Cytogenetic Characteristics of Chinese Hamster Ovarian Cell CHO-K1

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ABSTRACT

The Chinese Hamster Ovarian cells CHO-K1 are one of the most extensively used cells for the evaluation of gene expression and toxicology. However, these cells are frequently used for biomedical research without consideration of their cytogenetic characteristics. Therefore, we carried out to investigate the karyologic profiles, the frequency and type of chromosome aberration, and the distribution of telomeric DNA on chromosomes of the CHO-K1 cells. The GTG-banding and fluorescence *in situ* hybridization on CHO-K1 cells were performed to characterize the karyotype and the distribution of telomeric DNA. The present study revealed that the chromosome modal number of CHO-K1 cells was 2n=20; eight chromosomes appeared to be identical with those of the normal Chinese hamster, whereas the remaining 12 chromosomes were shown to be translocated, deleted, inversed, or rearranged from Chinese hamster chromosomes. The telomeric DNA on CHO-K1 chromosomes was intensively distributed at the centromeres rather than the ends of chromosomes. In addition, three chromosomes had interstitial telomeres and one marker chromosome entirely consisted of telomeric DNAs. The frequency and type of chromosome aberrations in CHO-K1 cells were examined. Of the 822 metaphase spreads, 68 (8.3%) cells resulted in chromosome aberrations of which the chromosome breakage was the most frequently occurred.

(Key words : CHO, Karyotype, G-banding, FISH, Telomere, Chromosome aberration)

INTRODUCTION

Cells established from cell lines can be widely used and preferred to study on *in vitro* biomedical researches, because of the ability to readily induce cell division and easy preservation with possessing their inherited characteristics in consecutive culture systems. Depending on the purpose of research, however, it is imperatively necessary to investigate cytogenetic and physiological characteristics of each cell line because some of them may lose their original traits during proliferation.

The Chinese Hamster Ovary cell lines (CHO) established from the ovary of Chinese hamsters have been usefully tooled in the fields of research involved in nutrition, cytogenetics, cell biology, virology, and toxicology. In particular, these lines are one of the most extensively used cells for evaluating *in vitro* toxicity, because they unlimitedly proliferate without tumorigenesis. There are also advantages for the popularity of CHO cell lines admitted by the FDA to use for manufacturing antibodies, for the reason that they are not allowed to duplicate deleterious viruses such as polio, herpes, hepatitis, measles, and adenovirus, and can be also used for large-scale production of antibodies. Due to their genetic stability and safety, *in vitro* genotoxicity tests with CHO cell lines have been developed for the evaluation of potentially mutagenic, anticarcinogenic, teratogenic, and recombinant agents (Kao and Puck, 1975; Thompson, 1983; Kang *et al*, 1997; Kim and Lee, 1999; Grillari *et al*, 2001; Schlatter *et al*, 2005). There are several hundreds of established commercial and private CHO cell lines, of which DON (Chinese hamster lung cell) and CHO are the most well known. It has been known that the CHO cell lines also have numerous subcultured cell lines such as CHO-K1, CHO-PV, CHP-DHFR, and P22. Thus, it is essential to select appropriate cell lines for the purpose of research.

Kao and Puck (1969) found that the modal number of CHO chromosomes was 21 (2n=21) from karyotypic analysis. They also revealed that 10 of the normal Chinese hamster chromosomes were absent in CHO cells and 9 of them were shown to be rearranged morphologically. Further karyotype of CHO chromosomes using G- and C-banding as well as autoradiographic techniques found that only 8 of the 21 CHO chromosomes were

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identical compared with Chinese hamster chromosomes (Deaven and Peterson, 1973). On the other hand, the karyotype of subcultured CHO-K1 was shown to be 20 chromosomes in modal number (2n=20), missing one small telocentric chromosome (Kao and Puck, 1970). Nevertheless, it still lacks of consistent information on the karyotype of CHO chromosomes, presumably due to various reasons such as the conditions of cell culture, the origin of cell lines, nomenclature, and subgrouped chromosomes. Although the CHO cells are the most extensively used cell lines for biomedical research, many studies are performed without considering their cytogenetic characteristics.

