Cytogenetic Features of Human Trophoblast Cell Lines SWAN-71 and 3A-subE

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Abstract
Immortalization of primary cells with telomerase is thought to maintain normal phenotypic properties and avoid chromosomal abnormalities and other cancer-associated changes that occur following simian virus 40 tumor antigen (SV40 Tag) induced immortalization. However, we report that the human telomerase reverse transcriptase (hTERT)-immortalized SWAN-71 trophoblast cell line has a near pentaploid 103~119,XXXX[cp20] karyotype. Additionally, DNA typing analysis indicated that SWAN-71 cells have acquired microsatellite instability. In comparison, the post-crisis SV40-transformed trophoblast cell line 3A-subE was hypertriploid 69~81,XX[cp20]. Both cell lines contained multiple specific clonal rearrangements. These findings emphasize the need to monitor for genetic instability in hTERT-immortalized cells.

Keywords
Trophoblast cell lines; cellular immortalization; telomerase; karyotype; polyploidy; short tandem repeat profile

Abbreviations
ATCC, American Type Culture Collection; hTERT, human telomerase reverse transcriptase; STR, short tandem repeats; SV40 Tag, simian virus 40 tumor antigen;

1. Introduction

Telomeres in viral oncogene transformed cells continue to shorten, resulting in chromosomal instability and crisis [1]. In contrast, cells that have been immortalized by reconstituting telomerase activity retain functional telomeres and tend to maintain a stable karyotype [2, 3]. However, hTERT-immortalized cells remain susceptible to growth arrest and DNA damage, particularly when culture conditions are not optimal [4]. Accordingly, several studies have reported that after long-term culture, cells with defects in cell cycle control, DNA damage response, and DNA repair mechanisms have emerged and overtaken the culture [5-7].

In the course of our validation studies on the first trimester extravillous SWAN-71 and term villous trophoblast 3A-subE cell lines, we performed DNA profiling and cytogenetic analysis. The SWAN-71 cell line was immortalized by exogenous expression of hTERT [8], whereas the 3A-subE line is a subclone that arose after the SV40 Tag-immortalized human 3A placental cell line underwent crisis [9]. We report that following 20 passages in culture, the hTERT-immortalized SWAN-71 cell line was no longer karyotypically normal. Additionally, the DNA profile indicated that these cells now exhibited a microsatellite instability phenotype. In comparison, the post-crisis 3A-subE cell line has developed additional karyotypic abnormalities compared to the parental 3A line, but the DNA profile has remained stable.

2. Materials and methods

2.1. Trophoblast cell lines.

The SWAN-71 cell line was derived from a 7-week normal placenta using the retroviral system consisting of the murine pA317-hTERT expressing cell line and the puromycin resistance gene [8]. Immortalization of this cell line occurred in September 2003 and the SWAN-71 clonal

isolate underwent 20 passages to confirm that it was an established cell line before it was shared with other laboratories. Numbering of the cell passages began in the recipient laboratories. One aliquot of cells used in this study came from a stock that was frozen in June 2004 at passage 7 (supplied by Dr. S. Guller, Yale University), and a second aliquot came from a frozen stock that was made in 2005 by Dr. G. Mor's lab (passage number not available). Viable aliquots of documented early passages of the SWAN-71 cell line could not be recovered for analysis. For this study, SWAN-71 cells were reselected for the presence of the hTERT expression construct by growing cultures in the presence of 800 ng/ml puromycin for 7 days.

The 3A-subE [post-crisis of 3A(tPA-30-1)] (ATCC CRL-1584) cell line was obtained from the American Type Culture Collection, Manassas, VA. Cell line authentication was performed within three passages of thawing frozen aliquots. DNA collection cards were spotted with 100,000 cells and were analyzed for a panel of 17 highly polymorphic short tandem repeat (STR) markers plus amelogenin for gender determination by DNA Diagnostics Center (Fairfield, OH).

2.2. Cytogenetic analysis.

Cells were exposed to 1 μg/ml colcemid for 1 hour and fixed in Carnoy's fluid (1:3 acetic acid: methanol). Metaphase spreads were prepared following standard cytogenetic procedures. Twenty metaphase cells from each cell line were analyzed and six metaphase spreads were karyotyped using ISCN 2013 nomenclature [10].

