Carcinogenesis vol.31 no.6 pp.1003–1009, 2010
doi:10.1093/carcin/bgq043
Advance Access publication February 22, 2010

Establishment and characterization of 13 human colorectal carcinoma cell lines: mutations of genes and expressions of drug-sensitivity genes and cancer stem cell markers

Ja-Lok Ku[1,†], Young-Kyoung Shin[1,†], Duck-Woo Kim[1,2,†], Kyung-Hee Kim[1], Jin-Sung Choi[1], Sung-Hye Hong[1], You-Kyung Jeon[1], Sung-Hee Kim[1], Hong-Sun Kim[1], Jae-Hyun Park[1], Il-Jin Kim[1] and Jae-Gahb Park[1,*]

[1]Laboratory of Cell Biology, Cancer Research Institute, Seoul National University College of Medicine, Seoul 110-744, Korea and [2]Department of Surgery, Seoul National University Bundang Hospital, Seongnam 463-707, Korea

*To whom correspondence should be addressed. Tel: +82 2 2072 3380; Fax: +82 2 742 4727;
Email: jgpark@plaza.snu.ac.kr

Thirteen human colorectal cancer (CRC) cell lines were established from 10 primary tumors and 3 metastatic tumors obtained from 13 Korean patients. Characteristics of the cell lines including morphology *in vivo* and *in vitro*; mutations of the *K-ras*, *p53*, *APC* and MMR genes and microsatellite instability (MSI) status *in vitro* were determined. Expression of drug-sensitivity genes including *MDR1*, *MXR*, *MRP1* and *COX2* was also analyzed. The cell lines were unique as judged by DNA fingerprinting using 16 short tandem repeats. Eleven of the cell lines grew as adherent populations and the remaining two as floating aggregates. None of the cell lines were contaminated with *Mycoplasma* or bacteria. All cell lines showed high viability with relatively long doubling times. Six cell lines contained mutations at *K-ras*. Seven cell lines displayed *p53* gene missense, nonsense and frameshift mutations. MSI was found in three cell lines and two cell lines with an MSI-high phenotype-possessed *hMLH1* mutations. Nine cell lines had an *APC* mutation. *MRP1* was highly expressed in all cell lines, and high expression of *MDR1*, *MXR* and *COX2* evident in eight, six and six cell lines, respectively. Embryonal stem cell markers (*MELK*, *SOX4* and *OCT4*) were expressed in most of cell lines. The cancer stem cell biomarkers *CD133*, *CD44* and *Lgr5* were expressed in 12, 13 and 13 cell lines, respectively. The presently well-characterized CRC cell lines should be useful in investigations of the biological characteristics of CRC, particularly for investigations related to gene alterations associated with CRC and biology of cancer stem cells.

Introduction

Colorectal cancer (CRC) was the fourth most prevalent cancer and cause of cancer-related deaths in the USA in 2009 (1). A marked increase in CRC prevalence has been noted in Korea. According to Korea Central Cancer Registry data on cancer incidence, between 2003 and 2005, CRC was the fourth most common cancer in men after cancer of the stomach, lung and liver, and the fourth most common in women after breast, thyroid and stomach cancer. In a total of 142 610 new cases of cancer registered by Korea Central Cancer Registry in 2005, CRC comprised 11.2% of all malignancies, representing a mean annual increase of 7.3% since 1999 (2).

Cell lines established from human CRCs are now widely used in various biological studies, including CRC biology and the development of new anticancer drugs. However, most established CRC cell lines have been obtained from Western countries, with only a few cell lines being from Korean patients (3). We previously reported the establishment and characteristics of 12 human CRC cell lines from Korean patients (4). However, epidemiological, clinical and molecular studies have indicated considerable differences in various aspects of CRC in Asians and patients from Western countries (5–7). Moreover, even in Asians, racial differences in clinical and histopathological aspects of CRC are evident (8). Therefore, more CRC cell lines from Korean patients are needed for the study of racial differences in CRC, increased understanding of CRC carcinogenesis and cell biology and the development and application of novel anticancer drugs.

In this study, we detailed the establishment and characterization of 13 CRC cell lines (SNU-70, SNU-254, SNU-479, SNU-796, SNU-977, SNU-1181, SNU-1235, SNU-1406, SNU-1411, SNU-1460, SNU-1544, SNU-1684 and SNU-1746) from 13 Korean patients. Cell line phenotypes including morphology *in vivo* and *in vitro*; growth characteristics and mutations of *K-ras*, *p53*, mismatch repair (MMR) and *APC* and microsatellite instability (MSI) status were described. The expressions of the drug-sensitivity genes *MDR1*, *MXR*, *MRP1* and *COX2* (which, respectively, encode multidrug resistance 1, mitoxantrone resistance protein, multidrug resistance-associated protein 1 and cyclooxygenase 2) were analyzed. Finally, the expression of *CD133*, *CD44* and *Lgr5* recently reported biomarkers of stem cells from human colorectal carcinoma were determined.

Abbreviations: CRC, colorectal cancer; FBS, fetal bovine serum; MSI, microsatellite instability; MMR, mismatch repair; PCR, polymerase chain reaction; RT–PCR, reverse transcription–polymerase chain reaction; SNU, Seoul National University.

[†]These authors contributed equally to this work.

Materials and methods

Establishment and maintenance of human CRC cell lines

Cell lines from pathologically proven colorectal carcinomas were established. Solid tumors were finely minced with scissors and dispersed into small aggregates by pipetting. Appropriate amounts of fine neoplastic tissue fragments were seeded into 25 cm^2 flasks. Most of the tumor cells were initially cultured in ACL-4 medium supplemented with 5% heat-inactivated fetal bovine serum (FBS) (AR5). ACL-4 is a fully defined specifically formulated for the selective growth of human lung adenocarcinoma cells (9) and has proven useful in the establishment of CRC and hepatocellular carcinoma cell lines (10,11). AR5 medium was prepared from RPMI 1640. ADF5 medium prepared by a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 supplemented with 5% heat-inactivated FBS was also used for the initial culture of tumor cells. Cultures were maintained in RPMI 1640 supplemented with 10% heat-inactivated FBS (R10). Initial passages were performed when heavy tumor cell growth was observed, and subsequent passages were performed every 1 or 2 weeks. Adherent cells were recovered while growth was subconfluent by treatment with trypsin, dispersed by pipetting and used for the passages. If stromal cell growth was noted in the initial cultures, differential trypsinization was used to obtain a pure tumor cell population. Cultures were maintained in humidified incubators at 37°C in an atmosphere of 5% CO_2 and 95% air. SNU-1, SNU-61, SNU-C2A and SNU-C4 cell lines obtained from the Korean Cell Line Bank (Seoul, Korea) were used as controls.

