Characterization of Three Early Passage Clinical Grade Human Embryonic Stem Cell Lines

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Introduction

Efficient and successful translation of pluripotent stem cell research into human therapeutics will require high quality, well characterized cell lines capable of supporting development from research through human clinical trials. Additionally, the ability to use cells not exposed to animal products during derivation and expansion offers a significant advantage by simplifying regulatory requirements. Accordingly, WiCell Research Institute has derived, banked, and fully characterized cell lines under xeno-free, feeder free conditions. They are valuable tools for furthering translational medicine goals.

Methods

Derivation and culture of cell lines:
Embryos used in this study were created for reproductive purposes prior to May 25, 2005 and donated to a UW-Madison, IRB approved protocol between September 2000 and January 2006. The embryos were thawed and cultured to blastocyst stage using G-Series™ medium (Vitrolife). The inner cell mass was isolated from trophectoderm using a laser (XYClone – Hamilton Thorn). Cells from the inner cell mass were cultured without albumin on a matrix of VTN-N variant of Vitronectin and Laminin-511 (BioLamina AB) in a modified version of E8 culture medium containing Poly Vinyl Alcohol (PVA: 0.1mg/mL) (Table 1). Initially, the cells were manually passaged using a stem cell cutting tool (Vitrolife), and later, passaged for expansion, banking and characterization studies using EDTA (Versene™). The cell lines produced were named WA25, WA26, WA27.

Characterization Studies:
The cell lines were expanded using the VTN-N variant of Vitronectin in the modified (+PVA) version of E8 culture medium. The cells were karyotyped by the WiCell Cytogenetics lab using standard protocols for hES cells, and STR profiles were done using Promega's PowerPlex 16 HS. Blood type data was obtained from genomic DNA by the New York Blood Center, and HLA status was determined from genomic DNA by the UW Molecular Diagnostics Lab. Cell counts taken using a ViCell™ were used to calculate population doubling times for each cell line. Marker expression was assessed by flow cytometry, and pluripotency confirmed by production of teratomas in SCID-Beige mice. Sterility testing (ST/07) was carried out by Apptec. Human virus testing including mycoplasma (Human Virus panel ID 91/0) was done by Charles River. SNP genotype analysis for copy number variation and LOH was done at WiCell Cytogenetics, using the Illumina Human CytoSNP-12v2.1 DNA Analysis BeadChip Array following Infinium HD Assay Ultra Protocol Guide. A diverse set of over 100 samples from HapMap populations was used for comparison. CGHfusion (infoQuant) and Genome Studio (Illumina) software was used to analyze the data.

Results

Full characterization reports including all results are available for review at the website www.wicell.org, as part of the Certificate of Analysis (COA) for each cell line. A summary of results is presented here.

WA25, WA26 and WA27 formed teratomas demonstrating all three germ layers upon injection into SCID-Beige mice (Figure 1), were greater than 90% positive for dual Oct4, SSEA4 expression when assessed via flow cytometry, and were confirmed sterile and mycoplasma free (data not shown). Further, each line exhibited appropriate doubling rates (Figure 2). Information on STR, ABO, and HLA has been gathered to further characterize these cell lines, and is available at www.wicell.org. Karyotype analyses found them to be normal (46,XX) at the effective g-band resolution of ~5Mb for greater than 40 passages. High-resolution SNP assays by Illumina HumanCytoSNP-12 v2.1 demonstrated that these xeno-free, feeder-independent cell lines (WA25 p7, WA26 p8, WA27 p8) carried few copy number changes, (none greater than 250kb) and no regions of loss/absence of heterozygosity (LOH) over 3.2Mb (WA26_chr19), 826kb (WA27_chr10), 3.2Mb (WA25_chr11), 2.1Mb (WA25_chr5) (Figures 3,4). Based on our analysis, all three cell lines were effectively characterized at high resolution and agreed with our karyotype analysis, indicating genomic stability. Importantly, these cell lines showed no significant copy number changes in regions of known import to pluripotent cell lines including the 20q amplicon noted by multiple studies, including Andrews *et al.* 2011 (ISCI study). This microarray data provides researchers with a higher level of confidence in the cytogenetic status of the cell lines than karyotype alone allows, and establishes a baseline characterization so future testing can more easily identify acquired changes.

