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Short communication

EVALUATION OF CHROMOSOMAL ANEUPLOIDY IN MOUSE FIBROBLASTS OF TWO DIFFERENT CELL LINES CD1 AND NIH - 3T3

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ABSTRACT

The aim of this study was cytogenetic analysis of CD1 and NiH-3T3 mouse embryonic fibroblast cell lines. Numerical chromosome aberrations were detected in chromosome sets at metaphase stage; metaphase arrest was achieved by the use of colcemid at the final concentration 5.10^{-7} M. Analysis of mouse fibroblast nuclei (90 metaphase plates per cell line) showed 72.22 % occurrence of correct diploid nuclei for CD1 and 62.22 % for NiH-3T3 cell line. Total aneuploidy detected in mouse embryonic fibroblasts was 17.78 % for CD1 in comparison to 27.78 % in NiH-3T3 cell line. According to obtained results, mouse embryonic fibroblasts of NiH-3T3 cell line exhibit higher aneuploidy level compared to CD1 cell line. Results from various types of analysis on different fibroblast cell lines including chromosomal studies may be helpful in selection of appropriate quality fibroblast cell lines for further embryonic stem cell culture.

Key words: mouse; fibroblast; CD1; NiH-3T3; chromosome; aneuploidy

INTRODUCTION

Chromosomal aberrations, presented by numerical and structural changes on chromosomal level are identified by standard cytogenetic methods. Their formation occurs as a result of mutational processes in the chromosome. In all populations there are numerical and structural variants of the standard karyotype (Parkanyi *et al*., 2004). The structural changes occur due to chromosome breaks and are accompanied by formation of abnormal combination of chromosomes. Numerical chromosomal changes are caused by excess or loss of whole chromosome. They may occur in the sex chromosomes or autosomes (Parkanyi *et al*., 2004).

Aneuploidy means the number of chromosomes, which are not euploid, and are presented by irregular number of chromosomes. It is a change in the number of chromosomes in chromosome sets (Weaver and Hedrick, 1995). The main cause is a non-disjunction process during meiotic and mitotic division. This cause uneven distribution of chromosome pairs in daughter cells. While one daughter cell contains two chromosomes of chromosome pair, the other one is without chromosome from the set (Bond and Chandley, 1983). Non-disjunction is common during meiosis I. It rarely occurs in meiosis II. If it occurs during the meiotic division, all cells that develop from aneuploid zygote will be aneuploid. Non-disjunction during mitosis affects only part of the somatic cells (Parkanyi *et al.*, 2004, Curlej *et al*., 2007). Aneuploidy is a frequent cause of various diseases, sterility, leads to the formation of tumours, or to the death of its carrier. The viability of aneuploid organisms is decreased by a number of reasons. Protein stoichiometry imbalance and reduction in function of selected genes are the main causes of errors associated with chromosomal loss. Since extra chromosomes are active in aneuploid cells,

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transcriptional and translational processes are affected due to the action of the chromosomes and this may lead to the reduced viability of the organism (Torres *et al*., 2007). Fibroblasts are connective tissue cells, which are found in all vertebrate organisms. The main function of fibroblasts is to maintain the structural integrity of connective tissue by continuous secretion of precursors of extracellular matrix. Mouse embryo fibroblasts are often used as cell culture in the research of human embryonic stem cells. Embryonic stem cells of animals require for their proliferation various factors, especially high-quality embryo fibroblasts. Inactivation of embryonic fibroblasts by mitomycin offers preparation of monolayer cell culture. This provides optimal conditions to embryonic stem cells for proliferation and targeted differentiation (Eghbali-Webb, 1995; Kanekar *et al*., 1998; Souders *et al.*, 2009).

The aim of this study was cytogenetic analysis of CD1 and NiH-3T3 mouse embryonic fibroblast cell lines.

MATERIAL AND METHODS

Biological material

Mouse fibroblasts of cell lines CD1 and NiH-3T3

NIH 3T3 and CD1 cells (permanent mouse fibroblasts) with a cell cycle of ~22 were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 medium (1:1 ratio; Gibco BRL, Eggenstein, Germany) supplemented with 1 % glutamine and 10 % fetal calf serum (Gibco BRL).

Colcemid treatment

Fibroblast cell culture was removed from CO_2 incubator (set on 38.5 °C) and under laminar box conditions colcemid (Gibco BRL, USA) was added to cells at the final concentration of 0.1 μg.ml^{-1}. Immediatelly after colcemid application cell culture was incubated in CO_2 incubator for 3 h. This allowed colcemid to block the process of mitosis at a stage when chromosomes are sufficiently condensed and, therefore, are visible under a light microscope.

