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Aneuploidy in immortalized human mesenchymal stem cells with non-random loss of chromosome 13 in culture

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Abstract Aneuploidy (an abnormal number of chromosomes) is commonly observed in most human cancer cells, highlighting the need to examine chromosomal instability in tumorigenesis. Previously, the immortalized human mesenchymal stem cell line UE6E7T-3 was shown to undergo a preferential loss of one copy of chromosome 13 after prolonged culture. Here, the loss of chromosome 13 was found to be caused by chromosome missegregation during mitosis, which involved unequal segregation, exclusion of the misaligned chromosome 13 on the metaphase plate, and trapping of chromosome 13 in the midbody region, as observed by fluorescence in situ hybridization. Near-diploid aneuploidy, not tetraploidy, was the direct result. The loss of chromosome 13 was non-random, and was detected by analysis of microsatellites and single nucleotide polymorphism-based loss of heterozygosity (LOH). Of the five microsatellite loci on chromosome 13, four loci showed microsatellite instability at an early stage in culture, and LOH was apparent at a late stage in culture. These results suggest that the microsatellite mutations cause changes in centromere integrity provoking loss of this chromosome in the UE6E7T-3 cell line. Thus, these results support the use of this cell line as a useful model for understanding the mechanism of aneuploid formation in cell cultures.

Keywords Aneuploidy · Human mesenchymal stem cell · Loss of chromosome 13 · Loss of heterozygosity · Microsatellite instability

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Introduction

In human tumors, aneuploidy (an abnormal number of chromosomes) occurs frequently, and has been proposed to drive tumor progression by enhancing genomic instability, as originally proposed by Boveri nearly a century ago. But even now, how aneuploidy arises in cells and whether it causes tumors remains to be fully elucidated (Pellman 2007). Currently, two principal mechanisms have been proposed to generate aneuploidy through chromosome missegregation (a chromosome distribution error) during mitosis. One hypothesis states that nondisjunction yields a tetraploid intermediate, and aneuploid cells are subsequently formed through chromosomal loss from the tetraploidy (Shi and King 2005). Recently, Shi and King, using fluorescence in situ hybridization (FISH) in a human cell line, proposed that nondisjunction-induced failure of cytokinesis, coupled with tetraploidization and subsequent aberrant mitosis, underlies the aneuploidy that is frequently found in human cancer cells (Shi and King 2005). Their hypothesis is supported by evidence showing that tetraploidy is frequently present with aneuploidy in many human cancers (Galipeau et al. 1996; Nigg 2002; Olaharski et al. 2006). However, the gain of a single chromosome during meiosis of a human egg or sperm is well known, as seen with trisomies involving chromosomes 13, 18, or 21, or the sex chromosomes (resulting in XXX, XXY, or XYY genotypes). Such chromosome missegregation is also frequently observed during mitosis of human cancer cells (Lengauer et al. 1997; Weaver and Cleveland 2006) and with mosaic variegated aneuploidy syndrome (Hanks et al. 2004). These observations can be explained by a different mechanism; nondisjunction through gain or loss of a single chromosome results directly in near-diploid aneuploidy, and does not involve a tetraploid intermediate (Weaver et al. 2006).

Chromosomal instability has been studied in human mesenchymal stem cells immortalized with human papillomavirus type 16E6/E7 (HPV-16E6/E7) and human telomerase reverse transcriptase (hTERT) genes. Preferential loss of one copy of chromosome 13, yielding near-diploid aneuploidy, occurred in all three cell lines examined (Takeuchi et al. 2007). This phenomenon is not unique to these three cell lines, and is observed frequently in cultured human vascular endothelial cells (Zhang et al. 2000; Kimura et al. 2004; Wen et al. 2006; Anno et al. 2007) suggesting that it might be associated with telomere length. However, cells immortalized with hTERT gene also lost chromosome 13 during culture as shown in our studies (Takeuchi et al. 2007) and those of others (Wen et al. 2006; Anno et al. 2007). These observations suggest that near-diploid aneuploidy with loss of chromosome 13 occurs independently of telomere length in most cases.

Many proteins are involved in the maintenance of correct chromosome number during cell division. A significant number have been identified, and include members of the kinetochore complex such as Mad1, Mad2, BubR1, Bub1, Bub3, and CEMP-E. The signaling pathways involving these proteins have been extensively investigated (Bharadwaj and Yu 2004; Weaver and Cleveland 2006). Recently, direct evidence has shown that the reduced levels of these components lead to chromosome instability: cells in which the mitotic checkpoint was completely inactivated by siRNA-mediated depletion of *Mad2* or *BubR1* or by knockout of *CENP-E* showed missegregation of large numbers of chromosomes during anaphase, and the cells died due to chromosome loss (Dobles et al. 2000; Putkey et al. 2002; Weaver et al. 2003; Kops et al. 2004; Meraldi et al. 2004; Michel et al. 2004), while partial loss or mislocation of these components led to weakened signal generation at an individual, unattached kinetochore; thus, the cells survived and exhibited chromosome instability (Michel et al. 2001; Weaver et al. 2003; Kops et al. 2004). Therefore, cells with reduced levels of these components became aneuploid due to the random missegregation of one or a few chromosomes, that is, random loss of any one of the chromosomes. In contrast, UE6E7T-3 cells lost only one copy of chromosome 13. This finding suggests that there was loss of heterozygosity (LOH) of chromosome 13, not loss of spindle checkpoint components.

This question could be elucidated by analyzing for alterations in microsatellite alleles (microsatellite instability: MSI) on chromosome 13. Based on the random occurrence of microsatellites on all chromosomes, such an analysis not only provide significant information about genomic instability, but also allows the detection of the corresponding allelic deletion (LOH) (Ionov et al. 1993; Powierska-Czarny et al. 2003). MSI and LOH have been detected in a wide variety of human neoplasmic tumors, both sporadic and hereditary cancers, and are therefore used as important indicators of genomic instability, during the progression of cancer (Miturski et al. 2002). Recently, analysis of single nucleotide polymorphism (SNPs) analysis has emerged as a promising method for detecting chromosome copy number changes and LOH in cancer (Lindblad-Toh et al. 2000; Zheng et al. 2002; Pfeifer et al. 2007). Using a high-resolution analysis, we explored an SNP array kit with 2×10^5 SNPs in order to detect LOH of chromosome 13 in UE6E7T-3 cells.

