P-22 Occurrences of Chromosomal Abnormalities in Prolonged Human Embryonic Stem Cell (hESC) Cultures

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Background & Objectives: Many hESC have been cultured for extended periods while retaining their diploid karyotypes. However, recent reports demonstrate genetic instabilities including chromosome abnormalities in some hESC. When any cell is cultured in vitro for prolonged periods, the cells tend to develop chromosome aberrations because of suboptimal culture conditions. Such aberrations would be unacceptable in hESC lines established for basic research. We investigated the karyotypes of SNUhES 3 cell line, which was cultured in four laboratories, including ours, to examine the occurrences of chromosomal abnormalities in extended in vitro cultures.

Method: We analyzed over 30 metaphases using Giemsa staining (G band by trypsin using Giemsa; GTG) and carried out fluorescence in-situ hybridization (FISH) to assess possible chromosomal abnormalities such as aneuploidies, translocations, or mosaicism.

Results: Normal karyotype (46,XY) was detected in SNUhES 3 (passage 121), which was cultured in our lab (lab I). 47,XY,+12 was detected in lab II (P 92) cultured cell line. Mosaicisms of 47,XY,+12[100]/46,XY,[6], and 47,XY,+12[35]/46,XY[2]/48,XY,+12,+12[1], were observed in lab III (P 51), and IV (P 87), respectively. The cell lines in labs III, and IV were converted completely to trisomy 12. These results were confirmed by carrying out FISH with specific probes against chromosome 12.

Conclusions: The emergence of karyotype changes in hESC lines, grown in a number of different laboratories, suggests that hESC are inherently predisposed to these aberrations. The occurrence of such potentially detrimental karyotypic changes will need to be carefully considered in the development of hESC basic research. Therefore, it is important to not only maintain optimal conditions for in vitro cultures, but also to maintain normal karyotypes by periodically testing for chromosomal abnormalities in hESC.

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