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Fetal colon cell line FHC exhibits tumorigenic phenotype, complex karyotype, and *TP53* gene mutation

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Abstract Stable cell lines obtained by spontaneous immortalization might represent early stages of malignant transformation and be useful experimental models for studies of mechanisms of cancer development. The FHC (fetal human cells) cell line has been established from normal fetal colonic mucosa. Detailed characterization of this cell line and mechanism of spontaneously acquired immortality have not been described yet. Therefore, we characterized the FHC cell line in terms of its tumorigenicity, cytogenetics, and *TP53* gene mutation analysis. FHC cells displayed capability for anchorage-independent growth in semisolid media in vitro and formed solid tumors after transplantation into SCID (severe combined immunodeficiency) mice. This tumorigenic phenotype was associated with hypotriploidy and chromosome number ranging from 66 to 69. Results of comparative genetic hybridization arrays showed that most chromosomes included regions of copy number gains or losses. Region 8q23~8q24.3 (containing, e.g., *MYC* proto-oncogene) was present in more than 20 copies per nucleus. Moreover, we identified mutation of *TP53* gene in codon 273; triplet CGT coding Arg was changed to CAG coding His. Expression of Pro codon 72 polymorphic variant of p53 was also detected. Mutation of *TP53* gene was associated with abolished induction of $p21^{Waf1/Cip1}$ and MDM-2 proteins and resistance to apoptosis after genotoxic treatment. Because of their origin from normal fetal colon and their relative resistance to the induction of apoptosis, FHC cells can be considered a valuable experimental model for various studies.

1. Introduction

Isolation of human immortalized cells and establishment of permanent cell lines from nontransformed cell populations are limited. However, it has been described in human prostate epithelial cells [1], human breast epithelial cells [2], and colon-derived fibroblasts [3]. Deregulation of several particular signaling pathways has been shown to be responsible for induction of senescence/immortalization [4]. It often includes defects associated with genome stability, epigenetic deregulation of gene expression, reactivation of telomerase activity, inactivation of cell cycle regulators, overexpression of oncogenes such as *MYC,* or viral oncogenes [4]. Mechanisms of spontaneous immortalization are not usually known. Events associated with spontaneous immortalization in breast epithelial cell line MCF10A [5–7] have been described in relative detail. This cell line shows several genomic alternations that include, for example, gain of *MYC* and *ERBB2*. Moreover, it shows increased telomerase activity and interestingly expresses wild-type p53 [7]. Cell lines established by spontaneous immortalization might represent early stages of malignant transformation and could be useful experimental models to study mechanisms of cancer development [8].

Colorectal cancer is a frequent disease in Western countries. It can be categorized according to type of genomic instability as chromosomally unstable (CIN+), characterized by aneuploid/polyploid karyotype, or as microsatellite

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unstable (MSI+), characterized by near-diploid karyotype and defective mismatch repair [9]. Approximately 40% of colorectal tumors contain point mutations in the *TP53* gene (http://www-p53.iarc.fr/Graph.asp). These point mutations are clustered in exons 4–9 with five hot spots: 273, 248, 175, 245, and 282 (http://www-p53.iarc.fr/Graph.asp). *TP53* gene product, the p53 tumor suppressor protein, is a transcription factor activated in response to stress signals, in particular to genotoxic stress. It is a crucial barrier against cancer development. Generally, detailed cell line characterization is considered necessary for validity of experimental studies. The FHC (fetal human cells) cell line has been established by Siddiqui and Chopra [10] from normal fetal colonic mucosa. This cell line has been used in various studies focusing on mechanisms of cancer progression, regulation of differentiation, and apoptosis [11–16]. However, to our knowledge, detailed characterization of this cell line has not yet been published. In this study, we present cytogenetic, array comparative genetic hybridization (CGH), and *TP53* gene characterization of this cell line.

2. Materials and methods

2.1. Cell lines, cultivation, and treatment

FHC was obtained from the American Type Culture Collection (CRL-1831, LGC Standards, Lomianki, Poland). Cells were cultured in 1:1 mixture of Ham F-12 medium and Dulbecco modified Eagle medium (Gibco, Invitrogen) supplemented with 25 mmol/L HEPES (N-[2-hydroxyethyl] piperazine-N′[2-ethanesulfonic acid]), 10 ng/mL cholera toxin (Calbiochem, Merck, Darmstadt, Germany), 0.005 mg/mL insulin, 0.005 mg/mL transferrin, 100 ng/mL hydrocortisone (all from Sigma-Aldrich, St. Louis, MO), and 10% fetal bovine serum (PAA, Pasching, Austria). HCT-116 p53+/+ and HCT-116 p53−/− colon carcinoma cells were obtained from B. Vogelstein (Johns Hopkins University, Baltimore, MD) and cultivated in McCoy media (Gibco, Invitrogen, Carlsbad, CA) with 2 mmol/L L-glutamine, streptomycin (0.1 mg/mL), and penicillin (100 U/mL) and supplemented with 10% fetal bovine serum (PAA). All cell lines were cultivated in Falcon (BD, San Jose, CA) and/or TPP (Trasadingen, Switzerland) cultivation dishes, flasks, and plates in a humidified incubator at 37 °C in an atmosphere of 5% CO_2. For induction of DNA damage, the cells were treated either by irradiation by a single 20-Gy dose with a γ-ray source (^{60}Co; Chisostat, Chirana, Prague, Czech Republic) or by dipenzo[a,l]pyrene (DBalP; Sigma-Aldrich, St. Louis, MO, 25 and 100 mmol/L).

