

Spontaneous induction of an homologous Robertsonian translocation, Rb(11.11) in a murine embryonic stem cell line

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(Received 10 October 1989 and in revised form 24 January 1990)

Summary

Karyotype analysis of a series of established mouse embryonic stem cell (MESC) lines showed that the majority were aneuploid by the 7th and 9th *passage* and that all lines contained a single Robertsonian (Rb) translocation chromosome with a symmetrical, homologous, arm composition Rb(11.11). Although the chromosomal imbalance makes these MESC lines unsuitable for genetic manipulation *in vitro* and hence for subsequent production of transgenic animals, the spontaneous occurrence and stable retention of the homologous Rb(11.11) as the only metacentric chromosome in an otherwise all acrocentric karyotype, provides potentially useful cell lines for gene assignment and recombinant DNA studies.

1. Introduction

Techniques for the establishment and maintenance of totipotent murine embryonic stem cell (MESC) lines are well established (Evans & Kaufman, 1981; Martin, 1981; Handyside *et al.* 1989) but relatively little is known about the stability of chromosomes within such lines (see below), although some insights can be obtained by extrapolation to chromosome behaviour in Embryonal Teratocarcinoma cell lines ([EC], Iles & Evans, 1977). The karyotypes of MESC lines become progressively unbalanced after a variable time *in vitro*, and the modal chromosome number in many MESC lines is aneuploid. Experiments which have resulted in the germ line transmission of MESC phenotypes/genotypes have been achieved mostly (but not exclusively) from newly established, i.e. low *passage* number MESC lines. However, aneuploid cell lines can contribute to the somatic lineages of chimaeric individuals (Epstein *et al.* 1982, 1984; Cox *et al.* 1984).

Reports of detailed systematic karyotypic studies of MESC lines are limited. Robertson *et al.* (1983), summarized the karyotypes of 21 MESC lines (mostly before the 15th *passage* generation) of which 13 were diploid, and 4 were either trisomic or double trisomic.

One of these was trisomy (Ts) 11, and included a *de novo* Rb (arm composition not defined). Of the remaining 3 unbalanced, 1 had a partial deletion of chromosome 5 and the other 2 were XO and XXY respectively. Four MESC lines karyotyped by Evans & Kaufman (1981) were normal, whereas, only 3 were diploid in 6 MESC lines karyotyped by Axelrod (1984). Suemori & Nakatsuji (1987), described 6 MESC grown on STO feeder layers as 'near tetraploid' in contrast to 11 MESC lines grown on a primary fibroblast feeder layer which 'had almost normal diploid number of chromosomes' [*sic*]. G-banding analyses of 4 of the second group were said to show no detectable abnormalities. Doetschmann *et al.* (1985), reported that the ES-D3 cell line had 62% 40, XY cells, with the remainder either hypo- or hyperploid.

2. Materials and methods

(i) Derivation of MESC lines

Pre-implantation mouse embryos were obtained by flushing the uteri of plugged F₁ hybrid females (C57BL/6 Lac × CBA/Ca Lac) on the afternoon of day 3 gestation (plug = d1). The subsequent manipulations of the pre-implantation embryos and the methods used for the derivation and maintenance of the undifferentiated embryonic stem cell lines are essentially as described by Handyside *et al.* (1989).

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(ii) *Cytogenetic methods*

Two or three days after trypsinization and sub-culturing (at the 7th to 9th passage) into a 25 cm² tissue culture flask, colcemid was added to the medium at a final concentration of 10 µg/ml. After incubation at 37 °C for 2 h, the cells were trypsinised from the flask, treated with hypotonic (0.7%) sodium citrate for 20 min at room temperature, and fixed in two changes of methanol:acetic acid (3:1). Chromosome spreads were made using standard cytogenetic methods. Slides were initially 'block-stained' with Giemsa and a minimum of 20 intact metaphases counted; further slides were GTG-banded using a modified trypsin-banding method (Seabright, 1971) and an average of 5 karyotypes were arranged from each MESC line according to the system described by Nesbitt & Franke (1973).