In the present study, aneuploidy with loss of chromosome 13 was examined in UE6E7T-3 cells after prolonged culture. Using FISH with a specific probe for chromosome 13, the loss of chromosome 13 was found to arise through chromosome missegregation, resulting directly in near-diploid aneuploidy. Moreover, analyses of the microsatellite DNA of chromosome 13 and the SNP-based LOH assay showed that one copy of chromosome 13 in UE6E7T-3 cells had MSI and the chromosome was lost after prolonged culture.

Materials and Methods

Cell culture. A human mesenchymal stem cell line, UE6E7T-3 (JCRB1136), was obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank (Osaka, Japan), which entailed no ethical problems. The UE6E7T-3 cells have been described previously (Takeuchi et al. 2007). The UE6E7T-3 cells were cultured in POWEREDBY10 medium (Med-Shirotori Co.; Tokyo, Japan). Cells were seeded at a concentration of 5×10^3 cells/ml and were cultured for 6–10 d. When culture plates were subconfluent, cells were treated with 0.25% trypsin and 0.5 mM EDTA (both from Invitrogen; Tokyo, Japan), before re-plating at a density of 5×10^3 cells/ml, as described previously (Takeuchi et al. 2007). All cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. The population doubling level (PDL) was calculated according to the following formula:

$$PDL = \log(\textit{cell output}/\textit{input})/\log 2$$

At the start of cultivation, the PDL of the UE6E7T-3 cells was 60.

Measurement of chromosome number. Metaphase chromosome spreads used for measurement of chromosome number and FISH were prepared from exponentially growing cells at various PDLs. The cells were treated in a hypotonic solution (0.075 M KCl) after exposure to 0.06 μg/ml colcemid (Invitrogen; Carlsbad, CA) for 1.5–2 h. After removal of the hypotonic solution, the cells were fixed in a solution of methanol and acetic acid (3:1). This

procedure was repeated several times with fresh fixative. The cells were then mounted on microscope slides.

In order to count the number of chromosomes, cells were stained with DAPI (4′-6-diaminido-2-phenylindol; Vector Laboratories, Inc.; Burlingame, CA) and examined using an Axioplan II imaging microscope (Carl Zeiss Microimaging GmbH; Jena, Germany) equipped with Leica QFISH software (Leica Microsystems Holding; Buckinghamshire, UK). In each assay, 50–54 metaphase spreads were scored.

Cell staining. For the staining of mitotic cells, on day 2 of culturing, the culture medium was removed and loosely adhered mitotic cells were collected in PBS using a shake-off protocol described elsewhere (Piel et al. 2001). The harvested cell suspension was fixed with 2% paraformaldehyde in PBS at room temperature for 10 min and centrifuged using a Cytospin (Shandon Cytospin 4; Thermo; Leicestershire, UK). The cells adhered to the glass coverslip were stained using a painting probe that was specific for chromosome 13 (XCP13 kit; MetaSystems; Atlussheim, Germany), described previously (Takeuchi et al. 2007).

Slides were analyzed using a motorized epifluorescence microscope (Axio Imager Z1; Carl Zeiss Microimaging) equipped with the appropriate filter sets and a MetaSystems Isis/mFISH imaging system (MetaSystems).

Microsatellite DNA assay. For detection of MSI and LOH of chromosome 13, polymerase chain reactions (PCR) were carried out using primers for specific microsatellite markers (D13S1493, D13S153, D13S788, D13S800, and D13S154) for the chromosomes 13. All primers are shown in Table 1. Microsatellite PCR was performed in a reaction volume of 20 μl that contained 10 ng of cell DNA and 10 ng of each oligonucleotide primer. AmpliTaq Gold DNA Polymerase (Applied Biosystems; Foster City, CA) was used for polymerase reaction of five markers. Reaction mixtures containing sample DNAs were heated at 96°C for 12 min, and cycled for 25 s at 95°C, and maintained for 30 s at 53°C for annealing, and for 50 s at 72°C for polymerase reaction. PCR products were applied to Capillary Electrophoresis equipment (Model HAD-GT12; eGene, Inc.; Irvine, CA) for molecular weight analysis using a QIAxcel DNA analysis kit.

SNP analysis. UE6E7T-3 DNAs were isolated using an isolation kit (Amersham BioSciences; Little Chalfont, UK). Hybridization of UE6E7T-3 DNA was performed according to a protocol of the Affymetrix Gene Chip Human Mapping 250K Nsp Array Set (Affymetrix, Inc.; Santa Clara, CA). Signal intensity of the fluorescence was gained using Gene Chip Operation Software GOS1.4 (Affymetrix). The SNP types indicating heterozygote or homozygote was estimated with Gene Chip Genotyping Analysis Software (GTYPE) 4.0 (Affymetrix).

Results

Changes in chromosomal number in long-term UE6E7T-3 cell cultures. Previously, aneuploid formation, accompanied by the loss of chromosome 13, was demonstrated in three immortalized human mesenchymal stem cell lines cultured for prolonged periods of time (Takeuchi et al. 2007). Changes in chromosomal numbers were, in detail, re-examined in a human mesenchymal stem cell line, UE6E7T-3, in order to ascertain whether or not the origin of aneuploidy in the UE6E7T-3 cells was diploid or tetraploidy.

