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 Ku1, Young-Kyoung Shin1, Duck-Woo Kim1,2, Kyung-Hee Kim1, Jin-Sung Choi1, Sung-Hye Hong1, You-Kyung Jeon1, Sung-Hee Kim1, Hong-Sun Kim1, Jae-Hyun Park1, Il-Jin Kim1 and Jae-Gahb Park1

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

Abbreviations: CRC, colorectal cancer; FBS, fetal bovine serum; MSI, microsatellite instability.

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

These authors contributed equally to this work.

Material 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 cm² flasks. Most of the tumor cells were initially cultured in ALC-4 medium supplemented with 5% heat-inactivated fetal bovine serum (FBS) (AR5). ALC-4 is a fully defined specifically formulated for the selective growth of CRC and hepatocellular carcinoma cell lines (10,11). AR5 medium was prepared from RPMI 1640. AD5 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% CO₂ 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 × 10⁴ to 3 × 10⁵ viable cells from each cell line were seeded into 14–20 identical 25 cm² flasks and the number of viable cells was determined daily for ≥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-Mycoplasm PC Detection Kit (Intron Biotechnology, Kyonggi, Korea). To examine cell morphology, cells grown in 75 cm² 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 complementay 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 AmpFISTR identifier 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 electrophorosed on a 7 M urea polyacrylamide gel for 2 h at 60 W. Gels were dried and visualized by autoradiography (4).

Growth curves for cell lines were also determined based on data from doubling time (supplementary Figure S6 is available at Carcinogenesis Online).

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). CRC specimens from metastatic colon cancers, grew as monolayers formed dense monolayers (Figure 1A; supplementary Figure S1F, S1H, S1I, S1J and S1L is available at Carcinogenesis Online).

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

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

aDoubling curves for cell lines were also determined based on data from doubling time (supplementary Figure S6 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 homotype mutation in exon 15 of the APC gene. Arrows indicate the mutated nucleotide.

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**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 TGFβR1 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 → TGT transition mutation in codon 12. SNU-1181 had a GGT → TGT mutation and SNU-1411 had a GGT → TGT mutation. Especially, SNU-1684 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 glycine. 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_846delG 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, TGFβR1, APC and β-catenin genes**

<table>
<thead>
<tr>
<th>MSI</th>
<th>Abnormalities of MLH1 and MSH2</th>
<th>TGFβR1</th>
<th>Abnormalities of APC</th>
<th>Abnormalities of β-catenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>Exon</td>
<td>Codon</td>
<td>Nucleotide change</td>
<td>a.a. change</td>
</tr>
<tr>
<td>SNU-70</td>
<td>MSS</td>
<td>A10</td>
<td>15</td>
<td>1294 c.3880C&gt;T</td>
</tr>
<tr>
<td>SNU-254</td>
<td>MSS</td>
<td>A10</td>
<td>15</td>
<td>1487 c.4461_4463delT</td>
</tr>
<tr>
<td>SNU-479</td>
<td>MSS</td>
<td>A10</td>
<td>13</td>
<td>554 c.1660C&gt;T</td>
</tr>
<tr>
<td>SNU-977</td>
<td>MSS</td>
<td>A10</td>
<td>15</td>
<td>1363 c.4087_4090insA</td>
</tr>
<tr>
<td>SNU-1181</td>
<td>MSS</td>
<td>A10</td>
<td>13</td>
<td>554 c.1660C&gt;T</td>
</tr>
<tr>
<td>SNU-1235</td>
<td>MSS</td>
<td>A10</td>
<td>15</td>
<td>1554 c.4661_4666insA</td>
</tr>
<tr>
<td>SNU-1411</td>
<td>MSS</td>
<td>A10</td>
<td>15</td>
<td>1554 c.4661_4666insA</td>
</tr>
<tr>
<td>SNU-1460</td>
<td>MSS</td>
<td>A10</td>
<td>15</td>
<td>1554 c.4661_4666insA</td>
</tr>
<tr>
<td>SNU-1544</td>
<td>MSI</td>
<td>MLH1</td>
<td>1</td>
<td>2 c.5C&gt;T</td>
</tr>
<tr>
<td>SNU-1684</td>
<td>MSI</td>
<td>A10</td>
<td>8</td>
<td>219 c.655A&gt;G</td>
</tr>
<tr>
<td>SNU-1746</td>
<td>MSI</td>
<td>MLH1</td>
<td>1</td>
<td>2 c.5C&gt;T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MLH1</td>
<td>14</td>
<td>523 c.1567G&gt;A, Homo</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Abnormalities of p53</th>
<th>Abnormalities of K-ras</th>
<th>Mutation of codon 484 at MXR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon</td>
<td>Codon</td>
<td>Nucleotide change</td>
</tr>
<tr>
<td>SNU-70</td>
<td>4</td>
<td>72*</td>
</tr>
<tr>
<td>SNU-254</td>
<td>8</td>
<td>282</td>
</tr>
<tr>
<td>SNU-479</td>
<td>5</td>
<td>162</td>
</tr>
<tr>
<td>SNU-977</td>
<td>5</td>
<td>175</td>
</tr>
<tr>
<td>SNU-1181</td>
<td>8</td>
<td>282</td>
</tr>
<tr>
<td>SNU-1235</td>
<td>6</td>
<td>213</td>
</tr>
<tr>
<td>SNU-1406</td>
<td>4</td>
<td>72*</td>
</tr>
<tr>
<td>SNU-1411</td>
<td>4</td>
<td>72*</td>
</tr>
<tr>
<td>SNU-1460</td>
<td>4</td>
<td>72*</td>
</tr>
<tr>
<td>SNU-1544</td>
<td>W</td>
<td>13</td>
</tr>
<tr>
<td>SNU-1684</td>
<td>W</td>
<td>12</td>
</tr>
<tr>
<td>SNU-1746</td>
<td>W</td>
<td>12</td>
</tr>
</tbody>
</table>

W, wild-type.
*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 particularly 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.

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 vitro 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 our 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 50–80% 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 (SN3-254 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 MRPI (48). Both MDR1 and MRPI 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, MRPI 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 ~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. MRPI, MDR1, MXR and COX2 genes are highly expressed in 13, eight, six and seven 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/

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**References**


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