Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells

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We have observed karyotypic changes involving the gain of chromosome 17q in three independent human embryonic stem (hES) cell lines on five independent occasions. A gain of chromosome 12 was seen occasionally. This implies that increased dosage of chromosome 17q and 12 gene(s) provides a selective advantage for the propagation of undifferentiated hES cells. These observations are instructive for the future application of hES cells in transplantation therapies in which the use of aneuploid cells could be detrimental.

Human embryonic stem cells have been cultured for extended periods while retaining a diploid karyotype[1–3]. They are strikingly similar to human embryonal carcinoma (hEC) cells, the stem cells of teratocarcinomas, in that undifferentiated hEC and hES cells display comparable expression profiles of genes and antigens[4]. However, hEC cells are typically aneuploid, showing distinctive chromosomal abnormalities including the gain of chromosome 17q and the presence of one or more isochromosomes 12p[5–7]. Long-term culture of mouse ES cells can lead to a decrease in pluripotency and the gain of distinct chromosomal abnormalities[8]. Here we show that similar chromosomal changes, which resemble those observed in hEC cells from testicular cancer, can occur in hES cells.

A culture of hES cell line H7 (designated H7.S0; karyotype 46,XX) was maintained in Sheffield for several months (see **Supplementary Methods** online). The cells retained an undifferentiated hES cell phenotype (SSEA3+, TRA-1-60+, Oct4+, Sox2+)[4,9], an ability to differentiate (judged by differentiation *in vitro* from embryoid bodies with the formation of cells such as neurons and beating cardiac muscle cells) and a normal karyotype. However, all the cells of a subline, H7.S6, defrosted from an early freezing of H7.S0, had acquired chromosomal changes by passage 60 (about 6 months): the cells all had a 46,XX, der(6)t(6;17)(q27;q1) karyotype. These cells expressed markers of undifferentiated hES cells and retained an ability to differentiate in culture. The translocation involved gain of the complete long arm of chromosome 17, translocated to 6q, without apparent loss of chromosome 6q or 17q material; the cells were trisomic for 17q (**Fig. 1**). A subgroup of cells also had trisomy 12.

Another diploid H7 subculture (H7.S9) was reestablished from a different early freezing. After four months, all the cells had become trisomic for the whole of chromosome 17. Over the same period, we studied a different hES cell line, H14 (designated H14.S0; 46,XY). This line was diploid, but a subsequent culture from a frozen stock, H14.S9, also became trisomic for chromosome 17. To further explore the recurrent gain of chromosome 17, we established yet another culture of H7 cells (H7.S14) from another early freezing. Interphase fluorescence *in situ* hybridization (FISH) analysis did not detect any cells with trisomy 17. After 22 passages (2 months), karyotyping revealed trisomy 17; interphase FISH indicated trisomy 17 in 76% of the cells and, after an additional 17 passages, in 95% of the cells.

In Sheffield, a gain of chromosome 17q was common to all hES cells in which chromosomal changes were detected, arising on four independent occasions in two different hES cell lines. A gain of chromosome 12 was also seen, but only in subpopulations of cells; no other consistent karyotypic changes were noted. Because the differentiated derivatives of ES and EC cells have limited growth potential, genetic changes that promote stem cell self-renewal at the expense of differentiation are likely to provide strong selective growth advantages for the variant sublines in which they occur. Thus, increased dosage of chromosome 17q gene(s) may provide hES cells with some strong advantage for maintenance *in vitro* or may inhibit apoptosis, and the evolution of hES cells in culture may parallel that of hEC cells in tumors. Indeed, when a subline of H7 carrying the t(6;17) translocation was cultured on Matrigel without fibroblasts or fibroblast-conditioned medium for over 25 passages, a clone of these feeder-independent H7 cells was found to carry an isochromosome, i(12p), the characteristic karyotypic marker of hEC cells (**Fig. 1**).

The original reports of hES cells indicated a stable diploid karyotype[1]. However, recently we have also seen karyotypic changes in H1 and H14 hES cell lines at the University of Wisconsin (**Table 1**). Mostly these changes involve gain of chromosome 12, although in one case amplification of part of chromosome 17q, together with a range of other changes, was seen. Why gain of chromosome 17 predominates in one laboratory and chromosome 12 in the other is unclear. Although initial culture protocols were, in principle, the same, the karyotypic changes observed in the Wisconsin cells were obtained after clonal selection or efforts to culture cells in the absence of feeders. Whether similar changes will be identified in other lines and other laboratories remains to be established.

