ELSEVIER

Cancer Genetics and Cytogenetics 155 (2004) 119–131

Cancer Genetics and Cytogenetics

Genome signatures of colon carcinoma cell lines

Kristine Kleivi[a], Manuel R. Teixeira[a,b], Mette Eknæs[a], Chieu B. Diep[a], Kjetill S. Jakobsen[c], Richard Hamelin[d], Ragnhild A. Lothe[a,*]

[a]*Department of Genetics, Institute for Cancer Research, The Norwegian Radium Hospital, Ullernschauseen 70, Oslo N-0310, Norway*
[b]*Department of Genetics, Portuguese Oncology Institute, Porto, Portugal*
[c]*Division of Cell and Molecular Biology, Biological Institute, University of Oslo, Norway*
[d]*INSERM, Paris, France*

Received 28 January 2004; received in revised form 22 March 2004; accepted 25 March 2004

Abstract In cancer biology, cell lines are often used instead of primary tumors because of their widespread availability and close reflection of the in vivo state. Cancer is a genetic disease, commonly caused by small- and large-scale DNA rearrangements. Therefore, it is essential to know the genomic profiles of tumor cell lines to enable their correct and efficient use as experimental tools. Here, we present a comprehensive study of the genomic profiles of 20 colon cancer cell lines combining conventional karyotyping (G-banding), comparative genomic hybridization (CGH), and multicolor fluorescence in situ hybridization (M-FISH). Major differences between the microsatellite instability (MSI) and chromosome instability (CIN) cell lines are shown; the CIN cell lines exhibited complex karyotypes involving many chromosomes (mean: 8.5 copy number changes), whereas the MSI cell lines showed considerably fewer aberrations (mean: 2.6). The 3 techniques complement each other to provide a detailed picture of the numerical and structural chromosomal changes that characterize cancer cells. Therefore, 7 of the cell lines (Colo320, EB, Fri, IS2, IS3, SW480, and V9P) are here completely karyotyped for the first time and, among these, 5 have not previously been cytogenetically described. By hierarchical cluster analysis, we show that the cell lines are representative models for primary carcinomas at the genome level. We also present the genomic profiles of an experimental model for tumor progression, including 3 cell lines (IS1, IS2, and IS3) established from a primary carcinoma, its corresponding liver- and peritoneal metastasis from the same patient. To address the question of clonality, we compared the genome of 3 common cell lines grown in 2 laboratories. Finally, we compared all our results with previously published CGH data and karyotypes of colorectal cell lines. In conclusion, the large variation in genetic complexity of the cell lines highlights the importance of a comprehensive reference of genomic profiles for investigators engaged in functional studies using these research tools.

1. Introduction

Tumor cell lines are an important resource in the role of understanding cancer initiation and progression. For most studies using cancer cell lines, information regarding their genomes is relevant, sometimes indispensable, to understand the biological events behind carcinogenesis. This is because they have chromosomal changes with potential effect at the molecular level, such as altered gene expression and regulation. Thus, it is surprising that many cell lines are left undefined with regard to their genomic profile, either by conventional karyotyping or fluorescence in situ hybridization (FISH)-based screening techniques. The combined use of karyotyping and molecular cytogenetic techniques is even more unusual, despite of the fact that the complexity of genomic rearrangements often requires such an approach to be able to describe it accurately.

Although the introduction of banding techniques [1] enabled the identification of chromosomes and chromosomal rearrangements, some marker chromosomes remain unidentified in complex karyotypes. Two main FISH-based screening techniques are now used to complement conventional karyotyping. The first technique, comparative genomic hybridization (CGH), gives an average genomic profile of

* Corresponding author. Tel.: +47-22-93-44-15; fax: +47-22-93-44-40.
E-mail address: rlothe@radium.uio.no (R.A. Lothe).

0165-4608/04/$ – see front matter
doi:10.1016/j.cancergencyto.2004.03.014

copy number gains and losses for all chromosomes in a single experiment, but it is unable to provide information on balanced chromosomal rearrangements [2]. The second methodology, based on simultaneous painting of all chromosomes, of which spectral karyotyping (SKY) [3] and multicolor fluorescence in situ hybridization (M-FISH) [4] are the most commonly used variants, is ideal for detecting interchromosomal rearrangements, but somewhat less effective for intrachromosomal changes.

The genetic aberrations in primary colorectal carcinomas are, as in many other human cancers, numerous and nonrandom [5,6]. The majority of primary colorectal carcinomas develops through the chromosome instability cell line (CIN) pathway and is characterized by aneuploidy with the presence of many numerical and structural cytogenetic abnormalities. About 15% show near-diploid indices but exhibit genome-wide instability at the nucleotide level. This is caused by a defect in the mismatch repair system that gives rise to the microsatellite instability (MSI) phenotype [7–10], which is also characteristic of 90% of tumors from patients with the hereditary non polyposis colon cancer syndrome (HNPCC) [11,12].

Here, we describe the genomic profiles of 20 colon cancer cell lines (11 with microsatellite stable (MSS) and 9 with MSI phenotype), combining the results obtained by 3 screening techniques. Some of the cell lines have not previously been cytogenetically described, and others are completely described for the first time in this study. The large differences in the genomic profiles among cell lines from the same tumor type demonstrate the importance of this knowledge when using cell lines as experimental tools. In addition, we add data from previous publications of CGH for colon cancer cell lines since the initial publication of this method [2]. For the commonly used colon cancer cell lines within our dataset, we also compare previously published karyotypes and identify the "core aberrations" for each cell line.

2. Materials and methods

Twenty different colon cancer cell lines, and 2 variants from 3 of them were included in this study. Information regarding their origin, *TP53* mutation, and MSI statuses is presented in Table 1 and in Gayet et al. [13]. Nine cell lines are known to exhibit MSI and 2 of these had a *TP53* mutation. None of the MSI cell lines showed loss of heterozygosity at chromosome arm 17p. The remaining 11 cell lines were MSS and 10 of these had both a *TP53* point mutation and loss of heterozygosity (LOH) of 17p sequences, whereas one cell line showed only LOH. Three of the cell lines were obtained from 2 laboratories to study pair-wise variability of the genetic pattern.

The cell lines Isreco1 (IS1), Isreco2 (IS2), and Isreco3 (IS3) were derived from the same patient, from a primary colon carcinoma, its corresponding liver- and peritoneal metastases [13,14]. The other 20 cell lines listed in Table 1 are derived from human primary colon carcinomas [13].

The copy number changes of the MSS cell lines were compared with 5 primary colon carcinomas (5TII, 6TI, 19T, 26P, 93P, and 136PIII) previously published [15].

2.1. Chromosome banding analysis

The cells were short-term cultured and analyzed cytogenetically as described by Pandis et al. [16]. Cells were cultured in coated 25 cm^2 flasks with RPMI 1640 growth medium supplemented with L-glutamine (Invitrogen, Pailey, Scotland), and harvested after 5–8 days. The cultures were treated with Colcemid (Invitrogen, Grand Island, NY) and trypsinised for 3–6 minutes. The cells were then exposed to a hypotonic solution treatment (0.05 M KCl) followed by fixation in 3:1 methanol/acetic acid. G-banding was obtained using Wright's stain.

2.2. Comparative genomic hybridization (CGH)

CGH was performed according to the method initially described by Kallioniemi et al. [2] using modifications as previously reported [17]. Tumor and reference DNAs (peripheral blood) were extracted using a standard phenol/chloroform procedure (nucleic acid extractor, Model 340A; Applied Biosystems, Foster City, CA). Tumor DNA was labeled by nick translation with Fluorescein-12-dCTP and Fluorescein-12-dUTP, and normal reference DNA was labeled with Texas Red-6-dCTP and Texas Red-6-dUTP (New England Nuclear, Boston, MA). Labeled tumor and reference DNA (800 ng each) were mixed together with 20 µg Cot-1 DNA (Life Technologies, Rockville, MD), precipitated in ethanol, dried, and dissolved in hybridization buffer (Vysis, Downers Grove, IL). The resulting mixture was denatured at 75°C for 5 minutes and applied to normal metaphase slides prepared from lymphocyte cultures from healthy donors or to commercial slides (Vysis). The hybridization was performed at 37°C for 2–3 days, after which the slides were washed, air-dried, and mounted in an antifade solution with DAPI (Vectashield; Vector Laboratories, Burlingame, CA).

Analyses were performed using a Zeiss Axioplan fluorescence microscope (Zeiss, Oberkochen, Germany) interfaced with the CytoVision system (Applied Imaging, Santa Clara, CA). Data for the chromosomes of 10 cells were used to generate average ratio profiles with 95% confidence intervals (CI). Losses of DNA sequences were defined by ratios less than 0.75, gains by ratios greater than 1.25, and amplifications by ratios higher than 2.0. A negative (normal versus normal DNA) and a positive control (the cell line Lovo) were included in every set of experiments.

2.3. Multicolor fluorescence in situ hybridization (M-FISH)

M-FISH was performed as described in the manufacturer's protocol (Vysis). The probe and hybridization mixture was denatured and applied to denatured metaphase cells.