Discussion

These results demonstrate that the chemically-defined, feeder-independent, xeno-free cell lines WA25, WA26 and WA27 established by WiCell are undifferentiated, self-renewing, pluripotent, genetically stable, and expand readily in culture. Initial characterization studies indicate that these lines perform similarly to lines derived using albumin and/or feeders. The lines were all produced from embryos created before May 25, 2005, making them exempt from FDA 21 CFR Part 1271 requirements for donor eligibility. Complete traceability on all raw materials used in derivation and production was maintained. Taken together, this data and documentation makes WA25, WA26 and WA27 excellent candidates for use in human clinical applications. They have been approved by the NIH for use with federal funding, and low passage material is currently available from WiCell for these lines and others established using these methods.

Composition of E8 + PVA *(Contains no Albumin)*
DMEM/F12
Sodium Bicarbonate
L-Ascorbic Acid
Sodium Selenium
Human Holo-Transferrin
rh-Insulin
rh-TGF-β1
rh-FGF-2
Poly Vinyl Alcohol (PVA)(0.1mg/ml)

Table 1: Composition of modified E8 medium used for cell line derivation (*Chen, 2011*).

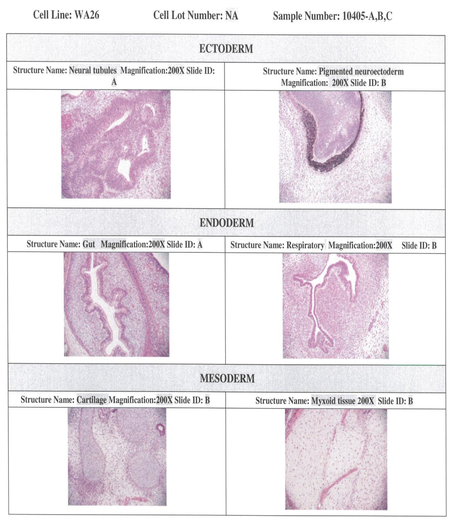

Figure 1: Example of teratoma studies showing the ability of WA26 to differentiate into cell types characteristic of the three embryonic germ layers.

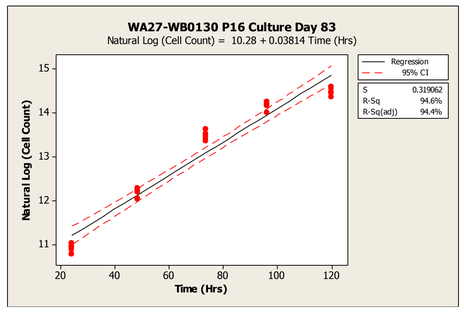

Figure 2: Example of population doubling time study, performed by WiCell, showing growth potential of WA27.

Regression Analysis:

Natural Log (Cell Count) versus Time (Hrs)

The regression equation is Natural Log (Cell Count) = 10.3 + 0.0381 Time (Hrs)

Predictor	*Coef*	*SE Coef*	*T*	*P*
Constant	10.2842	0.1371	75.00	0.000
Time (Hrs)	0.038136	0.001715	22.24	0.000

S = 0.319062 **R-Sq** = 94.6% **R-Sq(adj)** = 94.4%

Analysis of Variance

Source	*DF*	*SS*	*MS*	*F*	*P*
Regression	1	50.331	50.331	494.41	0.000
Residual Error	28	2.850	0.102		
Total	29	53.181			

Slope ± 95% C.I
0.0381 ± 0.0035
Apparent Doubling Time (hours) ± 95% C.I.
18.18 ± 2.05
Apparent Doubling Time (95% C.I.)
16.64 hours – 20.02 hours

