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Tottori University

Integration-Free iPS Cells Reprogrammed Using Human Artificial Chromosome.

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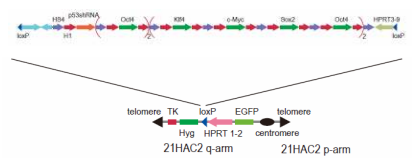
Summary

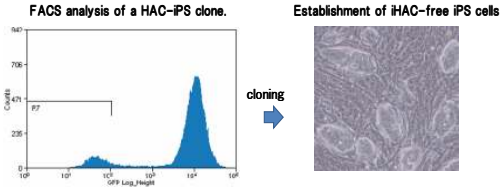
Human artificial chromosomes (HACs) have unique characteristics as gene-delivery vectors, including episomal transmission and transfer of multiple transgenes. Here we have demonstrated the advantages of HAC vectors for reprogramming mouse embryonic fibroblasts (MEFs) into induced pluripotent stem (iPS) cells. A HAC vector carried four reprogramming factors and a p53-knockdown cassette (iHAC). The iHAC efficiently reprogrammed MEFs. Under non-selecting conditions, we established iHAC-free iPS cells by isolating cells that spontaneously lost the iHAC. Analyses of pluripotent markers, teratomas and chimeras confirmed that these iHAC-free iPS cells were pluripotent. Thus, the HAC vector could generate integration-free iPS cells. Global gene expression patterns showed that the iHAC generated relatively uniform iPS cells.

Fig1. The map of an iHAC vector in CHO cells.



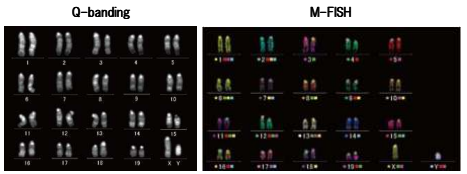
Construction of a HAC vector for inducing pluripotent stem cells.
We inserted reprogramming factors(2 copies of Klf4, c-Myc and Sox2, and also 4copies of Oct4). Furthermore, a siP53shRNA expression cassette was loaded into the HAC vector.

Fig3. Isolation of HAC-free iPS cells by FACS.



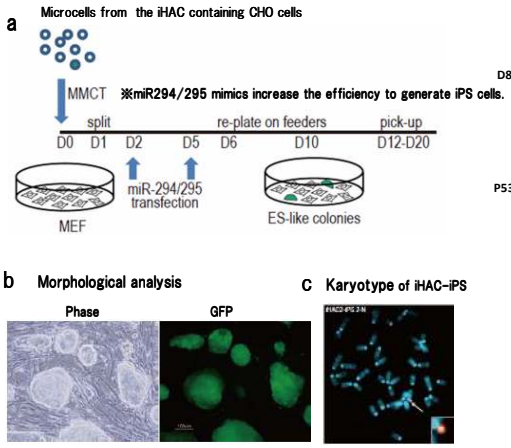
iHAC-iPS cells express GFP-fluorescence. Therefore, the iHAC-free iPS cells could be obtained from GFP-negative fraction. These morphologies of cellls were similar to ES cells.

Fig5. Karyotype analysis of iHAC-free iPS cells.



Q-banding and multi-color FISH analyses showed normal mouse karyotype. These cells were not observed the HAC vectors, indicating that then, we could generate iHAC-free iPS cells.

Fig 2. Generation of iPS cells by transferring the iHAC to MEFs.



(a) The iHAC was transferred by microcell-mediated chromosome transfer(MMCT) to MEFs. After MMCT, MEFs was seeded on feeder cells. iHAC-transferred MEFs were efficiently reprogrammed into iPS cells (23/33 = ES-like GFP(+) colonies / Total GFP(+) colonies.) Generated HAC-iPS cells expressed exogenous and endogenous reprogramming factors. (b) Morphology of the iPS clones was similar to ES cells. (c) FISH analysis of iHAC-iPS cells. Blue; DAPI, Red; hCOT-1, Green; Transgenes

Fig4. Analysis of HAC-free iPS cells.

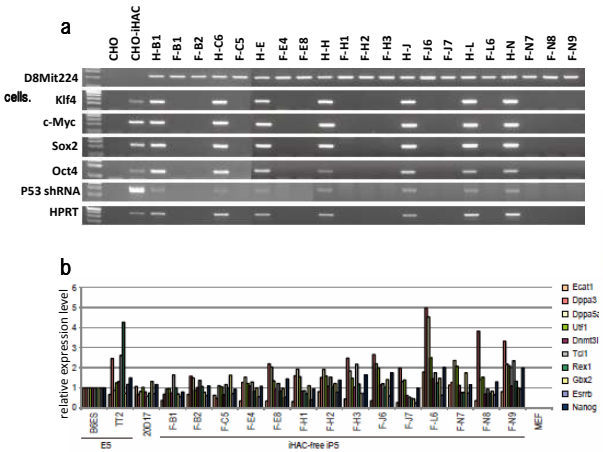
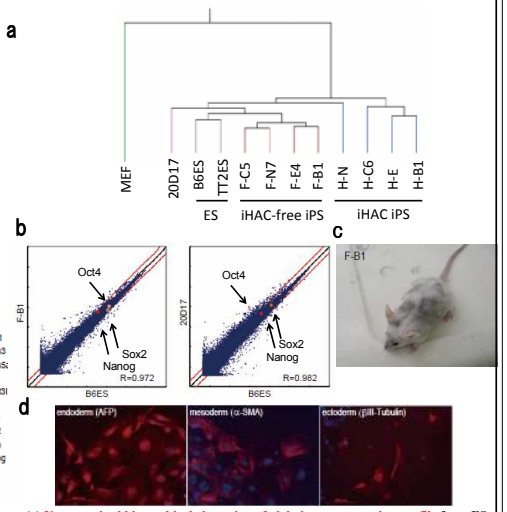


Fig4. Analyses of Pluripotency of iHAC-free iPS cells by molecular markers.
(a)Genomic PCR of individual transgenes in cells from a EGFP-negative fraction of iHAC-iPS cells was compared with that of the parental clones. D8Mit224 was used as an internal control of mouse genome. All transgene genome could not be detected. (b) Quantitative RT-PCR of pluripotency markers. Researched 12 pluripotent markers expressed at similar levels to mouse ES cells. Klf4, c-Myc, Sox2 and Oct4 also was detected.

Fig6. Global analyses by microarray and differentiation.



(a) Unsupervised hierarchical clustering of global gene expression profile from ES cells, iPS cells generated by retroviral or iHAC, and MEFs. (b) Scatter plots of microarray data comparing ES cell line B6ES to iHAC-free iPS and retroviral iPS 20D17. Highlighted marks indicate core pluripotent markers, Nanog, Oct4, and Sox2. Red lines indicate twofold changes in expression levels between samples. (c) Live-born chimeras from iHAC-free iPS clones. (d) in vitro differentiation mediated by EB formation. Spontaneous germ-layer differentiation of EBs was assayed using markers for endoderm (AFP), mesoderm (α- SMA), and ectoderm(βIII-tubulin).