RESEARCH ARTICLE

Comprehensive Molecular Cytogenetic Characterization of Cervical Cancer Cell Lines

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We applied a combination of molecular cytogenetic methods, including comparative genomic hybridization (CGH), spectral karyotyping (SKY), and fluorescence in situ hybridization (FISH), to characterize the genetic aberrations in eight widely used cervical cancer (CC) cell lines. CGH identified the most frequent chromosomal losses including 2q, 3p, 4q, 6q, 8p, 9p, 10p, 13q, and 18q; gains including 3q, 5p, 5q, 8q, 9q, 11q, 14q, 16q, 17q, and 20q; and high-level chromosomal amplification at 3q21, 7p11, 8q23–q24, 10q21, 11q13, 16q23–q24, 20q11.2, and 20q13. Several recurrent structural chromosomal rearrangements, including der(5)t(5;8)(p13;q23) and i(5)(p10); deletions affecting chromosome bands 5p11, 5q11, and 11q23; and breakpoint clusters at 2q31, 3p10, 3q25, 5p13, 5q11, 7q11.2, 7q22, 8p11.2, 8q11.2, 10p11.2, 11p11.2, 14q10, 15q10, 18q21, and 22q11.2 were identified by SKY. We detected integration of HPV16 sequences by FISH on the derivative chromosomes involving bands 18p10 and 18p11 in cell line C-4I, 2p16, 5q21, 5q23, 6q, 8q24, 10, 11p11, 15q, and 18p11 in Ca Ski, and normal chromosome 17 at 17p13 in ME-180. FISH analysis was also used further to determine the copy number changes of *PIKA3CA* and *MYC*. This comprehensive cytogenetic characterization of eight CC cell lines enhances their utility in experimental studies aimed at gene discovery and functional analysis.   © 2003 Wiley-Liss, Inc.

INTRODUCTION

Cervical cancer (CC) is the second most common gynecologic malignancy in the world in both incidence and mortality. Infection with human papilloma virus (HPV) is considered an initiating factor in CC carcinogenesis (Brisson et al., 1994). Although 95% of the patients with precancerous lesions harbor HPV, only a small fraction of the cases eventually progress to invasive cancer (zur Hausen, 2000). Therefore, HPV infection alone was considered insufficient for malignant conversion, suggesting a role of other genetic changes in the development of CC. Identification of such genetic alterations is critical for our understanding of the molecular basis of CC development. Thus, cell lines provide an excellent and continuous source of tumor material for such a genetic characterization.

Conventional cytogenetic analysis has identified several recurrent structural chromosomal changes involving chromosomes 1, 3, 5, 17, and the X chromosome in CC (Atkin, 1997). A precise cytogenetic characterization in CC was not feasible because of the difficulty in identifying complex chromosome changes in primary tumors. Recently, however, several molecular cytogenetic techniques, such as fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), and spectral

karyotyping (SKY)/multicolor-FISH (m-FISH), have been used to illustrate the precise genetic aberrations in tumor genomes (Kallioniemi et al., 1992; Schröck et al., 1996; Speicher et al., 1996; Singh et al., 2001; Rao et al., 2002). Several cell lines derived from patients with CC are available that provide a renewable source of material for genetic analysis and that have been used in various studies. Therefore, complete cytogenetic characterization of CC lines will provide useful genetic information for multiple studies.

Here, we characterized eight widely used CC cell lines by G-banding, CGH, SKY, and FISH, and we present their comprehensive molecular cytogenetic profiling. These cell lines have previously not been subjected to detailed cytogenetic characterization. We identified several chromosomal regions that may be important in tumor de-

velopment. Losses of 2q and gains of 3q, 5p, and 8q were significant. Notably, the combined approach of CGH, SKY, and FISH analysis defined the origin of 2q loss and gain of 3q, 5p, and 8q. In addition, we identified several amplified chromosomal regions: 3q21, 7p11, 8q23–q24, 10q21, 11q13, 16q23–q24, 20q11.2, and 20q13.1. Through FISH, we have also shown the integration of HPV16 sequences in three of the eight cell lines. This comprehensive characterization of these cell lines augments their utility in investigations targeted at gene discovery and functional analysis in CC.

MATERIALS AND METHODS

Cell Lines

The cell lines (Ca Ski, C-33A, C-4I, HT-3, ME-180, MS751, SiHa, and SW756) were obtained from the American Type Culture Collection (ATCC, Manassas, VA), and were grown according to the supplier's recommendations. Chromosome preparations and G-banded karyotypes were made by standard protocols. Clonal chromosomal abnormalities identified by G-banding were described according to An International System for Human Cytogenetic Nomenclature (ISCN, 1995). Clonality was described by detection of two cells with the same structural abnormality or chromosomal gain and loss of the same chromosome in three cells. The metaphase spreads were aged at room temperature for 1 week for SKY. The consensus karyotype was generated for each cell line on the basis of G-banding and SKY analysis.

CGH

High molecular weight DNA was isolated from cell lines and normal placenta by standard methods and subjected to CGH according to a previously published method, with some modifications (Kallioniemi et al., 1992). The metaphase preparations were captured and processed by use of the Quantitative Image Processing System (Applied Imaging, Santa Clara, CA).