2.2. Clonal growth of cells in semisolid media

For anchorage-independent clonal growth, a semisolid media colony-forming assay was performed in six-well plates. Each well contained 2 mL of 0.5% agar in complete medium as the bottom layer. The top layer contained 1 mL of 0.33% agar in complete medium and various concentrations (5×10^3, 5×10^2, 5×10^1) of FHC or HCT-116 p53+/+ cells. One milliliter of complete medium was added, and the cells were incubated at humidified incubator at 37 °C in an atmosphere of 5% CO_2 for up to 15 days. Fresh complete medium was added once a week. Live colonies were stained with 10 μL of the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 2.5 mg/mL in phosphate-buffered saline [PBS]; Sigma-Aldrich, St. Louis, MO) for 4 hours.

2.3. Flow cytometry

Surface expression of carcinoembryonic antigen (CEA, CD66e) was analyzed by flow cytometry (BD FACSCalibur, 488 nm argon–ion laser) after labeling with fluorescein isothiocyanate (FITC)–conjugated anti-CD66e antibody (MCA1744F, Serotec) or relevant isotype control (IgG_1–FITC). More than 1.5×10^4 cells were acquired per sample, and median fluorescence index was determined by CELLQuest Pro (BD). Cell debris and dead cells were excluded from analysis.

2.4. Detection of apoptosis

The samples for apoptotic cell quantification (nuclear morphology analysis) were washed with PBS, fixed in 70% ethanol for 30 minutes at 4 °C, and stained with DAPI (4′6-diamidino-2-phenylindole dihydrochloride; Fluka, Sigma-Aldrich, final concentration of 1 μg/mL for 5 minutes). After incubation, cells were centrifuged, mixed in MOWIOL 4-88 solution (polyvinyl alcohol, CAS No. 9002-89-5, Calbiochem, Merck, Darmstadt, Germany) with DABCO (1,4-diazabicyclo-[2.2.2]octane, 0.6%, Sigma-Aldrich), and mounted under coverslips. Apoptotic bodies incidence was evaluated by fluorescent microscopy (Olympus IX-70). The apoptotic index was based on counting more than 200 cells.

2.5. Electrophoresis and Western blot analysis

Subconfluent cells were washed twice in PBS and collected into RIPA buffer (50 mmol/L Tris–HCl pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mmol/L NaCl, protease and phosphatase inhibitor cocktail). The cell lysates were sonicated and spun. Equivalent quantities of protein (20 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis using established procedures. For detection of cytokeratins (CKs), 10 μg of proteins was used. Proteins separated by electrophoresis were electrotransferred with a TE22 Mini Transfer Tank (Hoefer, Holliston, MA) onto polyvinylidene diflouride membranes (Millipore, Billerica, MA). Membranes were blocked in Tris-buffered saline (TBS; 20 mmol/L Tris–HCl pH 7.6, 150 mmol/L NaCl) containing 0.1% Tween 20 and 5% nonfat milk for 1 hour. The blots were then washed with TBS–Tween, and incubated

with anti-pan-cytokeratin (Exbio), anti-cytokeratin 18 (Exbio), anti-p53 (sc-126, Santa Cruz, Santa Cruz, CA), anti-phospho-p53 (Ser15) (9284, CST), anti-phospho-H2A.X (Ser139) (9718, CST), anti-p21 (sc-397, Santa Cruz), or anti-MDM-2 (sc-965, Santa Cruz) primary antibodies overnight at 4 °C. The membrane was extensively washed in TBS–Tween and then incubated with secondary antibodies conjugated with horseradish peroxidase (HRP) for 1 hour. Detection of antibody reactivity was performed by Immobilon Western HRP Substrate (Millipore) and visualized on x-ray films (Agfa, Prague, Czech Republic). Equal sample loading was verified with immunodetection of β-actin (A5441, Sigma-Aldrich, St. Louis, MO).

2.6. Xenografts of FHC cells in SCID mice and histochemistry

Immunodeficient (NOD/NCrCrl-Prkdc^{scid}) female mice (12 weeks old) were purchased from Charles River Laboratories (Wilmington, MA) and were maintained under germ-free conditions. The use and treatment of these animals followed the European Community Guidelines as accepted principles for the use of experimental animals. The experiments were performed with the approval of the Institute Ethical Committee. FHC cells (5×10^7) were subcutaneously ($n = 8$) or intraperitoneally ($n = 8$) injected into SCID (severe combined immunodeficiency) mice. Clinical symptoms and animal survival were assessed daily; growth of subcutaneous tumors was assessed twice a week by measurement of the largest (a) and smallest (b) diameters, and tumor volume (V) was calculated by the following equation: $V = a \times (b)2/2$. Moribund animals or mice bearing tumors of $>20\%$ of body weight were humanely killed.

Histological analysis of solid subcutaneous tumors was performed after standard hematoxylin–eosin staining of sections of formaldehyde-fixed, paraffin-embedded tissues.

2.7. Chromosome preparation

Metaphase spreads were prepared by using standard conditions of 45 minutes' Colcemid treatment (KaryoMax, Invitrogen), followed by 20 minutes' incubation in hypotonic 0.075 mol/L KCl at 37 °C and multiple changes of Carnoy fixative. Cells were dropped onto slides and stained with trypsin and Giemsa stain (GTG banding).

2.8. DNA isolation and CGH array

DNA was isolated from one 60-mm dish of cultured cells (passages 13 and 40) by incubation overnight at 55 °C in 3 mL of single-strength Tris–EDTA (ethylenediaminetetra-acetic acid) buffer (pH 7.5) supplemented with 0.5% sodium dodecyl sulfate and 0.1 μg/μL proteinase K (Sigma-Aldrich, St. Louis, MO), followed by ethanol precipitation. The DNA was dissolved in 100 μL of water.