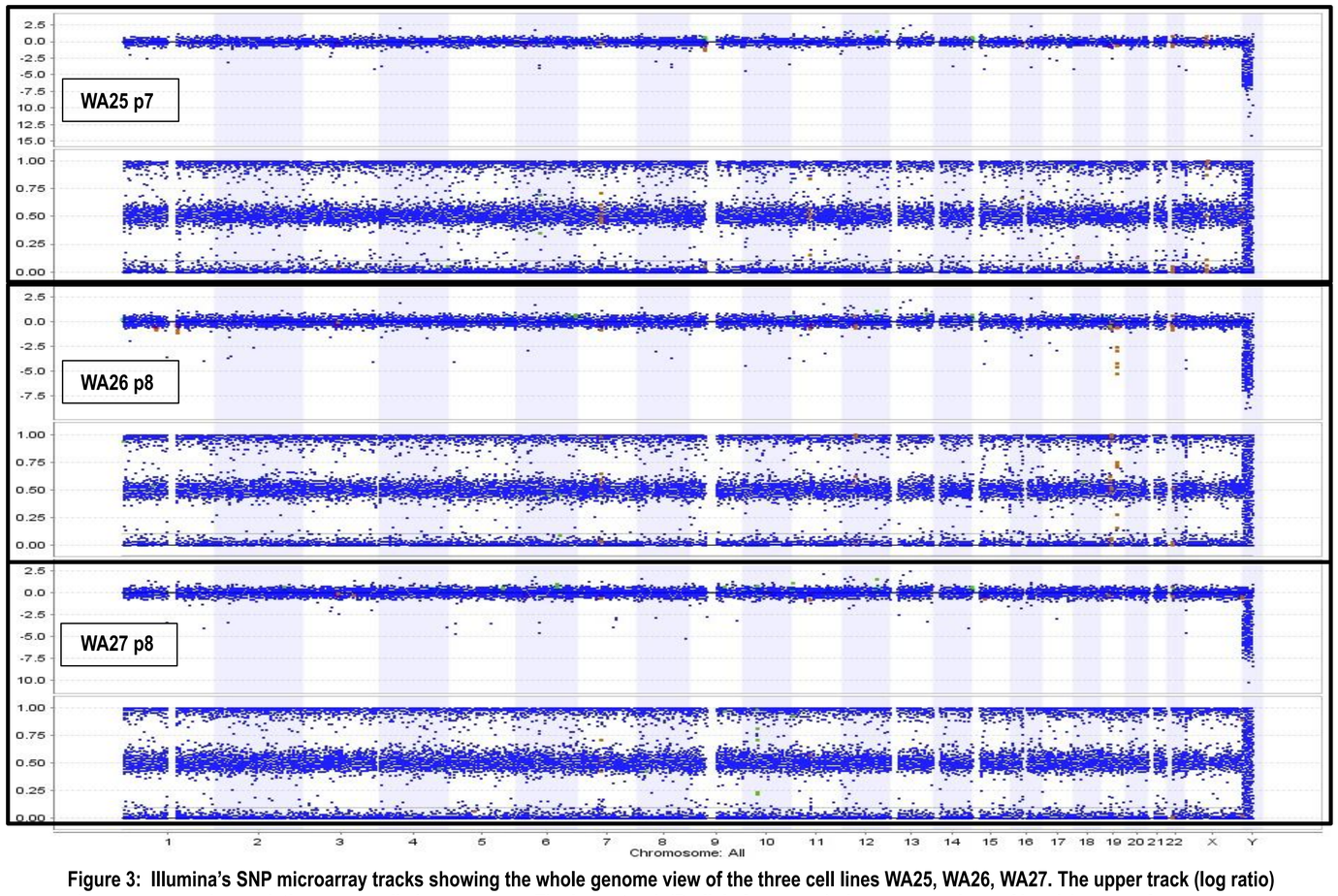

Figure 3: Illumina's SNP microarray tracks showing the whole genome view of the three cell lines WA25, WA26, WA27. The upper track (log ratio) shows copy number variation across all chromosomes. The lower track (B-allele frequency) shows any regions of loss/absence of heterozygosity.

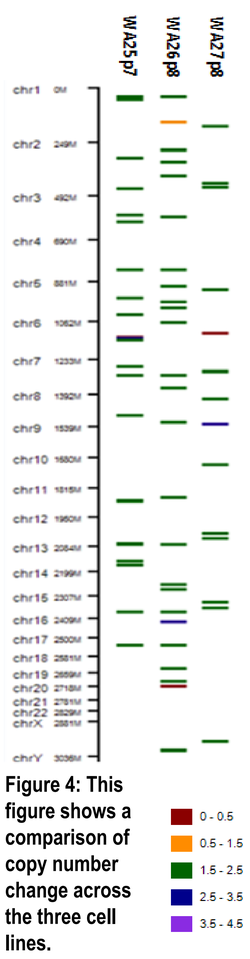

Figure 4: This figure shows a comparison of copy number change across the three cell lines.

References

1. Chen G, Gulbranson GR et al. Chemically defined conditions for human iPSC derivation and culture. Nature Methods 8(5): 424-429, 2011.
2. Andrews PW et al. (International Stem Cell Banking Initiative contributors): Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. Nature Biotechnology 29:1132-1146, 2011.

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