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Original article

Chromosomal Variation in Three Human-Mouse Hybridoma Cell Lines after Various Passaging Intervals as Assessed with Two Different Staining Methods

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Abstract

Objective(s)
The main objective of this study was to investigate the status of chromosome stability in 3 human-mouse hybridoma cell lines over a period of time in various passages.
Materials and Methods
Metaphase spreads from 3 human-mouse cell lines (HF2X653, SPMO-4 and F3B6) that had been cultured in 4 successive passages, from 1 to 4 weeks, were prepared and analyzed. Metaphase chromosomes stained in Giemsa and a fluorescent dye, Hoechst 33258, for differential staining. This staining was performed for differentiating human and mouse chromosomes.
Results
Numerical chromosome analysis showed that although in successive passages the total number of chromosomes in hybridoma cells remained unchanged, some changes occurred in the number of human and mouse metacentric and acrocentric chromosomes during different passages. These changes were detectable, using fluorescence staining method.
Conclusion
Since one of the main uses of human-mouse hybridoma cells is producing monoclonal antibody, chromosomal instability in these cells causes the loss of human chromosomes coding the antibody of interest occasionally. Therefore, cytogenetical analysis and characterization of these cells, especially by using the appropriate ways of chromosomal identification, is essential prior to use.

Keywords: Chromosomal instability, Hoechst 33258, Hybrid cells, Staining

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Introduction

The technique of somatic cell hybridization has provided a very useful tool for the investigation of a number of aspects of mammalian cells. Cells from different species have been used to obtain extensive information about gene location in specific chromosomes and produce monoclonal antibody (e.g. human-mouse hybridoma cells) (1). It has been known for a long time that interspecies somatic cell hybrids gradually lose chromosomes preferentially from one of the parent cell lines (2, 3). This property is responsible for a major part of the interest in somatic cell genetics because partial chromosome loss provides an opportunity for gene mapping. Moreover, although the loss of some chromosomes in interspecies hybridomas can be useful for genetic studies, it is most often a major problem, because it is the major mechanism (but not the only one) leading to the loss of the ability to produce the immunoglobine of interest (4). The loss of either the gene encoding H chain or the encoding L chain from the immune cells donor extinguishes antibody production. The extent to which this is a problem depends primarily on the species pair used in fusion. When using mouse myeloma cells as the immortalizing parent, chromosome loss always occurs in from the other species. The rate at which chromosomes are lost and the ultimate extent of the loss has been investigated for interspecies hybrids of different cell types from several species pairs most notably from human-mouse hybrids (5). The same initial studies, suggested that the human chromosome loss in these hybrids was random but subsequent studies suggested otherwise. In human-mouse myeloma hybrids, the chromosome loss was definitely not random; some chromosomes such as chromosomes 4, 16 were lost, whereas others were present with a much higher frequency than it would be expected (6-8). Under strictly random probabilities the average number of human chromosomes present in the hybrid cells was 7, although the range extended from 1 to 20. Once the hybrids were grown in mass culture and after the initial large human chromosome segregation had taken place the karyotypes appeared to be relatively stable. Few chromosomes less than two were lost during the periods of 8-12 months, in continuous cell culture (9-11). In a study Croce (1976) has reported that human-mouse B cell hybrids preferentially retain human chromosome 14, which carries the H chain gene complex. This finding may be functionally significant. In the same study chromosomes 22 and 2 which carry the L chain genes were also, preferentially retained (4).

Various techniques are being used for the study of chromosomes in hybridoma cell lines such as FISH (Fluorescence *in situ* hybridization) (12-14), PRINS (Primed *in situ* labeling) (15), SKY (Specteral karyotyping) (12, 16, 17), Giemsa-11, modified Giemsa-11 staining (18, 19), C-banding with fluorescent staining (20) and differential staining by fluorescent dyes (10). These studies are showing a continuous effort towards using an appropriate method to study chromosomes in hybrid cells. Most of these methods are very expensive and time consuming for routine use in laboratories. In the present study, we utilized Giemsa solid staining and Hoechst fluorescent dye for the study of chromosomal changes in hybridoma cells. The main objective of this study was to investigate the status of chromosome stability in the studied cell lines over a period of time in various passages.