Genomic DNA (800 ng) was labeled by random priming in a 25-μL reaction (Bioprime DNA labeling system, Invitrogen) to incorporate Cy3 or Cy5 deoxycytidine triphosphate. Labeled test DNA (FHC cell line) and reference DNA (isolated from white blood cells of a healthy donor) were precipitated together with 100 μg of human Cot 1 DNA (Roche Diagnostics) and resuspended in 50 μL of hybridization buffer consisting of 50% formamide, 10% dextran sulfate (molecular weight ~500,000, Fisher Scientific, Pittsburgh, PA), 2× standard saline citrate (SSC), 4% sodium dodecyl sulfate, and water. The hybridization solution was denatured at 72 °C and then incubated at 37 °C for 60 minutes to allow preannealing of Cot-1 DNA. Before applying the hybridization mixture containing labeled DNA to the microarrays, the hybridization buffer without labeled DNA was used to prewet slides. Hybridization to arrays of 2464 bacterial artificial chromosome (BAC) clones [HumArray3.2, UCSF Comprehensive Cancer Microarray Core (San Francisco, CA)] was performed for approximately 48 hours at 37 °C on a rocking table. After hybridization, excess of hybridization mixture was rinsed off with PN buffer (0.1 mol/L Na_2HPO_4/NaH_2PO_4 pH 8.0, 0.1% NP-40), and the arrays were washed at 45 °C with 50% formamide buffered with 2 × SSC for 15 minutes followed by incubation in PN buffer for 10 minutes at room temperature. The arrays were scanned with an Agilent scanner (Agilent Technologies, Santa Clara, CA), and images were quantified by UCSF Spot and Sproc software [17]. The estimate of experimental variability (ESD) was calculated as a standard deviation of $\log_2$ ratios of all BAC clones on chromosomes 1, 2, 9, 10, 13, and 16. Significant gains and loses were defined as regions of at least three consecutive $\log_2$ ratios, all above or below 1.5 times the estimate of experimental variability.

2.9. Sequencing of the TP53 locus

Mutation in *TP53* gene was checked by sequencing cDNA and genomic DNA (amplified exons 7 and 8). cDNA was prepared with 1 μg of total RNA by High-Capacity cDNA Reverse Transcription kits (Applied Biosystems, Foster City, CA). polymerase chain reaction (PCR) fragments and cDNA were sequenced by VBC-Biotech Service (Vienna, Austria).

2.10. Evaluation of MYC gene status

Status of chromosome 8 and *MYC* gene was confirmed by fluorescence in situ hybridization (FISH), performed with directly labeled probes: MYC (SpectrumOrange; IntellMed, Prague, Czech Republic) combined with centromeric probe for chromosome 8 (SpectrumGreen; IntellMed).

Metaphase spreads were codenatured with the probes for 1 minute at 75 °C and hybridized at 37 °C overnight. In the next step, slides were washed in the wash buffer I (0.4 × SSC, 0.3% NP40, pH 7.0, 73 °C) for 2 minutes, and then in the wash buffer II (2 × SSC, 0.1% NP40, pH 7.0, room temperature) for 30 seconds. Dried slides were counterstained by DAPI II (Vysis) and evaluated by fluorescence microscopy.

3. Results

3.1. Cytogenetics and array CGH analysis

Karyotype analysis of FHC cell line was made by conventional staining with Giemsa (GTG-banding) at passage 13 and 40; a representative karyotype is shown in Figure 1A. FHC is a hypotriploid cell line with a chromosome number ranging from 66 to 69. With regard to the complex rearrangements present in all tested metaphase spreads, karyotyping was supplemented with array comparative genomic hybridization analysis (Fig. 1B). With the exception of few chromosomes (1, 2, 10, and 16), all the other chromosomes included regions of copy number gains or losses (cells contain more or fewer than three copies). Although G-banding analysis showed hypotriploid cell line, FISH assay revealed that the region 8q23~8q24.3 (coding, e.g., *MYC* proto-oncogene) is present in more than 20 copies on isochromosome 8. The second chromosome did not show amplification of *MYC* oncogene (Fig. 2). There was no difference between copy number profiles for FHC cells at passages 13 and 40.

3.2. TP53 gene mutation analysis

By sequencing of PCR products of exons 8 and 7 and p53cDNA, we identified a mutation of *TP53* gene in codon 273; triplet CGT coding Arg was changed to CAG coding