In vitro growth and morphology

To determine the population doubling time, 5×10^4 to 3×10^5 viable cells from each cell line were seeded into 14–20 identical 25 cm^2 flasks and the number of viable cells was determined daily for $\geq$14 days. Cultures were fed every 3 or 4 days and 24 h prior to the viability determination. Cell viability was determined by the standard dye exclusion method using 0.4% trypan blue, with the number of dye excluding viable cells counted under a microscope using a hemocytometer. *Mycoplasma* contamination was tested by the 16S-rRNA-gene-based polymerase chain reaction (PCR) amplification method using e-Myco Mycoplasma PCR Detection Kit (Intron Biotechnology, Kyonggi, Korea). To examine cell morphology, cells grown in 75 cm^2 culture flasks were observed daily by phase-contrast microscopy and histopathologically compared with the original tumors.

Nucleic acid isolation and synthesis of complementary DNA

Genomic DNA was extracted from the cell lines using G-DEX genomic DNA Extraction Kit (Intron Biotechnology), and RNA was extracted using

the easy-BLUE total RNA Extraction Kit (Intron Biotechnology). For complementary DNA synthesis, 2 μg of total RNA was reverse transcribed using random oligo (dT) primer, deoxynucleoside triphosphates and 1 μl (200 U) of Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA) in a final volume of 20 μl for 75 min at 42°C, after a 10 min denaturation at 70°C. Eighty microliters of distilled water was then added to the reverse transcription reaction, which was stored −20°C until used.

DNA fingerprinting analysis

DNA was amplified using an AmpFlSTR identifiler PCR Amplification Kit (Applied Biosystems, Foster City, CA). A single round of PCR amplified 15 short tandem repeat markers (CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX and vWA) and an Amelogenin gender-determining marker at loci containing highly polymorphic microsatellite markers. Amplified products were analyzed using an ABI 3730 Genetic analyzer (Applied Biosystems). Additionally, DNA was PCR amplified at loci containing the highly polymorphic microsatellite markers D1S1586 and D3S1765. PCR products were denatured by 95% formamide and electrophoresed on a 7 M urea polyacrylamide gel for 2 h at 60 W. Gels were dried and visualized by autoradiography (4).

MSI and mutation analyses of MMR gene

For MSI analysis, BAT-25 and BAT-26 were evaluated by a capillary-based sequencing analysis. PCR was performed as described above, except that the forward primers were labeled with a fluorescent dye (FAM), and the labeled samples were run on an ABI 3730 genetic analyzer (Applied Biosystems). GeneMapper software (Applied Biosystems) was used to calculate the size of each fluorescent PCR product (12). For gel-based MSI analysis, the desired fragments were amplified in the presence of [α-P^{32}] deoxycytidine triphosphate. The PCR products were denatured and separated on 6 M urea/7% polyacrylamide gels run at 60 W (13). To identify mutation in *hMLH1* and *hMSH2*, we screened all coding regions and splicing sites by direct sequencing (14).

Mutational analysis of β-catenin, K-ras, p53, TGFβRII and MXR

From each sample, 100 ng of DNA was used as a template for PCR amplification. For mutations of the *β-catenin*, *p53*, *TGFβRII*, *K-ras* and *MXR* genes, exon 3 of *β-catenin*, exons 4–8 of *p53*, exon 3 of *TGFβRII*, exons 1 and 2 of *K-ras* and codon 482 in *MXR* were amplified and analyzed by bidirectionally direct sequencing using an ABI 3730 genetic analyzer.

Reverse transcription–polymerase chain reaction analyses

To check expressions of the *MDR1*, *MXR*, *MRP* and *COX2* genes in newly established CRC cell lines, reverse transcription–polymerase chain reaction (RT–PCR) was performed using gene-specific primers (14–17). To check the expression of stem cell biomarkers in the newly established CRC cell lines, RT–PCR of *Oct4*, *SOX4*, *MELK*, *CD44*, *CD133* and *Lgr5* was performed using specific primer sets (18–22). Primers for β-actin were used to confirm RNA integrity. The amplified DNA fragments were fractionated in a 2% agarose gel and visualized by ethidium bromide staining.

Results

General characteristics of cell lines

CRC specimens for cell line culture were collected from patients during surgeries conducted at Seoul National University (SNU) Hospital from 1988 to 2001. The 13 colorectal carcinoma cell lines (SNU-70, SNU-254, SNU-479, SNU-796, SNU-977, SNU-1181, SNU-1235, SNU-1406, SNU-1411, SNU-1460, SNU-1544, SNU-1684 and SNU-1746) were established in AR5 medium and maintained in RPMI 1640 medium supplemented with 10% heat-inactivated FBS. SNU-1235, SNU-1406, SNU-1460, SNU-1544 and SNU-1684 originated from primary colon cancers; SNU-70, SNU-254, SNU-796, SNU-977 and SNU-1411 originated from primary rectal cancers and SNU-479, SNU-1181 and SNU-1746 originated from metastatic colon cancers. Population doubling times ranged from 25 to 121 h. All lines were free of contamination by bacteria or *Mycoplasma*. The results are summarized in Table I.

