines has been rarely reported in the literature, these newly established and well characterised biliary tract cancer cell lines would be very useful for studying the biology of biliary tract cancers, particularly those related to hypermethylation of E-cadherin gene in biliary tract cancer.

Keywords: biliary tract cancer; cell line; cell culture; mutation; hypermethylation; E-cadherin

The prognosis of patients with biliary tract cancer is poor despite recent advances in diagnostic and therapeutic techniques (Yamada et al, 1995; Yoshida et al, 1995). An understanding of the biological nature of this neoplasm is needed to improve the prognosis of these patients. For this purpose, a permanently growing cell line can be the most suitable tool because it is applicable to a variety of experiments, including the understanding of tumour biology (Yano et al, 1992; Yamada et al, 1995). However, biliary tract cancer cell lines are very rare, a total of 13 cell lines have been reported in the literature. Gene alterations involving in carcinogenesis and cancer progress in the cancers of alimentary tract such as colorectal cancer and pancreatic cancer have been known. However, in biliary tract cancers, few reports have shown genetic changes responsible for oncogene and tumour suppressor gene (Yoshida et al, 1995). In the present study, we report the establishment and characterisation of six new human biliary tract cancer cell lines (designated SNU-245, SNU-308, SNU-478, SNU-869, SNU-1079 and SNU-1196). We described cell phenotypes including the in vivo and in vitro growth characteristics, and DNA profiles for authenticity of each line. We also checked genetic alterations of K-ras, p15, p16, p53, hMLH1, hMSH2, DPC4, STK11, E-cadherin, hOGG1, TGF-βRII genes and compared the genetic alterations in tumour cell lines and their corresponding tumour tissues. In these biliary tract cancer cell lines, the methylation status of promoter region in E-cadherin gene was also investigated by S-aza-2’-deoxycytidine treatment and methylation specific-polymerase chain reaction (MS-PCR) after sodium bisulphite treatment.

MATERIALS AND METHODS

Cell culture

Cell lines were established from pathologically proven primary biliary tract and ampulla of Vater cancer samples of six Korean patients. Of these, two cancer cell lines originated in extrahepatic bile duct cancer, one in intrahepatic bile duct cancer, and one in adenocarcinoma of gall bladder, and two in ampulla of Vater.
cancer. Solid tumours were finely minced with scissors and disassociated into small aggregates by pipetting. Appropriate amounts of finely minced neoplastic-tissue fragments were seeded into 25 cm² flasks. Tumour cells were initially cultured in ACL-4 medium supplemented with 5% heat-inactivated foetal bovine serum (AR5) (Park et al, 1987, 1995). After establishment, cultures were maintained in RPMI 1640 supplemented with 10% heat-inactivated foetal bovine serum. If stromal-cell growth was noted in initial cultures, differential trypsinisation was used to obtain a pure tumour-cell population. Cultures were maintained in humidified incubators at 37°C in an atmosphere of 5% CO₂ and 95% air. A-431, HeLa, and K-562 cell lines from the American Type Cell Culture (ATCC) and SNU-1 cell line from Korean Cell Line Bank (KCLB) were used for PCR controls. For growth properties and morphology study in vitro, population doubling times and cell viability were determined and cells grown on 75-cm² culture flasks were observed daily by phase-contrast microscopy (Park et al, 1995).

Nucleic acid isolation, cDNA synthesis and DNA profiles

Genomic DNA and total RNA were isolated from washed-cell pellets. cDNA was synthesised according to the manufacturer’s specifications (Power cDNA synthesis kit; Intront Biotechnology, Seoul, Korea) using 2 µg of total RNA. To compare the genetic alterations between established tumour cell lines and their corresponding tumour tissues, we obtained DNAs from microdissected tumour cells and stromal cells in H&E stained slides of corresponding tumour tissues of each tumour cell line. Approximately 500 – 1000 dissected tumour or stromal cells were digested using proteinase K method described previously (Hung et al, 1995). For DNA-profile analysis, DNA was amplified by PCR at loci containing highly polymorphic microsatellite markers: D1S1586 and D3S1765 (Ku et al, 1999).