To investigate cytogenetic characteristics of CHO-K1 cells, therefore, the present study was conducted to examine karyotypic profiles, the frequency and type of chromosome aberrations, and the distribution of telomeric DNA on chromosomes.

MATERIALS AND METHODS

Cells

Chinese hamster ovarian cells, CHO-K1 cell line (Cell No. KCLB 10061), were obtained from the Korean Cell Line Bank (Daejeon, Korea) to investigate their cytogenetic characteristics.

Culture and Chromosome Preparation

CHO-K1 cells were cultured as previously described by Sohn *et al.* (2002b). In brief, thawing cells were grown in a 25 cm^2 culture flask with RPMI 1640 medium supplemented with 10% fetal calf serum and 1% penicillin-streptomycin (Gibco, Invitrogen Corp. Grand Island, NY, USA) at 37℃ for 24-48 hrs in a humidified incubator containing 5% CO_2. The cultured cells grown in a monolayer were digested with 0.25% of trypsin-EDTA and the split cells were subcultured several times. Before they were harvested, 0.2 μg/ml of colcemid (Gibco, Invitrogen Corp. NY, USA) was added to induce metaphase chromosomes and continuously maintained for 1 hr. Then, the cultured cells were gathered by scraping and centrifugation at 200×g. The cells were treated with a hypotonic solution with 0.06 M KCl (Sigma-Aldrich, St Louis, Mo. USA) for 15 min and fixed in Carnoy's solution containing 3 parts of methanol and 1 part of acetic acid three times consecutively. Three to four drops of fixed cells were then dropped onto the fine wet glass slide for the specimen of GTG -banding.

GTG-banding

G-banding was performed using air-dried slides according to the method of Wang and Federoff (1974). Briefly, the obtained slides were immersed in 0.1% trypsin for 15 sec and washed in a cold D-PBS freed of Ca and Mg ions (Gibco, Invitrogen Corp. NY, USA). After washing, the slides were stained with 0.04% Leishman's solution (Sigma-Aldrich, St Louis, Mo. USA) for 5 min and examined by light microscopy.

Fluorescence *in situ* Hybridization using Telomeric DNA Probe

Fluorescence *in situ* hybridization (FISH) was performed using human telomeric DNA probe (TTAGGG)n to observe the distribution of telomeres on CHO chromosomes according to the modified method of Sohn *et al.* (2002a). In brief, the slides were incubated with 2×SSC solution containing RNase A (1 μg/ml) for 30 min to remove RNA, followed by washing with ddH_2O and dehydrating with an ethanol series. Hybridization buffer (2×SSC, 50% formamide and 10% dextran sulfate; Roche, Mannheim, Germany) containing 100 ng dig-labeled probe was applied onto the cytological preparations, covered with a coverslip and sealed with rubber cement. The specimens and probe were denatured for 10 min on a heating block at 72℃ and then allowed to hybridize for 12 hr at 38.5℃. The slides were washed with 2×SSC at 72℃ for 5 min and subsequently with a PN buffer (0.1% sodium phosphate containing 0.1% Nonidet 40; Roche, Mannheim, Germany) for 2 min at room temperature. The air-dried slides were incubated for 30 min at 38.5℃ with 20 μl of anti-dig-fluorescein isothiocyanate (FITC) conjugate (Roche, Mannheim, Germany). They were then washed with the PN buffer, counter-stained with propidium iodide (PI) and examined under a fluorescence microscope (Model AX-70, Olympus, Tokyo, Japan) equipped with a WIB 523 nm-pass filter. The images were captured by digital camera (Model DP-70, Olympus, Tokyo, Japan) and analyzed by an image analyzer software program (MetaMorph, Universal Imaging Co., PA, USA). Quantitative analysis of telomeric DNAs on chromosomes was carried out by the quantitative fluorescence *in situ* hybridization (Q-FISH) technique described as Slijepcevic (2001). Mean percentage of telomeric DNA of chromosomes were calculated as the ratio of the intensity of PI signals to FITC signals which conjugated with telomeric probe on a metaphase spread.