Morphology

The original tumors consisted of two well-differentiated tubular adenocarcinomas (SNU-70 and SNU-254), 10 moderately differentiated adenocarcinomas (SNU-479, SNU-796, SNU-977, SNU-1181, SNU-1235, SNU-1406, SNU-1411, SNU-1460, SNU-1544 and SNU-1684) and a signet ring cell carcinoma (SNU-1746). Gross morphology of the primary tumor revealed the ulcerofungating type most common in eight cell lines (SNU-70, SNU-796, SNU-1181, SNU-1235, SNU-1406, SNU-1460, SNU-1544 and SNU-1746), ulceroinfiltrative type in two cell lines (SNU-254 and SNU-1411) and polypoid type in SNU-977. Pathological data regarding gross type were unavailable in SNU-479 and SNU-1684. On *in vitro* cultivation, 10 cell lines (SNU-70, SNU-254, SNU-796, SNU-977, SNU-1181, SNU-1235, SNU-1411, SNU-1460, SNU-1544 and SNU-1684) grew as adherent populations and three cell lines (SNU-479, SNU-1406 and SNU-1746) grew as floating aggregates (Figure 1). The majority of tumor cells displayed a polygonal shape and had round-to-oval nuclei with prominent single-to-double nucleoli. SNU-254, SNU-796 and SNU-977, which were derived from rectal cancers, grew as monolayers composed of islands of epithelial cells (Figure 1B and D; supplementary Figure S1E is available at *Carcinogenesis* Online). SNU-70, SNU-1181, SNU-1235, SNU-1411, SNU-1460, SNU-1544 and SNU-1684 proliferated as islands of tightly packed epithelial cells and eventually formed dense monolayers (Figure 1A; supplementary S1F, S1H, S1I, S1J and S1L is available at *Carcinogenesis* Online). SNU-479, which was derived from colon cancer ascites, grew as irregular floating clumps (Figure 1C). SNU-1406 grew as tightly packed floating cell aggregates (supplementary Figure S1G is available at *Carcinogenesis* Online). SNU-1746, which was derived from

Table I. *In vivo* and *in vitro* characteristics of newly established 13 CRC cell lines

Cell line	Date of initiation	Sex/age	Primary tumor site/culture site	Original tumor differentiation	Gross type of growth	Doubling time (hours)[a]	Growth pattern
SNU-70	29 July 1988	M/61	Rectum/primary	Well	Ulcerofungating	118	Adherent
SNU-254	21 September 1989	F/61	Rectum/primary	Well	Ulceroinfiltrative	50	Adherent
SNU-479	7 August 1990	M/62	Sigmoid colon/ascites	Moderate	Unclear	44	Floating
SNU-796	23 December 1991	F/34	Rectum/primary	Moderate	Ulcerofungating	121	Adherent
SNU-977	11 November 1992	F/52	Rectum/primary	Moderate	Polypoid	63	Adherent
SNU-1181	7 January 1994	M/65	Transverse colon/liver	Moderate	Ulcerofungating	52	Adherent
SNU-1235	30 March 1994	F/68	Ascending colon/primary	Moderate	Ulcerofungating	45	Adherent
SNU-1406	9 October 1995	F/59	Ascending colon/primary	Moderate	Ulcerofungating	25	Floating
SNU-1411	1 November 1995	M/42	Rectum/primary	Moderate	Ulceroinfiltrative	38	Adherent
SNU-1460	2 October 1996	M/25	Sigmoid colon/primary	Moderate	Ulcerofungating	67	Adherent
SNU-1544	4 May 1998	F/38	Ascending colon/primary	Moderate	Ulcerofungating	42	Adherent
SNU-1684	13 June 2000	M/43	Ascending colon/primary	Moderate	Unclear	53	Adherent
SNU-1746	24 April 2001	M/43	Ascending colon/lymph node	Signet ring cell	Ulcerofungating	42	Floating

[a]Growth curves for cell lines were also determined based on data from doubling time (supplementary FigureS6 is available at *Carcinogenesis* Online).

the signet ring cell carcinoma, grew as loosely attached floating aggregates or grape-like clusters (supplementary Figure S1L is available at *Carcinogenesis* Online).

DNA profiles

DNA fingerprinting revealed that the 13 cell lines were unique and unrelated (supplementary Table S1 and Figure S2 are available at *Carcinogenesis* Online). These results definitely excluded the possibility of cell line cross-contamination.

MSI status and mutation analysis of hMLH1 and hMSH2 and screening of TGFβRII

A prominent MSI phenotype was evident in SNU-1544, SNU-1684 and SNU-1746 cell lines (23% of the total cell lines) and 10 cell lines exhibited a microsatellite stable phenotype (Figure 2A). Mutational analysis of the *hMLH1* and *hMSH2* genes was performed for SNU-1544, SNU-1684 and SNU-1746. Among these three cell lines, SNU-1544 harbored p.S2L in *hMLH1* and SNU-1746 harbored one missense mutation (p.E523K; a homotype mutation) and one

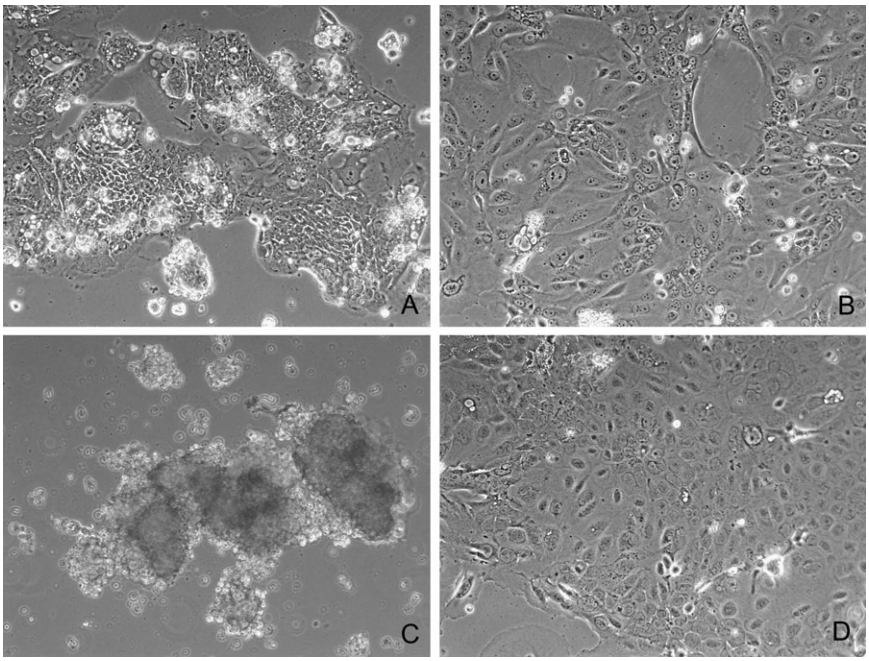

Fig. 1. Phase-contrast microscopy of newly established CRC cell lines. (**A**) SNU-70, (**B**) SNU-254, (**C**) SNU-479 and (**D**) SNU-796.

