The effects of culture methods on the genetic and phenotypic stability of human embryonic stem cells

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Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) hold great promise as tools and material for preclinical and clinical applications. The tremendous self-renewal and differentiation capacities of these pluripotent cell types make them potential sources of large quantities of differentiated cells for drug screening, toxicology studies, biomolecule production, and cell therapy. For cell therapy in particular, it is very important that the cell populations used are safe and do not contain genetic aberrations. In fact, common aneuploidies detected through standard karyotyping in hESC cultures, such as trisomies of chromosomes 1, 12, and 17, are also found in common clinical teratocarcinomas.

It is possible that hESC cultures can accumulate subchromosomal genetic changes undetectable by standard karyotyping but with potential adverse consequences on their utility and safety profiles. To detect these much smaller genomic changes, genome-wide SNP genotyping was used in a pilot study. SNP genotyping of hESC lines, somatic stem cell lines and normal human clinical samples was performed on the Illumina Hap550 SNP genotyping platform. Genetic aberrations ranging from 0.5 megabases to whole –chromosome size were detected, including copy number variants and loss of heterozygosity with preservation of copy number. Overall, hESCs demonstrated a higher level of genetic aberration than other cell types, with trisomies of chromosomes 12 and 17, and large duplications of chromosomes 1, 12, 17, and the X chromosome, only observed among the hESCs (Figure 1 and Table 1).

As a continuation of this study, the effects of commonly used methods for large-scale expansions, such as feeder-free culture and enzymatic passaging, will be analyzed on the genetic and phenotypic stability of hESCs. The accumulation of new aneuploidies, copy number variants (CNVs), and single nucleotide polymorphisms (SNPs) will be used as measures of genetic instability in hESCs cultures. The NIH-approved WA09 hESC line will be cultured under four different culture conditions with six replicates per condition (Figure 2). The cultures will be carried for a total of 35 passages with standard karyotyping by G-banding and immunocytochemistry performed every 5 passages (Figure 3). Cells from each replicate will be collected every 5 passages and subjected to genome-wide SNP genotyping. The genotyping data will be analyzed for CNVs and the overall rate of accumulation of new CNVs and SNPs among the four culture conditions will be compared and analyzed to detect specific regions of the genome that accumulate CNVs in more than one sample. At the end of the 35th passage, the pluripotency of the hESCs cultures will be determined by assaying for doubling time, plating efficiency, mitotic index, telomere length, telomerase activity, apoptosis rate, differentiation in vitro, teratoma formation, expression of pluripotence-associated genes and miRNAs. Growth curves for each of the growth conditions will also be determined with the xCELLigence System (Roche Applied Science) (Figure 4).

To conclude, high-resolution SNP genotyping can be used to identify the degree of genetic instability of hESC lines and the genetic changes discovered in this study could be used in the future to screen out cell preparations not appropriate for clinical use.

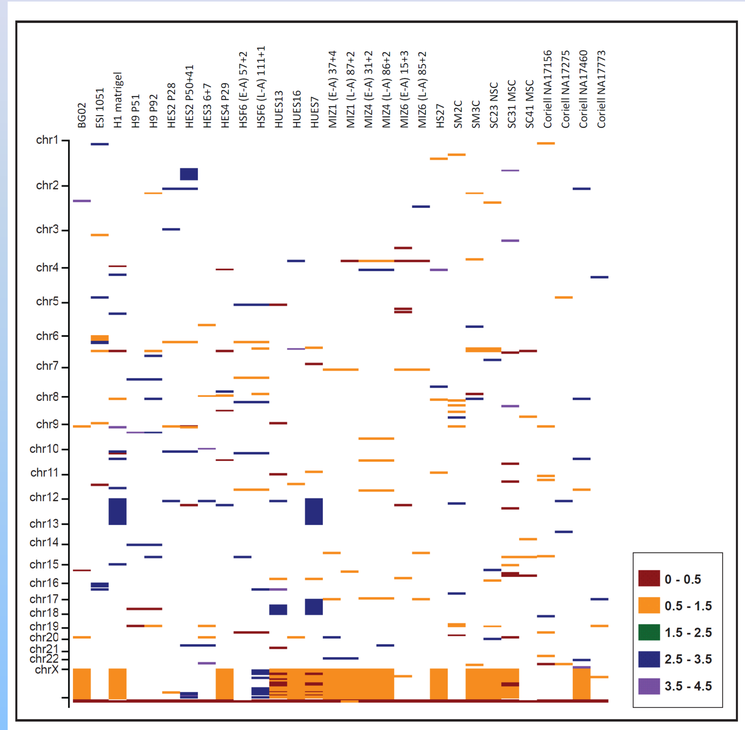


Figure 1. Autosomal Copy Number Variation.