Table 1
Genetic characteristics of a series of colon cancer cell lines

Cell lines	Ploidy[a]	Genetic phenotype[b]	*p53* mutation[b]	p53 LOH[b]	Copy number changes[c]	Rearranged chromosomes[a]
Co115	Hypo-diploid	MSI	−	−	4	4
HCT15[d]	Near-diploid	MSI	R241F	−	None	2
HCT15[e]	Diploid	MSI	nd	nd	None	2
HCT116[d]	Near-diploid	MSI	−	−	4	5
HCT116[e]	Near-diploid	MSI	nd	nd	4	4
Lovo[d]	Near-diploid	MSI	−	−	3	6
Lovo[e]	Near-diploid	MSI	nd	nd	4	5
LS174T	Near-diploid	MSI	−	−	2	3
RKO	Near-diploid	MSI	−	−	5	5
SW48	Near-diploid	MSI	−	−	3	3
TC71	Near-diploid	MSI	C176Y	−	4	5
TC7	Diploid	MSI	−	−	1	1
ALA	Hyper-diploid	MSS	301delC	+	24	25
Colo320	Near-diploid	MSS	R248W	+	17	19
EB	Hyper-triploid	MSS	−	+	14	15
FRI	Hyper-triploid	MSS	C277F	+	20	18
HT29	Hyper-triploid	MSS	R273H	+	20	18
Isreco1	Hyper-diploid	MSS	Y163H	+	19	13
Isreco2	Hyper-triploid	MSS	Y163H	+	11	17
Isreco3	Hyper-triploid	MSS	Y163H	+	17	17
LS1034	Hyper-triploid	MSS	G245D	+	21	21
SW480	Hyper-diploid	MSS	R273H	+	18	19
V9P	Hypo-diploid	MSS	G245D	+	18	14

Abbreviations: MSI, microsatellite instability; MSS, microsatellite stable; nd, not determined.
[a] Found by G-banding and M-FISH in the present study.
[b] Presented in [13].
[c] Found by CGH in the present study.
[d] INSERM.
[e] The Norwegian Radium Hospital.

Slides were then sealed with rubber cement and coverslip. The hybridization was performed at 37°C overnight, after which the slides were washed, air-dried, and mounted on an antifade solution with DAPI (Vectashield; Vector Laboratories). Metaphases were analyzed using a Zeiss Axioplan fluorescence microscope (Zeiss) interfaced with the CytoVision system (Applied Imaging).

2.4. Statistical analysis

The Mann-Whitney U test was used to compare the number of imbalances and rearranged chromosomes in the MSI and MSS cell lines. Two tailed P values < 0.05 were considered statistically significant. For hierarchical clustering the average-linkage method was used with Pearson's correlation similarity measure. The cluster analyses and the drawing of the dendogram were performed with J-Express Pro [18].

2.5. Nomenclature

The karyotypic findings by G-banding, M-FISH, and CGH were described in accordance to the nomenclature described in the ISCN [19].

3. Results

Various genetic characteristics of the 20 colon cancer cell lines analyzed in the present study are summarized in Table 1. The copy number changes and karyotypes of each cell line are presented in Table 2.

3.1. Comparative genomic hybridization

Here, we characterize the genome profiles of the 20 different cell lines by CGH to get an overview over the gains and losses of the whole genome (Tables 1 and 2). The 9 different MSI cell lines showed few copy number changes (Table 2; Fig. 1). We found copy number losses from 2 chromosomes in each of 2 MSI cell lines (Co115 and TC71). Copy number gains vary from 0 to 5 (mean: 2.6), with gains of 8q and chromosome 7 each being found in 5/9 MSI cell lines. One cell line, RKO, showed copy number amplification (ratio above 2.0) at 8q22~q24. HCT15 revealed no copy number changes by CGH.

We observed multiple copy number changes in the 11 MSS cell lines (Table 2; Fig. 2A). The number of gains detected by CGH varied from 5 to 14 (mean: 10) and the losses from 0 to 14 (mean: 7). The most frequent gains of chromosomes or chromosome arms were seen at chromosome 20 (83% of the MSS cell lines), chromosome 11 (72%),

Table 2
CGH copy number changes, and combined G-banding and M-FISH karyotype of colon cancer cell lines

Cell lines	CGH copy number changes	Combined G-banding and M-FISH karyotype
Co115	rev ish enh(8q22qter,18p11), dim(X,18q12qter)	44,X,−X,der(6)t(6;8)(p24;q22)x2,−18
HCT15[a]	none	44~47,XY,t(8;17)(p23;q21),inv(11)(p14q13)
HCT15[b]	none	46,XY,t(8;17)(p23;q21),inv(11)(p14q13)
HCT116[a]	rev ish enh(8q13qter,10q23q26,16q23qter,17q21qter)	45~46,X,−Y,dup(10)(q24q26),der(16)t(8;16)(q13;p13), der(18)t(17;18)(q21;p11),der(21)t(11;21)(q14;p13)
HCT116[b]	rev ish enh(8q13qter,10q24q26,16q24,17q21q25)	45~46,X,−Y,dup(10)(q24q26),der(16)t(8;16)(q13;p13), der(18)t(17;18)(q21;p11)
Lovo[a]	rev ish enh(5,7,15q)	43~49,XY,t(2;12)(q24;p13),+5,+7,t(7;18)(q31;q22), t(11;14)(p15;q23),+15
Lovo[b]	rev ish enh(5,7,12,15q)	48~49,XY,t(2;12)(q24;p13),+5,+7,+12,i(15)(q10)
LS174T	rev ish enh(7,15q)	47,X,−X,+7,+15
RKO	rev ish enh(7q21q36,8p11qter,9q22qter,10p15q11,20),amp(8q22q24)	46~50,XX,del(2)(p21),dup(7)(q21q36), +der(8)t(8;8)(p12;q21),+der(10)t(2;10)(p21;q11), +der(20)t(9;20)(q22;p13)
SW48	rev ish enh(7,10q22,14q13q32)	46~47,XX,+7,dup(10)(q22q24),der(22)t(14;22)(q11;q13)
TC71	rev ish enh(5q11qter,7,12p12qter),dim(X,3q29)	47~49,XX,der(3)t(3;5)(q28;q11),t(6;18)(q21;q21),+7, +9,+del(12)(p12)
TC7	rev ish enh(8q21q24)	46,XX,dup(8)(q22q24)
Ala	rev ish enh(1p11p22,1q23q42,2p13p16, 2q22q33,3p11p14,3q24q26,4q28q34,8p12pter, 13q14q31,14q21,16q,19p13.1qter,20p12q13), dim(Xp14qter,1p36,6p22pter,6q25qter, 7q33qter,9p21pter,10p13pter,10q21q22,10q26, 17p13,18q12qter),amp(8q13q21,8q22q24)	54~60<3N>,der(X)t(X;Y)(p22;?),−Y,−1, i(1)(q10),t(1;9)(q11;?),der(2)t(2;12)(q37;?),−4,−5,−6, der(7)t(7;14)(q21;q?),der(9)t(9;9)(p24;q11),−10, der(10)t(3;10),der(11)t(11;13)(p13;q11)x2,−12,−14,−15, i(16)(q10),del(17)(p12),−18,i(19)(q10), der(20)t(5;20)(?;q11),ider(20)t(X;Y;8;20),+2mar
Colo320	rev ish enh(1p12p13,1q21q23,2p16pter, 2p14q21,3q13qter,4q33qter,8q22q24,9q22qter, 12pterq12,13q,15q21qter,16p13qter,17q11, 17q22qter),dim(Xp,7p21pter,18q22q23), amp(8q23q24.1,13q11q31)	44~48,X,−X,der(X)t(X;4)(p11;q33), t(1;5)(p10;q10),der(2),+der(2)t(2;17)(p24;q21), der(3)t(3;?15),der(5)t(5;7)(p15;p15), der(7)t(2;7)(p23;p21),der(8)t(8;13)(p24;?),del(9), der(9)t(9;12)(p13;p11),der(10)t(3;10)(?;q26), ider(13)(q10)t(8;13),i(14)(q10),der(15)t(9;15)(?;p11)t(3;15)(?;q?), der(16)t(5;16)(p11;p11)t(13;16)(q11;q22), +der(16)t(2;7;8;16)(?;?;?;p13),der(17)t(17;18),−19
EB	rev ish enh(2p,6p25q12,6q22qter,7p22q33, 8q22q24,11p15q23,12p12q12,14q12q32, 17q21q23,19p12p13.2,20),dim(4,17p12pter,18p11q23),amp(20q)	76<3N>,XX,−X,del(2)(q11),−4,+del(6)(q12q22), +der(7)t(7;10)(p22;q22),+8,del(10),+11,+del(12)(q11), +14,dup(17)(q21q23),−18,+20,+20,+20
Fri	rev ish enh(1p11p31,1q23qter,3p24q26, 6q23qter,7,8p12qter,11p13p14,11q12, 11q22q23,20q),dim(Xq21q22,Xq26qter, 1p36.1,4q23qter,8p22pter,10p15,17p12pter,18,20p12pter,22q)	71~76,XX,−X,del(1)(p36)x2,+der(1)t(1;11)(p36;?), der(2)t(1;2)(?;q37),+3,del(4)(q21),+5,+7, der(8)t(8;8)(p12;q?),+9,+11, der(13)t(6;13)(q23q25;q34),+15,−17,−18,+i(20)(q10), −22
HT29	rev ish enh(3q12q26,5p13pter,8q,11, 12p11p13,13q,15q,17p21qter,18p11,19q, 20p11qter),dim(Xp22,3p12pter,4q33qter, 6q15q25,8p21pter,14q,17p11p13,18q11qter, 21q21qter),amp(8q22q24)	72~73,XXX,der(3)t(3;5)(p21;p?),i(3)(q10),del(4)(q33), −6,der(6)t(6;14)(q27;q11),der(8)t(7;8)hsr(8),+11, i(13)(q10),−14,+15,+der(15)t(15;15)(p13;?), +del(17)(p11),del(18)(q11),+19,+20,+20,−21,−22
IS1	rev ish enh(Xp11.3p21,Xp22,2p12q32,3q,5p, 5q31qter,7,10,11q,12p,13q12q14,15q,20, 22q11),dim(Xq12q21,4p15qter,9p23pter, 12q15q21,18q22qter),amp(12p)	51~53,X,der(X),+3,+i(3)(q10), der(6)t(6;11;22)(?;q12;?),der(8)t(X;8)(q11;p23),+10, der(11)t(5;11)(q22;q25),+der(11),hsr(12)(p11~13), dic(13;19)(p13;q13),der(14)t(X;14)(p?;p13),+15,+20, −21,+5mar
IS2	rev ish enh(X,6p12qter,7,13q,19p13,20p), dim(1p13p34,4,10pterq21,14q11qter,18,21q),amp(Xq28,13q33qter)	68~69,XXX,+X,der(1)t(X;1)(?;p11), der(2)t(X;2)(q?;p16),−4,+i(6)(q10),+7,−8,−10,+13,+13, −14,der(14)t(X;14)(?;p13),+15,−18,+20,−21,−22
IS3	rev ish enh(X,5,6q22q26,7p13pter,7q11q33, 9p13pter,13q,17q11qter),dim(6p12pter, 8p22pter,8q24.2qter,14q,16q,17p12pter,18, 21q,22q13),amp(Xq23q28)	69,XXX,+X,+X,+5,del(6),+del(7)x2,der(8)t(7;8),+9, +13,−14,der(14)t(X;14)(?;p13),−16,−17,i(17)(q10),−18, −21,−22
LS1034	rev ish enh(5q21q34,11,12pterq13,12q24,13q, 15q,20),dim(Xp22q28,1p22p34,1q42qter, 4q27q31,5p14pter,5q11q14,6p12pter,6q12q13, 6q24qter,8q12q22,17p12p13,18p11q23,21q, 22q13),amp(12p)	63~69,XY,−X,+Y,+Y,der(X)t(X;15)(p11;q22),der(1), der(1)t(1;5)(q22;?),−5,−5,t(5;7)(q13;q33),−6, der(7)t(5;7)(q13;q33),−8,−10,+11,+i(12)(p10), i(14)(q10),+15,−17,−18,+20,−21,+4~6mar