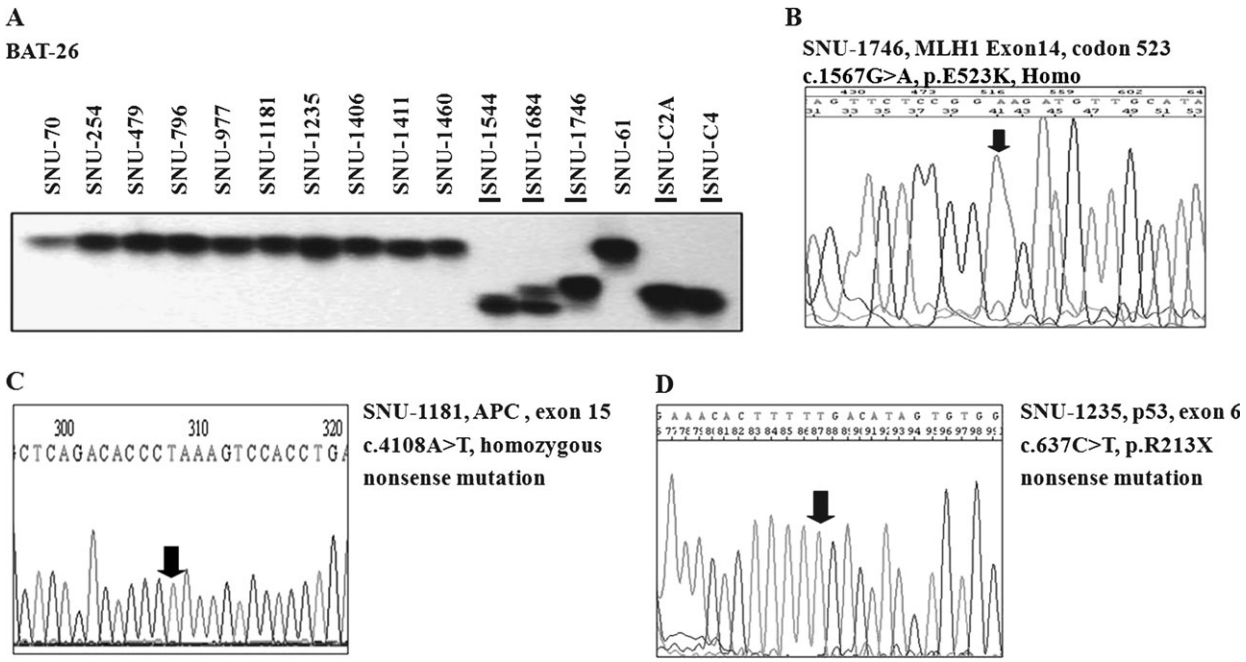

Fig. 2. (**A**) In MSI analysis, BAT-26 was evaluated by a capillary-based sequencing and gel-based analysis. We used three cell lines for control (SNU-61 for microsatellite stability control, SNU-C2A and SNU-C4 for MSI control). SNU-1544, SNU-1684 and SNU-1746 showed MSI-high phenotype. (**B**) SNU-1746 had homotype mutation at c.1567G>A in the *hMLH1* gene. Arrows indicate the mutated nucleotide. (**C**) Mutation analysis of the *APC* gene. SNU-1181 had a homozygous mutation in exon 15. (**D**) Mutation analysis of the *p53* gene in SNU-1235 cell line. Arrows indicate the mutated nucleotide.

single-nucleotide polymorphism (p.I219V) in *hMLH1*. No mutations in *hMLH1* and *hMSH2* were evident in SNU-1684 (Table II, Figure 2B). Among the MSI target genes, screening of poly A10 sequences in *TGFβRII* detected frameshift mutations in all three cell lines with MSI (Table II) (supplementary Figure S3 is available at *Carcinogenesis* Online).

Mutation analysis of K-ras, p53, APC, β-catenin and MXR genes

Among the 13 cell lines examined, six harbored missense mutations in *K-ras*. Two cell lines (SNU-254 and SNU-1746) had a GGT→GAT transition mutation in codon 12, SNU-1181 had a GGT→AGT mutation and SNU-1411 had a GGT→TGT mutation. Especially, SNU-1411 had a homotype mutation in codon 12. Mutations of SNU-1460 and SNU-1544 were found in codon 13 (Table III). Five mutations with SNU-1411 resulted from G→A transitions and substituted the wild-type glycin. Direct sequencing analysis revealed that 7 of 13 cell lines (53.8%) harbored the *p53* mutation, with a missense mutation in SNU-254, SNU-479, SNU-977 and SNU-1181; nonsense mutation in SNU-1235 and SNU-1411 and a frameshift mutation in SNU-70 (Table III, Figure 2D; supplementary Figure S5 is available at *Carcinogenesis* Online). Six cell lines (46.1%) harbored a single-nucleotide polymorphism at codon 72. Among these *p53* variations, six mutations and one single-nucleotide polymorphism were previously reported in the *p53* mutation database (http://www-p53.iarc.fr). The c.845_846delG and the 'G' deletion at codon 282 in SNU-70 are novel. All mutations showed the homo phenotype. Among the six cell lines with c.215G>C, only SNU-1684 had heterotype variation. Four of these seven mutations (c.845_845delG in SNU-70, R175F in SNU-977, R282W in SNU-1181 and R213X in SNU-1235) were located in hot spots containing CpG dinucleotides. Nine cell lines harbored *APC* mutations (five nonsense mutations and eight frameshift mutations) that resulted in a truncated protein (Table II, Figure 2C;

Table II. Abnormalities of the *hMLH1*, *hMSH2*, *TGFβRII*, *APC* and *β-catenin* genes