SKY

The cocktail of human chromosome paints was obtained from Applied Spectral Imaging (ASI, Carlsbad, CA). Hybridization and detection were carried out according to the manufacturer's protocol, with slight modifications. Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). For each case, at least five metaphase cells were analyzed by SKY. Images were acquired with a SD200 Spectra cube (ASI) mounted on a Zeiss

Axioplan II microscope (Zeiss, Jena, Germany) by use of a custom-designed optical filter (SKY-1; Chroma Technology, Brattleboro, VT) and analyzed by use of SKY View 1.2 software (ASI).

The breakpoints on the SKY-painted chromosomes were determined by comparison of corresponding inverted-DAPI banding of the same chromosome and by comparison with the G-banded karyotype for each cell line. By this method, we were able to define the breakpoints on *add* and *der* chromosomes, but were unable to assign the precise breakpoints of chromosomal segments from partner chromosomes that generated the *add* or *der* chromosomes. A breakpoint was considered to be recurrent if it was identified in two or more cases (Rao et al., 1998). A breakpoint cluster was defined as the occurrence of four or more breakpoints in the same chromosomal band.

FISH

FISH was performed to identify the HPV16 integration sites and copy number changes of *MYC* (8q24) and *PIKA3CA* (3q26.3) in eight CC cell lines. The plasmid containing HPV16 sequences and the BAC clone (386M7) containing *PIKA3CA* was labeled by nick translation with Spectrum Red/Spectrum Green (Vysis, Downers Grove, IL). The digoxigenin-labeled *MYC* probe was obtained from Ventana (Tucson, AZ). Hybridization and detection were performed according to the manufacturer's protocols.

RESULTS

DNA Copy Number Changes Identified by CGH

The most frequent chromosomal losses included 1p (38%), 2q (100%), 3p (62%), 4q (88%), 6q (63%), 8p (75%), 9p (38%), 10p (63%), 10q (38%), 11p (38%), 11q (38%), 13q (75%), and 18q (88%); and gains included 1p (38%), 3q (50%), 5p (63%), 5q (38%), 7p (63%), 7q (63%), 9q (88%), 11q (50%), 14q (50%), 15q (38%), 16q (50%), 17q (63%), 19q (50%), 20p (25%), 20q (88%), and Xq (38%) (Fig. 1A).