Mutation screening in K-ras, p53, p15, p16, hMLH1 and hMSH2 genes

Mutation screening of exons 1 and 2 of K-ras was performed by PCR sequencing analysis using oligonucleotide primers as previously described (Capon et al, 1983). Mutational screening of exons 5 to 8 of p53 was performed by PCR-based single strand conformation polymorphism (PCR-SSCP) analysis (Kang et al, 1996). Primers for PCR-SSCP for exons 1 and 2 of the p15 gene were synthesised (Orlow et al, 1996), and PCR-SSCP primers for exons 1 and 2 of p16 gene were carried out as described previously (Williamson et al, 1995). For deletion analysis of the p15 and p16 genes, we amplified the exons of each gene without [α-32P]-dCTP. To investigate the genetic status of hMSH2 and hMLH1 genes in biliary tract cancer cell lines, PCR-SSCP analysis was used to screen mutations (Liu et al, 1994; Han et al, 1996).

Mutation screening in β-catenin, DPC4, STK11, TGF-βRII, and hOGG1 genes

PCR-SSCP analysis to screen mutations of the β-catenin gene was carried out by designed-primers for amplification of exons 3, 5, and 6, since mutations reported earlier in the β-catenin gene are concentrated at these exons (Kitaeva et al, 1997; Iwao et al, 1998). To investigate the genetic alteration of the DPC4 gene, we screened 11 exons by PCR-SSCP (Hahn et al, 1998). For mutational analysis of STK11 gene, the PCR primer pairs were used as described previously (Jenne et al, 1998). Mutation screening of the full seven exons of TGF-βRII gene was also performed by PCR-SSCP analysis (Lu et al, 1996). For mutational analysis of hOGG1 gene, PCR primer pairs and conditions were described previously (Kohno et al, 1998).

Genetic alteration and methylation analysis of E-cadherin gene

To investigate mutation, all 16 exons were screened by PCR-SSCP (Bexx et al, 1996). For determination of the methylation status in E-cadherin gene, 5-aza-2′-deoxycytidine treatment and sodium bisulphite modification were employed. For 5-aza-2′-deoxycytidine treatment, cells were seeded at 2 × 10⁵ cells 75-cm² culture flask on day 0. The cells were treated with 10 µm 5-aza-2′-deoxycytidine for 24 h on days 2 and 5. The medium was changed 24 h after adding 5-aza-2′-deoxycytidine. Cells were harvested on day 8 for analysis of E-cadherin expression.

For mRNA expression analysis, cDNA was amplified in a 25 µl PCR reaction using 0.75 µl of the reverse-transcription reaction, the primers and 0.5 units of the Taq DNA polymerase. Both E-cadherin and β-actin RT–PCR reactions used the same cDNA synthesis. β-actin was amplified to control for RNA integrity. The primers for the amplification of E-cadherin gene mRNA were described previously (Melki et al, 2000). Products were electrophoresed on a 2% agarose gel with ethidium bromide.

The sodium bisulphite reaction was carried out for 16 h at 55°C. Following bisulphite modification, the DNA was ethanol precipitated, dried, and resuspended in 100 µl distilled water. A nested PCR was performed using the nested PCR primers as described previously (Melki et al, 2000).

Cloning and sequencing

Samples showing abnormal bands by SSCP were subjected to DNA sequencing analysis. Fresh PCR products were ligated into PCR™II vectors and subcloned using the TA cloning system (Invitrogen, San Diego, CA, USA). A minimum of 10 individual clones were then pooled and used for DNA isolation. Bi-directional DNA sequencing analysis performed by using dideoxy chain termination method with a T7 DNA polymerase sequencing kit (Pharmacia Biotech Inc., Piscataway, NJ, USA), or directly sequenced using a Taq dideoxy terminator cycle sequencing kit on an ABI 377 DNA sequencer (Perkin-Elmer, Foster City, CA, USA).