As shown in Fig. 1, nearly 90% of the cell population contained 46 chromosomes up to PDL62, but by PDL85 this proportion had decreased markedly to 35.3%, and a new population that contained 42–45 chromosomes appeared (31.3%). From PDL100 to PDL147, population patterns remained similar, and a population that contained 44 chromosomes became dominant (26–44% of the cell population). Near-tetraploidy appeared at PDL85 but was

Table 1. Sequence of primers used for PCR analysis

Marker	Location	Sequence of primer		PCR product (bp)
D13S1493	13q13.2	FW	ACCTGTTGTATGGCAGCAGT	223–248
		Rev	GGTTGACTCTTTCCCCAACT	
D13S153	13q14.2	FW	GACTCCTGTTTCTCCTCCCTG	155
		Rev	ATTTGTGGAAAGGAGCGTAT	
D13S788	13q14.3	FW	GATTGAGGTAGGGTCCCAAG	240–270
		Rev	GCTCCATAATTGTGTGAGCC	
D13S800	13q22.1	FW	AGGGATCTTCAGAGAAACAGG	295–319
		Rev	TGACACTATCAGCTCTCTGGC	
D13S154	13q32.1	FW	GTGCTATAAAGGCTTGCTGC	243–277
		Rev	CTCTTGCCCTGGTCTTGACT	

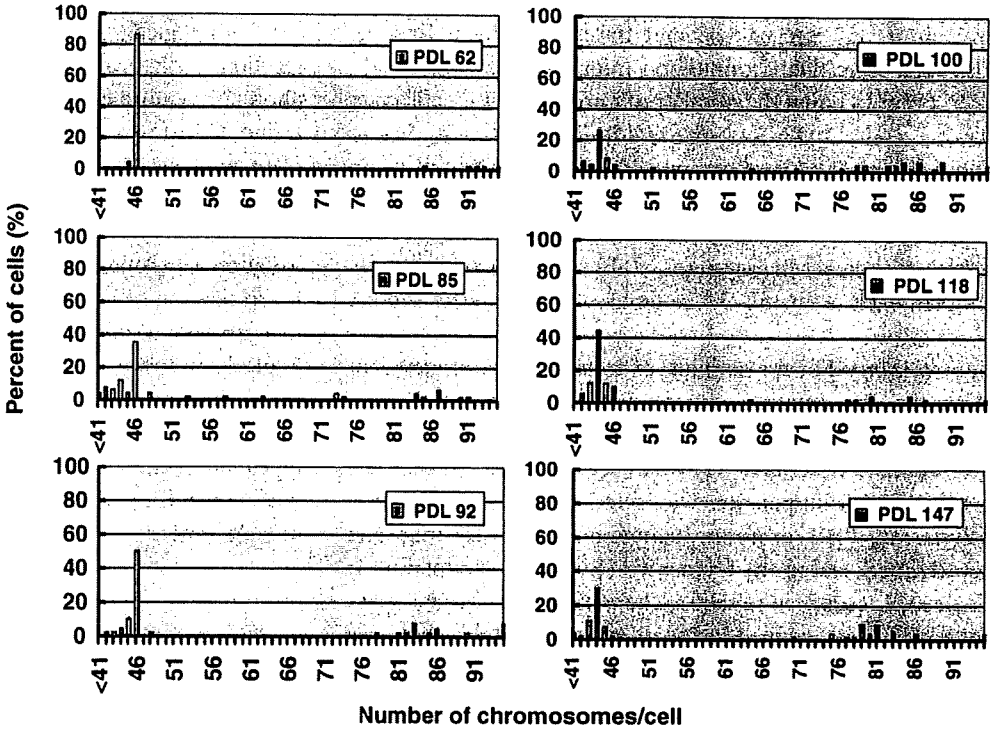

Figure 1. Changes in chromosomal numbers during prolonged cultivation of UE6E7T-3 cells. The chromosomal numbers at various culture stages (PDL62-147) were counted, using DAPI staining of 50–54 metaphase spreads for each PDL. Note the changes in chromosomal numbers from 46 per cell to 44–45 per cell in prolonged cultures. *PDL* population doubling level.

relatively infrequent. These results suggest that the loss of one or two chromosomes in UE6E7T-3 cells occurred in diploid cells at an early stage of culture.

Aneuploid cells are generated from diploids. In order to examine whether or not one of the lost chromosomes was in fact chromosome 13 and was generated directly from a diploid, whole chromosome painting–FISH analysis was applied using a DNA probe specific for chromosome 13. More than 80% of the UE6E7T-3 cells that contained 46 chromosomes had two copies of chromosome 13 at both interphase and metaphase at PDL 62, whereas the cells with 43–45 chromosomes at PDL118 contained only one copy of chromosome 13 (Fig. 2*B*). The near-tetraploidy increased gradually but remained infrequent even after a prolonged culture time, as shown in Fig. 1. If the tetraploid was generated after the loss of chromosome 13, the cells should have contained two copies of chromosome 13, and not four copies (Fig. 2*A*). In fact, the near-tetraploid cells, which constituted approximately 20% of the population at PDL118, contained two copies of chromosome 13 (Fig. 2*C*). These results indicate that near-diploid aneuploidy arises through the loss of one or two chromosomes (one of which is chromosome 13) from a diploid and that some diploid cells then spontaneously become tetraploid via cleavage failure during cell culture passage.

Chromosome missegregation during UE6E7T-3 mitosis. In order to investigate how near-diploid aneuploidy arises in UE6E7T-3 cells, segregation of chromosome 13 during anaphase and telophase of mitosis was followed by FISH analysis with a probe specific for chromosome 13. During mitosis, cells in culture detached from the glass coverslip. The loosely adhered cells were collected, fixed, and plated onto a glass coverslip using a Cytospin centrifuge (Shandon Cytospin 4).