	MSI	Abnormalities of *MLH1* and *MSH2*					*TGFβRII*	Abnormalities of *APC*				Abnormalities of *β-catenin*
		Gene	Exon	Codon	Nucleotide change	a.a. change		Exon	Codon	Nucleotide change	a.a. change	
SNU-70	MSS						A10	15	1294	c.3880C>T	Gln→STOP	W
SNU-254	MSS						A10	15	1487	c.4461_4463delT	Frameshift	W
SNU-479	MSS						A10					W
SNU-796	MSS						A10	13	554	c.1660C>T	Arg→STOP	W
								15	1363	c.4087_4090insA	Frameshift	W
SNU-977	MSS						A10	15	1354	c.4060_4063insT	Frameshift	W
SNU-1181	MSS						A10	15	1370	c.4108A>T, Homo	Lys→STOP	W
SNU-1235	MSS						A10	15	1554	c.4661_4666insA	Frameshift	W
SNU-1406	MSS						A10					W
SNU-1411	MSS						A10					W
SNU-1460	MSS						A10					W
SNU-1544	MSI	*MLH1*	1	2	c. 5C>T	Ser→Leu	A9, A11	15	1554	c.4661_4666insA	Frameshift	W
SNU-1684	MSI						A8, homo	13	554	c.1660C>T	Arg→STOP	W
								15	1557	c.4669_4670delAT	Frameshift	
SNU-1746	MSI	*MLH1*	8	219	c.655A>G	Ile→Val	A9, homo	13	560	c.1678_1682insA	Frameshift	W
		MLH1	14	523	c.1567G>A, Homo	Glu→Lys		15	1429	c.4285C>T	Gln→STOP	
								15	1554	c.4661_4666insA	Frameshift	

a.a. change: amino acid change; A10, 10 bp adenine repeat in exon 3; MSS, microsatellite stable; W, wild-type.

Table III. Abnormalities of the *p53*, *K-ras* and *MXR* genes

	Abnormalities of p53				Abnormalities of K-ras			Mutation of codon 484 at MXR
	Exon	Codon	Nucleotide change	Amino acid change	Codon	Nucleotide change	Amino acid change	
SNU-70	4	72[a]	c.215G>C	Arg→Pro	W			W
	8	282	c.845_846delG, Homo	Frameshift				
SNU-254	8	286	c.856G>A	Glu→Lys	12	GGT→GAT	Gly→Asp	W
	4	72[a]	c.215G>C	Arg→Pro				
SNU-479	5	162	c.484A>T	Ile→Phe	W			W
SNU-796	4	72[a]	c.215G>C	Arg→Pro	W			W
SNU-977	5	175	c.524G>A	Arg→His	W			W
SNU-1181	8	282	c.844C>T	Arg→Trp	12	GGT→AGT	Gly→Ser	W
SNU-1235	6	213	c.637C>T	Arg→Stop	W			W
SNU-1406	4	72[a]	c.215G>C	Arg→Pro	W			W
SNU-1411	4	72[a]	c.215G>C	Arg→Pro	12	GGT→TGT	Gly→Cys	W
	4	94	c.281C>A, Homo	Ser→Stop				W
SNU-1460	4	72[a]	c.215G>C	Arg→Pro	13	GGC→GAC	Gly→Asp	W
SNU-1544	W				13	GGC→GAC	Gly→Asp	W
SNU-1684	W				W			W
SNU-1746	W				12	GGT→GAT	Gly→Asp	W

W, wild-type.
[a]Six cell lines (46.1%) harbored single-nucleotide polymorphism at codon 72.

supplementary Figure S4 is available at *Carcinogenesis* Online). Two cell lines (SNU-796 and SNU-1684) harbored two different mutations, and SNU-1746 had three mutations at c.1678_1681insA, c.4285C>T and c.4661_4666insA. The frequency of *APC* mutations in all 13 cell lines was 69.2%. *β-catenin* mutation analysis did not detect a mutation in exon 3 by direct sequencing in any cell line (Table II). Similarly, no mutation at codon 484 in *MXR* was detected (Table III).

Expression of MDR1, MXR, MRP and COX2

We determined the expression of *MDR1*, *MXR*, *MRP* and *COX2* in the 13 colon cancer cell lines by RT–PCR (Figure 3A). Four cell lines (SNU-1406, SNU-1544, SNU-1684 and SNU-1746) expressed *MDR1*, *MXR*, *MRP* and *COX2* simultaneously. SNU-1406 in particular avidly expressed the four genes. *MRP1* was expressed in all 13 cell lines, followed by *MDR1* in 11 cell lines (84.6%) and *MXR* and *COX2* in eight cell lines (61.5%).

Expression of stem cell markers

We determined the expression of three embryonal cell markers (*Oct4*, *SOX4* and *MELK*) and three stem cell markers of CRC (*CD44*, *CD133* and *Lgr5*) in the 13 colon cancer cell lines by RT–PCR (Figure 3B). Lgr5 was weakly expressed in SNU-796, SNU-977 and SNU-1544. *Oct4*, *CD44* and *Lgr5* were expressed in all the cell lines. *SOX4* was expressed in cell lines except SNU-70, SNU-254 and SNU-1746 (76.9%). *MELK* and *CD133* were not expressed in SNU-1235 and SNU-1544, respectively.

Discussion

Cancer cell lines can be used for various oncological and biochemical studies that cannot be carried out using tissue specimens from surgical resection. In colon cancer, various CRC cell lines such as HCT-116, LoVo, SW-480, LS174T, Colo205 and WiDr have been established and used worldwide in many studies regarding colorectal carcinogenesis and biology (11). As more information is accumulated regarding the diversity of molecular changes in CRC, additional well-characterized CRC cell lines are needed.