RESULTS

Culture characteristics

Population doubling times ranged 48 to 72 h, and cell viability ranged from 85 to 94% (Table 1). All the cell lines were free of contamination with either bacteria or Mycoplasma. The culture success rate was 20% (six out of 30 attempts).

Morphologic studies

Six carcinoma cell lines derived from biliary tract system were established. The primary tumour of SNU-245 originated from the distal common bile duct. Microscopically, the tumour was composed of well-differentiated neoplastic glands and lined by a few rows of highly atypical cuboidal cells with lumina containing eosinophilic material or necrotic cell debris and infiltrated to the stroma. Cell line SNU-308 was established from an adenocarcinoma of a gall bladder. Microscopically, the tumour was composed of well-differentiated neoplastic glands or trabeculae. Two cases of ampulla of Vater carcinoma were obtained from primary tumours. Microscopically, the tumour of SNU-478 was poorly differentiated adenocarcinoma with signet ring cell feature and infiltrated to the pancreas along the interstitial space as a single cell or cell cords. The tumour of SNU-869 was composed of well differentiated adenocarcinoma with focal papillary feature. Five out of 10 peri-duodenal lymph nodes were involved by this tumour and the tumour infiltrated to the duodenal muscle layer and involved of
In vivo and in vitro characteristics of biliary tract cancer cell lines

| Cell line | Origin* | Degree of differentiation | Nodal status 1 | Growth characteristics** | Viability (%) | Doubling time (h) | Cell morphology | Nuclear morphology | Prior chemotherapy |
|-----------|---------|---------------------------|---------------|--------------------------|---------------|-----------------|-----------------|------------------|-------------------|
| SNU-245   | CBD     | Well                      | 0/9           | Ad                       | 85            | 54              | Polygonal       | Single           | None              |
| SNU-308   | GB      | Moderately                | –             | Ad                       | 88            | 48              | Polygonal       | Single           | None              |
| SNU-478   | AV      | Poorly                    | 0/8           | Ad                       | 83            | 52              | Polygonal/spherical | Single/multiple | None              |
| SNU-869   | AV      | Well                      | 5/10          | Ad                       | 91            | 48              | Polygonal       | Single           | None              |
| SNU-1079  | IHD     | Moderately                | –             | Ad                       | 89            | 72              | Pleomorphic     | Single/multiple  | None              |
| SNU-1196  | HDB     | Moderately                | 0/1           | Ad                       | 94            | 48              | Spindle to polygonal | Single           | None              |

*CBD=common bile duct, GB=gall bladder, AV=ampulla of Vater, IHD=intrahepatic duct, HDB=hepatic duct bifurcation. 

**Ad=adherent.

In hMLH1 mutational analysis, only SNU-478 harboured a heterozygous missense mutation from GAT to GTT at codon 384 of exon 12, with resultant amino acid change from aspartic acid to valine (data not shown).

Mutation analysis in β-Catenin, DPC4, hOGG1, STK11, and TGF-βRII genes

There were no abnormal band shift bands in the β-catenin, DPC4, STK11, and TGF-βRII genes by PCR-SSCP analysis. In hOGG1, 4 lines (SNU-308, SNU-478, SNU-869 and SNU-1079) were found to harbour abnormal band shift bands in exon 5. By the direct sequencing of DNA fragments corresponding to shifted bands, a C→G nucleotide change at -15 bp from exon 5 was found in all four lines (Table 2).