(*Continued*)

Table 2
Continued

Cell lines	CGH copy number changes	Combined G-banding and M-FISH karyotype
SW480	rev ish enh(2pterq13,3q23qter,5p15,5q23qter, 7pterq34,8q23q24.2,11,12p11pter,14q24qter, 17q25,18p,19p13qter,20),dim(X,4p13p15, 4q11qter,6q12q24,18q11qter),amp(7q21q22)	54~58<2N>,X,−Y,+der(X)t(X;15)(p22;q?), t(1;9)(q21;q11),+der(2)t(2;12)(q37;q13),+i(3)(q10), t(5;20)(q21;p12),+7,+der(7)dup(7)(q22)t(7;14)(q32;q24), der(8)t(8;9)(p21;q11),der(10)t(X;10)(?;p15),+11, i(12)(p10),+13,+17,i(18)(p10),der(19), der(20)t(5;20)(q21;p12),+21,+22
V9P	rev ish enh(4q28qter,7pterq11,8q11qter, 11p11q25,18p11q11,19,20p11q13,22q11q12), dim(X,4p,6q16qter,8p12pter,9pterq21,14q, 16p,17p,18q12q23,20p12pter),amp(7p22, 11q14q22)	40~41,X,−X or Y,t(1;16)(p10;q10), der(3)t(3;7;8)(p22;q?;q11),der(4;13)(q10;q10), der(6;16)(p10;q10),der(7)i(7)(p10)dup(7)(p?), der(8;17)(q10;q10),der(9;11)(q10;q10), +der(11)hsr(11)(q14~22)t(11;19)(q22;p11),−14, der(18)t(7;18)(q11;q21),der(19)t(3;19)(p22;p13) dup(19)(q11q13)t(4;19)(q28;q13),+i(20)(q10), der(21;22)(q10;q10),hsr(22)(q11~q12)

[a] INSERM.
[b] The Norwegian Radium Hospital.

and 8q (66%), whereas the most frequent losses were seen at 18q (100%), X chromosome (72%), 4q, and 17p (66%). Amplifications seen in at least 2 cell lines were found at 8q23~24, 12p, and Xq28.

3.2. Cytogenetic banding analysis

To identify structural rearrangements and obtain ploidy data on each cell line, we performed cytogenetic banding analyses on metaphases after short-term culturing. The 9 MSI cell lines were either diploid or near-diploid, and had few chromosomal aberrations (Table 2). One of these cell lines, HCT15, revealed only balanced aberrations, t(8;17)(p23;q21) and inv(11)(p14q13), and thus had a normal CGH profile (Fig. 1). The loss of 3q29 in TC71 found by CGH was in accordance with der(3)t(3;5)(q28;q11) seen by G-banding analysis.

All 11 remaining cell lines revealed a MSS phenotype and showed aneuploidy. Six lines were hypertriploid, 3 were hyperdiploid, one was near-diploid and one was hypodiploid (Table 1). We observed complex karyotypes (Table 2). One structural aberration, i(3)(q10), was found in HT29, IS1, and SW480, whereas i(12)(p10) was found in LS1034 and SW480, and i(20)(q10) was found in both Fri and V9P (Fig. 2B). The chromosomes most frequently involved in translocations were the X chromosome (18%), chromosome 8 (17%), chromosome 5 (15%), and chromosome 9 (14%). The most common numerical changes in the 11 different MSS cell lines were losses of chromosomes 18 (44%), 6, 14, and 21 (33%), and gains of chromosomes 11 (55%), 15, and 20 (44%).

3.3. Multicolor fluorescence in situ hybridization

We used a chromosome painting technique, M-FISH, to identify chromosomal rearrangements, particularly those not resolved by G-banding. Most of the markers and adhesions were solved, and we present a detailed karyotype for each cell line combining the results obtained by G-banding and M-FISH in Table 2 and Fig. 2C.

3.4. Statistical analysis

Using the Mann-Whitney U test, statistically significant differences regarding the number of imbalances (detected by CGH) and rearranged chromosomes (detected by G-banding and M-FISH) were found between MSI and MSS cell lines ($P < 0.0001$ and $P < 0.0001$, respectively).

3.5. Complete and novel karyotypes

In the present study, 7 cell lines (Colo320, EB, Fri, IS2, IS3, SW480, and V9P) were completely karyotyped for the first time. To our knowledge, EB, Fri, IS2, IS3, and V9P as well as Ala and IS1 have not at all been cytogenetically

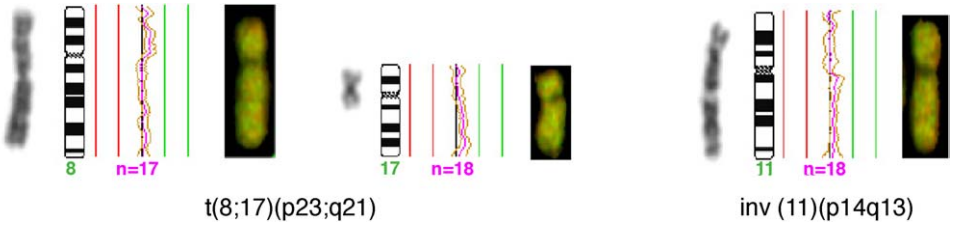

Fig. 1. Genome analyses of a MSI cell line. G-banding and CGH profile of the abnormal chromosomes of the MSI cell line HCT15. Only G-banding reveals the balanced chromosome aberrations.