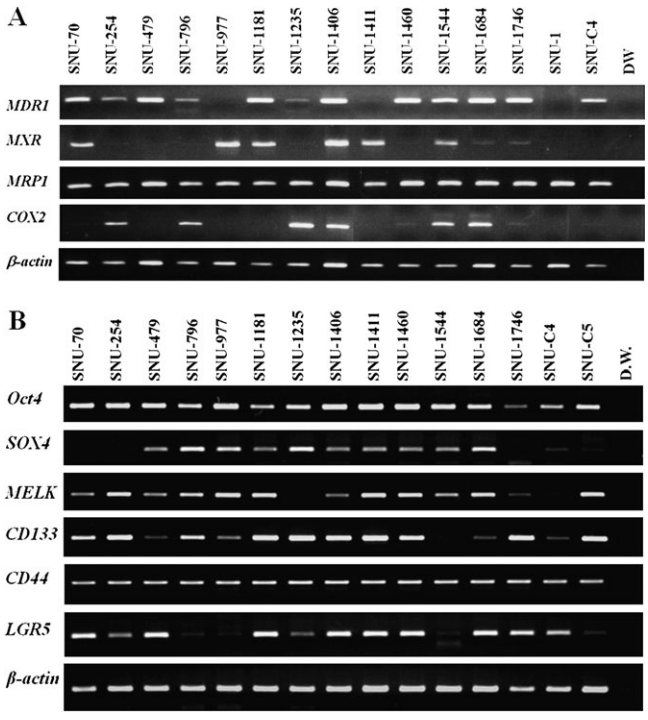

Fig. 3. (**A**) Expression analysis of *MDR1*, *MXR*, *MRP1* and *COX2* by RT–PCR. For control, we used the SNU-1 (gastric cancer cell line, negative control for *MDR1*), SNU-C4 (positive control for *MDR1*) and water only. (**B**) Expression analysis of *Oct4*, *SOX4*, *MELK*, *CD133*, *CD44* and *Lgr5* by RT–PCR. For control, we used the SNU-4, SNU-C5 and water only. β-actin was used to confirm RNA integrity.

The present study provides detailed information on 13 newly established CRC cell lines derived from primary colon cancers ($n = 5$), primary rectal cancers ($n = 5$) and metastatic colon cancers ($n = 3$) in Korean patients. The primary tumors revealed morphological heterogeneity, including different histological and gross growth patterns. *In vitro*, cell lines were heterogeneous in their cellular and nuclear morphology: three lines grew as floating aggregates and 10 lines as adherent monolayers. These *in vivo* characteristics had no effect on the *in vitro* growth pattern of the cell lines established.

There are two major pathways leading to colorectal carcinogenesis. MSI phenotype is observed in ∼15% of sporadic CRC and is caused by inactivation of MMR genes such as *hMLH1*, *hMSH2*, *hMSH6*, *hPMS2* or *hMSH3*. Germ line mutations in the MMR genes are also associated with hereditary non-polyposis CRC, the most common form of hereditary CRC accounting for 2–5% of all CRCs. About 90% of the identified mutations in the MMR genes are found in two genes, *hMLH1* and *hMSH2* (23,24). These MSI-high cancers display mutations in microsatellites (small genetic loci composed of 1–5 bp repeated 10–30 times). These cancers are also characterized by predominantly right-sided CRC proximal to the splenic flexure, tumor-infiltrating lymphocytes and mucinous or poorly differentiated differentiation (24). In the present study, three cell lines displayed a MSI phenotype and their primary tumor was located in the ascending colon. These three cell lines were from sporadic CRCs, not from patients with hereditary non-polyposis CRC. The same cell lines lacked *p53* gene mutations and SNU-1406 lacked mutations in the *APC*, *p53* or *K-ras* genes.

Recently, several studies showed that benefits from fluorouracil-based adjuvant chemotherapy depends on the MMR status in patients with stage II or stage III CRC, showing 5-fluorouracil adjuvant chemotherapy improves survival in patients with MMR competent tumors but that this chemotherapeutic benefit cannot be extended to patients with MMR-deficient tumors (25,26). MSI status of CRC cell lines is now more important, especially in the development of novel anticancer drugs.

Chromosomal instability phenotype is found in ∼85% of sporadic colon cancers. These cancers are characterized by a left-sided tumor location, predominantly moderate differentiation, less tumor-infiltrating lymphocytes, aneuploidy, multiple chromosomal rearrangements and accumulation of somatic mutations in oncogenes such as *APC*, *K-ras* and *p53* (27,28). These mutations are the most common genetic alterations in CRC, with *APC* mutations evident in 50–83% of sporadic CRCs (29–31), *p53* in 41–69% (32,33) and *K-ras* in 20–38% (34–37). Analysis of the 13 cell lines in our study revealed mutation frequencies *p53*, *APC* and *K-ras* in 54, 69 and 46, respectively, of the 13 cell lines, consistent with the previous reports. Mutations in all three genes are rare in the same tumor; in one study, only 6% of CRCs contained the mutations in both *p53* and *K-ras* (38). In our study, two cell lines (SNU-254 and SNU-1181) harbored mutations in *p53*, *APC* and *K-ras*.

The *APC* gene is composed of 8529 bp and encodes APC, a large protein whose diverse functions include cell adhesion, maintenance of cytoskeletal structure, signal transduction and regulation of cell proliferation (39). The majority of >800 *APC* germ line mutations identified to date are frameshift or nonsense mutations in the 5′ half of the gene, leading to production of a truncated protein, with very few missense changes (40). All *APC* mutations found in this study were nonsense or frameshift mutations, which resulted in truncation. *APC* mutations are significantly more probably to be located in the 5′ portion of the gene, which harbors the mutation cluster region (codons 1268–1513) (41). Curiously, in the 13 *APC* mutations found in this study, only 6(46.2%) were located in the mutation cluster region.

Seven of the 13 cell lines (53.8%) harbored the *p53* mutation in this study, and among the 25 CRC cell lines in the Korean Cell Line Bank,

52.0% (13/25) possess *p53* mutations (4). Data on the *p53* status of the primary tumor were not available in this study, but *p53* mutations between primary tumor samples and cultured cell lines are usually identical (33). For *p53* mutational analysis, we used bidirectional sequencing of exons 4–8 of the gene. According to the International Agency for Research on cancer *p53* mutation database, most *p53* mutations in CRC are limited to exons 4–8, and only 2% of CRCs harbor mutations outside these exons (42). A recent study reported that the determination of *p53* mutations are more relevant than MSI status as a predictor for response to fluorouracil-based adjuvant chemotherapy in stage III colon cancer patients, and *p53* status has become an important characteristic to determine in CRC (43).