Cell fixation

After 3 h culture with colcemid a solution of 0.05 % trypsin / EDTA was added to cover the whole surface of cell culture. The cells were then incubated for 5 minutes at room temperature (approx. 22 °C). Activity of trypsin enzyme was blocked by the addition of FCS serum to the culture medium with cells. The cell suspensions were transferred into 15 ml centrifuge tubes and centrifuged for 5 minutes at 800 rpm (approx. 122 x g). The supernatant was removed from the tube and about 200 ml of media with cells was gained. The cell pellet was resuspended gently by tapping on the tube bottom, and checked if there are no clusters of cells in suspension. Furthermore, under the constant rotation of the tube by hand 5 ml of preheated (37 °C) hypotonic solution (75 mmol.l^{-1} KCl) was slowly added. Then the cells were placed into the thermostat (at 37 °C) for 15 minutes. After 15 minutes of cell treatment by hypotonic solution, the tube with cells was removed from the thermostat, centrifuged for 5 minutes at 800 rpm and the supernatant was removed from the cell suspension. Approximately 0.5 ml of hypotonic solution was retained and at the beginning of fixation process, only a few drops of freshly prepared hypotonic solution (methanol / acetic acid in a ratio of 3:1) were added slowly to cell suspension. Tube was gently turned for mixing cell suspension with fixative solution, and the rest of 4 ml fixative solution was added to the tube. Tubes were centrifuged for 5 minutes at 800 rpm. The resulting supernatant was removed from tubes and approximately 0.5 ml of the solution with cells was retained. The cell pellet was re-suspended gently by tapping at the bottom of the tube and 4 ml of the fixative solutions were added to the tube. This steps including centrifugation process were repeated three times to obtain solution with properly fixated cells, ready for microscope slide preparation, painting and evaluation under light microscope.

Microscope slides cleaning

Prior the preparation of chromosome paints, the slides were properly cleaned in order to avoid unnecessary loss of some nuclei and metaphases. The slides were put into racks containing 6 mol.l^{-1} HCl for at least 3 hours at room temperature. After this time, the slides were washed under running water, rinsed in distilled water and dried at room temperature. Treated slides were stored in 96 % ethanol and dried with soft cloth immediately before use.

Sample preparation for microscopic evaluation

A few drops of solution (approx. three drops per slide) with fixed cells were applied on the surface of pre-cleaned slides by the use of Pasteur pipette. The slide with sample was treated by hot steam for 30 sec, immediately after sample application for „explosion" of the cell membrane. In this phase methanol from fixative solution was evaporated, resulting in increasing concentrations of acetic acid, which together with water stimulated the spreading of chromosomes on the slide surface. After this step, the slides with the sample were stained with 2 % Giemsa (Gibco BRL, USA) solution for 8 minutes at room temperature.

Sample evaluation

Stained slides were observed under the light microscopes (Zeiss and Leica Microsystems, Germany)

at 40 × magnification. Metaphase plates were recorded by camera placed on the microscope and images were transferred to computer for further selection according to the quality of metaphase spreads (Fig. 1). Finally, chromosomes were counted in each chromosome sets and the results were statistically processed by the use of χ^2 test.

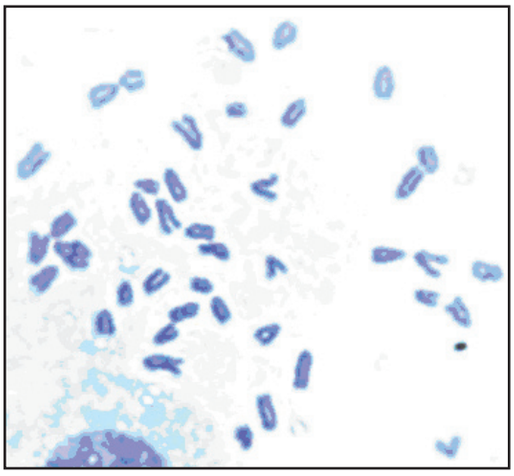

Fig. 1: Metaphase plate of CD1 mouse fibroblast cell line (2n=40)

RESULTS AND DISCUSSION

Chromosome analyzes were performed on two genetically different CD1 and NiH-3T3 mouse fibroblast cell lines to identify the incidence of numerical chromosomal aberrations. The cell line of CD1 mouse fibroblast is characterised by the presence of 40 chromosomes in diploid chromosome set and NiH-3T3 line by 68 chromosomes in diploid set. To assess potential changes in the number of chromosomes in the nucleus of cells, we analyzed 90 nuclei at the metaphase stage for both cell lines using a Leica fluorescent microscope.

The results of our study showed that in CD1 fibroblasts 72.22 % of the analyzed cell nuclei corresponded with diploid number of chromosomes, while the number of aneuploid cells was 17.78 % (Table 1, Fig. 2).

