A Retrospective Analysis of Cytogenetic Changes Seen in Human Pluripotent Stem Cell Cultures

Kimberly Leonhard[1], Erik McIntire[2], Seth Taapken[1], Zachary B. Abrams[3], Anna Lisa Larson[1], Erica Schutter[1], Kevin R. Coombes[3], Lynne V. Abruzzo[3], Kate Bird[1], Dawn Davis[1], Leah George[1], Timm Gonzales[1], Amber Kuhn[1], Robin McKnight[1], Pam Mill[1], Jennifer Pecos[1], Hannah Rueth[1], and Katrina Turner[1]

[1]WiCell, Madison, WI, USA, [2] University of Chicago, Chicago, IL, USA, [3] The Ohio State University, Columbus OH, USA

Introduction

Over a 10-year span (Jan 2009 - Jan 2019), we performed routine G-banded karyotype analysis on 2,665 human embryonic stem cell (hESC) cultures and 12,479 human induced pluripotent stem cell (hiPSC) cultures. By applying the CytoGPS software platform[1], we have identified new recurrent aberrations and their minimal overlapping regions in stem cell cultures. We have also explored the similarities and differences in the types of aberrations acquired by hESC and hiPSC in vitro and their rates of acquisition.

Materials and Methods

G-banded karyotyping was carried out using standard cytogenetic protocols modified for use with hPSC. G-banded karyotyping preparations were digitally captured and analyzed with Applied Spectral Imaging equipment. There were 15,144 stem cell karyotypes used in this study and these cases were gathered from between 1/5/09 and 1/3/19.

We used the CytoGPS software platform (www.cytogps.org) to process the karyotypes. CytoGPS automatically transforms ISCN karyotypes into a binary model, where each cytoband is represented by three bits of data recording a Loss, Gain, or Fusion at that location. The resulting binary vectors are used for large-scale computational analyses.

Results

- The overall acquisition rate of cytogenetic abnormalities was similar for hESCs (23%) and hiPSCs (21%)
- hESCs show a smaller range of aberration types but a higher rate of these abnormalities than hiPSCs
  - Whole or partial gains of 1, 8, and 17, and whole or partial losses of 7, 18, and 20 are more common in hESCs than hiPSCs (Figures 1 and 2)
- hiPSCs show a wider range of abnormalities but each type at a lower rate than hESCs
  - Gains of Y are more common in hiPSCs than hESCs (Figures 1 and 2)
- We have confirmed previously-known recurrent abnormalities[3]:
  - Whole and partial gains of X, 1, 8, 12, 17, 20, and partial losses 10 and 18[2,5,6] (Figure 3)
  - The minimal overlapping region has been narrowed to one cytoband for 1q32.1, 12p13.3, 17q25, 18q21, and 20q11.21[4]
- We have identified new recurrent abnormalities:
  - isochromosome 7p, gain of 14, gain of Y (Figure 3)
- We found no significant relationship between presence of cytogenetic events and passage number
- We saw no significant correlations for any individual abnormalities and passage number

Conclusions

The similar rate of acquisition of cytogenetic abnormalities between hESC and hiPSC suggests similar overall genetic stability in vitro. However, there was a difference in the rates and types of aberrations acquired. These differences may reflect the smaller number of hESC lines available for study and therefore less genetic diversity, and the persistence of an aberration in the original parental line through reprogramming of an hiPSC line.

The lack of correlation between passage number and presence of cytogenetic events may be explained by the removal of abnormal lines from use after an abnormality is detected. This routine cleaning of the population may result in a constant rate of new cytogenetic abnormalities occurring over time.

This data was developed from routine testing rather than through controlled experimentation and ascertainment bias is likely present. However, given both the quantity of samples and duration of data collection, these results provide valuable insight into genetic changes in hPSCs.

