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Chromosome rearrangements in sublines of human embryonic stem cell lines hESM01 and hESM03

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Abstract

Due to possible proliferative effects of karyotypic reorganization of human embryonic stem cell (hESC) lines detailed genetic analysis are indicated prior to any application of hESCs. Molecular cytogenetic analysis of two different hESC sublines was performed and revealed aberrant chromosomes in both of them, i.e. in hESM01r18 (46,XX,-18,+mar) and hESM0309 (46,XX,del(4),dup(9)). This study shows that microdissection and multicolor fluorescence *in situ* hybridization (mFISH) can be used to detect the chromosomal changes precisely of the derivative chromosomes that are difficult to identify by conventional G-banded chromosome analysis. In the present study chromosome microdissection and reverse FISH were applied using multicolor fluorescence *in situ* hybridization (mFISH) for detailed characterization of the derivative chromosomes. The karyotypes of hESC lines were described as: 46,XX,r(18)(::p11.31→q21.2::q21.2→p11.31::) and 46,XX,del(4)(q25q31.1),dup(9)(q12q33), respectively. The potential role of the chromosomal regions involved in rearrangements for cell proliferation is discussed.

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Introduction

Embryonic stem cells have attracted great interest both for their use in lab research and possible practical applications [1]. However, the genetic stability of human embryonic stem cell (hESC) lines *in vitro* is still poorly understood and the available data remains contradictory [2, 3]. Undoubtedly genetic instability has an impact on the differentiation abilities of hESCs and may also lead to tumor phenotype manifestations, thus, limiting further practical applications of such cells. Thus, strict control of the chromosome content and chromosomal rearrangements in human embryonic stem cells is of importance in improving their efficiency in research and safety in practical applications. Additionally, studies on chromosomal rearrangements in hESCs may be a helpful to obtain new insights into chromosome regions involved in pluripotency maintenance or in 'malignization' of the cells.

However, hESCs chromosome preparations frequently encounter certain difficulties, perhaps connected with hESCs biology and chromosome organization, thus making karyotyping problematic [4]. Consequently, specialized and sophisticated methods must be applied to characterize exactly chromosomal rearrangements

and involved breakpoints. In the present molecular cytogenetic study microdissection was used to generate specific probes derived from rearranged chromosomes in hESCs. These probes were used for reverse multicolor fluorescence *in situ* hybridization (mFISH) analysis of hESM01 and hESM03 sublines and human lymphocytes.

Materials and methods

Cell lines: Sublines hESM01r18 (46,XX,-18,+mar) and hESM0309 (46,XX,del(4),dup(9)) were derived from hESM01 and hESM03 parental cell lines, respectively, as described previously [5]. The used cell lines were cultivated on 35mm petri dishes (Costar) covered with gelatine in KO-DMEM (Invitrogen), with 20% serum replacement (Invitrogen), 0.1 mM β-mercaptoethanol (Sigma), 1 mM glutamine (Gibco), 1 mM non-essential amino acids (Gibco), 4 ng/ml basic fibroblast growth factor (bFGF) (Chemicon) and antibiotics in 6.5% CO_2 on mitotically inactivated mouse embryonic fibroblasts (MEF) feeder cells. Peripheral blood lymphocytes (PBL) were isolated and cultivated according to standard techniques [6].

Metaphase chromosome preparations: Chromosome samples were prepared according to standard procedures. In brief, PBL metaphase and prometaphase chromosomes for FISH analysis were performed as described by Henegariu [7].

DNA probe preparation: Chromosome microdissection was performed according to previously described methods [8, 9]. Briefly, rearranged chromosomes were identified by using an inverted microscope, the Axiovert 10 (Carl Zeiss, Jena, Germany) dissected with the aid of a micromanipulator MR (Carl Zeiss, Jena, Germany) and siliconized extended glass needles. Collected pieces were treated with (0.5 mg/ml) proteinase K (Roche) and amplified in a DOP-PCR, using degenerated MW6 primer [10]. Biotin-16-dUTP, dinitrophenyl-11-dUTP, or/ and digoxigenin-11-dUTP was used to label the amplified dissected chromosomes with an additional 20 cycles of PCR [9, 11].

A telomere-specific biotin-11-dUTP labeled (TTAGGG)n DNA probe was generated by PCR as described by Ijdo [12].