3. Results(i) *Counts on block-stained metaphases*

Metaphases from a total of 8 MESC lines were counted, 1 founder line and 7 sub-cell-lines; all were aneuploid and with the possible exception of 1 contained a single, symmetrical Rb. Two of the 8 lines [designated MESC 10 and 13 (see Table 1)] were non-mosaic with a stable count of 41, XY, 1Rb [nombre fundamental, = 42; (Matthey, 1949)]. All other lines were mixoploid but had a majority of cells with 41, XY, 1Rb. The one line which was thought to have a minor cell-line with a second, asymmetrical Rb was, therefore, removed from the study leaving seven MESC lines which were further characterized cytogenetically. No diploid cells were observed.

(ii) *G-banding studies (karyotype analyses)*

The consistent karyotypic feature of the MESC lines was trisomy (Ts) for chromosomes 1 and 11 together with a symmetrical homologous Robertsonian translocation chromosome, Rb(11.11), see Fig. 1. In every recorded example of trisomy 1 (except MESC 12 and 13; see below), one of the copies was consistently longer than the other two with the difference being attributable to the addition of a large C band to an apparently normal chromosome. On the basis of the listing of C-band polymorphisms in the mouse (Davisson, 1989) and the strains used in our study, the additional C band was too large to originate from a chromosome 1 and may have been derived from a rearrangement with another chromosome. It is probable that this chromosome is a 4 since a reciprocal translocation with breakpoints in 1A1/2 and in 4A2/3 would account for the increased length of chromosome 1 and the observed deletion in chromosome 4. All other lines were mixoploid and in addition had multiple anomalies (in addition to the Rb(11.11), Ts 1 and Ts 11) which are summarized in Table 1. There was an identifiable C-band polymorphism in the two no. 14s which was consistent with them being derived from the CBA and C57BL/6 strains (E. P. Evans, personal communication). The karyotypes of MESC 12 and 13 were not fully resolvable [particularly for the t(1;4)] because of multiple rearrangements, duplications and deletions; nevertheless both lines retained the Rb(11.11), and were trisomic for chromosomes 1 and 11.

4. Discussion

All of the MESC lines karyotyped (i.e. the founder and its sub-lines) were derived from a single F₁

Table 1. *Summary of MESC line karyotypes*

MESC line no.	Count	Trisomy	Robertsonian translocation	Other
8	40 ^a , 1Rb/41 1Rb (74%)	1 and 11	Rb(11.11)	Polymorphic 14s; t(1;4)
9	40, 1Rb/41, 1Rb (82%)	1 and 11	Rb(11.11)	Polymorphic 14s; t(1;4)
10	41, 1Rb (≈100%)	1 and 11	Rb(11.11)	Polymorphic 14s; t(1;4)
11	40, 1Rb/41, 1Rb (90%)	1 and 11	Rb(11.11)	Polymorphic 14s; t(1;4)
12	Mixoploid ^b	1 and 11	Rb(11.11)	Monosomy 14 Monosomy 4
13	41, 1Rb (≈100%)	1 and 11	Rb(11.11)	Polymorphic 14s
15	Mixoploid ^b	1 and 11	Rb(11.11)	Polymorphic 14s; t(1;4)

^a First number denotes the total chromosome count, i.e. 41 and 42 chromosome arms respectively. All lines are XY.

^b Wide variation in chromosome count, but lines contained a majority of metaphases with the Rb(11.11); 65% in line no. 12 and 67% in line no. 15.