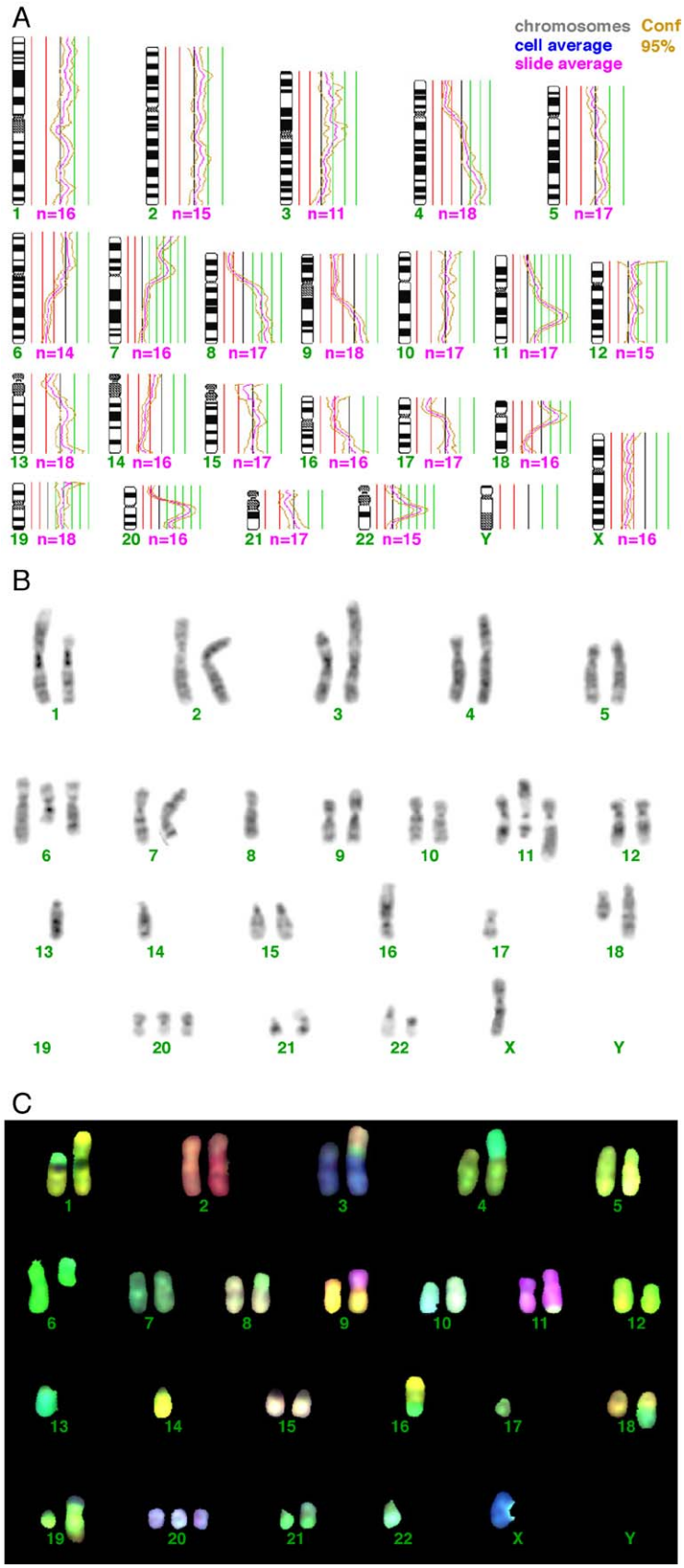

Fig. 2. Genome analyses of a MSS cell line. *(A)* CGH profile for copy number changes of MSS cell line V9P, with gains (profile deviated to the right) and losses (profile deviated to the left) along several chromosomes. *(B)* G-banding karyotype of the MSS cell line V9P. *(C)* M-FISH karyotype of the MSS cell line V9P.

described. In the karyotypes of the latter two, some markers remain unidentified. We confirmed several previously identified chromosomal aberrations in the remaining 11 cell lines, but more precise breakpoints were identified, as well as a copy number profile for each of them.

3.6. Clonal differences in tumor cell lines

Three MSI cell lines (HCT15, HCT116, and Lovo) were grown in two different laboratories prior to the present genome profiling. Each pair shares genetic changes, although a few differences were seen. The 2 HCT15 cell lines were identical. We detected small differences with G-banding at band level in HCT116; a der(21)t(11;21)(q14;p13) is found in a small proportion of metaphases in one of the two duplicate HCT116. The 2 Lovo cell lines have several differences with both techniques, one having 2 balanced translocations, t(7;8)(q31;q22) and t(11;14)(p15;q23), as well as trisomy 15, and the other having trisomy 12 and an i(15)(q10) (instead of trisomy 15, both giving rise to gain of 15q by CGH).

3.7. Experimental model for tumor progression

The cell lines IS1, IS2, and IS3 were established from 3 tumors from the same patient, and represent the tumor progression through different stages. Some of the many genetic changes were seen in all 3 cell lines: the structural aberration der(X)t(X;14)(?;p13) and the numerical changes +X, +20, and −21. IS1 and IS3 share gain of chromosome arm 5p, an uncommon change in primary colorectal carcinomas.

3.8. Comparison of the findings with previous studies

Since the initial publication of the method, CGH has been the most commonly used molecular genome screening technique of cancer tissue samples [20]. We performed a literature survey to assess the extent to which CRC cell lines have been previously studied. In addition, the karyotypes of some commonly used cell lines were examined from selected studies of the last 2 decades.

Table 3 summarizes the CGH results from 13 previously published colorectal cancer cell lines, 5 of which are included in the present series. Five cell lines have been published more than once. The most common changes shared in the previous publications and in the present series of all cell lines are in bold characters.

Table 4 shows the previously published G-banding and/or FISH karyotypes of each of 5 selected commonly used colon cancer cell lines, all of them analyzed in the present study. These cell lines have been karyotyped several times and 3 – 6 previous studies are included for each cell line. A core of changes (found in 3 or more cell lines) can be seen to be common among them, and other genomic aberrations were described in only some of them in addition to the present study (Tables 2 and 4).

3.9. Hierarchical cluster analysis

We performed a hierarchical cluster analysis on the 9 different MSS cell lines and 5 comparable primary colon carcinomas based on the alteration of 41 chromosome arms (Fig. 4). The cell lines and the primary tumors tend to cluster together, in particular, one cell line, Fri, clusters in close relationship to the primary carcinomas. The dendogram indicates that some cell lines cluster closer together than others, which cannot be explained by the ploidy stem line, but rather by specific chromosomal changes.

4. Discussion

4.1. Combination of genome screening methods

The combination of conventional G-banding, CGH, and M-FISH used in this study proved effective for characterizing the tumor genomic profiles. We took advantage of the particular strengths of each technique to obtain a detailed picture of the chromosomal changes that characterize this panel of 20 colon cancer cell lines. Whereas CGH only detects net gains and losses of chromosomes, we were able to identify structural aberrations such as translocations by G-banding and M-FISH analysis. For example, the MSI cell line HCT15 illustrates the strengths of complementing screening techniques. It showed only balanced rearrangements by G-banding analysis and a normal CGH profile (Fig. 1). The MSS cell lines presented complex karyotypes and the combination of G-banding and M-FISH was instrumental in revealing the identity of added material of unknown origin, homogeneously staining regions (hsr), and marker chromosomes (Figs. 2B and 2C).

The graphical comparison of the CGH copy number changes with the genome imbalances inferred by the combination of G-banding and M-FISH showed that the overall picture of gains and losses was not significantly different (Fig. 3). However, some small differences were seen: the number of gains detected by CGH was slightly higher at some chromosome regions, whereas the combined G-banding and M-FISH karyotype revealed somewhat higher frequencies of losses. These differences probably reflect the intrinsic strengths and weaknesses of each of these techniques.

We would like to highlight the importance of the use of conventional banding analysis, even though nowadays FISH techniques are readily available. The knowledge of genes involved in breakpoints in chromosome rearrangements can be of major importance, but without the complement of G-banding, it is difficult to detect the exact breakpoints of the chromosome bands involved in these rearrangements. There are few publications on colorectal cancer cell lines studied using a combination of CGH and other FISH techniques [21–24], and only one involving G-banding analysis [25]. Thus, we provide a comprehensive reference of the

Table 3
Literature survey of CGH data for colorectal cancer cell lines

Cell lines	CGH copy number changes	References
BE	rev ish enh(14q22qter,19q,**20q**),dim(8q21.2pter,9p21pter,10p15,**18q21qter**)	[47]
CACO-2	rev ish enh(10q21pter,**11q13qter**,12p,16q23qter,17q,**20q**),dim(1p,9p22pter,**18q)**	[47]
CACO-2	rev ish enh(10p,10q,**11q**,12p,16q,17q,**20q**),dim(1p,4,8,9p,9q,17p,18),amp(11q,20q)	[24]
CoCM-1	rev ish enh(7,8q,9p,11p,13q,20q,21q),dim(**4q**,8p,14,15q11q22,**17p,18**),amp(**8q**)	[24]
Colo201	rev ish enh(5p,6p12p21,6q24,9q,**11p**,13q,17q,**20**),dim(1p,3p,5q,6q12q22,10p,12p,14,**18**),amp(6p12p21,6q24)	[24]
Colo320DM	rev ish enh(2p,3q,**11p**,13q,16,17q),dim(**18q**,21q),amp(**8q22q24**)	[24]
Colo320HSR	rev ish enh(1cen,2p,**8q24,11p**,12p,13q),dim(**18q**,19),amp(**8q24**)	[48]
DLD-1	rev ish enh(2p,**11p**)	[23]
DLD-1	rev ish enh(1p35pter,9q34qter),dim(13q31,**18**)	[47]
H630	rev ish enh(2p13p23,5p,7p11.23,9p21p22,10p12pter,13q,18p,**20**),dim(1p22.2p31.1,**4q**, 5q15q31,9q33qter,10q25,**18q12.3qter**),amp(13q)	[47]
HCT116	rev ish enh(**8q,**10q,16q,17q),amp(16q)	[25]
HT29	rev ish enh(3q11.2qter,5p,7,**8p11.1q22.2,11,20q11.1qter**),dim(3p11pter,**4q32qter,**6q16qter,12q24.2qter, 14q21qter,**17p,18q**,19p,21,22),amp(**8q22.3**)	[22,58]
HT29	rev ish enh(**8q22qter,11**,12q24.1qter,13q12.2,**20q**),dim(3p12.3p13,8p21pter,**18q**,21q),amp(**8q22qter**)	[47]
Lovo	rev ish enh(5,7,12,15q)	[24]
Lovo	rev ish enh(5,7,12,15)	[25]
Lovo	rev ish enh(12q24.1qter)	[47]
RKO	rev ish enh(7q32q34,**8q21.1qter**),dim(2q37,6p23pter)	[47]
R10	rev ish enh(1q21,2p16pter,5p,9p,10p,**11q13qter**,13q,15q22q25,**20q12q13.2**),dim(1p,3p14q13.1,**4q**,5q,7p22,**18q**)	[47]
WiDr	rev ish enh(**8q22qter,11**,15q,19q),dim(3p,**4q32q35**,8p,14q,**17p,18q,**21q,22q),amp(**8q22qter**,19q)	[24]

The most common changes shared in the previous publications and in the present series of cell lines are in boldface. Some differences regarding breakpoint designation may be due to different thresholds used for CGH scoring.

genomic profile of colon cancer cell lines, using the combination of CGH, G-banding, and M-FISH.