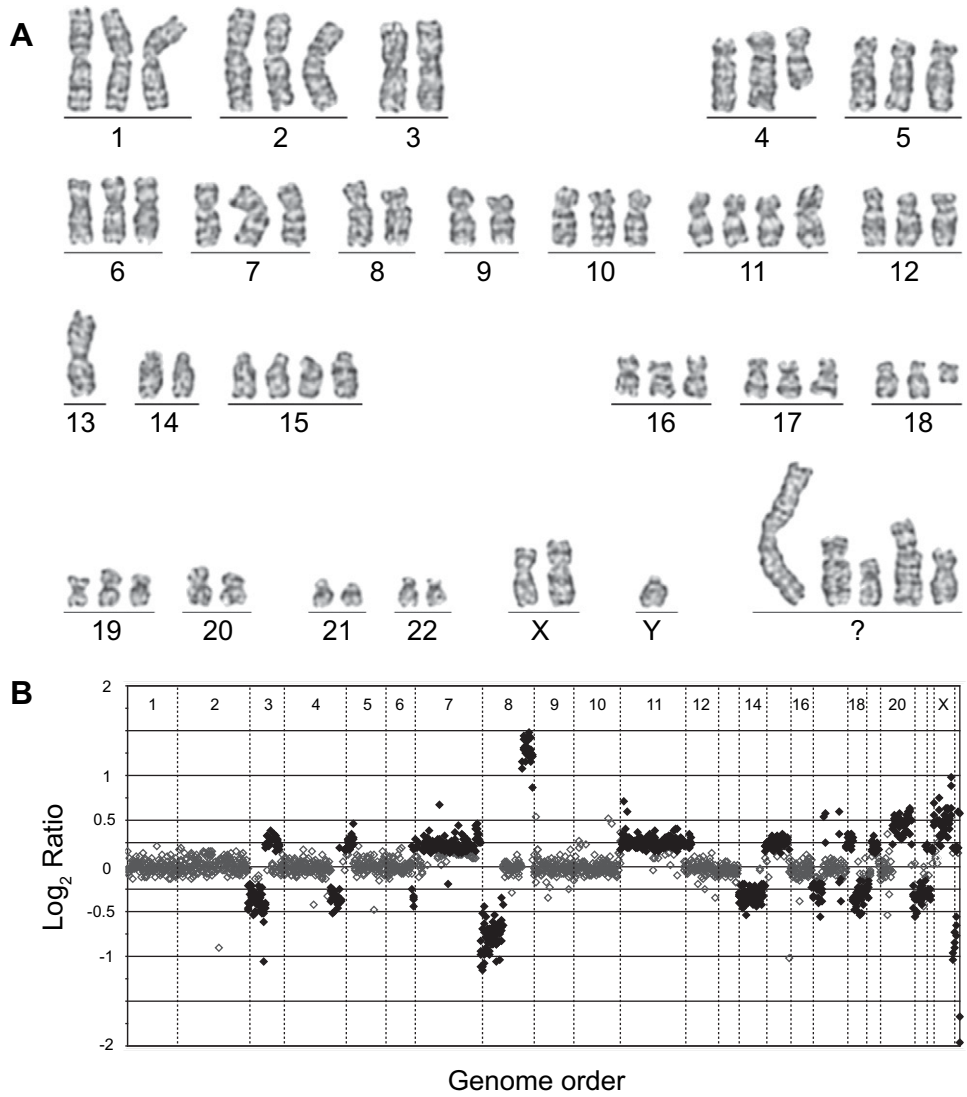

Fig. 1. (A) Trypsin-Giemsa staining for identifying individual human chromosomes in FHC (fetal human cells) cell line. All metaphase spreads screened (10 in total) contained characteristic chromosome rearrangements, which are highlighted in the representative karyogram [67,XXY,−3,del(4)(q28),add(6)(q?23),−8,−9,+11,−13,i(13)(q10),−14,+add(15)(q26),del(18)(q12),−20,−21,−22,+5mar]. (B) Array comparative genetic hybridization analysis of FHC cell line. The $\log_2$ ratios of BAC clones are shown, ordered according to position on the May 2004 freeze of the human genome sequence (UCSC Genome Browser). Closed diamonds, data points called above or below a threshold of 1.5xESD indicating areas of DNA copy gain or loss, detected across the genome; open diamonds, noncalled data points.

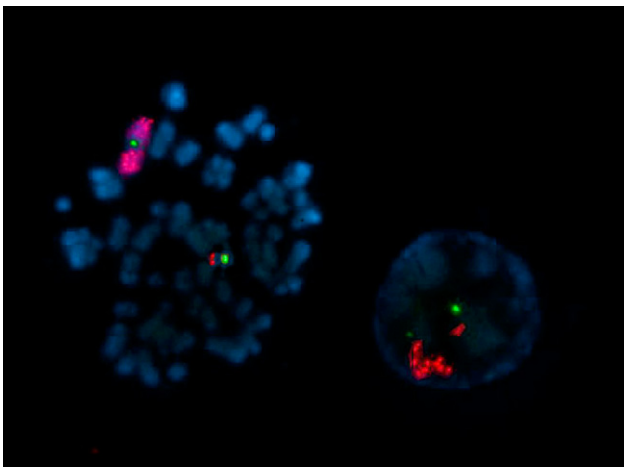

Fig. 2. Fluorescent in situ hybridization evaluation of *MYC* gene (SpectrumOrange) and chromosome 8 (SpectrumGreen) status revealed isochromosome 8, i(8q), with amplification of *MYC* gene. The second chromosome 8 bears only two copies of the *MYC* gene.

His (Fig. 3A). There were no more mutations in the remaining sequenced part of the *TP53*. We also analyzed polymorphism of the *TP53* gene in FHC cells. Our results demonstrate that p53 has Pro residue (CCC) at codon 72 (Fig. 3B).

3.3. DNA damage induced signaling pathways and apoptosis

After identification of mutation of *TP53* in FHC cells, our next step was to find out whether dysfunctions are expressed in p53-regulated genes after genotoxic stress. We treated FHC, HCT-116 p53+/+, and HCT-116 p53−/− cells with DBalP (25, 100 mmol/L) or γ radiation (20 Gy) and analyzed protein levels 24 hours after treatment (Fig. 4A). Phosphorylation of histone H2A.X on Ser139 in response to genotoxic treatments was detected in all cell lines. Similarly, phosphorylation of p53 on Ser15 was detected in both p53-expressing cell lines (FHC and HCT-116 p53+/+) after γ radiation and DBalP treatment. However, significant induction of $p21^{Waf1/Cip1}$ and MDM-2 proteins was detected only in control cells with wild-type p53 HCT-116 p53+/+. The number of apoptotic cells was determined by analysis of nuclear morphology 48 hours after genotoxic treatments. DBalP and γ radiation induced apoptosis predominantly in HCT-116 p53+/+ cells but not in HCT-116 p53−/− or FHC cell lines (Fig. 4B). We conclude that the defect in the induction of p53 target genes after genotoxic stress is associated with relative resistance to apoptosis induction in FHC cells. Thus, these cells demonstrate similar phenotype, as in the p53-negative clone of HCT—116 cells.