3. Results

Cell line authentication was performed to ensure that cross-contamination of cell cultures had not occurred. The STR analysis for 3A-subE cells was identical with the 8 loci reported on the product sheet (ATCC CRL-1584). We report here allele sizes for an additional 9 loci (Table 1, Fig S1). Since no DNA typing had been reported previously for SWAN-71 cells, we analyzed the DNA profiles of two cultures that were provided by separate laboratories. We found the allele sizes for 16 of 17 loci were identical between the two SWAN-71 stocks, which differed only at one of the FGA alleles (Table 1, Fig S1). The SWAN-71 profile did not match those of any other cell line in the STR database (https://www.atcc.org/STR_Database.aspx); however, it revealed that 13 loci had three or four alleles.

Cytogenetic analysis revealed that SWAN-71 cells were near pentaploid (5N) with a composite karyotype of 103~119,XXXX[cp20] (Fig. 1A and S2). Several normal chromosomes were present in six copies and 11 clonal rearrangements were found. In comparison, the composite karyotype for the post-crisis 3A-subE cell line was 69~81,XX[cp20] (Fig. 1B and S3); several normal chromosomes were present in four copies and 15 clonal rearrangements were identified. The cytogenetic and DNA profiling results from the amelogenin locus indicated that both SWAN-71 and 3A-subE cell lines are female.

4. Discussion

The cytogenetic analysis reported here indicates that both SWAN-71 and 3A-subE cell lines appear to have become tetraploid. Chromosome missegregation in tetraploid cells likely led

to further chromosomal aneuploidy. The ATCC product sheet for the parental SV40-transformed 3A(tPA-30-1) cell line reported that most cells contained 41-45 chromosomes including five or six non-clonal marker chromosomes, and that 20% of cells had a higher ploidy. Specific structural or numerical chromosome abnormalities in the 3A cells were not described, so comparison with the 3A-subE subclone was not possible; nevertheless, no apparent mutations occurred in the STR alleles following crisis.

STR typing that results in three to four peaks at the majority of loci typically is associated with cellular cross-contamination. However, we think that this explanation for SWAN-71 is unlikely because cytogenetic analysis revealed a homogeneous population of ~5N cells. In addition, the STR typing was identical in cell stocks that were obtained from separate laboratories and in cells where the exogenous hTERT construct had been reselected with puromycin. It is possible that like many other immortalized cell lines, SWAN-71 cells went through cellular crisis, resulting in both chromosomal and microsatellite instability. However, another interesting possibility is that these cells might have initially undergone endoreduplication to become 4N or higher ploidy. SWAN-71 cells express markers for extravillous trophoblasts and aneuploidy appears to be a normal phenomenon in this invasive cell type that limits their proliferation in maternal decidua [11-13]. Additional mutations in SWAN-71 cells likely occurred to overcome normal cell cycle checkpoints, similar to those required to overcome crisis.

The SWAN-71 cells characterized in this report came from frozen stocks that were made soon after the cell line was established. Thus, although the exact passage numbers of these stocks are not known, it appears that tetraploidization likely occurred before this cell line had been widely shared with other laboratories and before the first publications that reported on their original properties [8, 14]. While SWAN-71 and other trophoblast cell lines have served as useful models for investigating placental function, all have limitations and further characterization of their specific genetic and epigenetic mutations is warranted. These results emphasize the need for continued monitoring for genetic mutations in immortalized cell lines regardless of how they were derived.

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Conflict of interest

The authors have nothing to disclose and no conflict of interest.

Appendix A. Supplementary data

Figure S1. SWAN-71 and 3A-subE electropherograms and DNA typing reports

Figure S2. SWAN-71 additional karyotypes

Figure S3. 3A-subE additional karyotypes

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Figure Caption

Fig. 1. Cytogenetic analysis of trophoblast cell lines. Representative karyograms and composite karyotypes are shown for (**A**) SWAN-71 and (**B**) 3A-subE cell lines.