It should be noted that genomic arrays, first described in 1998 [26], are in principle a tool for the identification of high-resolution copy number changes. However, such arrays are still in development and the ones currently available do not cover the whole genome. Further, the same limitations associated with CGH apply to genomic arrays (i.e., inter- and intra-chromosomal changes that are found by different karyotyping techniques cannot be detected).

4.2. MSI and MSS tumor cell lines

The majority of colorectal carcinomas is aneuploid and reveals a MSS pattern, whereas tumors with MSI phenotype show a near diploid index with instability at the nucleotide level [27–29]. The copy number changes found by CGH in the MSI and the MSS cell lines in our series confirm this observation. The MSS cell lines showed a large number of gains and losses (mean: 8.5), and the MSI cell lines revealed considerably fewer changes (mean: 2.6) per cell line. Similarly, different patterns of genetic complexity between the MSI and MSS cell lines were found by the combined G-banding and M-FISH analysis. The karyotypes of the MSI cell lines revealed few aberrations, whereas the MSS cell lines displayed complex karyotypes with many changes, confirming previous reports in the literature [30]. The differences in the number of aberrations between MSI and MSS lines were found to be statistically significant for both methods.

There are two exceptions to these major observations. The cell line Colo320 showed a near-diploid karyotype and V9P showed a hypodiploid karyotype. Although these cell lines are derived from MSS tumors, they do not show a near triploid modal number as in the other MSS cell lines. Still, they clearly present a more complex karyotypic pattern than the MSI cell lines, thus confirming their MSS phenotype with mostly unbalanced aberrations.

4.3. Comparison of cell lines with primary tumors

The present series of cell lines display the most common aberrations in primary colorectal carcinomas using a combined methodological approach. A literature survey of CGH studies of 669 primary colorectal carcinomas revealed that the most common changes ($>$ 30%) include losses of chromosome arms 18q, and 17p, and gains of 20q, 13q, in descending order of frequency (Diep et al., unpublished data). The most common structural rearrangements found by banding analysis are i(8q), i(13q), del(1)(p22), i(17q), and i(1q) [6]. All but i(8q) were seen in our series of cell lines. Due to the relatively small number of cell lines, we do not expect the frequency of the genomic abnormalities to agree completely with those from large series of primary tumors. We observed no recurrent translocations, supporting the notion that fusion proteins and overexpression of oncoproteins caused by such aberrations do not play an important role in colorectal tumorigenesis. The numerical and structural similarities show that these cell lines are representative of

Table 4
Literature survey of karyotype information of selected colon cancer cell lines

Cell lines	Karyotype	References
HCT15	46,XY,**t(8;17)**(p23;q21),**inv(11)**(p15.3q13.1)	[59]
HCT15	47,XY,+Y,der(8)**t(8;17)**(p23;q21),**inv(11)**(p15.3q13.1),t(16;16)(q22;q24), del(17)(q21)	[60]
HCT15	46,XY,**t(8;17),inv(11)**(p15.3q13.1)	[30]
HCT116	45,X,**-Y,dup(10)**(q24q26),**der(16)t(8;16)**(q13;p13), **der(18)t(17;18)**(q21;p11.3)	[25]
HCT116	43~45,X,**−Y,**der(10)**dup(10)**(q?)t(10;16),**der(16)t(8;16),der(18)t(17;18)**	[21]
HCT116	45,X,**−Y,der(16)t(8;16),der(18)t(17;18)**	[30]
HT29	+**del(X)**(p11),+del(X)(q11),+1,+2,+der(3)t(3;?)(p21;?),+**del(4)**(q31),+5,−6, +del(6)(q13),+der(6)**t(6;14)**(q23;q13),+7,+del(7)(p15p22),+10,**+11**,+11, +12,−13,**−13,+i(13)(q10)**,**−14**,+der(14)t(6;14)(q23;q13),**+15**,+16,+17,−19, +der(19)t(19;?)(p11;?),+20,+6mar	[61]
HT29	del(1p),+2,der(3;6)(p10;q10),−4,add(6)(q27),del(7)(p15),−8,−9,−10,**+11**, i(11)(q10),**−13,i(13)(q10)**,add(13)(p11),**−14**,add(15)(p11),−16,−18,+19, add(19)(q13),−20,**−21**,−22,+6~16mar	[62]
HT29	70,XX,**del(X)**(?p21),+der(2)t(1;2)(q32;q11),**del(4)**(?q31), +der(5)t(5;6)(p10;?),+7,hsr(8)(p22p23),**+11,−13,i(13)(q10)**,**−14**,**+15**, del(18)(q12),+del(20),**−21**,+der(22)t(17;22)(?;q12),der(?)t(6;9)(p10;q10), der(?)t(17;19)(q10;?p10)	[21]
HT29	XX,**del(X)**(p11),del(3)(p14),der(3)del(3)(p14?)del(3)(p25?),**del(4)**(q31), +del(5)(q11),del(6)(q12),**t(6;14)**(q23;q13),+del(7)(p15),der(8;8)(q?;q?), der(9),**+11**,der(13;13)(q10;q10)del(13)(q14?),**i(13)(q10),−14**, **+15**,−17, i(18)(p10),add(19)(q13),+der(19)t(17;19)(q12/q21;q13),**−21,** ider(22)(p10)ins(22;22)(p11;?)	[54]
Lovo	49,XY,−2,+der(2)**t(2 ;12)**(q21;p21),**+5,+7**,+del(12)(q14q22),−15, +der(15)t(15;15)(p11;q11)	[63]
Lovo	49,XY,**t(2;12)**(q22;p12.1),**+5,+7**,t(7;18)(q31.3;q22),t(11;14)(p14;q21),+15	[64]
Lovo	49,XY,**t(2;12)**(q13;p11.2),**+5,+7**,+12,**i(15)(q10)**	[25]
Lovo	48~50,XY,der(2)**t(2;12),+5,+7,**+der(12)t(2;12),**i(15)(q10)**	[21]
Lovo	49,XY,+del(2)(?),der(2)**t(2;12),+5,+7,i(15)(q10)**	[24]
Lovo	49,XY,**t(2;12),+5,+7,i(15)(q10)**	[30]
SW480	+X,−Y,+der(2)**t(2;18)**(q21;q11),−5,+**der(5)t(5;20)**(q15;p11),+i(5)(p10),+7, +der(7)?ins(q22),−10,+der(10)**t(10;12)**(p15;q11),**+11**,−12,**+13**,−16, +der(16)?origin,−18,+**der(19)t(19;?)**(q13;?),+**der(20)t(5;20)**(q15;p11)x2, +21,+2mar	[61]
SW480	+X,−Y,−1,+der(1)**t(1;9)**(q25;q13),+der(2)**t(2;12)**(q37;q13),+del(3)(q11), +?i(4)(p10),−5,+**der(5)t(5;20)**(q15;p11),+ins(7)(7;?)(q22;??), +der(7)t(7;?)(q36;?),−8,+der(8)t(8;?)(p11.2;?),−9, +der(9)t(1;9)(q25;q13), −10,**+11**,−12, **+13**,+16,+17,−18,−19,+**der(19)t(19;?)**(q13;?), +**der(20)t(5;20)(q15;p11),+21**,+22	[65]
SW480	**t(1;9)**(q12;q11),dic(2;12)(q24;p13),der(4)t(4;14)(q21;q24), **der(5)t(5;20)**(q15;p11),+7,+der(7)inv(7)(q22q36)**t(7;14)**(q22;q22), der(8)t(8;9)(q11;q11),der(10)**t(10;12)**(p13;q12),**+11**,del(13)(q13), del(18)(q12.2),+**add(19)**(q13),+**der(20)t(5;20)**(q15;p11)x2	[66]
SW480	52~59,XX,−Y,der(1)**t(1;9)**,+der(2)**t(2;12)**,+del(3)(?),**der(5)t(5;20)**, +der(7)t(7;13),+der(7)**t(7;14)**,−8,der(9)**t(1;9)**,der(10)**t(10;12)**(3;12),**+11**, del(12)(?),**+13**,+del(17)(q?),del(18)(q?),−19,+**der(20)t(5;20)**,+21, der(?)t(8;9),der(?)t(8;19),der(?)t(5;19)t(8;19)	[21]

The core aberration for each cell line shared in the previous publications and in the present series are in boldface. Only chromosomes, not breakpoints, are in bold because some differences are most likely due to disparate interpretations and not real variation of the rearrangements.

in vivo tumors, although in vitro selection may occur (see later) for some chromosomal changes in individual cell lines.