mRNA expression and methylation analysis in E-cadherin gene

By PCR-SSCP for all 16 exons, abnormal band shifts were not found in all cases. To determine the expression of E-cadherin gene in six biliary tract cancer cell lines, we used RT–PCR analysis. SNU-1 and A-431 cell lines were used for negative and positive controls for the expression of E-cadherin mRNA. As shown in Figure 5, SNU-245, SNU-308, SNU-869, SNU-1196 and control (A-431) cell lines expressed E-cadherin mRNA, whereas SNU-478, SNU-1079 and control (SNU-1) cell lines showed absence of expression (Figure 5A). Therefore, we determined the methylation status by 5-aza-2′-deoxycytidine treatment and sodium bisulphite modification in these cell lines. After 5-aza-2′-deoxycytidine treatment, E-cadherin mRNA was repressed in SNU-478, SNU-1079 and control (SNU-1) cell lines (Figure 5B). Methylation specific PCR analysis for CpG island of promoter region in the E-cadherin gene after sodium bisulphite modification, DNA fragments were amplified in only SNU-478, SNU-1079 and SNU-1 cell lines (Figure 5C).

Comparison of genetic alterations in tumour cell lines and their corresponding tumour tissues

Of six biliary tract cancer cell lines, paraffin blocks from four cell lines (SNU-245, SNU-308, SNU-869 and SNU-1079) were available. Tumour cells and stromal cells were dissected, respectively, and DNAs were extracted from these samples. Genetic alterations in DNA of tumour cells were identical to those that had found in tumour cell lines and there were no mutations in constitutional DNAs (data not shown).

DISCUSSION

Advances in cell culture methods have made it possible to establish a variety of human carcinoma cell lines from surgical and autopsy tissues, peritoneum effusion, and biopsy specimens (Yano et al.
Moreover, because pure cells in cultures can be used for a variety of studies that cannot be carried out using tissue specimens, the study of permanent cell lines established from human cancers has played a major role in our understanding of the biology of cancers. However, cell lines originated from biliary tract cancers have rarely been reported. Ten human extrahepatic bile duct carcinoma cell lines (SK-ChA-1 (Knuth et al., 1985), KMBC (Yano et al., 1992), and OCUCh-LM1 (Yamada et al., 1995), TFK-1 (Saijyo et al., 1995), ICBD-1 (Takiyama et al., 1998), HBDC (Jiao et al., 2000), SCK, JCK, Cho-CK, Choi-CK (Kim et al., 2001a)) and three gall bladder carcinoma cell lines (MZ-ChA-1 and Mz-ChA-2 (Knuth et al., 1985), OCUG-1 (Yamada et al., 1997)) have been reported in the world literature.

In this paper, we present six newly established biliary tract cancer cell lines derived from histopathologically varied primary carcinomas including a distal common bile duct, gallbladder, two ampulla of Vater, an intrahepatic duct, and a hepatic duct bifurcation tumour. The culture success rate of 20%, reflecting the relative high efficiency of AR5 medium in selective growth of human biliary tract cancers, although the establishment of cell lines from biliary tract cancers, especially cell lines from primary tumours, is very difficult as described above.

Primary tumours revealed morphological heterogeneity including cellular and a growth pattern of original tumours in each tumour type. Cell lines derived from primary biliary tract cancers showed marked heterogeneity in cellular and nuclear morphology, and growth pattern in vitro. All six cell lines grew as adherent monolayer, of these, SNU-869 formed prominent domes.

In biliary tract cancers, the role of p53 is still controversial and mutation rate of this gene varied from 33 to 65% according to molecular and cellular pathology.
Table 2 Abnormalities of the p15, p16, p53, K-ras, hMLH1, E-cadherin, hOGG1, β-catenin, DPC4, STK11 and TGF-βRII genes in SNU biliary tract cancer cell lines

