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Genetic instability in human induced pluripotent stem cells

Classification of causes and possible safeguards

Uri Ben-David, Nissim Benvenisty* and Yoav Mayshar

Department of Genetics; Silberman Institute of Life Sciences; The Hebrew University; Jerusalem, Israel

In recent years much effort has been put to refine methods for derivation of human induced pluripotent stem cells (HiPSCs).[1-3] However, the issue of their genomic stability has been virtually sidelined. Recently, we have performed comprehensive analysis of genomic instability in 66 HiPSC lines, most of which have been previously published by other groups.[4] In order to assess aneuploidy we analyzed the gene expression profiles of these cell lines. Using a sufficient number of internal controls, we showed that aneuploidy could be inferred in regions that had undergone significant upregulation of genes within contiguous genomic regions. This analysis was performed in parallel to analysis of 38 human embryonic stem cell (HESC) lines. In this way we further validated the method and could also examine possible differences in selection for aneuploidy between these two highly similar pluripotent stem cell types. Our results showed that HiPSCs, like HESCs, may acquire genetic aberrations during culture and that some abnormalities provide such a strong selective advantage that they take over the culture rapidly, within several passages. However, a number of dissimilarities were also identified, pointing to the unique biology of HiPSCs due to their distinctive derivation process.

All together, chromosomal aberrations were detected in ~31% of all HESC lines (12 of 38) and in ~20% of all HiPSC lines (13 of 66). Although aneuploidy would thus seem to be less frequent in HiPSCs than in HESCs, taking into account the passage number at which the gene expression analyses were performed reverses this interpretation. Thus, although it is relatively rare to observe low passage HESC cultures that had acquired chromosomal aberrations, several such instances have been identified in low passage HiPSCs. It would thus seem that selective pressure during the reprogramming process could be a major contributor specifically to HiPSC aneuploidy. An additional source of aberrations unique to HiPSCs originates from possible aneuploidy present in the somatic cells from which they were derived. Lastly, 'traditional' aberrations arising due to culture adaptation were found to occur at surprisingly high frequency, as three of the four independent HiPSC lines that were analyzed at multiple passages acquired genetic aberrations. Interestingly, in all of these cases duplication of chromosome 12 was observed. This is especially significant because trisomy of chromosome 12 was previously demonstrated to be one of the most common aberrations occurring during HESC culture adaptation, as well as in germ cell tumors.[5,6] We further identified a minimal region that was duplicated in all samples. Among the genes residing within this region, the hallmark pluripotency genes *NANOG* and *GDF3* were found to be significantly overexpressed. This would suggest that overexpression of these genes may confer a major growth advantage to HiPSCs, possibly explaining the rapid rate in which cells with chromosome 12 trisomy took over a karyotypically normal culture. Moreover, functional analysis of regions found to be recurrently aberrant in HiPSCs revealed significant enrichment of cell cycle genes, which may be another source of selective advantage for these cells, in line with studies reporting that cell divisions are a limiting factor of reprogramming.[7]

The relatively high incidence in which aneuploidy was identified in HiPSC cultures raises an important caveat in the potential use of these cells. Aberrant lines may confound biological interpretation of studies preformed with them, particularly in the study of the reprogramming process itself, but also by affecting other aspects of their biology such as differentiation potential and tumorigenesis.[8] Thus, the unique origins of chromosomal aberrations in HiPSCs relative to HESCs raise new considerations in working with these cells. The first source of aberrations is those originating from the parental somatic cells. Two main factors may contribute to the establishment of such abnormalities. First, the tissue of origin itself may prove to be a significant factor as both the age of the tissue, and the tissue type, may dictate the frequency of abnormal cells in the parental somatic cell pool. Thus, such aberrations will probably be less frequent in HiPSC lines from newborn foreskin fibroblasts than from adult skin cells. Furthermore, the efficiency of the reprogramming process may play a vital role in reducing the incidence of somatic cell derived abnormalities. Because abnormal cells should be rare in a normal donor, highly efficient reprogramming will generate many clones, only a few of which would presumably be abnormal. Conversely,

*Correspondence to: Nissim Benvenisty; Email: nissimb@cc.huji.ac.il
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Source of aberration	Risk factors	Prevention methods
 Somatic cell of origin	Type and age of somatic tissue: cell of origin may harbor genetic abnormalities	Generation of HiPSCs from embryonic tissues
	Minimal number of cell divisions required, leading to strong selection for proliferating cells within a generally slow replicating culture	Use of normally proliferating cells as the parental somatic cells
 Early passage HiPSC	Low efficiency of somatic cell reprogramming increases selective pressure in culture	Use of high efficiency reprogramming methods
	Stress induced by the reprogramming process, such as: a. ectopic expression of oncogenes b. viral integrations c. drastic non-concerted gene expression changes	Integration-, viral- & oncogene-free reprogramming methods
 High passage HiPSC	Low replating efficiencies, mostly in single cell passaging	Improving replating efficiency by apoptosis inhibitors
	Culture adaptation: selective advantage to cells that proliferate faster or are more resistant to apoptosis	More gentle passaging techniques Use of cells at an early passage
	Growth of large cultures increases the chance for spontaneous aberrations	Growth of smaller cultures for routine use

inefficient reprogramming may lead to selection of those few aberrant clones. The second source of chromosomal abnormalities in HiPSCs lies in the essence of the reprogramming process itself, i.e., in the intense selective pressure involved and the drastic gene expression changes and epigenetic modifications required of the cells. It remains to be seen, however, how certain factors in the method of reprogramming, such as the method of ectopic gene expression, the identity of the genes used in reprogramming, and the possible use of small molecules will affect chromosomal instability at this stage. Lastly, the third source of chromosomal aberrations is adaptation to culture, in a similar manner to HESCs, providing the cells with selective advantage to in vitro growth or survival during routine culture. The main factors that have been previously proposed to affect the emergence of such aberrations are time in culture (i.e., number of passages), and culture techniques.[9] Thus, passaging techniques that subject the cells to less selective pressure, such as manual passaging versus single cell dissociation and growing smaller size cultures, may reduce the rate of adaptation.[10] Similarly, use of apoptosis inhibitors, such as the ROCK inhibitor that greatly improves cloning efficiency,[11] may act in a similar fashion. For schematic representation of the various causes and possible safeguards regarding HiPSC genomic instability see **Table 1**.

Finally, despite the abovementioned methods that could serve to reduce chromosomal instability in HiPSC lines, there is no alternative to directly test individual cultures for chromosomal aberrations. As aneuploidy may arise within a few passages in culture, clones should be analyzed as close as possible to their actual use in experiments, and re-evaluated upon continued culture. This is important as aneuploidy may affect the differentiation capacity and increase tumorigenicity of HiPSCs.

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