RESULTS

To investigate the karyotype of CHO-K1 cells, GTG-banded metaphase spreads were prepared as shown in Fig. 1. The results showed that the karyotype of CHO-K1 cells was 2n=20 which consisted of some normal Chinese

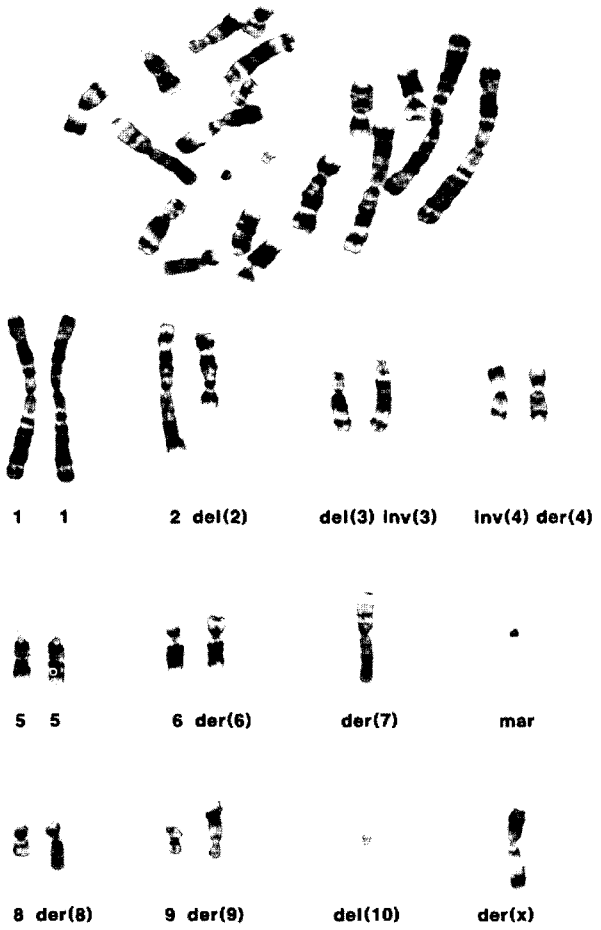

Fig. 1. G-banded metaphase spread and it's karyotype of the CHO-K1 cell.

hamster chromosomes and newly rearranged chromosomes. Eight chromosomes including two pairs of homologous chromosomes 1 and 5, and one of chromosomes 2, 6, 8 and 9 appeared to be identical with Chinese hamster chromosomes, whereas the remaining twelve chromosomes were shown to be translocated, deleted, inversed and/or rearranged chromosomes from the Chinese hamster chromosomes. The newly rearranged 12 chomosomes were del (2), del (3), inv (3), inv (4), der (4), der (6), der (7), der (8), der (9), der (10), der (X), and mar chromosome. The karyotype of CHO-K1 cells presented here was established by referring and comparing with the high resolution banded karyotype of Chinese hamster chromosomes presented by Shibaski and Ronne (1988). Table 1 showed the designation and nomenclature of CHO-K1 karyotype, which was followed by the ISCN system (1995).

To observe the distribution of telomeres on CHO-K1 chromosomes, FISH using a human telomeric DNA probe was performed. Fig. 2 showed the pattern of telomeric DNA distribution on CHO-K1 metaphase chromosomes. As shown in Fig. 2, the telomeric DNA on CHO-K1 chromosomes were intensively distributed at the centromeres rather than the ends of chromosome to arms, while that on one marker chromosome appeared be evenly spread out on the whole chromosome. In particular, a markedly strong signal of interstitial telomere was observed in chromosomes inv (3), der (8), and der (9). We also analyzed the amount of telomeric DNA on CHO-K1 chromosomes by the Q-FISH technique. Table 2 exhibited the relative amount of telomeric DNA on CHO-K1 chromosomes. The amount of telomeres on each CHO-K1 chromosome was highly variable, ranging from 0 to 100% and the distributional site of telomeric DNA on each chromosome was also diverse. The relative amount of telomeric DNA was the highest (100%) on chromosome mar, followed by chromosomes der (8), der (9), and inv (3). In contrast, telomeric DNA did not exist on a pair of homologous chromosome 1 and one of chromosome 2, del (2), inv (4), and 9.