Materials and Methods

Cell lines

Three human-mouse cell lines, namely HF2X653 and SPMO-4 and F3B6 were obtained and maintained in National Cell Bank of Iran, Pasteur Institute (Tehran, Iran). HF2X653 is a cell line produced from fusion of mouse myeloma cells and human normal lymphocytes; this cell line is sensitive to HAT (hypoxanthine aminopetrine). SPMO-4 is a cell line produced from the fusion of X-irradiated mouse myeloma cells and human lymphocytes; these cells are also sensitive to HAT culture medium and resistant to Oaubin. F3B6 is a cell line, as above, produced from

the fusion of mouse myeloma cells and human normal lymphocytes; the cells are sensitive to HAT culture medium and resistant to Oaubin.

Slide preparation and staining

The cells were cultured routinely in RPMI-1640 medium supplemented with 10% fetal calf serum and antibiotics (penicillin 100 IU/ml and streptomycin 100 µg/ml, Sigma, USA) and kept in a 37 °C incubator with humidified atmosphere containing 5% CO_2. These cells maintained for 1 month and their viability and proliferation assessed at the onset of the culture. Samples were taken weekly from each cell line to prepare metaphase spreads. To arrest cells in metaphase, colchicine at a final concentration of 4 µg/ml was added to the cultures 2 hr before harvesting. Then cells in the medium transferred into a centrifuge tube and centrifuged at 1200 rpm for 10 min, the supernatant was removed and the cell palette re-suspended in the remaining medium. The cells then subjected to a hypotonic solution of 0.075 M KCl (Merck, Germany) at 37 °C for 20 min and subsequently fixed in a mixture of methanol and acetic acid (1:3 v/v, Merck). Slides were made using standard air-drying method and chromosomes stained either in 10% Giemsa stain (Merck, Germany) or Hoechst 33258 fluorescent dye (Merck, Germany) to differentiate between human and mouse chromosomes (10).

Metaphase analysis

One hundred well spread metaphases were analyzed for each sample using a Ziess (Germany) light microscope with a 100× objective lens for Giemsa stained samples. Thirty fluorescent stained metaphases were analyzed using an E800 epi-fluorescence microscope (Nikon, Japan) with a 100× objective lens. Figure 1 shows typical photomicrographs of chromosomes stained either with Giemsa (A) or Hoechst 33258 fluorescent dye (B).

Statistical analysis

Results were analyzed using SPSS (version 11.2) software and one way analysis of variance (ANOVA) to determine the significant of difference between studied groups. *P*-value of less than 0.05 was considered as significant.

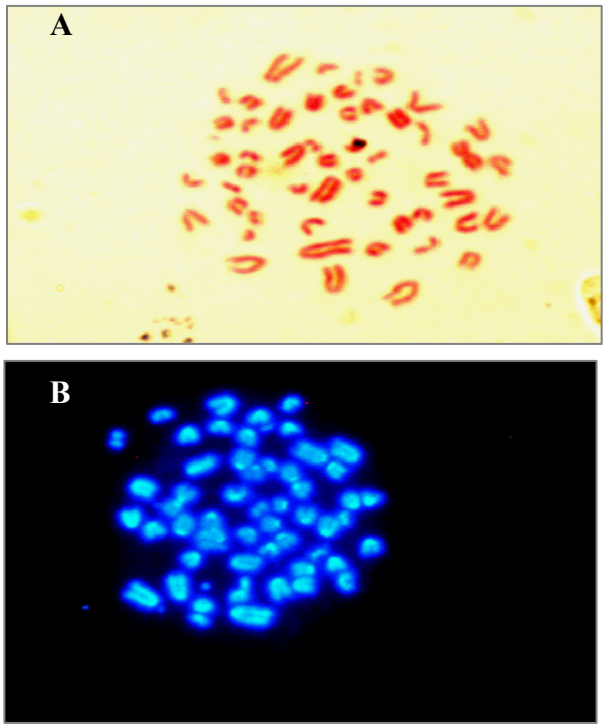

Figure 1. **A.** Giemsa stained HF2X653 cell chromosomes. Differentiating between human and mouse chromosomes is not possible with this method of staining. **B**. Fluorescence staining of HF2X653 cell chromosomes using Hoechst 33258 dye. Arms of human chromosomes and centromere of mouse chromosomes shows intense fluorescent.

Results

Results of HF2X653 cell line

Results obtained for HF2X653 cell line are shown in Tables 1 and 2. As seen in Table 1, after Giemsa staining, metacentric, acrocentric, and the total chromosome numbers were unchanged in 4 different passages in that cell line, but fluorescent staining analysis (Table 2) shows that changes in human metacentric chromosomes between the first week and the next three weeks were significantly different from each other ($P<0.05$). Regarding mouse chromosomes, the changes in metacentric chromosomes observed for all passaging times were significantly different from each other ($P<0.05$). Mouse metacentric and the total chromosome numbers in different passages were unchanged in this cell line.