3.4. Expression of CEA and CKs

The CEA gene family represents a large number of different genes, including CD66e (CEACAM5) [18]. It has been described that some members of CEA have structural features of the immunoglobulin superfamily and functions similar to the cadherin family of cell adhesion

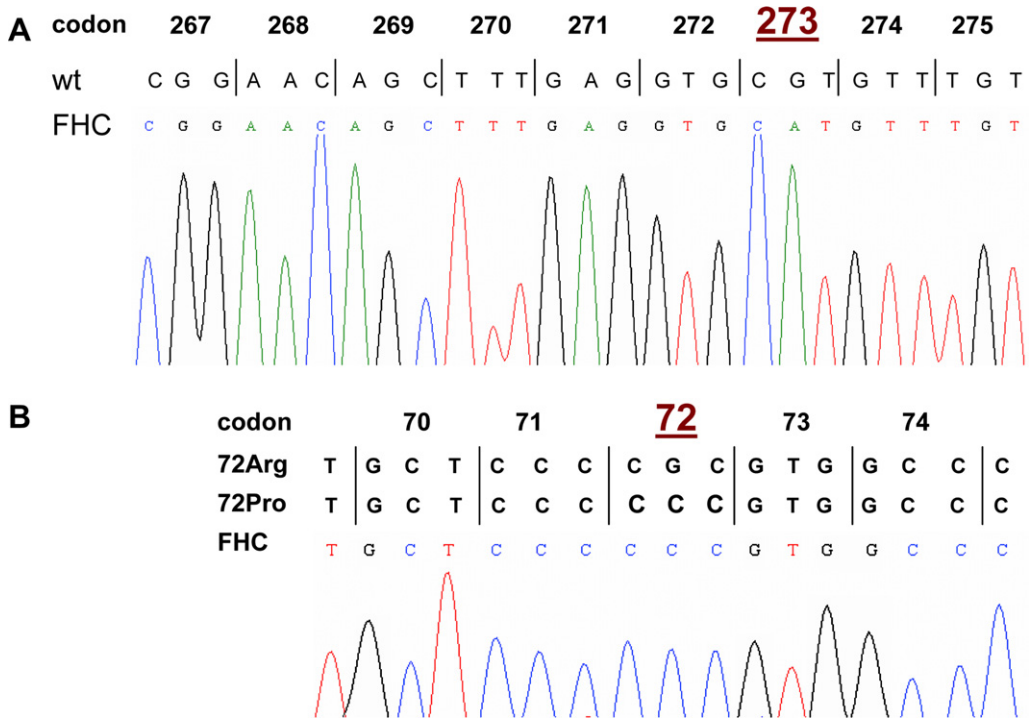

Fig. 3. Mutation and 72 codon analysis of *TP53* gene in FHC (fetal human cells) cell line. (A) Visualization of sequencing data of part of exon 8 of *TP53* gene obtained by sequencing of polymerase chain reaction product from genomic and cDNA. (B) The p53 cDNA sequencing of part of exon 4 shows expression of Pro (CCC) variant of 72 codon of p53 in FHC cells.