Figure 1. **A:** Ideograms showing DNA copy number changes identified by CGH in the cell lines. Thin vertical lines on either side of the ideogram indicate losses (left) and gains (right) of the chromosomal region. High-level amplifications are shown as thick lines (right). **B:** Ideograms showing all of the breakpoints noted in the cell lines identified by SKY (inverted-DAPI) in combination with G-banding (★ represents translocation breakpoint, ● represents deletion breakpoint, ✗ represents duplication breakpoint). The number of breakpoints in each chromosome that were identified by SKY/G-banding, but could not precisely be assigned to a chromosomal band, are noted in the box on top of the chromosome.
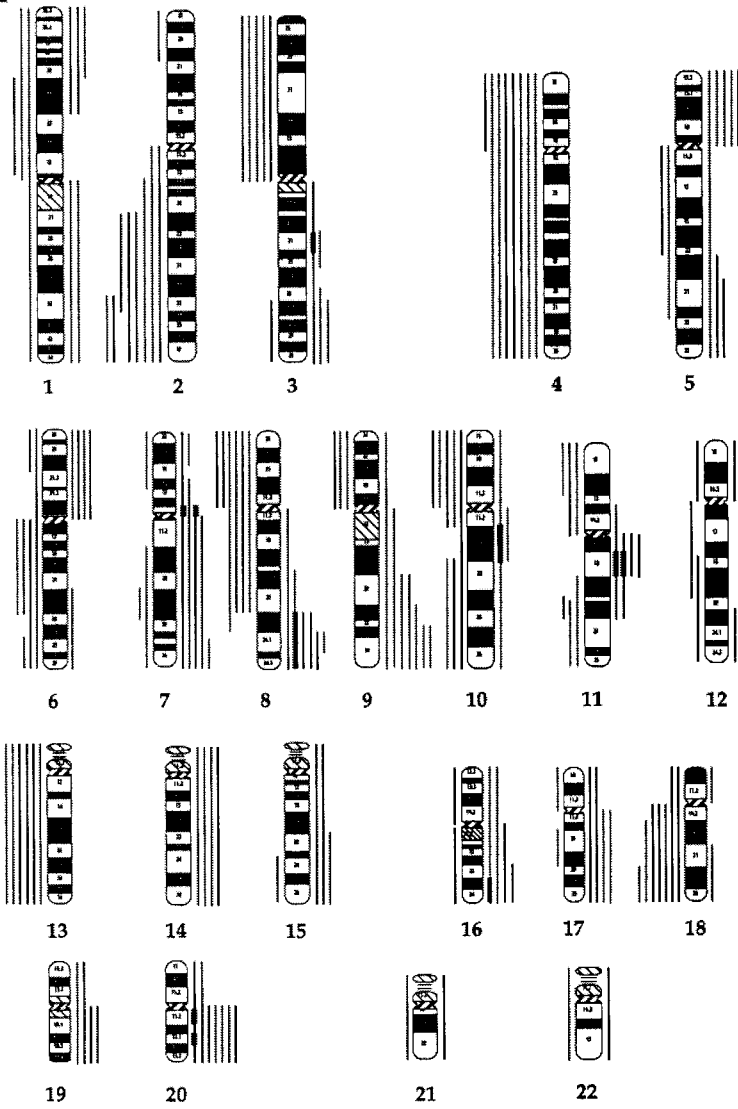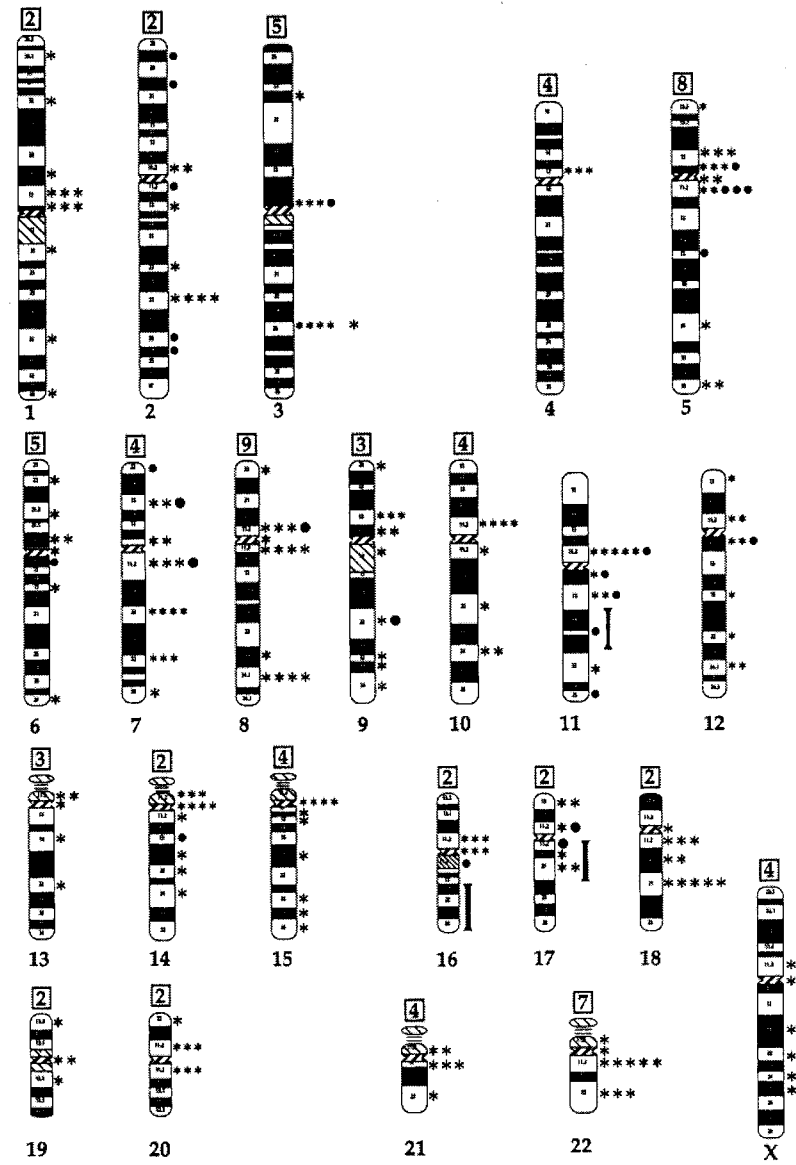
Figure I.

TABLE I. Combined G-Banded and SKY/Inverted DAPI Karyotypes of Eight Cervical Cancer Cell Lines