To evaluate the cytogenetic characteristics of CHO-K1 cells, the frequency and type of chromosome aberrations at metaphase were analyzed. Of the 822 metaphase spreads, 68 (8.3%) cells resulted in chromosome aberrations, which were mostly structural aberration rather than numerical aberration as shown in Table 3. The chromatid-type aberration such as endoduplication, breakage, and chromatid exchange was significantly higher than the chromosome-type aberration. The highest frequency of chromosome aberration was chromosome breakage reaching 6.1%, followed by dicentric chromosomes. Fig. 3 showed the representative chromosome aberrations such as chromosome breakage (Fig. 3a), endoduplication (Fig. 3b), dicentric (Fig. 3c), and complex euploidy (Fig. 3d) from CHO-K1 cells.

DISCUSSION

To reveal the karyotype of CHO-K1 cells, we analyzed the G-banding patterns of metaphase chromosomes. G-banding using proteolytic digestion and Giemsa staining as described previously is a powerful technique to identify the homologues and structural aberration of chromosomes, showing the specific and identical bands of chromosomes within species. The pattern of G-bands was occurred by the selective loss of specific proteins in the chromatin consisting of histone protein, non-histone protein and DNA (Holmquist and Comings, 1976; Comings, 1978; Burkholder and Duczek, 1980). Since the G-banding markers were first adapted to establish the human karyotype (Paris Conference, 1972), these markers have been widely used for the characterization of karyotypes in animal species (Reading Conference, 1980).

The karyotypic characteristics and the number of chromosomes in CHO cells have been reported to be differ-

Table 1. The karyotype designation and description of CHO-K1 cell

Chromosome number and symbol	Karyotype designation	Description
1		Normal Chinese hamster chromosome
2		Normal Chinese hamster chromosome
del(2)	del(2)(q10q31) i.e. del(2)(pter→ q10::q31→ qter)	Interstitial deletion of a small segment from 2q10 to 2q31
del(3)	del(3)(p31) i.e. del(3)(qter→ p31:)	Terminal deletion with a break in band 3p31
inv(3)	inv(3)(p28q12)	Pericentric inversion in which breakage and reunion have occurred at bands 3p28 and 3q12
inv(4)	inv(4)(p12q12)	Pericentric inversion at bands 4p12 and 4q12
der(4)	der(4)t(4;?)(q10;?) i.e. der(4)(4qter→ 4q10::?)	The derivate chromosome 4 has result from a translocation of unknown chromosome segment to the long arm of chromosome 4 at band 4q10
5		Normal Chinese hamster chromosome
6		Normal Chinese hamster chromosome
der(6)	der(6)t(6;?)(p13;?) i.e. der(6)(6qter→ 6p13::?)	The derivate chromosome 6 has result from a translocation of unknown chromosome segment to the long arm of the chromosome 6
der(7)	der(7)t(7;X)(p10;q10) i.e. der(7)(7pter→ 7p10:: Xq10→ Xqter)	The derivate chromosome 7 has result from a translocation of the chromosome X segment distal to Xq10 to the short arm of chromosome 7 at band 7p10
8		Normal Chinese hamster chromosome
der(8)	der(8)t(8;?)(q10;?) i.e. der(8)(8pter→ 8q10::?)	The derivate chromosome 8 has result from a translocation of unknown chromosome segment to the p-arm of chromosome 8 at band 8q10
9		Normal Chinese hamster chromosome
der(9)	der(9)t(2;9;?) i.e. der(9)(:2q10→ 2q31:: 9pter→ 9p10::?)	The derivate chromosome 9 has result from multiple translocation of unknown chromosome segment and a partial segment of chromosome 2. to the short arm of chromosome 9
del(10)	del(10)(p10) i.e. del(10)(pter→ p10:)	Terminal deletion with long arm of chromosome 10
der(X)	der(X)t(X;4)(q14;p21) i.e. der(X)(Xpter→ Xq14:: 4p21→ 4pter)	The derivate chromosome X has result from a translocation of the chromosome 4 segment distal to 4q21 to the short arm of chromosome X
mar	marker chromosome	One additional marker chromosome