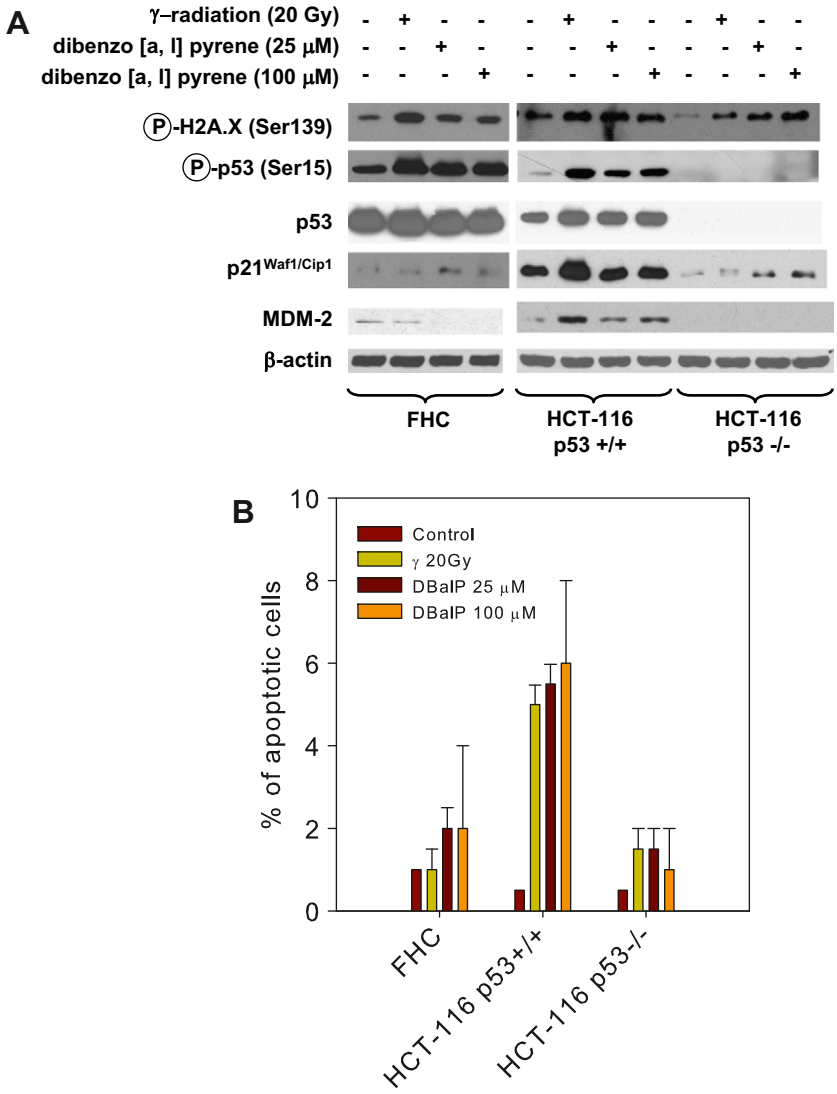

Fig. 4. Genotoxic treatment of FHC cells leads to phosphorylation of p53 at Ser15, but is nevertheless not associated with increase of p21$^{Waf1/Cip1}$ expression. FHC (fetal human cells) and HCT—116 p53−/− cells are relatively resistant to the induction of apoptosis, compared to HCT-116 p53+/+ cells. FHC, HCT-116 p53+/+, and HCT-116 p53−/− cells were irradiated (20 Gy) and cultivated for the next 24 hours or treated by dipenzo[a,l]pyrene (DBalP; 25 and 100 mmol/L) for 24 hours. (A) Expression of phospho-H2A.X (Ser135), phospho-p53 (Ser15), total p53, p21$^{Waf1/Cip1}$, and MDM-2 protein was analyzed by Western blot test. Detection of β-actin served as loading control. (B) Nuclear morphology characteristic for apoptotic cells was analyzed by fluorescent microscopy 48 hours after treatment of the cells.

molecules; their expression correlates with tumor invasion and stage of colorectal carcinoma [19]. Here, we analyzed surface expression of CD66e in FHC, HCT-116 p53+/+, and HCT—116 p53−/− cells. Our results show that FHC cells express approximately four times more CD66e in comparison to HCT-116 cells (Figs. 5A and 5B). There were no differences in expression of CD66e in HCT-116 wild-type and knockout clones because both HCT-116 p53+/+ and HCT-116 p53−/− were negative. Western blot detection of CKs with pan-reactive antibody and cytokeratin 18−specific antibody showed no difference in expression of CKs in both HCT-116 clones and FHC cells (Fig. 5C). Moreover, pattern of CKs expression and level of CK18 remained unchanged during FHC cells' cultivation between passages 5 and 23.

3.5. Growth in semisolid media and tumor formation in vivo

To characterize FHC cells in the term of tumorigenicity, we first determined their potential for anchorage-independent growth in semisolid agar media (Fig. 6A). FHC cells formed visible colonies after 15 days of cultivation. The number of colonies was higher than in the HCT-116 cancer cell line, which was used as a positive control for this assay. Moreover, both subcutaneous and intraperitoneal injection of FHC cells to immunodeficient SCID mice revealed their potential to form tumors in vivo. Figure 6B demonstrates aggressive formation of solid tumors in 40 days after subcutaneous injection to mice. Both subcutaneous and intraperitoneal injections of FHC cells resulted in the death of all animals