In the NiH-3T3 mouse fibroblasts, the majority of total assessed cell nuclei (62.22 %) contained diploid chromosome number and aneuploidy was identified in 27.78 % of cell nuclei (Table 1, Fig. 2). In comparison to CD1 mouse fibroblasts (3.33 %), aneuploidy was occurred in 22.22 % of hyperdiploid chromosomal sets (Table 1, Fig. 3).

The most common cause of chromosomal aneuploidy is the non-disjunction process that occurs during meiotic or mitotic cell division (Goepfert *et al*., 2000; Shi *et al*., 2004). These results confirm the findings of Zartman and Fechheimer (1967), who mentioned that the incidence of aneuploidy in mammalian cells is partially genetically determined. Moreover, it was found that several factors contribute to the occurrence

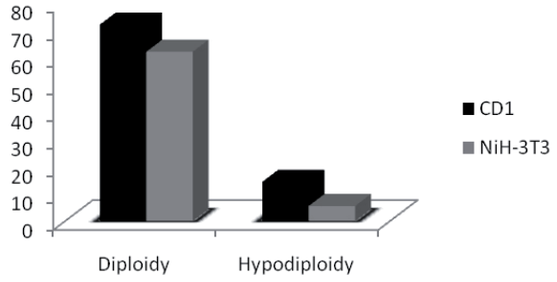

Fig. 2: Percentage of diploid and aneuploid cell nuclei in mouse fibroblast of CD1 and NiH-3T3 cell lines

Table 1: Numerical analysis of chromosomes isolated from two different mouse fibroblast cell lines CD1 and NiH-3T3

Cell line	Diploidy	Aneuploidy		Aneuploidy Total	Polyploidy (≥ 3n)
		Hypodiploidy (2n < 40)	Hyperdiploidy (2n > 40)		
CD1 (2n = 40)	65 (72.22 %)	13 (14.44 %)	3 (3.33 %)	16 (17.78 %)	9 (10 %)
NiH-3T3 (2n=68)	56 (62.22 %)	5 (5.56 %)	20 (22.22 %)	25 (27.78 %)	9 (10 %)

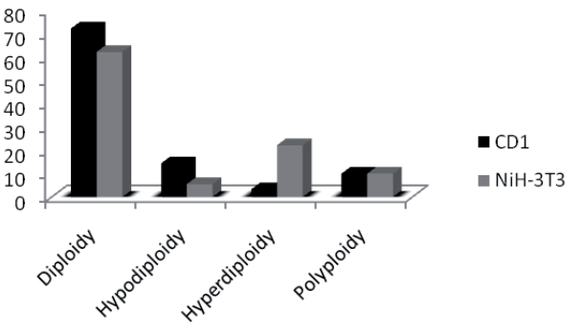

Fig. 3: Percentage of cell nuclei with the number of chromosomes in mouse fibroblast of CD1 and NiH-3T3 cell lines

of aneuploidy, such as influence the dynamics of mitotic spindle, changes in the centromere regions, duplication, changes in chromosome condensation and cohesion, including changes at the level of proteins associated with the centromere area influencing the process of mitosis (Goepfert *et al.*, 2000; Cleveland *et al*., 2003; Kalitsis *et al*., 2005). The incidence of aneuploidy also depends on the origin of cells (Parkanyi *et al*., 2004). Knock-out studies of genes in humans and mice suggest that mitotic checkpoint proteins, including Mad2, BubR1 and Bub3 have essential importance for proper process of cell division (Dobles *et al*., 2000; Kalitsis *et al*., 2000; Babu *et al*., 2003; Baker *et al*., 2004; Wang *et al*., 2004). It was found that rare mutations of Bub1 and BubR1 genes are the cause of instabilities on chromosomal level and carcinogenesis (Cahill *et al*., 1998, Hanks *et al*., 2004). Kalitsis *et al.* (2005) identified the correct number of chromosomes (40) in 65 % occurrence in mouse embryonic fibroblasts of Bub3 wild type.

The above mentioned results are consistent with diploidy identified in a group of NiH-3T3 fibroblasts (62.22 %) from our experiment. On the other hand Kalitsis *et al.* (2005) identified 58 % occurrence in heterozygous mouse embryonic fibroblasts. The results are in accordance with other similar cytogenetic studies.

CONCLUSION

Mouse embryo fibroblasts are often used as cell culture in the research of human embryonic stem cells. Embryonic stem cells of animals require for their proliferation various factors, especially high-quality embryo fibroblasts.

Although, according to our results, NiH-3T3 mouse fibroblast cell line exhibited higher proportion of aneuploid nuclei in comparison to CD1 line, both types of mouse fibroblasts are suitable for embryonic stem cell culture.

Results from various types of analyses of different fibroblast cell lines, including chromosomal studies may be helpful in selection of appropriate quality fibroblast cell lines for further embryonic stem cell culture.

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