References

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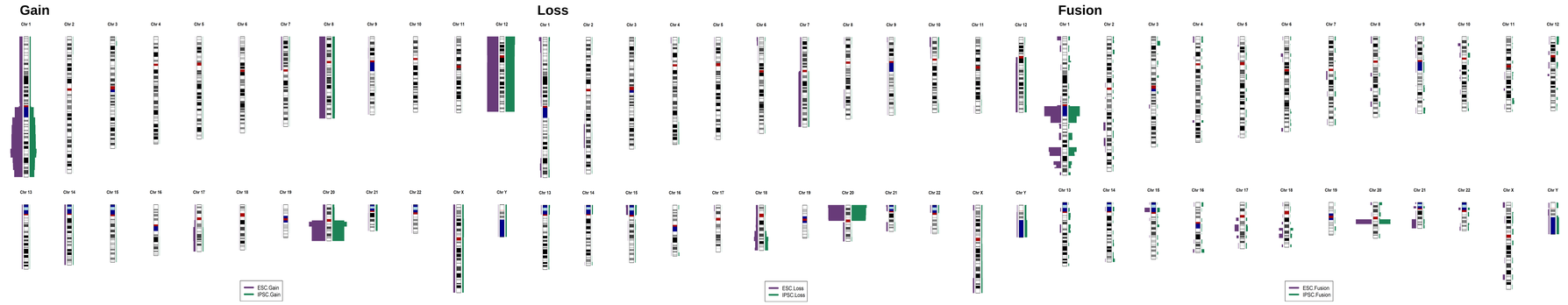
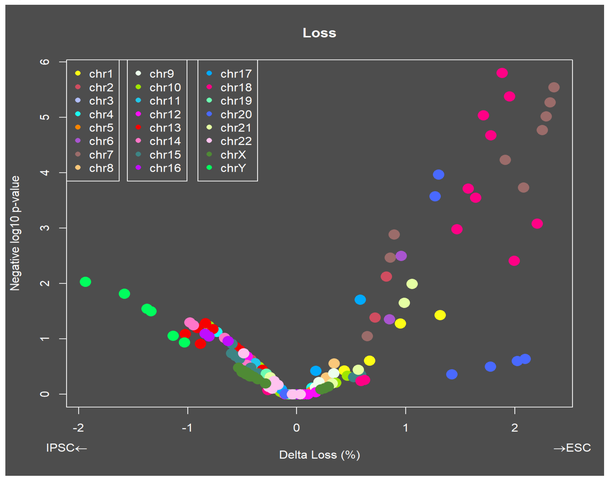


a)



b)

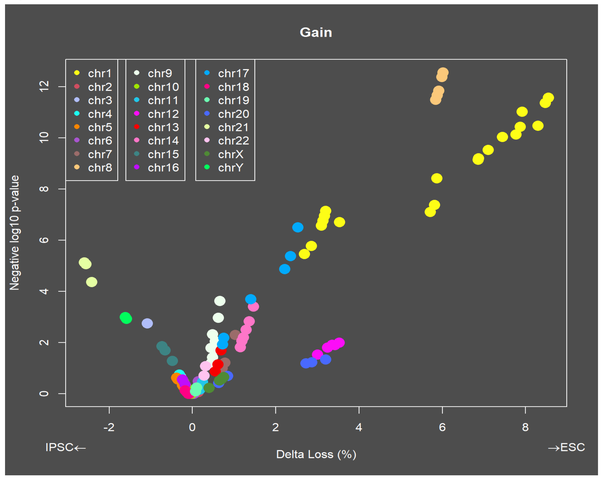


Figure 1 (top): Genome-wide plots of hESC and hiPSC events by chromosome. Each figure is plotted on the same scale so that the relative heights within each figure are uniform and comparable.
Gain events in hESCs (purple) and hiPSCs(green)
Loss events in hESCs (purple) and hiPSCs(green)
Fusion events in hESCs (purple) and hiPSCs (green)

Figure 2 (middle): The volcano plot is a scatterplot that shows statistical significance (p value) versus magnitude of change. This volcano plot enables quick visual identification of chromosomes with large differences in the rate of aberrations between cell types that are also statistically significant. In the volcano plots, the chromosomes with higher abnormality rates in hESC vs hiPSCs are towards the right, the chromosomes with higher abnormality rates in hiPSCs vs hESCs are towards the left. The most statistically significant chromosomes are towards the top.

a) Whole or partial gains of chromosomes 1, 8, and 17 are more common in hESC and gains of Y are more common in hiPSC.
b) Whole of partial loss of chromosomes 7, 18, and 20 are more common in hESC.

Figure 3 (bottom): Genome-wide plots of combined stem cell events by chromosome.
Gain events with cell types merged.
Loss events with cell types merged.
Fusion events with cell types merged.