By hierarchical cluster analysis, we confirm that cell lines are representative models for primary carcinomas at the genome level (Fig. 4). One cell line in particular, Fri, is closely related to the primary tumors by chromosomal changes. The common changes such as loss of 18q, and gain of 20q and 13q cluster together across the sample set. Although conclusions cannot be drawn, due to the limited number of tumors, none of the 4 outlier cell lines showed gain of chromosome 7, which is commonly seen in primary carcinomas.

4.4. Target genes for amplifications

Amplifications of oncogenes, corresponding to hsr, are common in different cancer types, and associated with poor prognosis [31]. We observed amplifications (defined as ratios above 2.0) at 8q23~q24, 12p, and Xq28 in at least 2 tumors. There are several candidate oncogenes at these chromosomal locations. At distal 8q, *C-MYC* shows high-level amplification [32] and *PRL-3* is highly expressed in the metastasis of colorectal cancer [33]. At chromosome arm 12p *CCND2 (cyclin D2)* and *K-RAS* are among other oncogenes known to be involved in colorectal tumorigenesis [34,35].

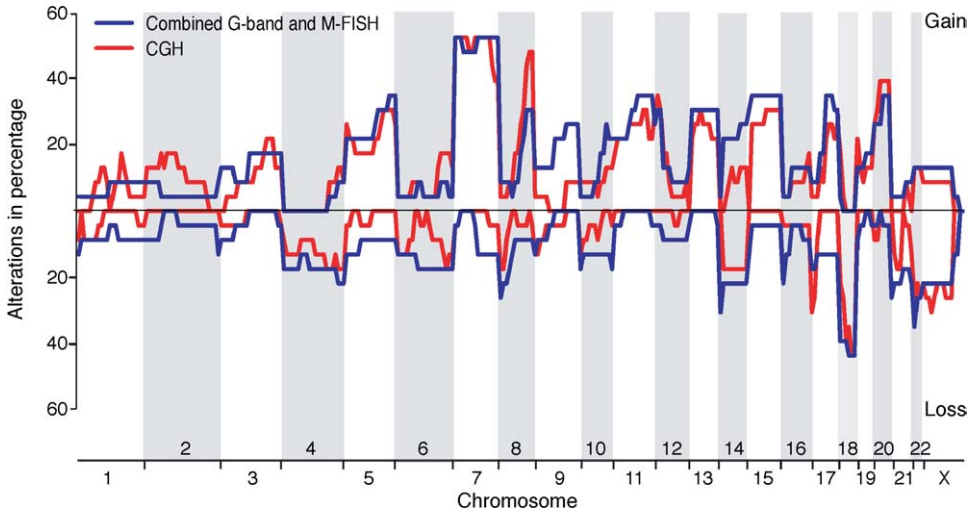

Fig. 3. Genome profiles of 20 colon cancer cell lines identified by CGH (red) and combined G-banding and M-FISH (blue) along the chromosomes from band 1p36 to Xq28. Gains of chromosomes or chromosome bands are graphically illustrated with lines above the X-axis, whereas losses are seen below. Although CGH is a quantitative technique and G-banding and M-FISH are mainly qualitative methods, the overall imbalances detected by each approach do not differ significantly. However, the numbers of gains detected by CGH are slightly higher at some chromosome regions, whereas the combined G-banding and M-FISH karyotype reveal somewhat higher frequencies of losses.

Potential target genes of the Xq28 amplification remain to be identified.

4.5. Target genes for common deletions

Loss of chromosome arm 18q was seen in all aneuploid cell lines, leading to loss of several TSGs including *DCC, SMAD4, SMAD2,* and *SMAD7*. Inactivation of *TP53* is the target gene for the observed losses at 17p [36]. All but one MSS cell lines showed both *TP53* point mutation and LOH at 17p13, suggestive of complete inactivation of the gene (Table 1) [13]; this confirms previous studies on primary colorectal carcinomas [21,37–39]. Loss of 17p or 18q in colorectal cancer is associated with poor prognosis and losses of both predict an even worse disease outcome [40,41]. The loss of chromosome 4 was found in over half of the MSS cell lines, and is frequent in primary colorectal carcinomas [31,42–45]. Deletions of chromosome 4 are associated with tumor progression and poor survival for several cancer types. The apoptosis related genes such as *CPP32* and *MCH2* might be candidate suppressor genes for this region [45,46].

4.6. Clonal divergence in tumor cell lines

For cell lines to be useful, they must retain the relevant genetic and biological characteristics of the tumors from which they derive. However, there is a possibility that clonal evolution may occur during in vitro culturing due to stochastic factors and varying selective forces operating at different laboratory environments. We investigated this issue by analyzing 3 cell lines obtained from 2 laboratories. Most of the genomic features were observed by both CGH and the combination of G-banding and M-FISH analyses, but two cell lines (HCT116 and Lovo) displayed clonal differences. This probably reflects the fact that these cell lines have undergone numerous sub-cultures, allowing time and opportunity for new genetic changes to occur during growth. Thus, the importance of knowing the source and checking the genomic profile of a cell line before it is used as an in vitro model is demonstrated. A few differences, however, may be due to disparate interpretation of the same rearrangements.

4.7. Comparison of the findings with previous studies

We evaluate our results by comparing our findings with previously published CGH data and karyotypes of colorectal cell lines. There are only a few publications of CGH on colorectal cancer cell lines [21–25,47,48], and in some cases, the results were described incompletely, indicating only the number of aberrations or the average CGH ratio profile. In these reports, 13 cell lines were described with detailed copy number changes (Table 3). Comparing these findings with our data, 5 cell lines showed similarities pointing to their common origin and differences indicating divergent clonal evolution. Among the studies that report the cut-off values used, there were losses defined from ratios fewer than 0.75–0.80, gains by ratios greater than 1.20–1.25, and amplifications by ratios higher than 1.4–2.0. The different choices of cut-off values may contribute to variations in the CGH scorings.

We also compared 3–6 previous publications of karyotypes of the commonly used cell lines HCT15 [49], HCT116 [50], HT29 [51], Lovo [52], and SW480 [53] with our karyotypic data. Due to absent or non-interpretable banding analysis in earlier reports, we chose to refer to karyotypes from 1987 and later. Identical translocations as well as disparate structural aberrations were seen (Table 4). Strikingly,

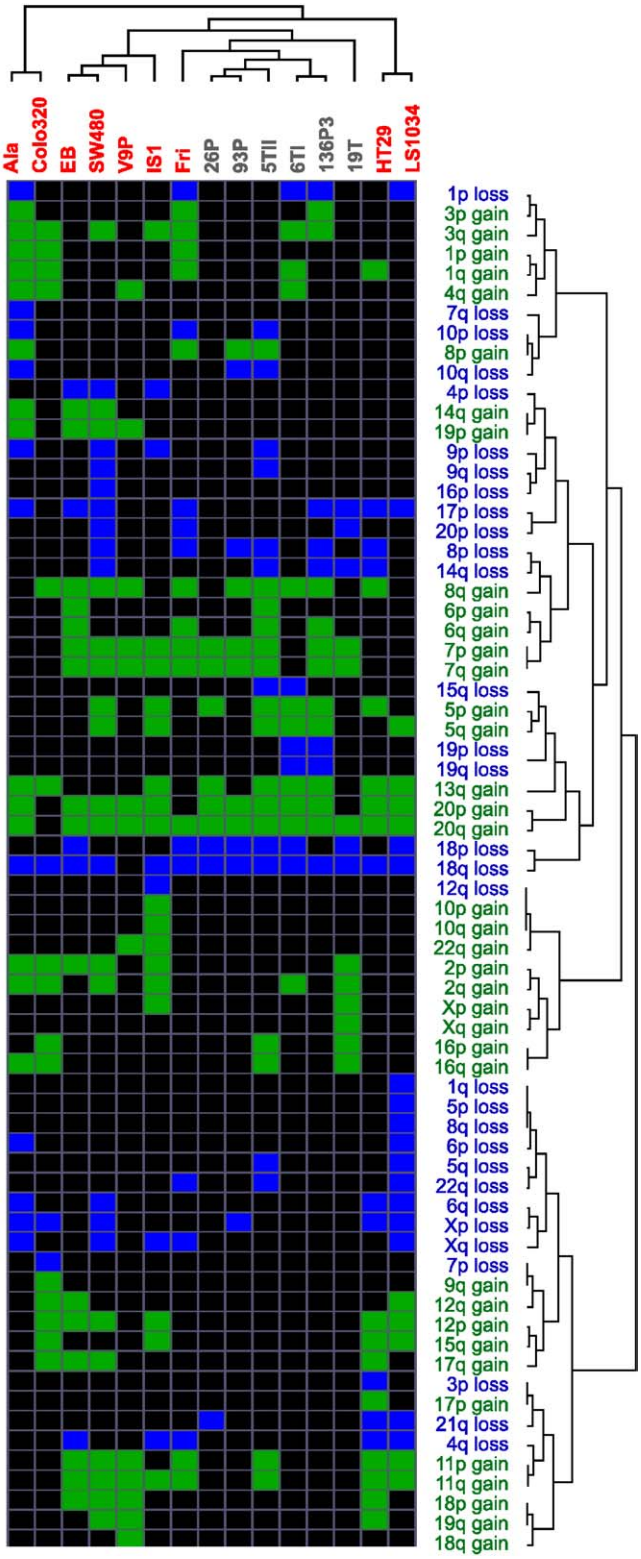

Fig. 4. Hierarchical clustering of cell lines and primary carcinomas. The hierarchical clustering is based on the level of chromosome aberration of 41 chromosome arms by CGH. Chromosome arms 13p, 14p, 15p, 21p, 22p, and the Y chromosome were excluded from the clustering analyses due to large repetitive sequences. The chromosome arms are depicted in the right dendogram. The cell lines (red) and primary tumours (grey) are depicted in the top dendogram. Each row represents the changes from a separate chromosome arm over all samples, and each column represents the changes from all chromosome arms in a separate cell line/primary carcinomas.

there are more differences of numerical aberrations than structural changes, suggesting that the former occur more easily during in vitro growth than the latter.