The mechanism by which gain of chromosome 17q confers proliferative advantage is a matter of conjecture. In germ cell tumors,

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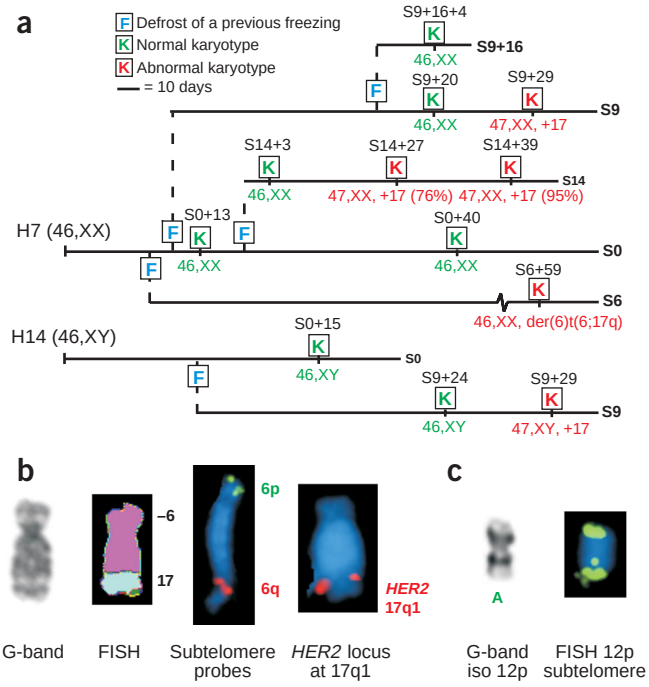

Figure 1 Karyotypic changes affecting chromosomes 17 and 12 in sublines of H7 hES cells. (**a**) The relationship between the different cultures of H7 and H14 cells that were maintained in Sheffield. H7 was originally cultured from a vial frozen in passage 15 at the University of Wisconsin. Cultures were then provided with an 'S' number to designate subsequent passages in Sheffield. Subcultures derived from subsequent freezings were designated by the 'S' number the cells had reached at the time of freezing. Different cultures were frozen and karyotyped as shown (see **Supplementary Figure 1** online). A minimum of 20 metaphase spreads was analyzed for all karyotypes. The latter two H7.S14 karyotypes were confirmed by analyzing 200 interphases with FISH. (**b**) The derivative chromosome, der(6)t(6;17), present in the subline of H7 showing, respectively, the chromosome stained by G-banding, M-FISH for chromosome 17q marker, subtelomere probes for chromosome 6 and locus-specific probes (*HER2*, also known as *ERBB2*) for chromosome 17q. These data indicate the presence of a complete extra copy of 17q, translocated to chromosome 6, apparently without loss of chromosome 6 material. (**c**) An isochromosome 12p present in a clonal subline of the H7 cells adapted to culture on Matrigel in the absence of feeders or feeder-conditioned medium (subline H7.S6-5ANF), shown by G-banding and by FISH using a 12p subtelomeric probe. These cells retained the der(6)t(6;17)(q27;q1) chromosome.

Table 1 Examples of karyotypic changes in hES cells seen at the University of Wisconsin

Cell line	Passages under standard culture conditions	Passages under feeder-free conditions	Karyotype
H1.1 A	75	0	47,XY, +12
H1.1 B[a]	75	≈ 30	44,X,–Y,der(6)t(6;17;17)(6pter→6q15::17q25.1→17q25.3::17q11.2→17qter), del(10)(p11.2),+12,–17, der(18)t(17;17;18)(17qter→17q11.2::17q25.3→17q25.1::18q23→18pter)
H14	41	13	47,XY,+12 (40%); 46,XY (60%)

hES cell lines H1 (subclone H1.1) and H14 were maintained initially as previously described[2], and subsequently under feeder-free conditions (see **Supplementary Methods** online). Classical G-banding karyotypes (20 metaphases) were obtained after the specified number of passages. [a]B is a subclone from A.

overexpression of *GRB7*, located on chromosome 17q, is a common feature of hEC cells[7]. Gain of 17q is also associated with neuroblastoma[10], and genes implicated in apoptosis and differentiation are sited on 17q; for example, *Survivin*, encoding an inhibitor of apoptosis, lies at 17q25 (ref. 11). STAT3 and GRB2, the homologs of which regulate self-renewal and differentiation in mouse ES cells[12], are encoded by human 17q. With respect to chromosome 12, *Nanog*, a master controller of pluripotency in murine ES cells[13], lies on the boundary of the minimal 12p amplicon associated with hEC cells[14]. Karyotypic change occurs in mouse ES cells, correlating with a reduced ability to colonize the germ line in chimeric mice[8]. These changes commonly affect chromosome 8, but also occasionally chromosome 11, which is partly syntenic with human chromosome 17q[8,15].

Apart from clues they offer regarding the genetic control of stem cell self-renewal, our observations of chromosomal changes in hES cells suggest that caution is warranted when designing culture conditions, and especially feeder-free conditions, for the cells; *in vitro* evolution may select for adaptive genetic changes. The occurrence and potential detrimental effects of such karyotypic changes will need to be considered in the development of hES cell–based transplantation therapies.

Note: Supplementary information is available on the Nature Biotechnology website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Biotechnology* website for details).

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