Fluorescence *in situ* hybridization (FISH): The chromosomal location of the dissected chromosome fragments was assessed by reverse painting as previously described [13]. Biotin-labeled DNA probes were detected by avidin-Alexa488 (Molecular Probes), or avidin-Cy5 (Jackson ImmunoResearch); digoxigenin (DIG) labeled DNA probes were detected using Cy3-conjugated anti-digoxin mouse antibodies (Jackson ImmunoResearch); dinitrophenyl (DNP) labeled DNA probes were detected by fluorescein isothiocyanate (FITC)-conjugated anti-DNP rabbit antibodies (Invitrogen). To visualize telomeres a biotin-11-dUTP labeled (TTAGGG)n probe was used avidin-Alexa488 (Molecular Probes).

Chromosomes were counterstained with 1 µg/ml 4',6-diamidino-2-phenylindole (DAPI) and analyzed using an AXIOPlan2 Imaging (Carl Zeiss, Jena, Germany), equipped with CCD camera, filter sets, and ISIS5 image-processing package of MetaSystems GmbH. Chromosomes and chromosomal regions were identified by inverted DAPI-banding using human an International Systems for Human Cytogenetic Nomenclature [14].

Results

Chromosomal rearrangements in hESM0309 cell line

Using DAPI staining, it was shown previously that in all cells of hESM0309 cell line chromosomes 4 and 9 were rearranged [5]. The patterns revealed by the staining allowed to assume that chromosome 4 contained a partial deletion and chromosome 9 a partial duplication. These chromosomes were described as del(4) and dup(9). To confirm these results and assign precisely the breakpoints region-specific microdissection DNA-probes were obtained from those derivatives:

(i) The breakpoints involved in chromosome 4 deletion formation were characterized as 4q25 and q31.1 (Fig. 1). Based on the data abnormal chromosome 4 was described as del(4)(q25q31.1).

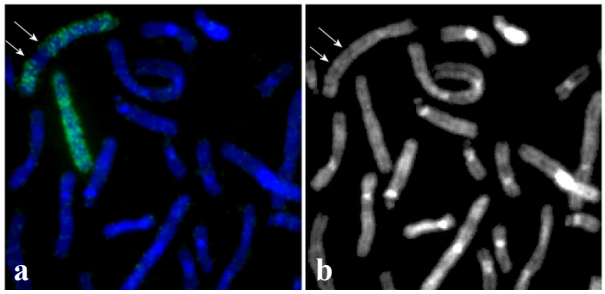

Figure 1. Molecular cytogenetic analysis of chromosomes of hESM0309 line. a – FISH of microdissection DNA probe del(4) (green) with metaphase chromosomes lymphocytes of a healthy donor. b – Inverted DAPI banding of lymphocytes of healthy donor.

(ii) Another abnormal chromosome found in hESM0309 cells was previously described as dup(9)(pter→q3::q12→qter) by DAPI banding [5]. C-banding revealed two C-positive regions confirming the location of one of the breakpoints in a C-positive region 9q12 (Fig. 2a). To describe chromosome dup(9) more precisely the set of DNA probes were generated by microdissection from this chromosome and normal chromosome 9 as shown schematically in Fig. 2b. E.g. DNA probes WCP9 and WCPder(9) (Fig. 2b) completely painted chromosome 9 and dup(9), however, the intensity of the FISH staining of these chromosomes was different (Fig. 3). Further studies summarized in Fig. 3 revealed a dup(9)(pter→q33::q12→qter) in hESM0309.

Thus, the karyotype of hESM0309 cells was described 46,XX,del(4)(q25q31.1),dup(9)(q12q33).

Chromosomal rearrangements in hESM01r18 cells

A previously performed hESM01r18 karyotype analysis allowed to hypothesize that the marker, most likely an abnormal ring chromosome was a derivative of normal chromosome 18 and hESM01r18 cells