Many of the MSS cell lines with complex karyotypes are incompletely described with unsolved markers and without breakpoints. In 2002, Kawai et al. [54] reported a complete karyotype of HT29, by the combination of SKY and G-banding. Their results are in line with our findings. Seven of the cell lines in the present study were completely karyotyped for the first time. Among these, two are well known, Colo320 and SW480, and have previously been studied by several groups. Although Abdel-Rahman et al. [21] showed by CGH and SKY a karyotype of SW480 without markers in a panel of cell lines, the specific breakpoints remained undefined for several of the rearrangements, which are best explained by the lower resolution level of the SKY technique in breakpoint identification compared with that of G-banding analysis.

4.8. Experimental model for tumor progression

The cell lines Isreco1 (IS1), Isreco2 (IS2), and Isreco3 (IS3) were derived from the same patient, from a primary colon carcinoma, its corresponding liver- and peritoneal metastases, respectively [14]. The 2 metastases share many of the numerical changes and both have a near-triploid modal number. Furthermore, all 3 cell lines share one structural aberration and 2 numerical changes, indicating the sharing of a common founder cell. The spread of a primary colorectal tumor to the peritoneum is rare compared with its ability to metastasize to the liver, and the prognosis for these patients is poor [55,56]. We have previously shown that the type and frequency of chromosomal imbalances differ at different stages of colorectal carcinogenesis, with gain of chromosome arm 5p being potentially involved in the development of carcinomatoses [15]. A possible candidate gene at 5p is *SKP2,* which is implicated in the ubiquitination and degradation of the cyclin dependent kinase inhibitor *CDKN1B* (p27) [57]. In these 3 related cell lines, we saw increased copy number changes of 5p in both the primary tumor and the peritoneal metastasis, whereas this aberration was absent in the liver metastasis. These data corroborate the suggestion that a gene(s) located on 5p is involved in the development of peritoneal carcinomatosis.

5. Conclusions

Here, we present a genetic study of 20 cell lines and a review of relevant data from other sources. We emphasize the importance of using complementary genome screening techniques and show that the combination of CGH, G-banding, and M-FISH is an effective way to characterize the genomic profiles of tumor cell lines. We provide a combined reference for some of the most commonly used colon cancer cell lines. In addition, copy number profiles are presented

for the first time for most of the cell lines, and a complete resolved karyotype is obtained for 7 cell lines. Finally, the large variation of genetic complexity of the cell lines clearly shows the importance of this knowledge in the use of cell lines as experimental tools.

Acknowledgments

Thanks to Nicholas M. Luscombe at the Department of Molecular Biophysics and Biochemistry at Yale University for critically reading the manuscript. K.K. is a research fellow funded by a grant from the Norwegian Cancer Society A95068 (RAL).

References

[1] Caspersson T, Zech L, Johansson C, Modest EJ. Identification of human chromosomes by DNA-binding fluorescent agents. Chromosoma 1970;30:215–27.

[2] Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992;258:818–21.

[3] Schrock E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T. Multicolor spectral karyotyping of human chromosomes. Science 1996;273:494–7.

[4] Speicher MR, Gwyn BS, Ward DC. Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat Genet 1996;12:368–75.

[5] Loeb LA. Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 1991;51:3075–9.

[6] Heim S, Mitelman F. Cancer cytogenetics. USA: Wiley-Liss, 1995.

[7] Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Powell SM, Jen J, Hamilton SR. Clues to the pathogenesis of familial colorectal cancer. Science 1993;260:812–6.

[8] Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 1993;363:558–61.

[9] Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. Science 1993;260:816–9.

[10] Lothe RA, Peltomaki P, Meling GI, Aaltonen LA, Nystrom-Lahti M, Pylkkanen L, Heimdal K, Andersen TI, Moller P, Rognum TO. Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. Cancer Res 1993;53:5849–52.

[11] Vasen HF, Mecklin JP, Khan PM, Lynch HT. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). Dis Colon Rectum 1991;34:424–5.

[12] Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993;75:1027–38.

[13] Gayet J, Zhou XP, Duval A, Rolland S, Hoang JM, Cottu P, Hamelin R. Extensive characterization of genetic alterations in a series of human colorectal cancer cell lines. Oncogene 2001;20:5025–32.

[14] Cajot JF, Sordat I, Silvestre T, Sordat B. Differential display cloning identifies motility-related protein (MRP1/CD9) as highly expressed in primary compared to metastatic human colon carcinoma cells. Cancer Res 1997;57:2593–7.

[15] Diep CB, Teixeira MR, Wiig JN, Eknæs M, Nesland JM, Giercksky KE, Johansson B, Lothe RA. Genome characteristics of primary carcinomas, local recurrences, carcinomatoses, and liver metastasis from colorectal cancer patients. Mol Cancer 2004;3:6.

[16] Pandis N, Heim S, Bardi G, Limon J, Mandahl N, Mitelman F. Improved technique for short-term culture and cytogenetic analysis of human breast cancer. [published erratum appears in Genes Chromosomes Cancer 1992;5:410] Genes Chromosomes Cancer 1992;5:14–20.

[17] Kraggerud SM, Szymanska J, Abeler VM, Kaern J, Eknaes M, Heim S, Teixeira MR, Trope CG, Peltomaki P, Lothe RA. DNA copy number changes in malignant ovarian germ cell tumors. Cancer Res 2000;60:3025–30.

[18] Dysvik B, Jonassen I. J-Express: exploring gene expression data using Java. Bioinformatics 2001;17:369–70.

[19] Mitelman F, editor. An international system for human cytogenetic nomenclature. Basel: S. Karger, 1995.

[20] Rooney PH, Murray GI, Stevenson DA, Haites NE, Cassidy J, McLeod HL. Comparative genomic hybridization and chromosomal instability in solid tumours. Br J Cancer 1999;80:862–73.

[21] Abdel-Rahman WM, Katsura K, Rens W, Gorman PA, Sheer D, Bicknell D, Bodmer WF, Arends MJ, Wyllie AH, Edwards PA. Spectral karyotyping suggests additional subsets of colorectal cancers characterized by pattern of chromosome rearrangement. Proc Natl Acad Sci USA 2001;98:2538–43.

[22] Ghadimi BM, Sackett DL, Difilippantonio MJ, Schrock E, Neumann T, Jauho A, Auer G, Ried T. Centrosome amplification and instability occurs exclusively in aneuploid, but not in diploid colorectal cancer cell lines, and correlates with numerical chromosomal aberrations. Genes Chromosomes Cancer 2000;27:183–90.

[23] Roschke AV, Stover K, Tonon G, Schaffer AA, Kirsch IR. Stable karyotypes in epithelial cancer cell lines despite high rates of ongoing structural and numerical chromosomal instability. Neoplasia 2002;4:19–31.

[24] Tsushimi T, Noshima S, Oga A, Esato K, Sasaki K. DNA amplification and chromosomal translocations are accompanied by chromosomal instability: analysis of seven human colon cancer cell lines by comparative genomic hybridization and spectral karyotyping. Cancer Genet Cytogenet 2001;126:34–8.

[25] Masramon L, Ribas M, Cifuentes P, Arribas R, Garcia F, Egozcue J, Peinado MA, Miro R. Cytogenetic characterization of two colon cell lines by using conventional G-banding, comparative genomic hybridization, and whole chromosome painting. Cancer Genet Cytogenet 2000;121:17–21.

[26] Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 1998;20:207–11.

[27] Lengauer C, Kinzler KW, Vogelstein B. Genetic instability in colorectal cancers. Nature 1997;386:623–7.

[28] Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 1998;396:643–9.

[29] Lothe RA. Microsatellite instability in human solid tumors. Mol Med Today 1997;3:61–8.

[30] Melcher R, Koehler S, Steinlein C, Schmid M, Mueller CR, Luehrs H, Menzel T, Scheppach W, Moerk H, Scheurlen M, Koehrle J, Al Taie O. Spectral karyotype analysis of colon cancer cell lines of the tumor suppressor and mutator pathway. Cytogenet Genome Res 2002;98:22–8.

[31] De Angelis PM, Clausen OP, Schjolberg A, Stokke T. Chromosomal gains and losses in primary colorectal carcinomas detected by CGH and their associations with tumour DNA ploidy, genotypes and phenotypes. Br J Cancer 1999;80:526–35.