Cell line	Karyotype
Ca Ski	73~83, X, −X,der(X)t(X;5)(?;?),der(X)t(X;8)(p11;p11),der(1)t(1;4)(q44;?),del(2)(p22–p24),del(2)(q11), der(2)t(2;7)(q31;q22),der(3)t(3;5)(p11;?),der(3) t(3;5)(p22;q35),−4, del(5)(p11),del(5)(q11), der(5)t(5;8)(p15;q23)x2,der(5)t(5;16)(q11;?),del(6)(q11),der(6)t(6;9)(p11;?)t(6;7)(q27;?),der(7)t(7;8) (p11;q23),der(7)t(7;17)(q11.2;q21),der(10)t(5;10)(?;p11),der(10)t(10;20) (p11;?),der(11)t(11; 14)(p11.2;?), der(11)t(11;14) (p11;q22)t(11;13)(q13;?) t(13;15)(?;q21), del(12)(q11), −13,del(13)(q?), −14,der(15)t(6;15)(?;q26),der(14;15)(q10;q10),i(16)(q10),del(16)(q11),der(18)t(12;18)(q11;q11), der(18)t(15;18) (?;q11),−21,der(21)t(10;21)(?;p11.1) [cp 5]
C-33A	46,XX,t(X;10)(q25;q24),der(1)t(1;18)(p36;q21),der(1)t(1;18)(p13;q21),der(1)t(1;10)(q32;q11.2), der(2)t(2;7)(?;p15)del(2)(q21),der(3)t(1;3)(p21;p?)t(3;8)(q25;?),der(3)t(3;7)(q25;q32),der(8)t(2;8) (p11;q11),der(9)t(2;9)(q31;q34),der(13)t(13;19)(q22;?),der(14)t(4;14)(?;?),der(14) t(7;14) (?;p11), der(18)t(18;20) (q12;?),der(21)t(1;21)(p13;p11) [cp 5]
C-4I	45,XX,+X,del(2)(q33),der(4)t(4;22)(p11;q11),der(5)t(5;8)(p15;q23),i(5)(p10),−8,del(11)(p11),dup(11) (q14q22),der(12)t(8;12)(q11;p11),del(14)(q13),der(15)t(15;15)(q10;q10)t(15;16)(q24;?),del(17)(p11), der(17)t(17;19)(p13;?),ins(18;14)(q21;q21–q24),i(18)(p10) [cp 5]
HT-3	63~72, XX,−X,der(1)t(1;12)(p32;q24), der(1)t(1;12)(p11;q11),der(1)t(1;9)(p11;p11),del(2)(q34),der(3)t(3;21)(q25;q?)t(6;21)(q?;q?),der(3) t(3;12)(p11;p11),−4,der(4)t(3;4)(?;?),der(5)t(X;5)(q21;p11)t(5;12)(q?;q15),der(6;13)(p10;q10),der(7) t(7;22)(p11;q11)t(7;5)(q32;q35), der(7)t(4;7)(p11;q11),der(8)t(4;8)(?;p23)t(8;9)(q24;p13),del(9)(?), del(9)(q22),der(10)t(8;10)(?;p11)t(8;10)(?;?),der(10)t(10;15)(q24;q11),del(11)(q11),del(11)(q23), der(13)t(4;13)(p11;p11),der(14)t(14;14)(q10;q10),der(15)t(2;15)(?;q24),der(16)t(9;16)(q11;p11)x2, der(17)dup(17)(q11.2q21) t(1;17)(?;q25),der(18)t(X;18)(p15;q11),der(18)t(8;18)(?;q21),der(18)t(11; 18)(q12;q21),der(19)t(5;19)(q31;q13), der(16;19)(p10;q10)x2,+der(20)t(6;20)(q15;p13),der(19)t(8; 19)(?;?),der(22)t(11;22)(q13;q13)
ME-180	61~62, XX,−X,−1,−2,del(3)(p11),i(3)(q10),−4,del(5)(q11),−6,−7,der(7)t(7;9)(p15;q33)t(3;7)(q25; q22),der(8;22)(p10;q10),der(8)t(5;8)(q?;q24),+der(9)t(5;9)(p13;p13),+der(9)t(7;9)(q22;p13), der(9)t(8;9)(q?;p24),der(10)t(10;10)(?;?),−12,der(12;15)(p10;q10),−13,der(14)t(7;14) (q32;p11), der(16)t(2;16)(q32;p11),−18,−21,der(22)t(17;22)(?;q13) [cp 5]
MS751	39~79,X,der(X)t(X;5)(q?24;q?),i(X)(q10),der(1)t(1;2)(p13;q?13),der(1)(1;8)(p11;?),der(2)t(2;22) (q?31;q11),der(3)t(3;8)(p11;q11),−4,der(5)t(5;8) (q11;?),del(5)(q11),der(6)t(6;18)(p23;p?),der(7)del(7)(p15p22)t(7;22)(q36;q?) t(3;22)(?;q?),−8, der(8)t(8;18)(p11;p?),der(11)t(11;22)(p11;q?)t(14;22)(q11;?),der(11)t(11;22)(p11;q?) t(1;22)(p?;?), der(11)t(11;22)(p11;q11),del(11)(q21–q25),der(12)t(8;12)(p13;?),−13,der(14)t(7;14)(?;p11.1),der(16) t(16;20)(q11;q11),der(17)t(3;17)(?;p13),−18,der(18)t(X;18)(?;q23),−19,der(20)t(15;20)(q?;p11), der(20)t(20;22)(p11;q11)t(15;22)(q?;q13),der(21)t(6;21)(?;?),der(21)t(20;21)(q11;q10),der(22)t(3; 22)(?;p11.1)t(15;22)(q13;q?)
SiHa	61~72, XXX,der(X)t(X;9)(q22;q32),der(2)t(2;10)(q23;q22),der(3)t(3;6)(q11;p21),−4,+der(5)t(5;7)(p?; q22),i(5)(p10),del(5)(q15),der(8)del(8)(p11) del(8)(q?),der(9)t(9;10)(p11;p11),−10,der(12)t(6;12)(?; q22),der(12)t(6;12)(?;q24),−13,der(16)t(7;16)(q?;p11),dup(16)(q21q24), del(17)(q12q?), der(18)t(18; 20)(q12;?),der(19)t(15;19)(q?;p11),der(19)t(19;20)(p11;q11),idic(21;21)(q10;q10) [cp 5]
SW756	67~80,XXXX,+X,+X,+del(X)(?),−1,−2,−3,−4,der(5)t(1;5)(q21;p15),+del(5)(p11),+i(5)(p10), +der(6)t(6;17)(p11;q21),del(7)(q11),der(8)t(2;8)(p11;p11),−9,der(11)t(7;11)(q11;q13),i(12)(p10), der(13)t(13;13)(p11;q14),der(14)t(14;20)(p11;p11),−15,der(21)t(5;21) (?;?),der(21) t(9;21) (q22; q22) [cp 5]

High-level amplification was detected in eight chromosomal sites in seven cell lines at 3q21, 7p11.2, 8q23–q24, 10q21, 11q13, 16q23–q24, 20q11.2, and 20q13.1. Recurrent amplifications were noted in two sites at 7p11 (Ca Ski and MS751) and 11q13 (MS751 and HT-3). Non-recurrent amplifications were noted at 3q21 (SiHa), 8q23–q24 (Ca Ski), 10q21 (ME-180), 16q23–q24 (SiHa), 20q11.2 (SW756), and 20q13.1 (C-33A).