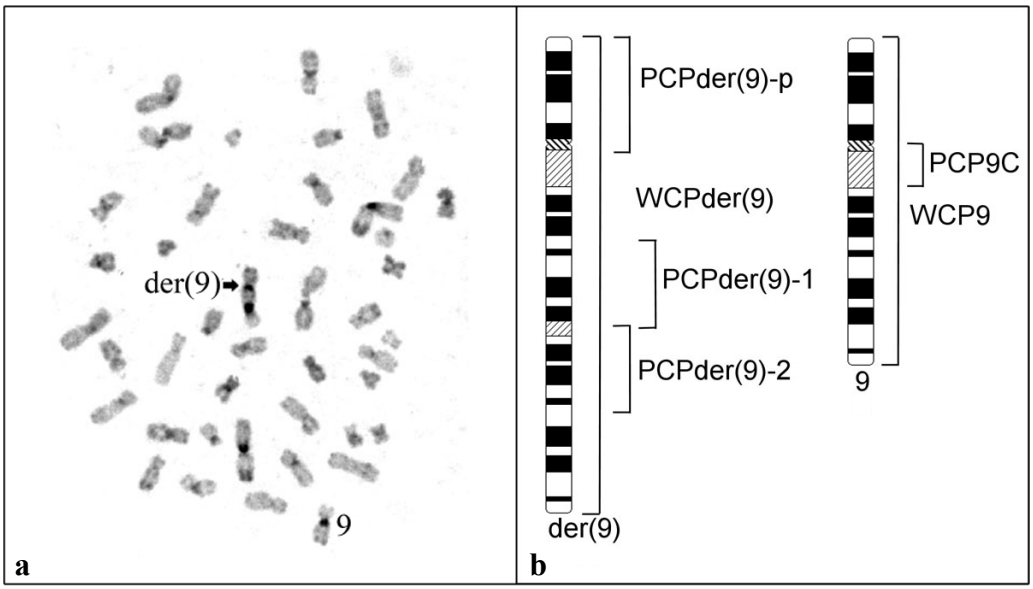

Figure 2. a – C banding of chromosomes of hESM0309 cell line. The arrow indicates additional C-positive region in the chromosome der(9). b – the scheme of generation of microdissection probes from chromosomes der(9) and 9. Names of DNA probes are shown. Chromosome regions from which probes were generated are indicated by square brackets.

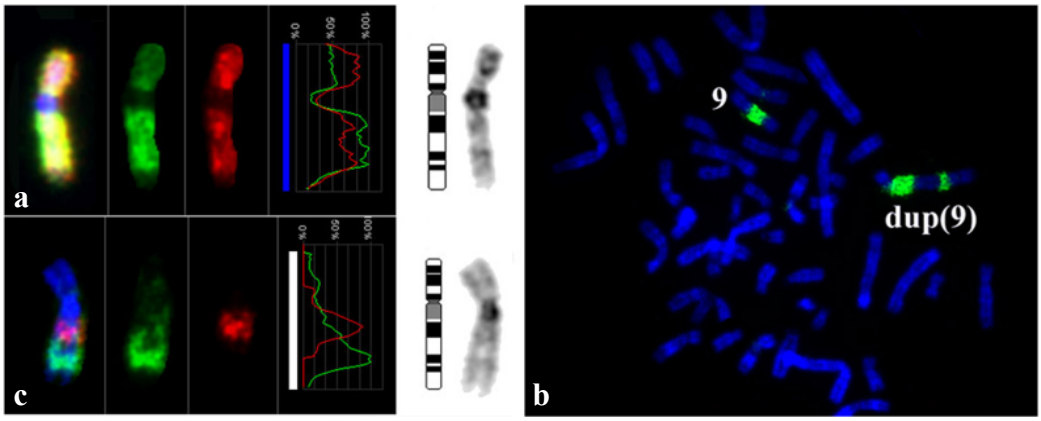

Figure 3. Molecular cytogenetic analysis of hESM0309 by FISH with microdissection probes. a – dual hybridization of microdissection probes WCPder(9), (green) and WCP9, (red) with chromosomes of normal human lymphocytes. b – hybridization of PCP9C DNA probe (green) with chromosomes of hESM03der9. c – hybridization of PCPder(9)-1 (red) and PCPder(9)-2 (green) with chromosomes of normal human lymphocytes. Profiles of signals intensity along the chromosome are shown on the right. Red line – signal from PCPder(9)-1, green line – signal from PCPder(9)-2. Ideograms and C-banding of chromosome 9 are shown to the right of a and c.

were described as 46,XX,-18,+?r(18) [5]. The ring organization of the marker chromosome was confirmed by the telomere specific probe (TTAGGG)n, which labeled termini of all normal chromosomes but gave no signal on the derivative chromosome (Fig. 4a). Chromosome 18 origin of the ring was confirmed by WCP18 and reverse FISH using a microdissected probe derived from ring chromosome (WCP18r). WCP18r painted the r(18) and the region 18p11.31 to q21. of normal chromosomes 18 (Fig. 4b). The profile of signal intensity along chromosome 18 indicated that ring chromosome18 represented inverted duplication of the part of chromosome 18 (Fig. 4c).

Thus r(18) contain two copies of the indicated region and the karyotype of hESM01r18 cells was 46,XX,r(18) (::p11.31→q21.2::q21.2→p11.31::).