Table 1. Mean frequency of chromosomes scored following Giemsa staining in three different hybridoma cell lines at various sampling times.

Cell line	Sampling time (Week)	Acrocentric chromosomes	Metacentric chromosomes	Total chromosomes
HF2HX	1	48.6 ± 0.6	1.4 ± 0.8	49.4 ± 0.6
	2	47.3 ± 0.6	1.4 ± 0.8	48.7 ± 0.6
	3	48.6 ± 0.6	1.1 ± 0.8	49.8 ± 0.6
	4	48.8 ± 0.6	1.9 ± 0.8	49.9 ± 0.6
SPMO4	1	70.1 ± 1.3	0.6 ± 0.1	70.8 ± 1.3
	2	61.5 ± 1.3	0.8 ± 0.1	62.3 ± 1.3
	3	61.6 ± 1.3	0.9 ± 0.1	62.2 ± 1.3
	4	62.3 ± 1.3	0.3 ± 0.1	62.5 ± 1.3
F3B6	1	47.0 ± 0.6	2.8 ± 0.2	50.4 ± 0.6
	2	42.6 ± 0.7	2.0 ± 0.1	44.5 ± 0.7
	3	44.1 ± 0.6	1.2 ± 0.9	45.3 ± 0.6
	4	43.2 ± 0.6	0.9 ± 0.1	44.1 ± 0.6

Table 2. Mean frequency of mouse and human chromosomes scored following fluorescent staining (Hoechst 33258) in three different hybridoma cell lines at various sampling times.

Cell line	Sampling time (Week)	Human acrocentric chromosomes	Human metacentric chromosomes	Mouse acrocentric chromosomes	Mouse metacentric chromosomes	Total chromosomes
HF2HX	1	2.1 ± 0.4	0.3 ± 0.1	45.2 ± 1.6	0.9 ± 0.1	48.6 ± 1.1
	2	0.9 ± 0.4	0.2 ± 0.1	47.7 ± 1.6	0.7 ± 0.1	49.8 ± 1.1
	3	0.9 ± 0.4	0.2 ± 0.1	45.5 ± 1.6	0.7 ± 0.1	47.3 ± 1.1
	4	0.5 ± 0.4	0.4 ± 0.1	45.9 ± 1.6	0.4 ± 0.1	47.3 ± 1.1
SPMO4	1	1.6 ± 0.4	0.0 ± 0.0	66.0 ± 2.5	0.3 ± 0.2	67.8 ± 2.5
	2	1.7 ± 0.4	0.0 ± 0.0	62.5 ± 2.5	0.4 ± 0.2	64.6 ± 2.5
	3	1.1 ± 0.4	0.0 ± 0.0	57.8 ± 2.5	0.8 ± 0.2	59.7 ± 2.5
	4	1.5 ± 0.4	0.0 ± 0.0	56.4 ± 2.5	0.4 ± 0.2	58.4 ± 2.5
F3B6	1	2.0 ± 0.3	0.6 ± 0.1	44.9 ± 1.5	1.8 ± 0.2	48.6 ± 1.3
	2	0.7 ± 0.1	0.6 ± 0.1	44.1 ± 0.8	0.9 ± 0.2	45.8 ± 0.9
	3	0.4 ± 0.1	0.5 ± 0.1	43.4 ± 1.0	0.7 ± 0.1	44.9 ±1.0
	4	0.5 ± 0.1	0.3 ± 0.1	42.0 ± 1.3	0.6 ± 0.1	43.4 ± 1.4

Results of SPMO-4 cell line

Results of the study of SPMO-4 cell line are shown in Tables 1 and 2. Metaphase analysis of Giemsa stained chromosomes in different time intervals show that the frequency of metacentric chromosomes changed after the week 1 and remained relatively unchanged at later passaging time intervals ($P>0.05$). Similarly the total number of chromosomes number in this cell line was statistically different from weeks 2 to 4 ($P<0.05$). Frequency of acrocentric chromosomes and the total number of chromosome were statistically significant between the first week and next three weeks' passages ($P<0.05$).

Fluorescent staining analysis (Table 2) shows that there is no change in the number of human metacentic and acrocentric chromosomes, but there is a significant change in the number of mouse metacentric chromosomes between the first and the third week passages ($P<0.05$). Moreover, there is a statistically significant difference between the number of mouse acrocentric chromosomes and the total chromosome number of first, third and the forth week passages ($P<0.05$).