During anaphase, the two sister chromatids separated and moved to opposite poles of the cell. Following normal segregation, two copies of chromosome 13 were observed in each daughter nucleus (Fig. 3*A*), whereas only one copy of chromosome 13 was detected in the two daughter nuclei derived from the parental cell that contained only one copy of chromosome 13 (Fig. 3*B*). When improper segregation of chromosome 13 occurred, the pattern of segregation fell into three categories. The first pattern, as shown in Fig. 3*C* and *D*, involved unequal segregation and nondisjunction whereby one daughter nucleus contained one copy and the other daughter nucleus contained two or three copies of chromosome 13. Notably, however, very few cells that contained three copies of chromosome 13 were observed at late passages. The second pattern of improper segregation, as shown in Fig. 3*E*, involved lagging of the chromosome during metaphase. The lagging chromosome did not align with the duplicated chromatid pairs on the metaphase plate, most likely resulting in loss of chromosome 13 through exclusion. The third pattern of segregation (Fig. 3*F*) involved a lagging anaphase bridge chromosome. Here, chromosome 13 was trapped in the midbody region between the dividing daughter cells. It is possible that the lagging chromosome 13 was excluded from the reforming

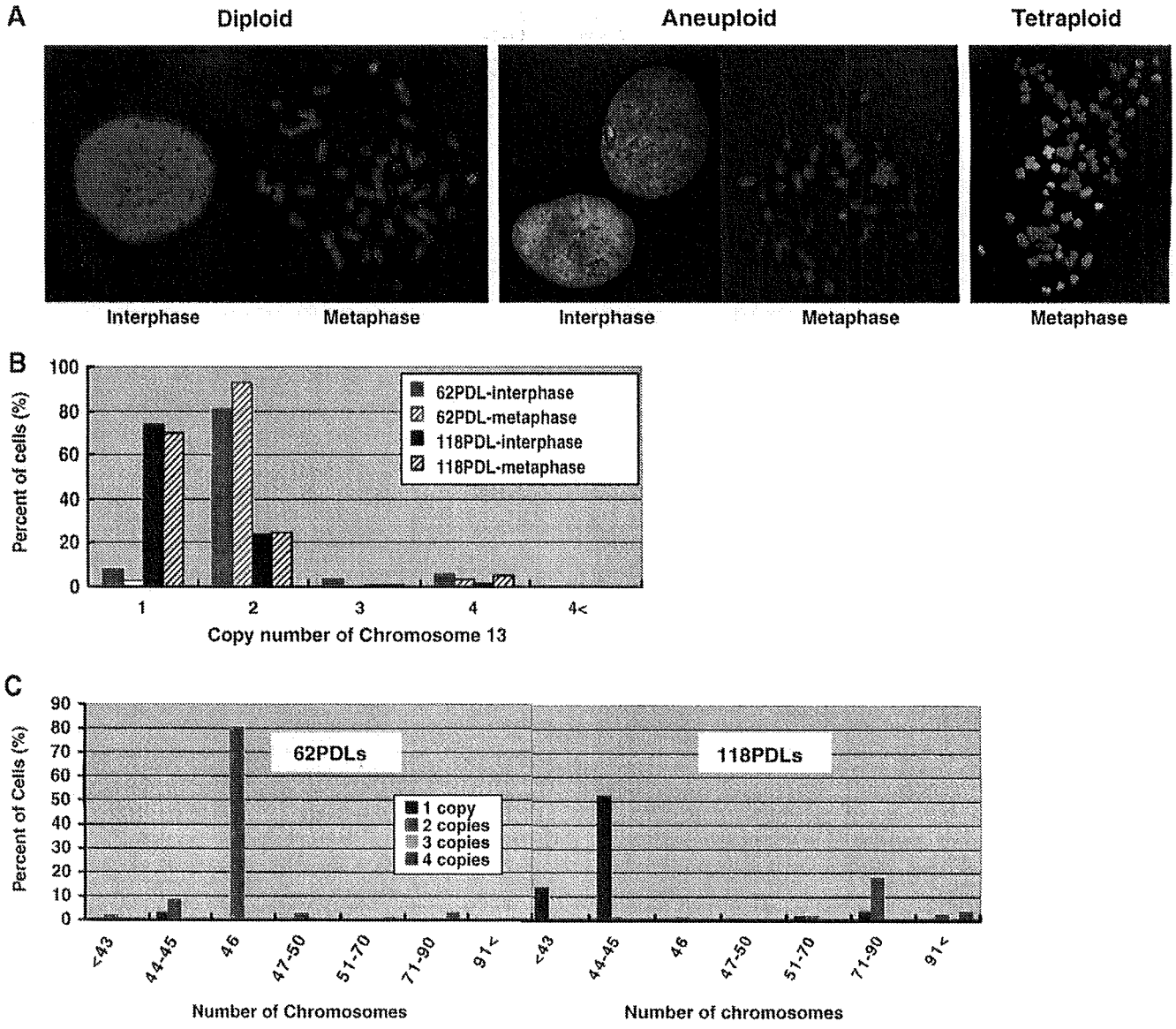

Figure 2. FISH analysis of chromosome 13 in the UE6E7T-3 cell line at early and late passages. (*A*) FISH images using a DNA probe specific for chromosome 13 (*red*) at interphase and metaphase in diploid, aneuploid, or tetraploid cells. *Blue fluorescence* shows other chromosomes. (*B*) Chromosome 13 copy numbers obtained by direct counting in metaphase spreads or interphase nuclei using FISH at PDL62 (*red*) and PDL118 (*blue*). (*C*) Analysis of chromosome 13 copy numbers in each metaphase chromosome spread. UE6E7T-3 cells contained two copies of chromosome 13 within the set of 46 chromosomes, showing a normal karyotype at PDL62, but contained one copy of chromosome 13 within the set of 44–45 chromosomes at PDL118. *PDL* population doubling level.

daughter nucleus, resulting in its loss. Together, these results suggest that near-diploid aneuploidy in UE6E7T-3 cells can arise through three different mechanisms, each of which confers errors in segregation of chromosome 13 during mitosis.