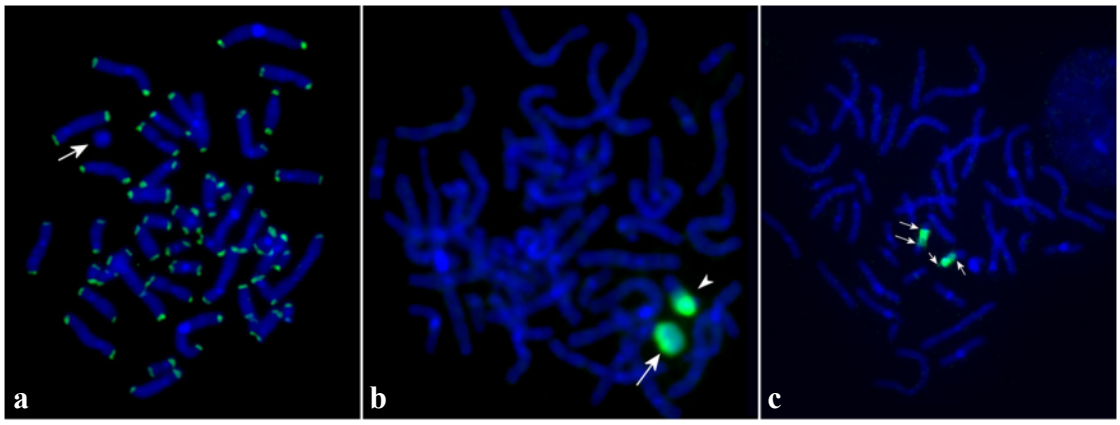

Figure 4. Analysis of hESM01r18 cells. a – FISH of telomere specific probe (green) with chromosomes of hESM01r18 cells. The arrow indicates ring chromosome 18. b – FISH of microdissection der(18) probe with chromosomes of hESM01r18 cells. Arrow indicates chromosome der(18), arrow head indicates normal chromosome 18. c – FISH of microdissection der(18) probe with chromosomes of healthy donor's lymphocytes. Arrows indicate sites of DNA breakage during the formation of r(18)(p11.31q21.2). Chromosomes were counterstained with DAPI.

Discussion

Since their isolation in 1998 [15] hESCs have attracted a tremendous interest, primarily because of their potential practical applications in regenerative medicine. However, during the last twelve years only a limited number of mechanisms involved in self-maintenance of hESCs or directed differentiation to the particular lineage have been deciphered. Even the available information concerning hESCs genetic stability *in vitro* is controversial [2].

Karyotyping of hESM01r18 and hESM0309 cell lines demonstrated that chromosomes r(18) and dup(9), respectively, were present in all cells from given cell lines [5]. hESM01r18 and hESM0309 cells showed a higher proliferation rate and more robust cultivation conditions compared with their parental cell lines. Consequently, these cell lines could be used as a model to study the effects of some particular chromosomal regions on the pluripotency maintaining or differentiation potential, in future.

The breakpoints in chromosome 9 revealed in hESM0309 cell line coincides with the breakpoints quite often observed during loss of heterozygosity in transitional cell carcinoma of urinary tract tumor (9q12, 9q22.3, 9q33-34) [16]. Possibly, hESM0309 cells could be a valuable model to study causal mechanisms of genetic instability and chromosomal abnormalities. So, to look for effective remedy of its prevention. The breakpoint 18q21.2 observed in hESM01r18 cells was also detected in some cases of lymphomas [17] and acute lymphoid leukemia [18]. The other breakpoint in chromosome18p11.31 was not seen in tumors yet.

The existence of chromosomal abnormalities in the stem cells are often associated with carcinogenesis [2]. Loss of chromosome 4 is a common feature of many tumor entities summarized in Gebhart and Liehr [19]. Thus, 4q25 to 4q31.1 deleted in cell line hESM0309 could be a critical region for advanced cell proliferation. For the region 9q12 to 9q33 duplicated in the same cell line, similar gains of copy numbers were observed in tumor entities like head and neck cancer, histiocytoma, esophagus-, lung-, stomach- and uterus-carcinoma [19]. Chromosome 18p11.31 to 18q21.2, however, present in hESM01r18 is rather involved in loss of copy numbers in human tumors than in gain – exceptions are lung cancer and neuroblastoma [19].

Overall, this study shows that hESCs should be (i) molecular cytogenetically characterized in detail and (ii) that such studies may be extremely helpful in understanding tumor initiation and progression, as well.

Acknowledgments

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