SKY Analysis

A total of 116 translocations, 28 deletions, three duplications, and an insertion were identified by SKY in the eight cell lines. Recurrent deletions affecting chromosomal bands were noted at 5p11 (Ca Ski, SW756), 5q11 (Ca Ski, MS751, SW756), and 11q23 (HT-3, MS751). Table 1 summarizes the karyotypes of the cell lines generated by SKY/G-banding. One reciprocal translocation was noted [t(X;10)(q25;q24) in C-33A] and a recurrent translocation [der(5)t(5;8) (p13;q23)] was noted in Ca Ski and C-4I. Robertsonian translocations involving der(14;15)(q10;q10) in Ca Ski, der(14;14)(q10; q10) in HT-3, and der(21;21)(q10;q10) in SiHa were identified. Translocations involving whole chromosomal arms were identified in two cell lines:
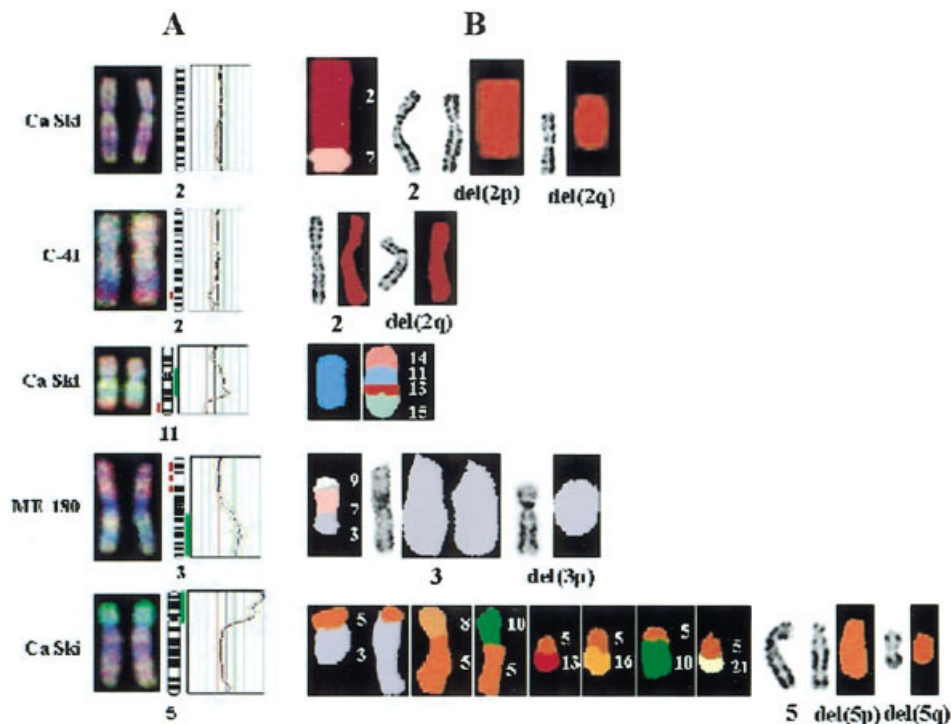
Figure 2. **A:** Partial CGH karyotypes for chromosome 2 (Ca Ski, C-4I), 11 (Ca Ski), 3 (ME-180), and 5 (Ca Ski) (left) and corresponding ratio profiles (right) showing loss of 2q, 11q, and 3p and gain of 3q. The vertical red and green bars on the right of the ideogram indicate the threshold values of 0.80 and 1.20 for loss and gain, respectively. **B:** Partial spectral karyotypes (classified images) of del(2q), del(2p), and der(2) in Ca Ski; del (2q) in C-4I; der(11) in Ca Ski; del(3p) and der(3) in ME-180; and del(5p), del (5q), and der(5) in Ca Ski.

der(8;22)(p10;q10) and der(12;15)(p10;q10) in ME-180 and der(6;13)(p10;q10) and der(16;19) (p10; q10) in HT-3. Six isochromosomes were noted: i(X)(q10) in MS751; i(3)(q10) in ME-180; i(5)(p10) in C-4I, SW756, and SiHa; i(12)(p10) in SW756; i(16)(q10) in Ca Ski; and i(18)(p10) in C-4I, of which i(5)(p10) is recurrent.

SKY/G-banding identified a total of 232 breakpoints in rearranged chromosomes, with another 79 assigned to chromosomes on the basis of the identification of chromosomal segments attached to or inserted into *add* or *der* chromosomes, but whose specific breakpoints could not be determined. Overall, recurring breaks (two or more) were noted in 51 sites. The breakpoint clusters (four or more) were seen at 2q31, 3p10, 3q25, 5p13, 5q11, 7q11.2, 7q22, 8p11.2, 8q11.2, 10p11.2, 11p11.2, 14q10, 15q10, 18q21, and 22q11.2 (Fig. 1B).