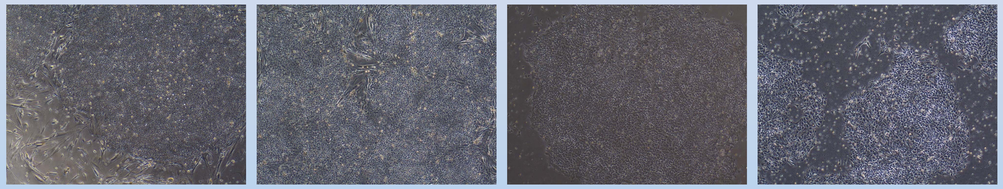
CNV regions were called using CNVPartition1.0.2. Sample names are listed at the top of the CNV region display, and chromosome numbers are shown at the left. The legend shows the color code used to indicate the number of copies in a given region of CNV.

Trisomic regions are visualized as large blocks of blue. Aneuploidies commonly seen in hESC lines duplications of portions of chromosomes 1, 12, 17, and X) are represented in this set of samples.

Sample Name	Sample Description
BG02	ESC BresaGen lines
ESI 051	ESC from ESI
H1 matrigel	ESC WA01 WiCell
H9 P51	ESC WA09 WiCell early passage
H9 P92	ESC WA09 WiCell late passage
HES-2 P28	ESC Melbourn Lines
HES-2 P50/41	ESC Melbourn Lines
HES-3 P6/7	ESC Melbourn Lines
HES-4 P29	ESC Melbourn Lines
HSF6 (E-A) 57+2	ESC UC06 from UCSF early passage
HSF6 (L-A) 111+1	ESC UC06 from UCSF late passage
Hues 13	ESC Harvard lines
Hues 16	ESC Harvard lines
Hues 7	ESC Harvard lines
MIZ1 (E-A) 37+4	ESC MizMedi Hospital (Korea) early passage
MIZ1 (L-A) 87+2	ESC MizMedi Hospital (Korea) late passage
MIZ4 (E-A) 31+2	ESC MizMedi Hospital (Korea) early passage
MIZ4 (L-A) 86+2	ESC MizMedi Hospital (Korea) late passage
MIZ6 (E-A) 15+3	ESC MizMedi Hospital (Korea) early passage
MIZ6 (L-A) 85+2	ESC MizMedi Hospital (Korea) late passage
HS27	human foreskin fibroblast
SM3C	fetal neural progenitor cells
SM2C	fetal neural progenitor cells
SC23 NPC	neonatal neural progenitor cells
SC31 MSC	bone marrow mesenchymal stem cells
SC41 MSC	bone marrow mesenchymal stem cells
NA17156	Coriell EBV-transformed B lymphocyte DNA
NA17275	Coriell EBV-transformed B lymphocyte DNA
NA17773	Coriell EBV-transformed B lymphocyte DNA
NA17460	Coriell EBV-transformed B lymphocyte DNA

Table 1. Description of all samples

Figure 2: WA09 hESC line cultured under four different conditions



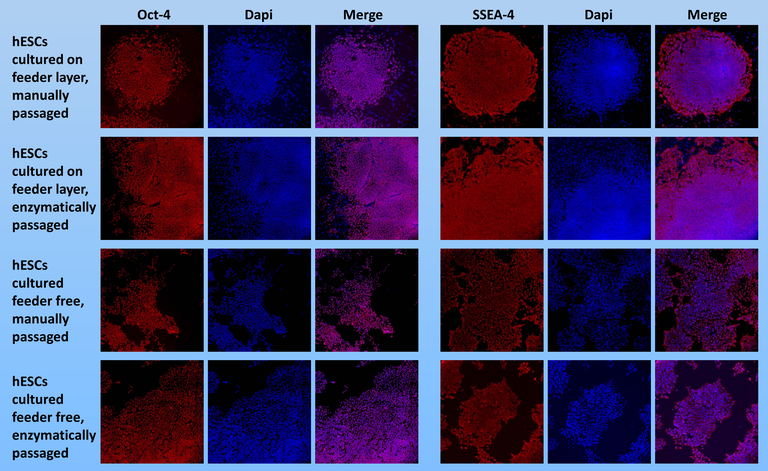
hESCs cultured on a feeder layer, manually passaged (p13)

hESCs cultured on a feeder layer, enzymatically passaged (p13)

hESCs cultured feeder free, manually passaged (p12)

hESCs cultured feeder free, enzymatically passaged (p19)

Figure 3: Immunocytochemistry of WA09 hESCs grown under four different conditions



Preliminary titration experiment on the xCELLigence System (Roche Applied Science) of the WA09 hESC line grown under four different conditions. Five different concentrations of cells 60000-3750 cells/well were grown on a 96-well format. The growth of the cells was monitored for over a week and the media of the cells was changed everyday. On one of the conditions (with feeders and manual passage) there was almost no growth observed. This could have been due to a low initial attachment of the stem cells to the feeder layer.

Figure 4: Growth curves of WA09 hESC lines determined with the xCELLigence System (Roche Applied Science)