| Cell line | p15 gene | p16 gene | p53 gene | K-ras gene (exon 1) | MLH1 gene | Mutation | Expression | 5-aza | MS-PCR | hOGG1 |
|-----------|----------|----------|----------|---------------------|------------|----------|------------|-------|--------|-------|
| SNU-245   | WT       | WT       | WT       | WT                  | WT         | +        | +          | –     | –      | WT    |
| SNU-308   | WT       | WT       | WT       | WT                  | WT         | +        | –          | +     | –      | IVS4-15C>G |
| SNU-478   | Deletion | Deletion | 266-267insT | WT | Asp384Val | WT | –          | +     | –      | IVS4-15C>G |
| SNU-869   | WT       | WT       | Asp48Gly | WT                  | WT         | +        | –          | –     | –      | IVS4-15C>G |
| SNU-1079  | Deletion (exon 1) | Deletion | IVS4-15C > G | WT | IVS4-15C>G | WT | –          | +     | –      | IVS4-15C>G |
| SNU-1196  | Deletion | Deletion | Arg273Cys | WT | WT         | WT | +          | +     | –      | WT    |

WT=mutations was not detected. 5-aza=RT – PCR after 5-aza-2-deoxycytidine treatment. MS-PCR=methylation specific PCR. +=expression. –=no expression. In the p15 gene, the exon 1 was only detected in SNU-1079 cell line. Mutations were not detected in K-ras, β-catenin, DPC4, STK11 and TGF-βRII genes.

Figure 4 PCR-based deletion analysis of the p15 and p16 genes. (A,B) PCR amplification of the p15 gene. (C) PCR amplification of exon 2 in the K-ras gene used as internal control for separate PCR amplification. (D,E) PCR amplification of the p16 gene. (F) K-ras gene for internal control for separate PCR amplification. Lane numbers 1 to 9 show cell lines SNU-245, SNU-308, SNU-478, SNU-869, SNU-1079, SNU-1196, HeLa, K-562, and water only.

Figure 5 Hypermethylation of E-cadherin gene in SNU-biliary tract cancer cell lines. (A) RT – PCR analysis of E-cadherin gene. Lane numbers (1–9) indicate cell lines: SNU-245, SNU-308, SNU-478, SNU-869, SNU-1079, SNU-1196, SNU-1 (gastric carcinoma cell line, positive control for methylation of E-cadherin gene, A-431(negative control), and water only. β-actin is RT – PCR control for mRNA expression. (B) RT – PCR analysis after 5-aza-2′-deoxycytidine treatment. It is note that E-cadherin gene is re-expressed after 5-aza-2′-deoxycytidine treatment. β-actin is RT – PCR control for mRNA expression. (C) Methylation specific PCR analysis after sodium bisulphite modification. It is evident that SNU-478 and SNU-1079 lines are methylated in CpG island of promoter region in the E-cadherin gene.
E-cadherin gene on chromosome 16q22.1 encodes a protein product important in the maintenance of the epithelial phenotype mediated by a Ca²⁺-dependent, homotypic cell–cell adhesion. The gene has been termed a ‘metastasis suppressor’ gene, because the E-cadherin protein can suppress tumour cell invasion and metastasis. E-cadherin gene expression is reduced or silenced in carcinomas of the breast and liver and many cell lines including those from colon, stomach, and prostate (reviewed by Melki et al., 2000). Alterations in DNA methylation patterns are commonly found in essentially all cancers, often with concomitant changes in gene expression. In our study, we have not found mutations in all 16 exons of E-cadherin gene by PCR-SSCP. However, we found that E-cadherin gene expression was silenced in two cell lines (SNU-478 and SNU-1079) by RT–PCR analysis (Figure 5A). After demethylating agent (5-aza-2’-deoxycytidine) treatment, E-cadherin was successfully reexpressed in these two cell lines (Figure 5B). Hypermethylation of E-cadherin gene in two cell lines have also been confirmed by methylation specific PCR (MS-PCR) analysis (Figure 5C). These results would be the first report about hypermethylation of E-cadherin gene in biliary tract cancers.

Since the establishment of biliary tract cancer cell lines has been rarely reported in the literature, these well-characterised biliary tract cancer cell lines will be useful tools for investigating the biological characteristics of biliary tract cancer, especially those related to the hypermethylation of E-cadherin gene in biliary tract cancers.