Comparison of CGH and SKY Analysis

There was a good concordance between the number of genomic imbalances identified by CGH and unbalanced structural aberrations identified by SKY. An example of the comparison is depicted in Figure 2, which displays a loss of 2q (Ca Ski and

C-4I) and 11q (Ca Ski) and loss and gain of 3p and 3q (ME-180) and 5p and 5q (Ca Ski).

An isochromosome for the short arm of chromosome 5 [i(5)(p10)] was noted by SKY in C-4I, SW756, and SiHa. The corresponding CGH profiles showed only a gain of chromosome arm 5p and no loss of 5q. This may be attributable to the translocation of 5q chromosomal material to other chromosomes in the genome. The loss of Xp and gain of Xq in cell line MS751 and loss of 16p and gain of 16q in Ca Ski can be correlated with the presence of i(X)(q10) and i(16)(q10), respectively. SKY identified i(12)(p10) in SW756, but the corresponding CGH profiles were normal; this discrepancy may be ascribed to the low abundance of this clone in the tumor genome.

The partial or total loss of 2q was identified by CGH in all cell lines analyzed in the present study. The loss of 2q coincides with the presence of unbalanced structural rearrangements: der(2)t(2; 7)(?;p15)del(2)(q21) in C33-A, der(2)t(2;22)(q?31; q11) in MS751, der(2)t(2;10)(q23;q22) in SiHa and SW756, del(2)(q34) in C-4I, and del(2)(q33) and der(2)t(2;9)(q?31;p?) in HT-3. Both deletion [del(2)(q11)] and unbalanced structural rearrange-

ment [der(2) t(2;7)(q31;q22)] affecting 2q were noted in cell line Ca Ski. In cell line ME-180, CGH detected a loss of 2q, but the corresponding SKY analysis failed to show any aberration. This discrepancy may be ascribed to the limitation of sampling size by SKY.

In the majority of cases, the chromosomal amplification results from the unbalanced translocations material from one region to the other chromosomes in the genome except for SIHA, where it displayed the translocation, der(16)t(7;16)(q?;p11), and duplication of the 16q21q24 region. However, the amplification at 10q21 in ME-180 and 11q13 in MS751 was visible only by CGH.

FISH Analysis

We detected integration of HPV16 sequences in C-4I, Ca Ski, and ME-180 by FISH (Fig. 3). The HPV16 integration was noted at 18p10 on i(18)(p10) and at 18p11 on ins(18;14) (q21;q21q24) in C-4I, and on normal chromosome 17 at 17p13 in ME-180. In Ca Ski, HPV16 was integrated at 8q24 on der(X)t(X;8)(p11;p11), at 2p16 on del(2)(p22–p24), at 5q21 and 5q23 on der(5)t(5;8)(p13;q23), at 6q? on der(6)t(6;9)(p11;?), at 11p11 on der(11)t(11;14)(p11;q22) and der(11)t(11;14)(p11.2; ?)t(11;13)(q13;?)t(13;15)(?;q21), at 18p11 on der(18)-t(12;18)(q11;q11), at 15q? on der(18)t(15;18)(?;q11), and at 10? on der(21)t(10;21)(?;p10) (Table 2 and Fig. 3). The HPV16 and *MYC* signals were detected as closely spaced signals on der(X)t(X; 8)(p11;p11)(p13;q23) in Ca Ski. On the basis of fluorescent signal intensity, we estimated the HPV16 copy number to be two to seven copies in Ca Ski and two to four copies in C-4I.

Because we identified recurrent gains at 3q and 8q by CGH, we performed FISH analysis to estimate copy numbers in interphase nuclei and metaphase spreads through use of *PIK3CA* (3q26.3) and *MYC* (8q24) as probes (Table 2). The distribution of copy number for *MYC* ranged from two to six copies, with more than five copies being noted in Ca Ski, ME-180, MS751, and SiHa. In the ME-180 cell line, we identified der(5)t(5;8)(p13;q23) by SKY, but FISH analysis identified this as a cryptic duplication of *MYC* on the der(5) chromosome (Fig. 3). This was consistent with the increased copy number identified by CGH at 8q23–q24.1. By use of *PIK3CA* (3q26.3) as a probe, FISH analysis revealed a copy number increase in C-4I (three to five copies), Ca Ski (two to four copies), and ME-180 (two to three copies). These cell lines exhibited gain of chromosome arm 3q by CGH.

DISCUSSION

We report here a comprehensive molecular cytogenetic description of eight CC cell lines by G-banding, SKY, CGH, and FISH. CGH identified the most frequent chromosomal losses including 2q, 3p, 4q, 6q, 8p, 9p, 10p, 13q, and 18q; and gains including 3q, 5p, 5q, 8q, 9q, 11q, 14q, 16q, 17q, and 20q. The DNA copy number changes identified in this study are consistent with previously published CGH results for primary tumors (Heselmeyer et al., 1996; Dellas et al., 1999; Kirchhoff et al., 1999; Allen et al., 2000; Umayahara et al., 2002). High-level chromosomal amplifications at 3q21, 7p11, 8q23–q24, 10q21, 11q13, 16q23–q24, 20q11.2, and 20q13.1 were noted in seven of the eight cell lines.