The *K-ras* oncogene encodes a small (21 kDa) protein involved in signal transduction by virtue of its intrinsic guanosine triphosphatase activity. It remains inactivated in the normal cellular environment, but activating mutations occur early in colorectal carcinogenesis, resulting in unregulated cellular proliferation and malignant transformation (44). The presence of *K-ras* mutations is associated with poorer prognosis in CRC (45,46). Most *K-ras* mutations have been identified at codons 12, 13, 59 or 61 (4). Consistent with this, *K-ras* mutations were presently detected in codon 12 in four cell lines and codon 13 in two cell lines.

Drug resistance is an important obstacle to the success of chemotherapy. The resistance is caused by various reasons including inhibited drug transport, target alteration and metabolic changes (47). The most common gene alteration in drug resistance is the increased expression of *MDR1* and *MRP1* (48). Both *MDR1* and *MRP1* are expressed in colon cancers with a *p53* mutation, and these cancers are resistant to chemotherapy (49). However, presently SNU-1406, SNU-1544, SNU-1684 and SNU-1746, which expressed *MDR1*, *MXR*, *MRP* and *COX2*, did not possess *p53* mutations.

Recently, tumor-causing cells in CRC were identified CRC in a CD133-positive subpopulation (50). These cells accounted for $\sim$2.5% of tumor cells and could induce xenograft tumors in immunodeficient mice that resembled the original tumor. Several studies have provided evidence supporting a stem cell origin for CRC (51). However, the mechanism of stem cell involvement remains obscure. Moreover, cancer stem cells are thought to be the cause of failure to effectively cure CRC. Current chemotherapeutic agents kill actively proliferating cells, but these agents are not curative because cancer stem cells proliferate relatively slowly. Stem cells tend to be increased in more advanced tumors and more resistant to chemotherapeutic agents than mature cancer cells (50–52). As a result, it is necessary to develop agents that kill stem cells more selectively and effectively.

In summary, the present study describes 13 newly established CRC cell lines with their distinct growth characteristics *in vitro* and tumor phenotypes *in vivo*. Of these cell lines, six harbor mutations in the *K-ras* gene. Mutations in *p53* and *APC* were detected in seven and nine cell lines, respectively. The MSI phenotype was evident in three cell lines, of which two possess mutations in *hMLH1* genes. *MRP1*, *MDR1*, *MXR* and *COX2* genes are highly expressed in 13, eight, six and six cell lines, respectively. The CRC stem cell markers *CD44*, *CD133* and *Lgr5* are highly expressed in most of the cell lines. These cell lines will be widely available to the scientific community through the Korean Cell Line Bank (http://cellbank.snu.ac.kr.). And also, these cell lines could serve as useful tools for investigating the biological characteristics of CRC.

Supplementary material

Supplementary Figures S1–S6 and Table S1 can be found at http://carcin.oxfordjournals.org/

Funding

Korean Cell Line Research Foundation; National Research Foundation of Korea grant, Ministry of Education, Science and Technology (2008-0061491); Priority Research Centers Program National Research Foundation of Korea grant, Ministry of Education, Science and Technology (2009-0093820).

Acknowledgements

Conflict of Interest Statement: None declared.

References

1. Jemal,A. *et al*. (2009) Cancer statistics, 2009. *CA Cancer J. Clin.*, **59**, 225–249.
2. Shin,H.R. *et al*. (2005) Nationwide cancer incidence in Korea, 1999–2001; first result using the national cancer incidence database. *Cancer Res Treat.*, **37**, 325–331.
3. ATCC. (2007) ATCC Cell Biology Printed Catalog, ATCC. http://www.atcc.org/CellBiologyPrintedCatalog/tabid/578/Default.aspx
4. Oh,J.H. *et al*. (1999) Establishment and characterization of 12 human colorectal-carcinoma cell lines. *Int. J. Cancer*, **81**, 902–910.
5. Whittemore,A.S. *et al*. (1990) Diet, physical activity, and colorectal cancer among Chinese in North America and China. *J. Natl Cancer Inst.*, **82**, 915–926.
6. Qing,S.H. *et al*. (2003) Racial differences in the anatomical distribution of colorectal cancer: a study of differences between American and Chinese patients. *World J. Gastroenterol.*, **9**, 721–725.
7. Yu,H. *et al*. (1991) Comparative epidemiology of cancers of the colon, rectum, prostate and breast in Shanghai, China versus the United States. *Int. J. Epidemiol.*, **20**, 76–81.
8. Lin,M. *et al*. (1995) Differences in colorectal cancer between China and Japan: a clinical and histological study of two groups of cases. *Eur. J. Cancer Prev.*, **4**, 195–199.
9. Brower,M. *et al*. (1986) Growth of cell lines and clinical specimens of human non-small cell lung cancer in a serum-free defined medium. *Cancer Res.*, **46**, 798–806.
10. Park,J.G. *et al*. (1995) Characterization of cell lines established from human hepatocellular carcinoma. *Int. J. Cancer*, **62**, 276–282.
11. Park,J.G. *et al*. (1987) Characteristics of cell lines established from human colorectal carcinoma. *Cancer Res.*, **47**, 6710–6718.
12. Kim,I.J. *et al*. (2003) Robust microsatellite instability (MSI) analysis by denaturing high-performance liquid chromatography (DHPLC). *J. Hum. Genet.*, **48**, 525–530.
13. Yoon,S.N. *et al*. (2008) Hereditary nonpolyposis colorectal cancer in endometrial cancer patients. *Int. J. Cancer*, **122**, 1077–1081.
14. Logashenko,E.B. *et al*. (2002) Short double-stranded RNA suppresses multiple drug resistance gene expression in tumor cells. *Dokl. Biochem. Biophys.*, **386**, 296–297.
15. Stein,U. *et al*. (2002) Impact of BCRP/MXR, MRP1 and MDR1 /P-Glycoprotein on thermoresistant variants of atypical and classical multidrug resistant cancer cells. *Int. J. Cancer*, **97**, 751–760.
16. Pascolo,L. *et al*. (2003) Effects of maturation on RNA transcription and protein expression of four MRP genes in human placenta and in BeWo cells. *Biochem. Biophys. Res. Commun.*, **303**, 259–265.
17. Hase,T. *et al*. (2003) Cyclooxygenase-1 and -2 in human testicular tumours. *Eur. J. Cancer*, **39**, 2043–2049.
18. Cantz,T. *et al*. (2008) Absence of OCT4 expression in somatic tumor cell lines. *Stem Cells*, **26**, 692–697.
19. Yokota,N. *et al*. (2004) Identification of differentially expressed and developmentally regulated genes in medulloblastoma using suppression subtraction hybridization. *Oncogene*, **23**, 3444–3453.
20. Hemmati,H.D. *et al*. (2003) Cancerous stem cells can arise from pediatric brain tumors. *Proc. Natl Acad. Sci. USA*, **100**, 15178–15183.
21. Pasquinelli,G. *et al*. (2009) Architectural organization and functional features of early endothelial progenitor cells cultured in a hyaluronan-based polymer scaffold. *Tissue Eng. Part A*, **15**, 2751–2762.
22. Tanese,K. *et al*. (2008) G-protein-coupled receptor GPR49 is up-regulated in basal cell carcinoma and promotes cell proliferation and tumor formation. *Am. J. Pathol.*, **173**, 835–843.
23. Thibodeau,S.N. *et al*. (1993) Microsatellite instability in cancer of the proximal colon. *Science*, **260**, 816–819.
24. Wheeler,J.M. *et al*. (2000) DNA mismatch repair genes and colorectal cancer. *Gut*, **47**, 148–153.
25. Jover,R. *et al*. (2006) Mismatch repair status in the prediction of benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. *Gut*, **55**, 848–855.
26. Ribic,C.M. *et al*. (2003) Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N. Engl. J. Med.*, **349**, 247–257.