ent, depending on the origin of subcultured cells and culture conditions. Kao and Puck (1969) first reported the karyotype of CHO cells whose modal number was 2n=21. They also recognized that 10 of the normal Chinese hamster chromosomes were absent in CHO cells, and 9 of them were identified as having altered morphology. In nomenclature, 9 altered chromosomes were classified as Z group, while the remaining 12 chromosomes were designated as 1 to 10 and X chromosomes. On the other hand, Deaven and Petersen (1973) conducted G- and C-banding as well as autoradiographic analysis to exhibit the exact karyotype of CHO cells. As a result, they revealed that the chromosome modal number of CHO cells was 2n=21; 8 of the 21 chromosomes including 1, 2, X, 6, 8, 9, 10 and 11 turned out to be identical compared with those of the Chinese hamster, but the remaining rearranged 13 chromosomes were classified as Z group. Park and Lee (1984) found that the chromosome modal number of CHO cells was 2n=20; 7 of the 20 chromosomes including two pairs of homologous chromosomes 1 and 6, and one of chromosomes 2, 7 and 10 were identical with the Chinese hamster chromosomes, whereas the remnant 13 were shown to be deleted or added chromosomes. In addition, Bertoni et al. (1992) reported that the modal number of chromosomes of CHO-PV cells established from CHO-K1 cells was 19; 8 of them, which were two pairs of homologous chromosomes 1 and 6, and one of chromosomes 2, 4, 8, and 9 appeared to be

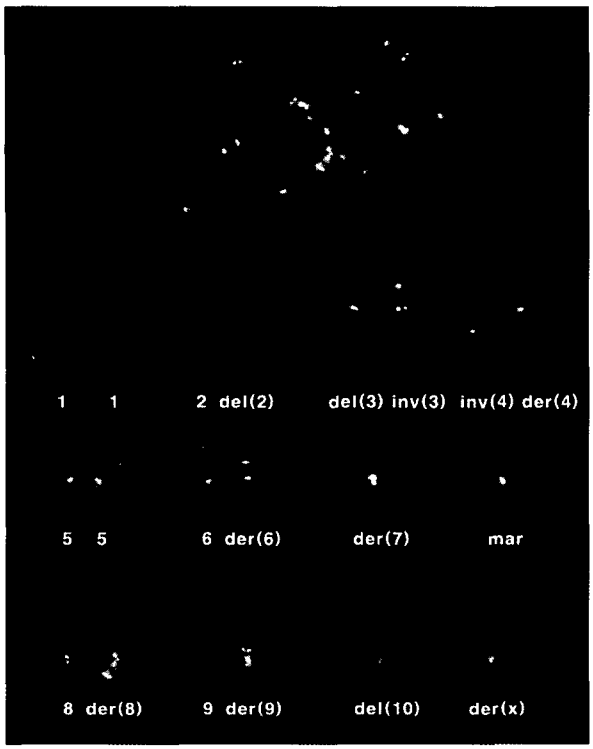

Fig. 2. Metaphase spread and it's karyotype after FISH on chromosomes of CHO-K1 cell using telomeric DNA probe.

identical with the chromosomes observed in the Chinese hamster. They also found two deleted chromosomes such as chromosomes 3p- and 6q-, five translocated chromosomes designated as Z set including Z2, Z4, Z7, Z8, and Z13, and four newly rearranged chromosomes as R set including R1, R2, R3, and R4. Taken together, it could be concluded that the modal number of chromosomes derived from CHO cells was 2n=19~21; at least 7 chromosomes were identical with the Chinese hamster chromosomes but the remaining chromosomes resulted in morphological alterations consisting of translocated, deleted, inversed, and/or rearranged chromosomes. In this study, the karyotype of CHO-K1 cells was analyzed as 2n=20. Of 20 chromosomes, 8 chromosomes coincided with the chromosomes of the Chinese hamster, whereas the remaining 12 chromosomes were found to be translocated, deleted, inversed, and/or rearranged from normal Chinese hamster chromosomes. Our observation here was very similar to the previous studies, although there were some differences in morphological aspects and designation order of chromosomes in the Z and R set. In particular, one marker chromosome which was not previously observed by other studies seemed to be generated by the fragmented chromosomes, although their origin was not known. Even though numerous studies were conducted to establish the karyotype of CHO cells, there is little information on the rearranged chromosomes.