An increased copy number for 3q was noted in four of the eight cell lines, and the common region mapped to 3q26–q27. The 3q gain was identified in a diverse group of tumors including head and neck, cervix, lung, and esophagus, and it has been shown to associate with a progression from high-grade CIN to invasive CC (Heselmeyer et al., 1996). Although previous studies have identified several candidate genes on 3q26–q27, controversy still exists regarding the gene(s) that are the target of amplification in CC. The *PIK3CA* locus, which encodes a catalytic subunit of phosphatidylinositol 3-kinase, has been implicated as a candidate oncogene at 3q26 in carcinomas of the cervix, ovary, and head and neck (Shayesteh et al., 1999; Ma et al., 2000; Singh et al., 2001). We recently showed that the negative effect of *TP53* induction on cell survival involves the transcriptional inhibition of *PIK3CA* that is independent of *PTEN* activity in squamous cell carcinomas (Singh et al., 2002). Another gene, *eIF-5A2*, a member of the eukaryotic initiation factor, which plays a role in translation initiation, has been implicated as a candidate oncogene in ovarian cancer (Guan et al., 2001). Gains of 5p were noted in five of eight cell lines studied. This is suggestive of the presence of i(5)(p10) in the tumor genome and is consistent with previously published cytogenetic studies (Atkin, 1997). The telomerase reverse transcriptase (*TERT*) that maps to 5p15 has been shown to control the telomerase activity by an expression of its catalytic component. The *TERT* gene has been shown to be a putative oncogene and was amplified in several solid tumors, including cervical cancer (Greider, 1999; Zhang et al., 2002).

The loss of 2q was noted in 100% of the cell lines, and the common region of deletion was
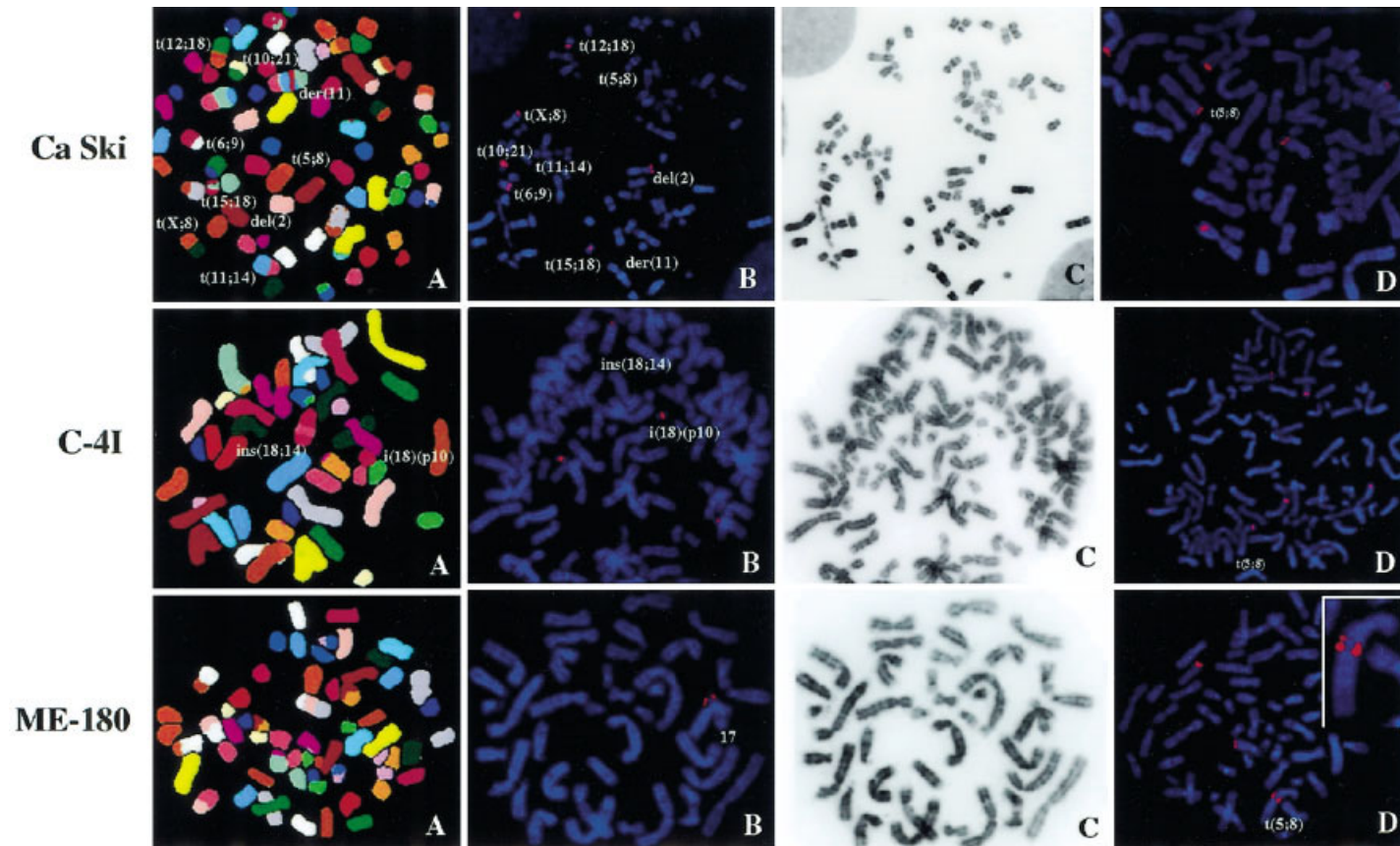
Figure 3. Metaphase spreads from Ca Ski, C-4I, and ME-180 hybridized with SKY painting probes, HPV16, and *MYC* probes. For each image from left to right are spectral classified images (**A**), FISH images with HVP16 integration sites (**B**), and corresponding DAPI (**C**) and *MYC* signals (**D**). The integrated HPV16 sites are numbered in all metaphase spreads. **Inset:** Magnified image of duplicated *MYC* in the ME-180 cell line.