27. Lengauer,C. *et al*. (1997) Genetic instability in colorectal cancers. *Nature*, **386**, 623–627.
28. Fearon,E.R. *et al*. (1990) A genetic model for colorectal tumorigenesis. *Cell*, **61**, 759–767.
29. Powell,S.M. *et al*. (1992) APC mutations occur early during colorectal tumorigenesis. *Nature*, **359**, 235–237.
30. Rowan,A.J. *et al*. (2000) APC mutations in sporadic colorectal tumors: a mutational ''hotspot'' and interdependence of the ''two hits''. *Proc. Natl Acad. Sci. USA*, **97**, 3352–3357.
31. De Filippo,C. *et al*. (2002) Mutations of the APC gene in human sporadic colorectal cancers. *Scand. J. Gastroenterol.*, **37**, 1048–1053.
32. Slebos,R.J. *et al*. (1996) Clinical and pathological associations with p53 tumour-suppressor gene mutations and expression of p21WAF1/Cip1 in colorectal carcinoma. *Br. J. Cancer*, **74**, 165–171.
33. Baker,S.J. *et al*. (1990) p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. *Cancer Res.*, **50**, 7717–7722.
34. Andreyev,H.J. *et al*. (1998) Kirsten ras mutations in patients with colorectal cancer: the multicenter ''RASCAL'' study. *J. Natl Cancer Inst.*, **90**, 675–684.
35. Lee,J.C. *et al*. (1996) K-ras gene mutation is a useful predictor of the survival of early stage colorectal cancers. *Anticancer Res.*, **16**, 3839–3844.
36. Samowitz,W.S. *et al*. (2001) Inverse relationship between microsatellite instability and K-ras and p53 gene alterations in colon cancer. *Am. J. Pathol.*, **158**, 1517–1524.
37. Brink,M. *et al*. (2003) K-ras oncogene mutations in sporadic colorectal cancer in The Netherlands Cohort Study. *Carcinogenesis*, **24**, 703–710.
38. Conlin,A. *et al*. (2005) The prognostic significance of K-ras, p53, and APC mutations in colorectal carcinoma. *Gut*, **54**, 1283–1286.
39. Polakis,P. (1997) The adenomatous polyposis coli (APC) tumor suppressor. *Biochim. Biophys. Acta.*, **1332**, F127–F147.
40. Nagase,H. *et al*. (1992) Correlation between the location of germ-line mutations in the APC gene and the number of colorectal polyps in familial adenomatous polyposis patients. *Cancer Res.*, **52**, 4055–4057.
41. Cetta,F. *et al*. (2000) Germline mutations of the APC gene in patients with familial adenomatous polyposis-associated thyroid carcinoma: results from a European cooperative study. *J. Clin. Endocrinol. Metab.*, **85**, 286–292.
42. Olivier,M. *et al*. (2002) The IARC TP53 database: new online mutation analysis and recommendations to users. *Hum. Mutat.*, **19**, 607–614.
43. Westra,J.L. *et al*. (2005) Determination of TP53 mutation is more relevant than microsatellite instability status for the prediction of disease-free survival in adjuvant-treated stage III colon cancer patients. *J. Clin. Oncol.*, **23**, 5635–5643.
44. Bos,J.L. (1989) ras oncogenes in human cancer: a review. *Cancer Res.*, **49**, 4682–4689.
45. Andreyev,H.J. *et al*. (2001) Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. *Br. J. Cancer*, **85**, 692–696.
46. Goh,H.S. *et al*. (1999) p53 point mutation and survival in colorectal cancer patients: effect of disease dissemination and tumour location. *Int. J. Oncol.*, **15**, 491–498.
47. Johnstone,R.W. *et al*. (2000) Multiple physiological functions for multidrug transporter P-glycoprotein? *Trends Biochem. Sci.*, **25**, 1–6.
48. Borst,P. *et al*. (2000) A family of drug transporters: the multidrug resistance-associated proteins. *J. Natl Cancer Inst.*, **92**, 1295–1302.
49. Sampath,J. *et al*. (2001) Mutant p53 cooperates with ETS and selectively up-regulates human MDR1 not MRP1. *J. Biol. Chem.*, **276**, 39359–39367.
50. Barker,N. *et al*. (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature*, **449**, 1003–1007.
51. McClanahan,T. *et al*. (2006) Identification of overexpression of orphan G protein-coupled receptor GPR49 in human colon and ovarian primary tumors. *Cancer Biol. Ther.*, **5**, 419–426.
52. Boman,B.M. *et al*. (2007) Symmetric division of cancer stem cells–a key mechanism in tumor growth that should be targeted in future therapeutic approaches. *Clin. Pharmacol. Ther.*, **81**, 893–898.

Received November 2, 2009; revised February 4, 2010; accepted February 15, 2010