[32] Schleger C, Verbeke C, Hildenbrand R, Zentgraf H, Bleyl U. c-MYC activation in primary and metastatic ductal adenocarcinoma of the pancreas: incidence, mechanisms, and clinical significance. Mod Pathol 2002;15:462–9.

[33] Saha S, Bardelli A, Buckhaults P, Velculescu VE, Rago C, St Croix B, Romans KE, Choti MA, Lengauer C, Kinzler KW, Vogelstein B. A

phosphatase associated with metastasis of colorectal cancer. Science 2001;294:1343–6.
[34] Forrester K, Almoguera C, Han K, Grizzle WE, Perucho M. Detection of high incidence of K-ras oncogenes during human colon tumorigenesis. Nature 1987;327:298–303.
[35] Bartkova J, Thullberg M, Slezak P, Jaramillo E, Rubio C, Thomassen LH, Bartek J. Aberrant expression of G1-phase cell cycle regulators in flat and exophytic adenomas of the human colon. Gastroenterology 2001;120:1680–8.
[36] Vogelstein B, Fearon ER, Kern SE, Hamilton SR, Preisinger AC, Nakamura Y, White R. Allelotype of colorectal carcinomas. Science 1989;244:207–11.
[37] Georgiades IB, Curtis LJ, Morris RM, Bird CC, Wyllie AH. Heterogeneity studies identify a subset of sporadic colorectal cancers without evidence for chromosomal or microsatellite instability. Oncogene 1999;18:7933–40.
[38] Eshleman JR, Casey G, Kochera ME, Sedwick WD, Swinler SE, Veigl ML, Willson JK, Schwartz S, Markowitz SD. Chromosome number and structure both are markedly stable in RER colorectal cancers and are not destabilized by mutation of p53. Oncogene 1998;17:719–25.
[39] Clausen OP, Lothe RA, Borresen-Dale AL, De Angelis P, Chen Y, Rognum TO, Meling GI. Association of p53 accumulation with TP53 mutations, loss of heterozygosity at 17p13, and DNA ploidy status in 273 colorectal carcinomas. Diagn Mol Pathol 1998;7:215–23.
[40] Kern SE, Fearon ER, Tersmette KW, Enterline JP, Leppert M, Nakamura Y, White R, Vogelstein B, Hamilton SR. Clinical and pathological associations with allelic loss in colorectal carcinoma. JAMA 1989; 261:3099–103.
[41] Diep CB, Thorstensen L, Meling GI, Skovlund E, Rognum TO, Lothe RA. Genetic tumor markers with prognostic impact in Dukes′ stages B and C colorectal cancer patients. J Clin Oncol 2003;21:820–9.
[42] Diep CB, Parada LA, Teixeira MR, Eknaes M, Nesland JM, Johansson B, Lothe RA. Genetic profiling of colorectal cancer liver metastases by combined comparative genomic hybridization and G-banding analysis. Genes Chromosomes Cancer 2003;36:189–97.
[43] Bardi G, Sukhikh T, Pandis N, Fenger C, Kronborg O, Heim S. Karyotypic characterization of colorectal adenocarcinomas. Genes Chromosomes Cancer 1995;12:97–109.
[44] Malkhosyan S, Yasuda J, Soto JL, Sekiya T, Yokota J, Perucho M. Molecular karyotype (amplotype) of metastatic colorectal cancer by unbiased arbitrarily primed PCR DNA fingerprinting. Proc Natl Acad Sci USA 1998;95:10170–5.
[45] Shivapurkar N, Maitra A, Milchgrub S, Gazdar AF. Deletions of chromosome 4 occur early during the pathogenesis of colorectal carcinoma. Hum Pathol 2001;32:169–77.
[46] Arribas R, Ribas M, Risques RA, Masramon L, Tortola S, Marcuello E, Aiza G, Miro R, Capella G, Peinado MA. Prospective assessment of allelic losses at 4p14–16 in colorectal cancer: two mutational patterns and a locus associated with poorer survival. Clin Cancer Res 1999;5: 3454–9.
[47] Rooney PH, Boonsong A, McKay JA, Marsh S, Stevenson DA, Murray GI, Curran S, Haites NE, Cassidy J, McLeod HL. Colorectal cancer genomics: evidence for multiple genotypes which influence survival. Br J Cancer 2001;85:1492–8.
[48] Wessendorf S, Fritz B, Wrobel G, Nessling M, Lampel S, Goettel D, Kuepper M, Joos S, Hopman T, Kokocinski F, Dohner H, Bentz M, Schwaenen C, Lichter P. Automated screening for genomic imbalances using matrix-based comparative genomic hybridization. Lab Invest 2002;82:47–60.
[49] Dexter DL, Barbosa JA, Calabresi P. N,N-dimethylformamide-induced alteration of cell culture characteristics and loss of tumorigenicity in cultured human colon carcinoma cells. Cancer Res 1979;39: 1020–5.
[50] Brattain MG, Fine WD, Khaled FM, Thompson J, Brattain DE. Heterogeneity of malignant cells from a human colonic carcinoma. Cancer Res 1981;41:1751–6.
[51] von Kleist S, Chany E, Burtin P, King M, Fogh J. Immunohistology of the antigenic pattern of a continuous cell line from a human colon tumor. J Natl Cancer Inst 1975;55:555–60.
[52] Drewinko B, Romsdahl MM, Yang LY, Ahearn MJ, Trujillo JM. Establishment of a human carcinoembryonic antigen-producing colon adenocarcinoma cell line. Cancer Res 1976;36:467–75.
[53] Leibovitz A, Stinson JC, McCombs III WB, McCoy CE, Mazur KC, Mabry ND. Classification of human colorectal adenocarcinoma cell lines. Cancer Res 1976;36:4562–9.
[54] Kawai K, Viars C, Arden K, Tarin D, Urquidi V, Goodison S. Comprehensive karyotyping of the HT-29 colon adenocarcinoma cell line. Genes Chromosomes Cancer 2002;34:1–8.
[55] Culliford AT, Brooks AD, Sharma S, Saltz LB, Schwartz GK, O'Reilly EM, Ilson DH, Kemeny NE, Kelsen DP, Guillem JG, Wong WD, Cohen AM, Paty PB. Surgical debulking and intraperitoneal chemotherapy for established peritoneal metastases from colon and appendix cancer. Ann Surg Oncol 2001;8:787–95.
[56] Sadeghi B, Arvieux C, Glehen O, Beaujard AC, Rivoire M, Baulieux J, Fontaumard E, Brachet A, Caillot JL, Faure JL, Porcheron J, Peix JL, Francois Y, Vignal J, Gilly FN. Peritoneal carcinomatosis from non-gynecologic malignancies: results of the EVOCAPE 1 multicentric prospective study. Cancer 2000;88:358–63.
[57] Demetrick DJ, Zhang H, Beach DH. Chromosomal mapping of the genes for the human CDK2/cyclin A-associated proteins p19 (SKP1A and SKP1B) and p45 (SKP2). Cytogenet Cell Genet 1996;73: 104–7.
[58] SKY and CGH database. Available at: http://www.ncbi.nlm.nih.gov/sky/skyweb.cgi, Accessed January 2004.
[59] Chen TR, Nierman WC. Chromosome painting and quantitative karyotyping of colon adenocarcinoma cell lines, DLD-1 and HCT-15. Anticancer Res 1994;14:109–12.
[60] Chen TR, Dorotinsky CS, McGuire LJ, Macy ML, Hay RJ. DLD-1 and HCT-15 cell lines derived separately from colorectal carcinomas have totally different chromosome changes but the same genetic origin. Cancer Genet Cytogenet 1995;81:103–8.
[61] Yaseen NY, Watmore AE, Potter AM, Potter CW, Jacob G, Rees RC. Chromosome studies in eleven colorectal tumors. Cancer Genet Cytogenet 1990;44:83–97.
[62] Bertrand V, Couturier-Turpin MH, Louvel A, Panis Y, Couturier D. Relation between cytogenetic characteristics of two human colonic adenocarcinoma cell lines and their ability to grow locally or metastasize or both: an experimental study in the nude mouse. Cancer Genet Cytogenet 1999;113:36–44.
[63] Slovak ML, Hoeltge GA, Trent JM. Cytogenetic alterations associated with the acquisition of doxorubicin resistance: possible significance of chromosome 7 alterations. Cancer Res 1987;47:6646–52.
[64] Soulie P, Poupon MF, Remvikos Y, Dutrillaux B, Muleris M. Distinct chromosomal alterations associated with TP53 status of LoVo cells under PALA selective pressure: a parallel with cytogenetic pathways of colorectal cancers. Oncogene 1999;18:775–81.
[65] Tomita N, Jiang W, Hibshoosh H, Warburton D, Kahn SM, Weinstein IB. Isolation and characterization of a highly malignant variant of the SW480 human colon cancer cell line. Cancer Res 1992;52:6840–7.
[66] Gagos S, Iliopoulos D, Tseleni-Balafouta S, Agapitos M, Antachopoulos C, Kostakis A, Karayannakos P, Skalkeas G. Cell senescence and a mechanism of clonal evolution leading to continuous cell proliferation, loss of heterozygosity, and tumor heterogeneity: studies on two immortal colon cancer cell lines. Cancer Genet Cytogenet 1996;90:157–65.