TABLE 2. FISH Analysis of Eight Cervical Cancer Cell Lines

Cell line	Number of copies		HPV16 integration sites[b]	Previous HPV status[c]
	PIK3CA[a]	MYC[a]		
Ca Ski	2–4 (3q25–q29)	6 (8q21.3–q24.3)	**8q24** on der(X)t(X;8)(p11;p11)	HPV16
			2p16 on del(2)(p22–p24)	
			5q21 and **5q23** on der(5)t(5;8)(p13;q23)	
			6q? on der(6)t(6;9)(p11;?)	
			11p11 on der(11)t(11;14)(p11.2;q22)	
			11p11 on der(11)t(11;14)(p11.2;?)t(11;13)(q13;?)t(13;15) (?;q21)	
			18p11 on der(18)t(12;18) (q11;q11)	
			15q? on der(18)t(15;18)(?;q11)	
			10? on der(21)t(10;21)(?p10)	
C-33A	2	2–4	None	Negative
C-4I	3–5 (3q26–q29)	2–3	**18p10** on i(18)(p10)	HPV18
			18p11 on ins(18;14)(q21;q21q24)	
HT-3	2	2 (8q23–q24)	None	Negative
ME-180	2–3 (3q)	4–6 (8q23)	**17p13** on 17	HPV39
MS751	2	3–6 (8q)	None	HPV18
SiHa	2 (3q21–q23)	3–5 (8q24)	None	HPV16
SW756	2	3–5 (8q)	None	No information

[a]Gains identified by CGH are presented in parentheses.
[b]HPV16 integration breakpoints are in bold.
[c]Source: American Type Culture Collection (ATCC).

mapped to the 2q33–q36 region. SKY analysis also confirms the deletions as unbalanced structural rearrangements affecting the 2q region in seven of the eight cell lines. These data strongly suggest that this region harbors a tumor-suppressor gene(s). We recently identified two common regions of deletions at 2q34–q35 and 2q36.3–q37.1 by high-resolution deletion mapping studies (data not shown). Other frequently deleted chromosomal regions were 1p, 3p, 4p, 4q, 6q, 7q, 8p, 11p, 11q, 13q, 17p, 17q, 18q, 19p, and 19q. Some of these regions (e.g., 3p, 4q, 5p, 6p, and 11p) were previously shown to be deleted by loss of heterozygosity studies (Atkin, 1997; Luft et al., 1999; Pulido et al., 2000; Chatterjee et al., 2001).

SKY analysis allowed the identification of novel breakpoints and recurrent translocations. Previous cytogenetic studies in primary tumors and cell lines identified several recurrent changes involving chromosomes 1, 3, 5, 11, 17, and the X chromosome (Atkin, 1997). The recurrent chromosomal rearrangements identified by SKY were translocation der(5)t(5;8)(p13;q23), i(5)(p10), and deletions affecting chromosomal bands 5p11, 5q11, and 11q23. SKY identified several breakpoint clusters at 2q31, 3p10, 3q25, 5p13, 5q11, 7q11.2, 7q22, 8p11.2, 8q11.2, 10p11.2, 11p11.2, 14q10, 15q10, 18q21, and 22q11.2. Further, characterization of genes in the breakpoint clusters may provide clues for understanding the biology and clinical behavior of CC.

CC is shown to associate with HPV infection, particularly the high-risk types HPV16 and HPV18 (Canavan and Doshi, 2000). It has been established that the oncogenic HPV viral proteins E6 and E7 interact with and inactivate the tumor-suppressor proteins p53 and pRb1, thereby initiating a malignant transformation (zur Hausen, 2000). The HPV integration was found in a limited number of CC cell lines or primary tumors at normal or abnormal chromosomes. Recent molecular cytogenetic characterization of the HeLa cell line revealed HPV18 integration on normal chromosome 8 (8q24) and two derivative chromosomes involving the 8q24 band (Macville et al., 1999). In the present study, we noted HPV16 integration on normal chromosome 17 at 17p13 in the ME-180 cell line and two derivative chromosomes involving chromosome bands 18p10 and 18p11 in C-4I. In cell line Ca Ski, HPV integration was observed on one derivative chromosome involving the 8q24 band and another eight derivative chromosomes involving 2p16, 5q21, 5q23, 6q, 10, 11p11, 15q, and 18p11. This strongly suggests that the genomic reorganization is caused by HPV viral integration in cervical cancer. This is consistent with previously published correlation studies between the breakpoints in chromosomal rearrangements and HPV integration sites in cervical cancer (De Braekeleer et al., 1992).

In conclusion, we report here the precise and detailed characterization of eight frequently used

CC cell lines by a combination of molecular cytogenetic methods, including CGH, SKY, and FISH. This comprehensive genetic characterization further augments experimental studies aimed at gene discovery.

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