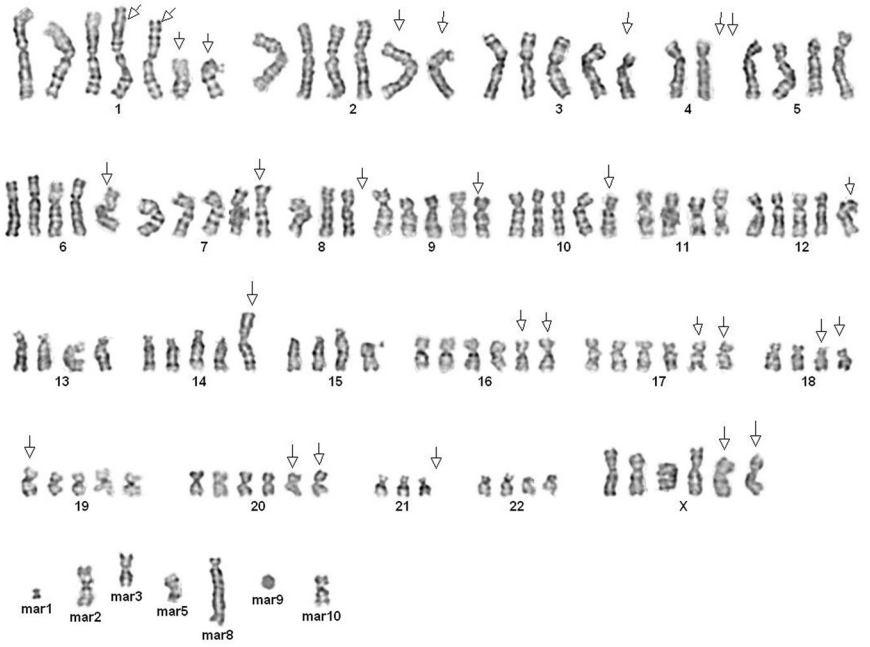
Table 1
DNA Typing Profiles

Locus	SWAN-71	3A-subE [a]
CSF1PO	10, 11, 12	*10, 12*
D13S317	11, 12	*11*
D16S539	9, 11, 12, 13	*9, 12*
D5S818	11, 12, 13	*11, 13*
D7S820	10, 11	*10*
TH01	8, 9, 9.3	*9, 9.3*
TPOX	6, 8, 11	*8, 11*
vWA	14, 15, 16, 17	*16, 17*
D3S1358	15, 16, 17	15
D21S11	28, 29, 30	28, 29
D18S51	12, 14, 20	12, 20
Penta E	5, 7, 9, 14	7, 14
Penta D	8, 9, 11, 13	8, 9
D8S1179	13, 14	13, 14
FGA	(20, 21), 25	21, 25
D19S433	12, 14, 16.2	14, 16.2
D2S1338	17, 18, 20, 22	20, 22
Amelogenin	X	X

[a] Alleles reported by ATCC are italicized.

A

SWAN-71 Composite Karyotype:
103~119,<4n>,XXXX,+X,+X,+del(1)(p13),+del(1)(q21),+iso(1)(q10),+2,+del(2)(p11.2),+del(3)(p13),
+del(3)(p21),-4,+6,+7,+7,+8,-8,+9,+9,+10,+12,add(14)(p11.2),-15,+16,+16, +17,+17,
+del(18)(q21.1q21.3)x2,+add(19)(q13.3),+der(19)t(1;19)(q23;p13.1),+20,+20,-21,+22,
der(22)t(1;22)(p13;p11.2)x2,+mar1,+mar2,+mar3,+mar4,+mar5,+mar6,+mar8, +mar10, +mar11[cp20]



B

3A-subE Composite Karyotype:
69~81,XX,+X,+add(X)(p22.3),der(1;1)(q10;q10)add(1)(q32),iso(1)(q10),+2,+del(2)(p15),+3,+del(3)(p21),
+del(3)(p13),+del(3)(q21),+4,+5,+del(5)(q13q33),+6,+7,+7,+add(7)(q32),+8,+8,+9,+9,+10,+10,
+add(11)(p11.2),+add(11)(q23),add(11)(q23),+12,-13,+15,+15,+16,+16,+17,+17,+del(18)(q21q21),+19,
+20,+20,+20,+21,+der(22)t(1;22)(p13;p11.2),der(22)t(1;22)(p13;p11.2), +mar1,+mar2,+mar3,+mar5,
+mar6,+mar9,+mar13[cp20]