Assessment of LOH and MSI. MSI often causes chromosome instability and is a common phenomenon observed in many kinds of tumor cells (Mitурski et al. 2002). MSI for DNA samples was assessed as positive in cases in which there were additional bands in the test sample that were not observed in the corresponding reference sample (Powierska-Czarny et al. 2003). By PCR analysis employing five microsatellite markers of chromosome 13 (D13S1493, D13S153, D13S788, D13S800, and D13S154), the instability of chromosome 13 was evaluated (Fig. 4*A*). In general, microsatellite markers of heterozygotes generate two bands during PCR analysis. However, as shown in Fig. 4*B*, more than two bands were detected for four of the microsatellite marker products obtained from the UE6E7T-3 cells at PDL78. It has been reported that mismatch repair during the process of DNA replication results in MSI, which produces more than two bands (Mitурski et al. 2002). MSI was detected for all four microsatellite markers of

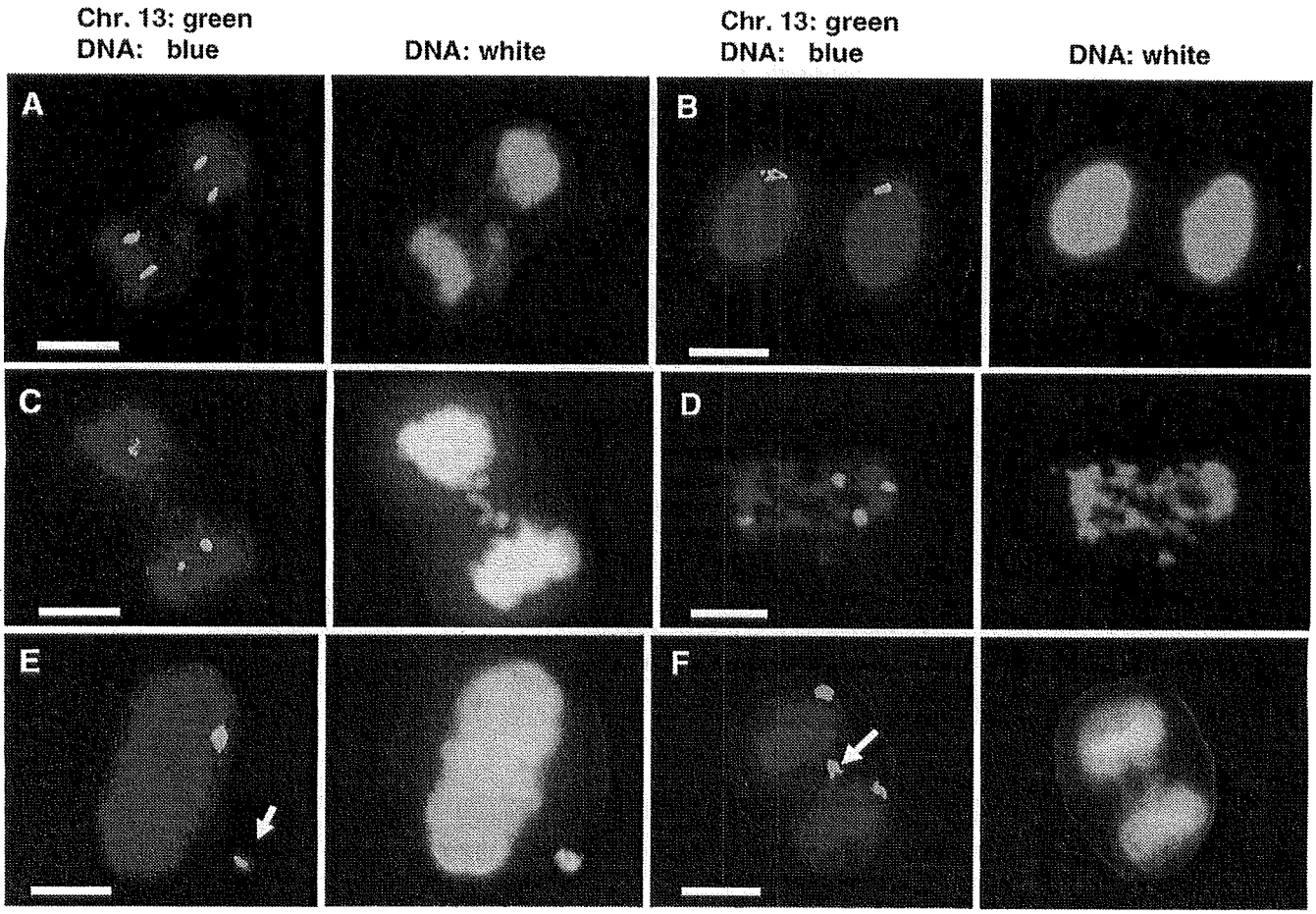

Figure 3. Segregation of chromosome 13 at anaphase or telophase. UE6E7T-3 cells were analyzed by FISH using chromosome 13-specific probes. All *left-hand panels* (*A–F*) show chromosome 13 (*green*) and DNA (*blue*); all *right-hand panels* show DNA (*white*). There was normal segregation of cells with (*A*) two copies or (*B*) one copy of chromosome 13. Abnormal segregation of cells in which the copies of chromosome 13 unequally segregate into daughter cells with (*C*) one and two copies or (*D*) three copies of the chromosome also occurred. Abnormal segregation of cells displaying one misaligned copy of chromosome 13 in a metaphase cell (*E*, *arrow*) and trapping of a lagging chromosome 13 in the anaphase bridge (*F*, *arrow*) are shown. *Scale bar*, 10 μm; *Chr* chromosome.

chromosome 13 (Fig. 4*Ba*) and may subsequently result in chromosome instability. We were especially interested in the observations that one of the two copies of chromosome 13 was lost non-randomly and that loss of chromosome 13 might occur via MSI. Specifically, three, two, four, and four bands were observed for each of the four PCR products obtained for the UE6E7T-3 cells with the microsatellite markers D13S1493, D13S153, D13S788, and D13S800, respectively (Fig. 4*B*). However, the PCR product for D13S154 was a single homologous band (data not shown). As shown in Fig. 2, one copy of chromosome 13 was observed for UE6E7T-3 cells at PDL118. Each PCR product obtained from this cell by using the microsatellite markers was one band (Fig. 4*Bb*). For the UE6E7T-3 cells at PDL101, LOH was also detected for chromosome 13 with any of the four markers (D13S1493, D13S153, D13S788, and D13S800) which were widely localized to chromosome 13.