Table 2. The relative amount of telomeric DNA on CHO-K1 chromosomes by Q-FISH

Chromosome number	Relative amount(%) of telomeric DNA on				
	chromosome	p-arm	q-arm	Centromere	Interstitial
1	0.00±0.00				
2	0.00±0.00				
del(2)	0.00±0.00				
del(3)	7.28±0.15			7.28±0.15	
inv(3)	23.93±0.24			14.93±0.33	9.00±0.15
inv(4)	0.00±0.00				
der(4)	4.63±0.52			4.63±0.52	
5	8.11±0.45			8.11±0.45	
6	9.70±0.19			9.70±0.19	
der(6)	17.65±0.41	11.58±0.21		6.07±0.20	
der(7)	9.66±0.38			9.66±0.38	
8	13.22±0.24			13.22±0.24	
der(8)	43.91±0.54			21.11±0.26	12.80±0.28
9	0.00±0.00				
der(9)	33.88±0.59			11.41±0.31	22.47±0.28
del(10)	22.66±0.21			22.66±0.21	
der(X)	8.00±0.34			8.00±0.34	
mar	100.00±0.00				

Values are means±SD from 10 metaphase chromosomes.

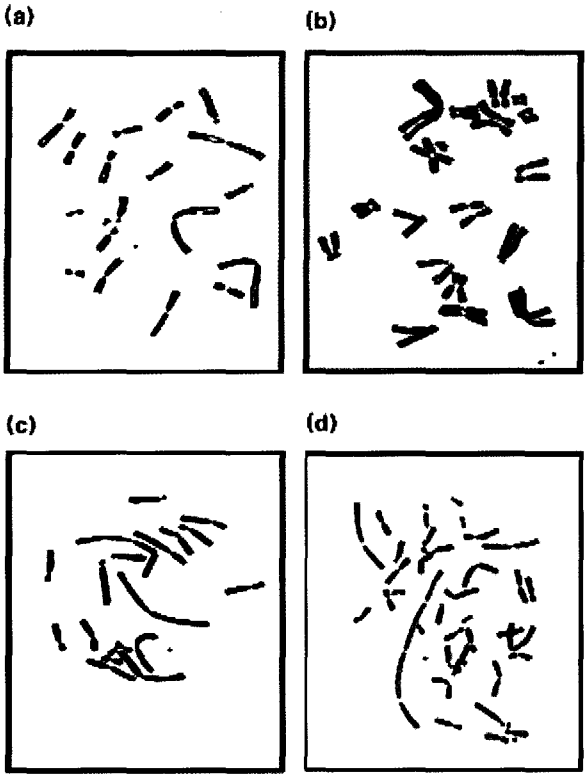

Fig. 3. The representative chromosome aberration metaphase spreads prepared from CHO-K1 cells. (a) A metaphase spread showing a chromatid breakage at one chromosome.. (b) A metaphase spread showing endoduplication. (c) A metaphase spread showing a dicentric chromosome at one chromosome. (d) A metaphase spread showing euploidy with a telomeric association.

Table 3. Frequencies and types of chromosome aberrations in CHO -K1 cells

	Number of observed cells	Frequencies (%)
Analyzed cells	822	100
Cells with normal karyotype	754	91.7
Cells with chromosomal aberrations	68	8.3
- Dicentrics	8	1.0
- Acentric fragments	4	0.5
- Ring chromosomes	1	0.1
- Breakage	50	6.1
- Chromatid exchange	2	0.2
- Endoduplication	1	0.1
- Euploidy	2	0.2

Therefore, in this study, we tried to establish the origin of each chromosome by means of G-band markers and the distribution of telomeric DNA on chromosomes. According to the karyotype of CHO-K1 cells, we provided the nomenclature and designation of these chromosomes as described in Table 1. The generation of rearranged chromosomes of CHO-K1 cells could be explained by non-disjunction of the specific chromosomes and by inducing deleted and translocated chromosomes during repeated cellular differentiation of Chinese hamster ovarian cells to establish immortal cell lines.