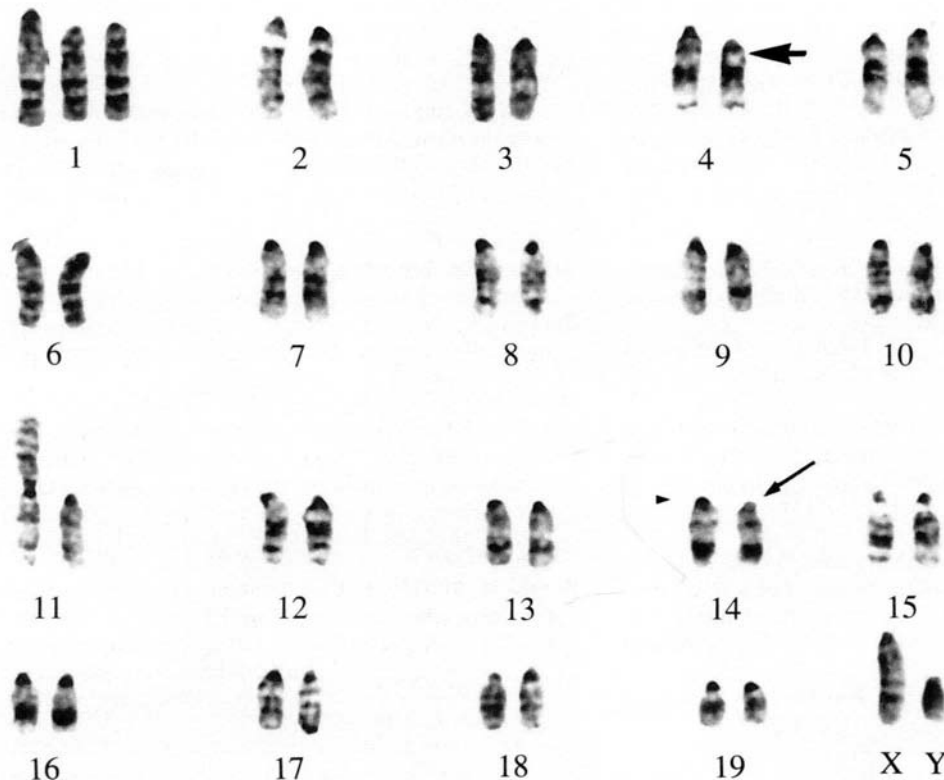


Fig. 1. GTG-banded karyotype from MESC line no. 9 [41, XY, Rb(11.11), +1, +11, t(1;4)], showing the heteromorphic centromeric regions of the 14s (small

arrow and arrowhead), and presumed position of the reciprocal translocation breakpoint in the proximal region of one of the 4s (large arrowhead).

blastocyst (CBA \times C57BL/6). The most significant karyotypic finding was the presence of a single, homologous Rb(11.11) in all lines. Although non-homologous Rb translocations occur with high spontaneous frequency in feral mouse populations (Gropp & Winking, 1981), Rb involving homologues are lost *in vivo* because carriers produce only disomic and nullisomic gametes which if fertilized with chromosomally balanced gametes, result in trisomic and monosomic zygotes or fetuses, thus rendering the Rb-carrier reproductively sterile.

The pattern of karyotypic changes and imbalances observed in our MESC lines are similar to those observed in mouse teratocarcinoma (EC) cell lines (Iles & Evans, 1977), and in this respect it is interesting to note that trisomy 11 was a frequently encountered karyotypic change in EC lines with reduced differentiative capacity.

The Rb(11.11) may have arisen as a *de novo* centric fusion translocation in one of the F_1 parental stock and was 'rescued' by explantation and transformation into an MESC line, or may have been an *in vitro* translocation event early in the development of the founder MESC line. Certainly, Rb chromosomes frequently occur spontaneously in transformed cell lines of rodent origin and may involve homologous as well as non-homologous combinations. The particular advantage of the present Rb rests in its homologous arm-composition together with its apparent integrity and stability within the otherwise karyotypically

unstable MESC lines. Furthermore, it is the only metacentric chromosome in an otherwise all acrocentric chromosome cell line and in this context, the Rb is a clearly defined chromosome marker which may be of considerable interest to groups working on mouse chromosome 11. For example, this Rb, would provide excellent material for chromosome sorting with a Fluorescence Activated Cell Sorter, for *in situ* hybridization studies using mouse chromosome 11 specific probes, or could be used as the target for microdissection of specific chromosome 11 regions and enzymatic amplification of the micro-dissected DNA (Ludecke *et al.* 1989).

We are grateful to Dr E. P. Evans for his help and advice with the karyotyping.

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