In order to confirm non-random loss of chromosome 13, SNP analysis of chromosome 13 in UE6E7T-3 cells at PDL101 was accomplished using the Affymetrix Gene Chip Human Mapping 250K Nsp Array Set (Fig. 5). The total number of SNPs detected for the genome was 262,262 in UE6E7T-3 cells (at PDL78) which retained two copies of chromosome 13 (Fig. 5). Of the 11,117 SNPs on chromosome 13 in UE6E7T-3 cells (PDL 78), 10,586 SNPs were detected by the array kit. The call rate of SNPs analyzed on chromosome 13 was 95.2% in this assay. The number of heterozygous SNPs among the 11,117 SNPs on chromosome 13 in UE6E7T-3 cells at PDL78 was determined to be 2,359. These 2,359 SNPs were subsequently analyzed at PDL101 and 2,069 of the corresponding SNP sites (88%) were detected as homozygous sequences. These results show that specific one copy of chromosome 13 was lost non-randomly. A partial strand of chromosome 16 changed from heterozygous to homozygous, as shown in Fig. 5. In a

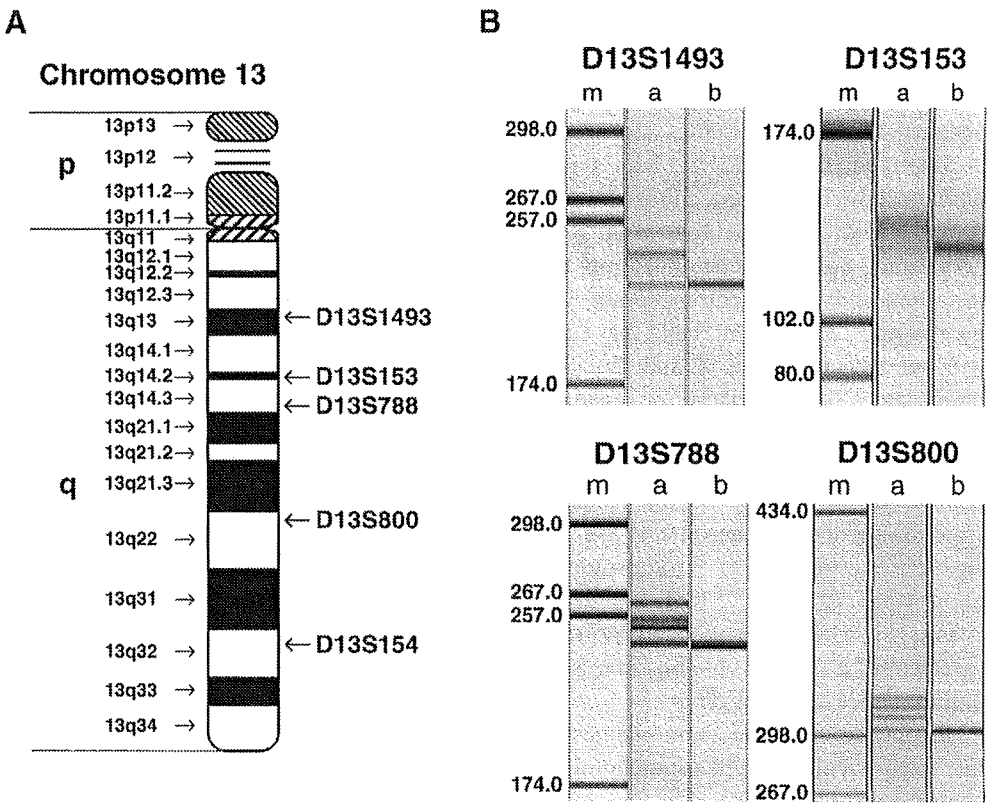

Figure 4. Microsatellite analysis of chromosome 13 in UE6E7T-3 cells. (*A*) Map of chromosome 13. The five microsatellite markers used in this study are indicated by *arrows* (*right*). (*B*) *Middle* and *right* column patterns in each microsatellite marker group show PCR products obtained from UE6E7T-3 cells at (*a*) PDL78 or (*b*) PDL147, respectively, with each of the four microsatellite primer sets shown in Table 1. *Ba*, PDL78 DNA. *Bb*, PDL147 DNA. *Bm* indicates molecular weight markers of DNA.

previous report (Takeuchi et al. 2007), loss of the q-arm of chromosome 16 was observed in UE6E7T-3 cells at PDL101 by array CGH analysis. This loss of chromosome 16 was due to a partial q-arm change. In this report, we did not further investigate the loss of chromosome 16 by microsatellite analysis.