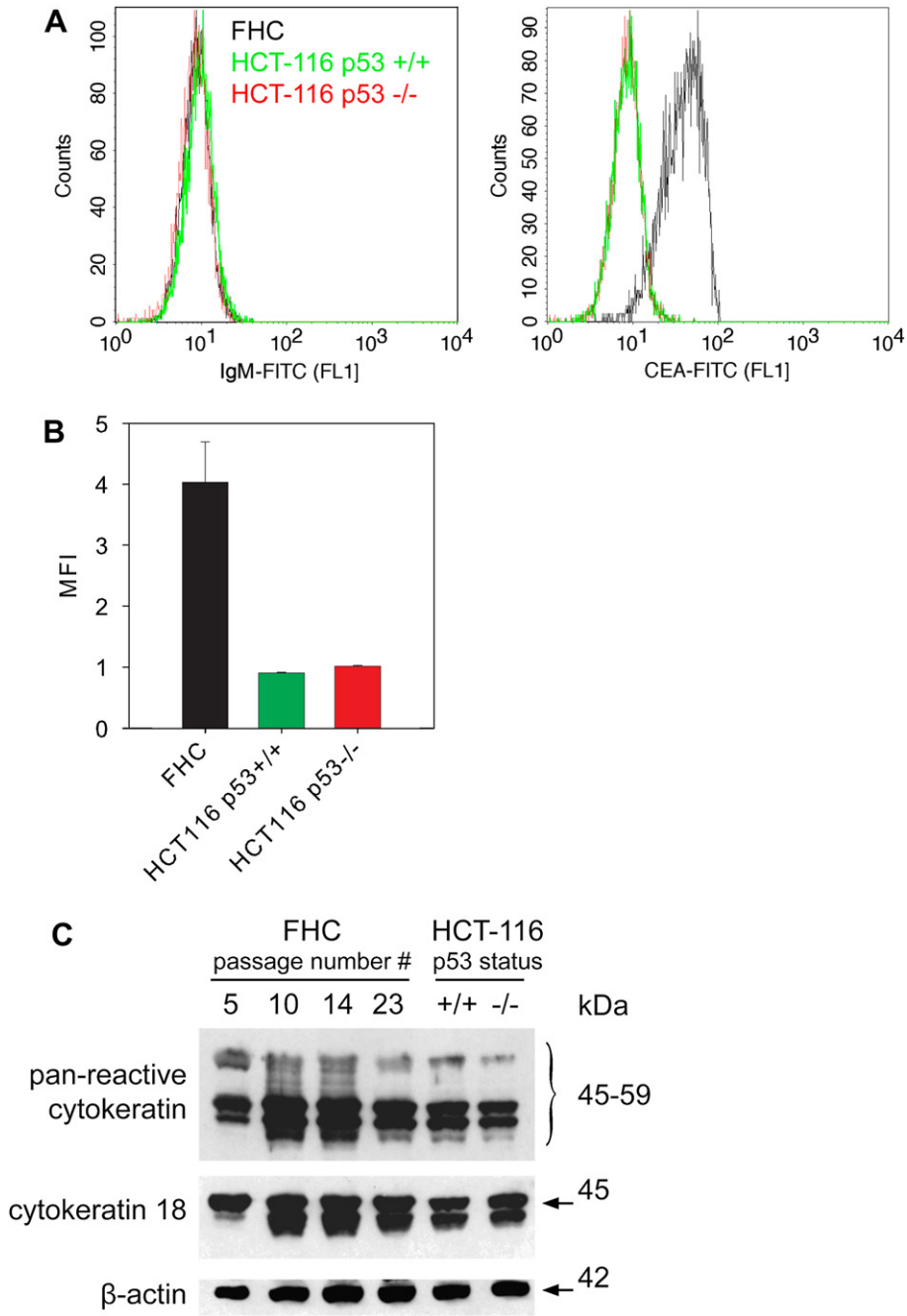

Fig. 5. Carcinoembryonic antigen (CEA, CD66e) is expressed on the surface of FHC (fetal human cells), but not HCT-116 cells. However, all cell lines express the epithelial marker cytokeratin 18. (A) Data represent the typical histogram plots of samples stained with monoclonal antibodies anti-CD66e-FITC or isotype control and analyzed by flow cytometry. (B) Bars represent the median ± standard deviation of median intensity index (MFI) of flow cytometric measurements. (C) The expression of cytokeratins and cytokeratin 18 (CK18) in various passage numbers of FHC cells was analyzed by Western blot test by pan-reactive anti-cytokeratin antibody or monoclonal anti-CK18 antibody, respectively. HCT-116 p53 wild-type and knockout cells were used as positive controls. Data represent typical results of three independent experiments.

within 67 days (Fig. 6C) as a result of tumor progression. Necroscopies were carried out on all animals; tumor was always progressing locally and invading surrounding tissues and/or organs, but without evidence of distant metastases. Histologically, subcutaneous FHC tumors were present as invasive high-grade adenocarcinomas with polymorphic nuclei, frequent mitotic figures, and extensive necrosis (Fig. 6D).

4. Discussion

We analyzed tumorigenic characteristics and molecular cytogenetic features of spontaneously immortalized cell line FHC isolated from normal fetal colonic mucosa. FHC cells exhibit epithelial morphology and were originally established by Siddiqui and Chopra [10]. Basic

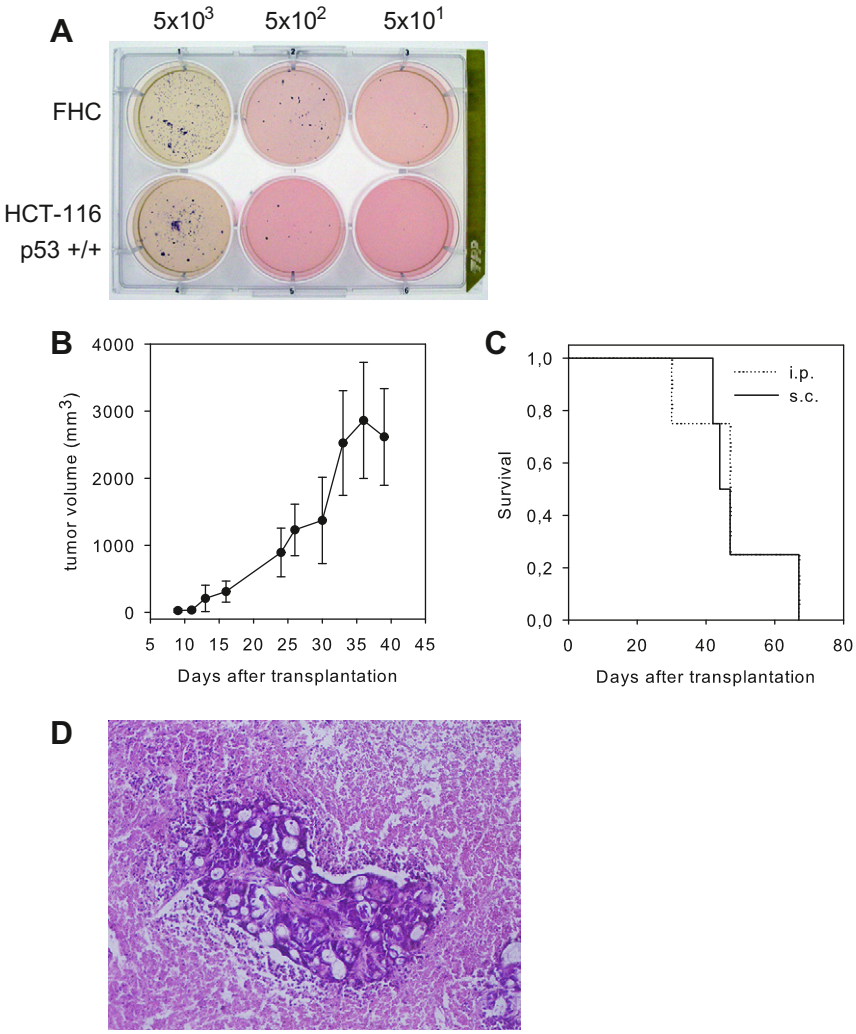

Fig. 6. Cells of the FHC (fetal human cells) cell line grow in semisolid media in vitro and form aggressive solid tumors in vivo. (A) FHC cells were seeded in various concentrations on semisolid agar as described in Materials and Methods. Live colonies were stained by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium salt after 15 days of cultivation and photographed. HCT-116 cells were used as positive control. (B) SCID (severe combined immunodeficiency) mice were subcutaneously injected with 5×10^7 of FHC cells in cultivation media. Tumor volume was measured twice a week. (C) SCID mice were injected subcutaneously (s.c.) or intraperitoneally (i.p.) with 5×10^7 FHC cells. Data represent survival analysis. (D) Histological analysis of solid tumors formed after subcutaneous injection of FHC cells. Tumor is present as invasive high-grade adenocarcinoma with extensive necrosis.