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REFERENCES

Berg G, Cleton-Jansen AM, Strumane K, de Leeuw WJ, Nollet F, van Roy F, Cornelisse C (1996) E-cadherin is inactivated in a majority of invasive human lobular breast cancers by truncation mutations throughout its extracellular domain. Oncogene 13: 1919–1925
Capon DJ, Seeburg PH, McGrath JP, Hayflick JS, Edman U, Levinson AD, Capon DJ, Seeburg PH, McGrath JP, Hayflick JS, Edman U, Levinson AD, Hahn SA, Bartsch D, Schroers A, Galehdari H, Becker M, Ramaswamy A, Knuth A, Gabbert H, Dippold W, Klein O, Sachsse W, Bitter-Suermann D, Park J-G, Oie HK, Sugarbaker PH, Henslee JG, Chen TR, Johnso BE, Gazdar AF (1995) Allele-specific chromosome 3p deletions occur at an early stage in the pathogenesis of lung carcinoma. J Am Med Assoc 273: 558–563
Iwao K, Nakamori S, Kameyama M, Imaoka S, Kinoshita M, Fukuji T, Ishiguro S, Nakamura Y, Miyoshi Y (1998) Activation of the beta-catenin gene by interstitial deletions involving exon 3 in primary colorectal carcinomas without adenomatous polyposis coli mutations. Cancer Res 58: 1021–1026
Jenne DE, Reimann H, Nezu J, Friedel W, Loff S, Jeschke R, Muller O, Back W, Zimmer, M (1998) Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. Nat Genet 18: 38–43
Jiao W, Yakushiji H, Kitajima Y, Ogawa A, Miyazaki K (2000) Establishment and characterization of human hilar bile duct carcinoma cell line and cell strain. J Hepatobiliary Pancreas Surg 7: 417–425
Kang MS, Lee HJ, Ku JL, Lee KP, Kelly MJ, Won YJ, Kim ST, Park J-G (1996) Mutation of p53 gene in hepatocellular-carcinoma cell lines with HBV DNA. Int J Cancer 67: 898–902
Kim DG, Park SY, You KR, Lee GB, Kim H, Moon WS, Chun YH, Park SH (2001a) Establishment and characterization of chromosomal aberrations in human cholangiocarcinoma cell lines by cross-species color banding. Genes Chromosomes Cancer 30: 48–56
Kim YT, Kim J, Jang YH, Lee WJ, Ryu JK, Park YH, Kim SW, Kim WH, Yoon YB, Kim CY (2001b) Genetic alterations in gallbladder adenoma, dysplasia and carcinoma. Cancer Lett 169: 59–68
Kitaeva MN, Glogan L, Williams JP, Dimond E, Nakahara K, Hausner P, DeNobile JW, Soballe PW, Kirsch IR (1997) Mutations in beta-catenin gene in colon, colorectal cancer, and extracellular biliary adenocarcinoma. Int J Cancer 76: 617–623
Knuth A, Gabbert H, Dippold W, Klein O, Sachse W, Bitter-Suermann D, Prellwitz W, Meyer ZNHK (1985) Biliary adenocarcinoma characterization of three human new tumor cell lines. J Hepatol 1: 579–596
Kohno T, Shinmura K, Tosaka M, Tani M, Kim SR, Sugimura H, Nohmi T, Kasai H, Yokota J (1998) Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. Oncogene 16: 3219–3225
Ku JL, Kim WH, Lee JH, Park HS, Kim KH, Sung MW, Park JG (1999) Establishment and characterization of human laryngeal squamous cell carcinoma cell lines. Laryngoscope 109: 976–982
Liu B, Parsons RE, Hamilton SR, Petersen GM, Lynch HT, Watson P, Markowitz S, Willson JKV, Green J, de la Chapelle A, Kinzler KW, Vogelstein B (1994) hMSH2 mutations in hereditary nonpolyposis colorectal cancer kindreds. Cancer Res 54: 4590–4594