Discussion

Here, we have presented data to show that the loss of one or two chromosomes in the UE6E7T-3 cell line directly caused near-diploid aneuploidy and that this occurred independently of tetraploid intermediates. Evidence for this

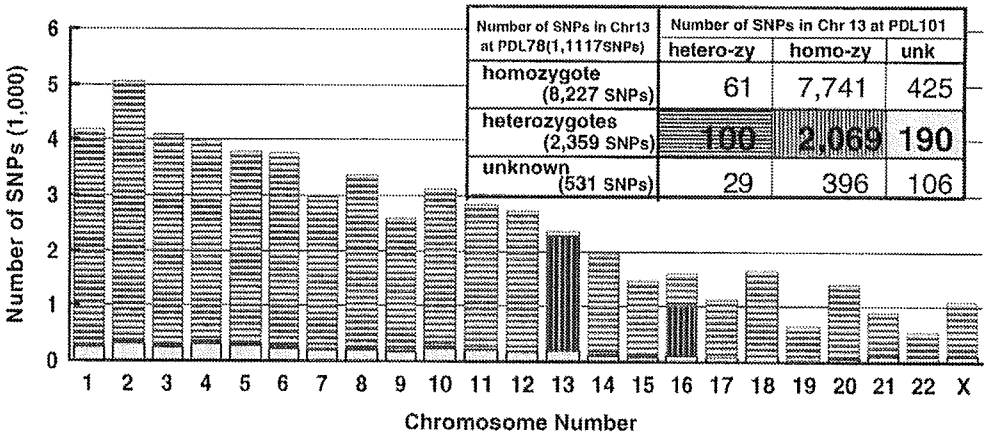

Figure 5. Heterozygosity of SNPs on chromosome 13 at PDL101 that correspond to those at PDL78. The total number of SNPs detected using the Affymetrix Gene Chip Human Mapping 250K Assay Set was 262,262 in whole genome at PDL78 for the UE6E7T-3 cells. There were 11,117 SNPs on chromosome 13 in the cell. The distribution of SNPs at PDL101 corresponded to 2,359 of the heterozygous SNPs at PDL78 was shown as homozygote (*vertical line*), heterozygote (*horizontal line*), or unknown (no call; *white*) SNPs. *SNP* single nucleotide polymorphism, *Chr* chromosome, *PDL* population doubling level, *hetero-zy* heterozygotes, *homo-zy* homozygotes, *unk* unknown.

includes the following: (1) At early stages of long-term culture (e.g., PDL85), the population of cells that contained 46 chromosomes decreased and a new population that contained 42–45 chromosomes appeared, but no tetraploidy was observed (Fig. 1). (2) At later stages (e.g., PDL118), approximately 70% of the cultured cells contained 42–45 chromosomes and had lost one or more copies of chromosome containing chromosome 13, and 18% of the cells contained 71–90 chromosomes with two copies of chromosome 13. However, cells that contained four copies were not detected (Figs. 1 and 2). (3) FISH images of chromosomes in metaphase or anaphase showed three patterns through which UE6E7T-3 cells lost a copy of chromosome 13. These were unequal segregation of chromosome 13 during mitosis (Fig. 3*C*, *D*), exclusion of the misaligned chromosome 13 on the metaphase plate (Fig. 3*E*), and trapping of chromosome 13 in the midbody region (Fig. 3*F*). The FISH images provide the first instance in which it has been possible to follow the movement of a specific chromosome, such as chromosome 13, throughout cell division.

Missegregation of the chromosomes during mitosis will most likely yield near-diploid aneuploidy. Several molecular mechanisms through which aneuploidy may be generated have been recently proposed, but the precise mechanism remains largely unclear. Many studies have suggested that aneuploidy arises from defects in the conserved spindle checkpoint in which a large number of gene products participate, including BUB1, BUBR1, BUB3, MAD1, MAD2, MPS, Aurora B, and CENP-E. It has been shown that decreased expression of one or more checkpoint components leads to near-diploidy, and that a complete inactivation of these checkpoint genes results in embryonic lethality. Weaver et al. (2003) reported that mouse fibroblasts with a heterogeneous CENP-E gene, induced by knockout of the mitosis-specific motor protein CENP-E, tended to gain or lose only one or two chromosomes, without inducing failed cytokinesis, resulting in near-diploid aneuploidy and promotion of tumorigenesis (Kops et al. 2005). Similarly, Mad2, BuBR1, or BuB3 haploinsufficient mice exhibit an impaired mitotic checkpoint response and develop near-diploid aneuploidy (Michel et al. 2001; Babu et al. 2003; van Deursen 2007). Our data cannot, however, be explained by near-diploid aneuploidy generated through decreased expression of the checkpoint proteins because chromosome loss occurred specifically for one copy of chromosome13 and not for just any chromosome as observed in the cases of reduced checkpoint proteins.

Overexpression of Mad2 or BuBR1 blocks an essential step in cell division and induces failure of cytokinesis, resulting in tetraploidization, and the formation of a wide variety of tumors (Sotillo et al. 2007). Recently, a challenging proposal was made that nondisjunction of chromosomes during mitosis does not produce near-diploid aneuploid cells directly, but instead gives rise to mitotic cleavage failure, resulting in tetraploidy. These tetraploid cells could subsequently become aneuploid through further cell division, being transient intermediates during tumorigenesis in human cell lines (Shi and King 2005). However, the mechanistic basis for the tetraploid formation still remains to be elucidated. In this study, we also observed tetraploid UE6E7T-3 cells at late PDLs, but these cells most likely arose from the fusion of near-diploid cells after the loss of one copy of chromosome 13, because two copies, not four, were observed in the tetraploid cells (Fig. 2*A*).

Missegregation can also occur as a consequence of the inappropriate attachment of microtubules to spindle poles, as a monotelic attachment. The lagging of one copy of chromosome 13 might be produced by such a mechanism, resulting in its exclusion from daughter cells (Fig. 3*E*, *F*). The microsatellite analysis of chromosome 13 suggests that the structural mutation of microsatellite DNA leads to the mutation of essential loci on chromosome 13 for microtubule attachment. At early culture stage, UE6E7T-3 cells had a normal karyotype in which a copy of chromosome 13 contained various microsatellites different from the corresponding allele at four loci (microsatellite instability), and lost the chromosome 13 with these microsatellites in prolonged culture, indicating LOH (Fig. 4*B*). In addition, whole genome SNP assay with nearly 262,000 SNP markers confirmed the non-random loss of chromosome 13 (Fig. 5).