To investigate the distribution of telomeres on CHO-K1 chromosomes, the FISH analysis was applied to metaphase chromosomes using human telomeric DNA probe (TTAGGG)n. The telomeric DNA on the normal Chinese hamster chromosomes was known to be distributed at the ends of arms as well as the centromeres of chromosomes (Slijepcevic and Bryant, 1995). By contrast, Bertoni et al. (1994) revealed that telomeric DNA on CHO-PV chromosomes was intensively distributed at the centromeric regions with a little telomeric DNA at the ends of chromosomes. Faravelli et al. (2002) reported that telomere-like repeats were distributed at the regions of pericentromeric and short arrays were localized at interstitial sites in Chinese hamster fibroblast, CHO-PV, and V79 cells. They also found that the internal telomeric sequences (ITs) were located at the regions of 3q13-15, 3q21-26 and 1p26, which were highly polymorphic in size. This observation is similar to our result relating to telomeric DNA distribution on CHO-K1 chromosomes. In accordance with the result of Bertoni et al. (1994), we also observed a strong signal of telomeric DNA at the centromeric regions of chromosomes without telomeric signal at the ends of chromosomes. In addition, ITs clearly existed on the same site of 3q13-15 in chromosome inv (3). However, our observations regarding ITs patterns in short-arms of chromosome der (6), chromosome der (8), chromosome der (9), and in the whole region of chromosome mar were not previously observed by other studies. One of the reasons why the ITs occurred might be that the chromosomes with interstitial telomeres such as der (8) and der (9) were newly rearranged chromosomes derived from reciprocal translocation involving a part of the telomeric site. The mar chromosome might be formed from a deleted chromosome fragment which mostly consisted of telomeric DNAs. In addition to the observation regarding the distribution of telomeres on CHO-K1 chromosomes, the amount of telomeric DNA on chromosomes was also analyzed by Q-FISH. The relative percentage of telomeric DNA in each chromosome was highly variable, ranging from 0 to 100% with different distributional regions. The average amount of telomeric DNA per genome was calculated to be 8.1% with 100 interphase cells (Data was not shown), which was slightly higher than that of our previous study (5.5%) with DON cells (Sohn *et al.*, 2002b). In addition, this result had a relatively higher percentage than that of other studies obtained from the genome of Chinese hamsters (5%, Berto-

ni et al., 1994), chickens (3~4%, Delany *et al.*, 2003), mice (1.4~1.5%, Lavoie *et al.*, 2003), and human (1.3%, Krejci and Koch, 1998). Thus, our data supported that the amount of telomeric DNA in the immortal cells or cancer cells was relatively higher than that of the normal cells (Engelhardt and Martens, 1998; Shay and Wright, 2001).

To assess cytogenetic characteristics of CHO-K1 cells, we examined the frequency and type of naturally occurred chromosome aberrations. The frequency of total chromosomal aberration of CHO-K1 cells was 8.3%, which was significantly higher in value compared with other studies. The result of previous studies showed that the frequency of naturally occurred chromosome aberration in metaphase chromosomes was about 4% in CHO cells (Park and Lee, 1984), and a similar percentage of aberration in CHEL cells and CHO cells (Biondi *et al.*, 2000). Although we observed a higher frequency of total chromosome aberration compared with other studies, it was in agreement with the ratio of abnormal types of chromosomes. In our results, the chromosome breakage was the highest rate among chromosome aberrations, which was similar to the others reports. Because the established immortal cell lines were consecutively subcultured for several generations and subsequently lost some of their typical cytogenetic characteristics, the cell lines had a high frequency of chromosome aberrations in comparison with the normal cells. The CHO cells are one of the most widely used cell lines for studying cancer, mutagenesis, toxicology, and bioactive test substances. In particular, CHO cells are the most frequently applied and well accepted to evaluate effectiveness and genotoxicity in response to pharmacological agents. Thus, the results of this study regarding the frequency and type of naturally occurred chromosome aberrations in CHO-K1 cell lines would be beneficial for the evaluation and the assessment of genotoxic substances.

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