Lu SL, Zhang WC, Akiyama Y, Nomizu T, Yusa Y (1996) Genomic structure of the transforming growth factor beta type II receptor gene and its mutations in hereditary nonpolyposis colorectal cancers. Cancer Res 56: 4595–4598
Melki JR, Vincent PC, Brown RD, Clark SJ (2000) Hypermethylation of E-cadherin in leukemia. Blood 95: 3208–3213
Myeroff LL, Parsons R, Kim SJ, Hedrick L, Cho KR, Orth K, Mathis M, Kinzler KW, Lutterbaugh J, Park K, Bang YJ, Lee HY, Park J-G, Lynch HT, Roberts AB, Vogelstein B, Markowitz SD (1995) A transforming growth factor beta receptor type II gene mutation common in colon and gastric but rare in endometrial cancers with microsatellite instability. Cancer Res 55: 5545–5547
Orlow I, Lacombe L, Hannon GJ, Serrano M, Pellicer I, Dalbagni G, Reuter VE, Zhang ZF, Beach D, Cordon-Cardo C (1995) Deletion of the p16 and p15 genes in human bladder tumors. J Nation Cancer Inst 87: 1524–1529
Park J-G, Lee JH, Kang MS, Park KJ, Jeon YM, Lee HJ, Kwon HS, Park HS, Yeo KS, Lee KU (1995) Characterization of cell lines established from human hepatocellular carcinoma. Int J Cancer 62: 276–282
Park J-G, Oie HK, Sugarbaker PH, Henslee JG, Chen TR, Johnso BE, Gazdar AF (1987) Characteristics of cell lines established from human colon carcinomas. Cancer Res 47: 6710–6718
Saijo S, Kudo T, Suzuki M, Katayose Y, Shinoda M, Muto T, Fukushima K, Suzuki T, Matsuno S (1995) Establishment of a new extrahepatic bile duct carcinoma cell line, TFK-1. Tohoku J Exp Med 177: 61–71
Takizayama I, Terashima M, Ikeda K, Kawamura H, Kashiwaba M, Tamura G, Suto T, Nakashima F, Sasaki R, Saito K (1998) Establishment and characterization of a human extrahepatic bile duct carcinoma cell line (ICBD-1). Oncol Rep 5: 463–467
Vincent F, Nagashima M, Takenoshiba S, Khan MA, Gemma A, Hagiwara K, Bennett WP (1997) Mutation analysis of the transforming growth factor-beta type II receptor in human cell lines resistant to growth inhibition by transforming growth factor-beta. Oncogene 15: 117–122
Williamson MP, Elder PA, Shaw ME, Devlin J, Knowles MA (1995) p16(CDKN2) is a major deletion target at 9p21 in bladder cancer. Hum Mol Genet 4: 1569–1577
Yamada N, Chung YS, Arimoto Y, Sawada T, Seki S, Sowa M (1995) Establishment of a new human extrahepatic bile duct carcinoma cell lines (OCUCH-LM1) and experimental liver metastatic model. Br J Cancer 71: 543-548
Yamada N, Chung YS, Ohtani H, Ikeda T (1997) Establishment and characterization of a new human gallbladder carcinoma cell line (OCUG-1) producing TA-4. Int J Oncol 10: 1251-1257
Yano H, Maruiwa M, Iemura A, Mizoguchi A, Kojiro M (1992) Establishment and characterization of a new human extrahepatic bile duct carcinoma cell line (KMBC). Cancer 69: 1664-1673

Yazumi S, Ko K, Watanabe N, Shinohara H, Yoshikawa K, Chiba T, Takahashi R (2000) Disrupted transforming growth factor-beta signaling and deregulated growth in human biliary tract cancer cells. Int J Cancer 86: 782-789
Yoshida S, Todoroki T, Ichikawa Y, Hanai S, Suzuki H, Hori M, Fukao K, Miwa M, Uchida K (1995) Mutations of p16Ink4/CDKN2 and p15Ink4B/MTS2 genes in biliary tract cancers. Cancer Res 55: 2756-2760