Loss of a copy of chromosome 13 has also been frequently reported in human endothelial cells from umbilical cord veins (Zhang et al. 2000; Kimura et al. 2004; Anno et al. 2007) and bone marrow (Wen et al. 2006). A number of studies have showed that LOH on chromosome 13 is a common feature of malignancies such as retinoblastoma and breast tumors, arising after the loss of the remaining normal allele in heterozygotes (Hagstrom and Dryja 1999; Berwick et al. 2007). LOH on chromosome 13 has also been observed in normal human vascular endothelial cells in culture (Kimura et al. 2004), but the mechanism as to why only one copy of chromosome 13 is lost remains unknown. In light of the current results, it is possible that MSI may play an important role in the loss of chromosome 13 in the UE6E7T-3 cell line.

MSI is caused by a failure of the DNA mismatch repair system to repair errors that occur during DNA replication and is characterized by the accelerated accumulation of single nucleotide mutations and alteration in the length of simple, microsatellite sequences (Miturski et al. 2002). If a strand-alignment error remains unrepaired, it will result in a frameshift mutation, leading to adjacent DNA sequence error(s) including centromere and coding regions. Centro-

meres serve both as the sites of association of sister chromatids and as attachment sites for microtubules of the mitotic spindle. They consist of specific DNA sequences to which a number of centromere association proteins bind, forming a special structure called the kinetochore. Taken together with our results, these findings suggests that the loss of a single chromosome 13 with MSI probably occurs by mutation of essential centromere DNA-containing microtubule attachment sites and does not involve the mutation of the known tumor suppressor gene (RB1) on chromosome 13.

In conclusion, we showed by FISH with a probe specific for chromosome 13, that the preferential loss of chromosome 13 in UE6E7T-3 cell line is caused by chromosome missegregation, which results directly in near-diploid aneuploidy. In addition, microsatellite and SNP analyses showed non-random loss of a single copy of chromosome 13 that had MSIs at four loci. This finding suggests the mutation of essential loci on chromosome 13 for microtubule attachment. Thus, the results may contribute significantly to a better understanding of how aneuploid cells arise and help to elucidate the mechanisms of chromosomal instability in carcinogenic progression and in the non-random loss of chromosomes.

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Cell line cross-contamination initiative: an interactive reference database of STR profiles covering common cancer cell lines

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Dear Sir,

Recent reports[1–4] demonstrate the growing perception in the scientific community that cross contamination (CC) of mammalian cell lines represents a major risk for generating false scientific data. The level to which research has been compromised by the use of contaminated or misidentified cell lines has become a major concern for scientists, granting agencies, and, increasingly, scientific journals. In 2007, a group of cell biologists led by Roland M. Nardone petitioned the United States Secretary of Health and Human Services to develop an active program for cell line authentication.[5] They stressed that research and teaching tools in diverse fields of science and industry would be unimaginable without cell cultures. Despite the key importance of cell cultures, only little consensus exists regarding the technical means by which cell line identity can be controlled and how to follow through the results of any such testing.

The key problems of CC are known and chronic in nature: neglecting guidelines for quality control and disregarding adequate cell culture techniques are the main reasons why cell lines have been misidentified or cross contaminated. The incidence of CC in directly and indirectly provenanced cell lines alike[1,3] implies that the majority of false cell lines are perpetrated in originators' own laboratories, presumably by failures during the establishment of new cell lines. A plethora of reports unmasking bogus cancer cell lines, including members of the NCI-60 panel used to generate reference baseline transcriptional drug responses has triggered calls for remedial action.[5,6] Nevertheless, standard authentication procedures for testing cell line identity have yet to be defined.

Short tandem repeat (STR) microsatellite sequences are highly polymorphic in human populations, and their stability throughout the lifespan of individuals renders STR profiling (typing) ideal for forensic use. STR typing has served as a reference technique for identity control of human cell lines at Biological Resource Centers (BRCs) since the turn of the millennium.[7] Ideally, authentication involves coincident STR typing of paired donor and derived cell line samples. However, this ideal is met by a few recently established cell lines only. Most widely used cell lines are decades old and their identification is largely retrospective and multidisciplinary, combining diverse criteria such as uniqueness and the congruence of STR profiles across independent samples with the consistency of observed karyotypes with those reported by the originators.

The DSMZ as well as the ATCC, JCRB, and RIKEN repositories have generated large databases of STR cell line profiles. By using the same microsatellite loci at these BRCs, individual databases could be merged, thereby facilitating interactive searches. This work was piloted at the DSMZ to generate an international reference STR profile database for human cell lines. To render it user friendly, a simple search engine for interrogating STR cell line profiles has now been made available on the homepages of JCRB and DSMZ (http://cellbank.nibio.go.jp/cellbank_e.html, http://www.dsmz.de/STRanalysis). Registered users simply login at the search-site on the DSMZ homepage and will be guided. Aided by simple prompts, users can input their own cell line STR data to retrieve best matches with authenticated cell lines listed on the database.

Once the problem of false negatives due to discrepant representation of single STR alleles, *e.g.*, by losses of heterozygosity and bottlenecking selection—has been tackled and unambiguous search results are produced, human cell lines will need to be consistent with consensus STR reference data sets. STR profiles of all human cell lines distributed by DSMZ, JCRB, and RIKEN and one-third of the cell lines distributed by ATCC are now publicly accessible on interactive databases where match criteria have been arbitrarily set to 95%. Inevitably, reference profiles remain subject to revision until all commonly held cell lines have been STR typed across participating repositories. At present, about 2,342 such cell lines have been STR typed and are represented as reference sets on the database.

The authors of this article are currently participating in an international workgroup organized by the ATCC Standards Development Organization, (ATCC SDO) to develop a standardized methodology (protocols and procedures for STR analysis) for authenticating human cell lines. An additional

goal of the workgroup is to establish a global database for STR profiles of human cell lines. The development of the consensus standard offers a new tool to the cell biology community that will foster reproducibility and comparability of cell lines used in different laboratories. Armed with these tools, online verification of cell line identity should prove a vital weapon to combat the havoc of cell line cross contamination which has dogged cancer research since inception.

Yours sincerely,
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