characteristics of these cells have been published [10]; however, other detailed molecular cytogenetic characteristics are missing. Through their origin from normal colonic mucosa and capability to differentiate after various treatments, this cell line might represent a unique experimental model.

FHC cells exhibit a large number of chromosomal aberrations, which is characteristic for a group of chromosomally unstable (CIN+) colorectal cancer cell lines [20]. For example, gain of chromosome 20 and 8q24.21, and loss of chromosome 18q and 8 p have been previously reported by other authors as representative chromosomal changes for CIN+ cell lines [21]. Moreover, p53 mutation accompanied by loss of 17p13.1 is also a common feature in CIN+ colorectal cell lines [22] and enables cells to acquire additional chromosomal defects. Therefore, we hypothesize that most of the chromosomal changes found in this cell line might more likely be a consequence of a generally unstable karyotype. In addition to genomic instability and p53 mutation, other critical factors important for cell immortalization have been observed. There are several connections between p53 inactivation and telomerase activity. Telomerase plays a crucial role in process of cellular immortalization [23]. Wild-type and mutant p53 proteins play opposite roles in the regulation of hTERT (human telomerase reverse transcriptase) expression. Repression of hTERT by endogenous wtp53 is mediated by p21 and E2F [24]. Several hot spot mutations of p53 (including R273H) activate hTERT promotor and hTERT expression [25]. Previously, we described telomerase reactivation in FHC cells [16]. But telomerase activity of FHC cells was more sensitive to sodium butyrate treatment in comparison to adenocarcinoma cell line HT-29

(also with R273H mutation of *TP*53), and these cells produced differently spliced mRNA of hTERT [16].

Another activator of hTERT transcription and genomic instability is the MYC protein [26]. By array CGH, we found amplification of *MYC* proto-oncogene coding sequence located at 8q23~8q24.3 in FHC cells. It is possible that overexpression of MYC during initial stages of immortalization helped induce genomic destabilization and predispose cells to tumorigenesis [27]. By sequencing of PCR products of exon8 and p53cDNA, we identified *TP53* mutation R273H in FHC cells. Codon 273 is the most frequently mutated *TP53* codon in colorectal cancer (http://www-p53.iarc.fr/Graph.asp). Together with increased levels of CEA, p53 overexpression and mutation belong to typical colorectal tumor markers and indicate poor prognosis [28]. Expression of mutant p53 is connected with abolishment of tumor suppressor functions [29]. In contrast to HCT116 (p53+/+) cells, genotoxic treatment with γ irradiation or DBalP did not induce p53 target genes p21 and MDM2. FHC cells were also more resistant to apoptosis. Generally, it is accepted that mutant p53 exerts a gain of oncogenic function (resistance to genotoxic treatment, activation of proliferation, tumorigenicity) [30]. In comparison to HCT116 (p53−/−), and in agreement with the observation of others [31], we suppose that resistance to induction of p21, MDM2, and apoptosis can be connected with mutant p53 ability to block p73 substitution function of the p73 protein. It has been shown in several studies that expression of mutant p53 is connected to tumorigenicity [30]. It is in good agreement with our observation of higher potential of cells for anchorage-independent growth of FHC cells in comparison to HCT116 (p53+/+) cells and potential to form tumors in vivo. It was shown that p53 has two major polymorphic variants at codon 72; they differ in p53 functional activity [32]. For example, Arg72 variant of wild-type p53 induces apoptosis markedly better than Pro72. Genotype of codon 72 of p53 may alter susceptibility to colorectal tumor [33].

So far, it is not fully clear whether the Pro-carrying genotypes, which also occur in investigated FHC cells, are associated with a higher risk of cancer incidence [34,35]. Interestingly, Schneider-Stock and coworkers [36] reported that association with malignant potential for colorectal cancer is connected to preferential loss of Pro72 or mutation of Arg72 allele. To further characterize phenotype of FHC cells in more detail, we analyzed CEA surface expression and pattern of expression of CKs by pan-CKs-specific antibody and by CK18-specific antibody. CKs belong to the intermediate filaments subfamily expressed dominantly in epithelial tissues. Pattern of CKs and CK18 expression in FHC cells was similar to expression in HCT—116 colon cancer epithelial cells. CD66e (CEACAM5) belongs to the CEA gene family [18]. Expression of CEA is often deregulated in cancer cells and correlates with tumor invasion and stage of the patients with colorectal carcinoma [19]. Significantly higher basal expression of CD66e in FHC cells in comparison to HCT-116 cells could be associated with their normal origin. It has been demonstrated that normal glandular cells from colon and rectum show very high positivity for CD66e expression [37].

We conclude that in contrast to the nontumorigenic fetal origin of FHC cells, these cells represent a highly tumorigenic cell line with numerous genomic abnormalities, as well as an ability to grow in semisolid media and to form invading solid tumors in SCID mice. Through their origin from normal fetal colon and relative resistance to the induction of apoptosis, FHC cells are a valuable experimental model.

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