The Results of F3B6 cell line study

The results of the study of F3B6 cell line are summarized in Tables 1 and 2. Metaphase analysis of Giemsa stained chromosomes show that the difference between the frequency of metacentric chromosomes and the total number of chromosomes was significantly different in comparing the the first week with the next three weeks passages ($P<0.05$). Metacentric chromosome changes between the four week passages cells and other passage time was significantly different ($P<0.05$). Fluorescent staining analysis showed that there was no change in the number of human metacentric and mouse acrocentric chromosomes. However, there was a significant change in the number of mouse metacentric chromosomes and the total number of chromosomes between the first week and the next three weeks passages ($P<0.05$) (Table 2).

Discussion

Hybridoma cells are made from the fusion of somatic cells. The origin of fused cells can be from one or two species. When the fused cells are from one species, the cells, like other cells, maintain their chromosome stability. It means that during successive cell divisions the number of chromosomes remains unchanged. Usually when the fused cells are from two different species, chromosome loss occurs mostly in the early cell divisions after the fusion and stabilizes after 1 to 6 weeks (20). Once the hybrids were grown to mass culture, and after the initial large human chromosome segregation had taken place the karyotypes largely appeared to be relatively stable. Few chromosomes were reported to be lost in the continuous cell cultures (8, 9). The mechanism for human chromosomes loss is not clear; however, it might be due to the early mouse DNA replication because of the preferential activities of enzymes involved in, or factors specific for, mouse DNA replication. Human DNA is then replicated, but not all of the genome is replicated before mitosis ensues one of the daughter cells ending up with reduced human DNA (21). Malfunction in mitotic spindle leading to chromosome non-disjunction might be another possibility (22). Despite the position selection for mouse chromosomes and the absence of selection for human chromosomes, it appears that some of the either sets of the chromosomes are essential for these hybrids (23). It seems reasonable to say replication time difference between human and mouse chromosomes causes loss of human chromosomes in mitotic division.

In fact one of the major uses of hybrid cells is producing monoclonal antibody. Numerical instability in hybrid cells sometimes causes the loss of the human chromosomes coding an antibody of interest is lost, therefore these cells can not synthesize and secret antibody. It has been previously reported the loss of chromosome complement stabilizes after 1 to 6 weeks. Cell lines used in this study have had previously passed several passages and are thought to have reached chromosomal stability. In metaphase spreads that were stained in the Giemsa, acrocentric, metacentric and total chromosome number were analyzed altogether, while in fluorescent stained metaphases human and mouse chromosomes were analyzed separately because the differentiation between these chromosomes was possible.

All chromosomes were stable in HF2X653 cell line in metaphase spreads that stained by Giemsa (Table 1) and fluorescence dye (Table 2). Therefore, apparently in different passages the chromosome number remained unchanged. Analysis of SPMO-4 and F3B6 metaphase spread stained in Giemsa and fluorescent dye showed significant differences in chromosome variation after the first passage but remained unchanged for the next three passages (Tables 1 and 2). Results shown in tables 1 and 2 indicate that the number of metacentric and acrocentric chromosomes in different passages was changing. For example, human or mouse acrocentric and metacentric chromosomes were either increased or decreased in different passages while the total chromosome number remained unchanged. These findings are similar to the findings previously reported by Coffino *et al* for other hybridoma cell lines (24).

There are different reasons for variation in the number of chromosomes in hybridoma cells (25). One of the reasons for the increase in metacentric chromosomes and the decrease in acrocentric chromosomes might be due to the Robertsonian translocations between acrocentric chromosomes to form metacentric chromosomes as seen in the fluorescent stained metaphases. Since one of the main uses of human-mouse hybridoma cells is producing monoclonal antibody, chromosomal instability in these cells sometimes causes loss of human chromosomes that code specific antibody, therefore, cytogenetical analysis is essential for these cells prior to use for antibody production. Using fluorescent Hoechst 33258 differential staining could be a suitable and inexpensive method for human-mouse interspecies chromosome characterization.

Conclusion

Hybridoma cell lines are valuable tools for the production of the monoclonal antibodies, however, because of the repeated usage of these cells in laboratories, chromosome loss might occur for both human and mouse chromosomes complementation. Results obtained in this study clearly indicate that, although the total numbers of chromosomes remain unchanged, changes occurred in the number of human and mouse chromosomes during different passages. Having a method for identification of such chromosomal changes might help those using hybridoma cell lines to be sure of the presence of the specific chromosome. Using fluorescent Hoechst 33258 differential staining method showed to be a suitable and inexpensive method for human-mouse interspecies chromosome characterization.

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