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Investigation of regeneration of damaged CNS and neurological diseases mechanisms using iPS cells

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Induced pluripotent stem (iPS) cells are pluripotent stem cells directly reprogrammed from cultured mouse fibroblast by introducing *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4* (Takahashi and Yamanaka, 2006). Cells obtained using this technology, which allows the ethical issues and immunological rejection associated with embryonic stem (ES) cells to be avoided, might be a clinically useful source for cell replacement therapies. In our recent paper (Miura et al., 2009; Tsuji et al., 2010), we demonstrated that murine iPS cells formed neurospheres that produced electrophysiologically functional neurons, astrocytes, and oligodendrocytes. On the other hand, secondary neurospheres (SNSs) generated from various mouse iPS cell lines showed distinct teratoma forming propensities after transplantation into the brain of immunodeficient NOD/SCID mice, depending the content of persistent presence of *Nanog*-GFP-positive undifferentiated cells within SNS. These *Nanog*-GFP-positive are likely to be differentiation-resistant cells, which could act as a tumor-initiating cells. In order to obtain safeness of iPS cells-derived NS/PCs, we examined various factors who could affect the tumorigenic ability of iPS cells-derived NS/PCs, including the origin of iPS cells, usage of c-Myc transgene or usage of genetic selection using drug resistant gene. In this experiment, we transplanted SNS cells (0.5×10^6 cells) derived from totally 36 mouse iPS cell lines, which differ in terms of i) origin (cellular source) of the iPS cells, ii) presence or absence of c-Myc transgene upon production of iPS cells, and iii) presence or absence of *Nanog*-puromycine resistance genetic selection upon production of iPS cells. First, we noticed that persistent presence of pluripotent stem cell marker-positive undifferentiated cells within SNSs results in tumorigenesis (teratoma-formation). We also found that the origin of the iPS cells is the crucial factor for predicting the safety issue and that the content of undifferentiated cells (i.e. differentiation-resistant cells) within NS/PCs highly correlated with their teratoma-forming propensity (Miura et al., 2009). On the other hand, teratoma-forming propensity of mouse ES-derived neural stem/progenitor cells was very low in the same condition, indicating the substantial difference between ES and iPS cells. Notably, it is proposed that genes differentially expressed between iPS and ES cells are referred as "reprogramming-recalcitrant" genes, because they resist the induction of transcriptional state identical to that seen in ES cells (Sakurada 2010). The fact that mouse iPS-derived NS/PCs showed substantially varied teratoma-forming propensities depending the iPS cells' tissue of origin (Miura et al., 2009) might indicate insufficient suppression of somatic cell-specific genes could result in the emergence of "reprogramming-recalcitrant" genes associated with tumorigenic propensities.

Furthermore, our recent results suggest the potential application of mouse and human iPS-derived neural stem/progenitor cells for the regeneration of damaged CNS including spinal cord injury (SCI) (Tsuji et al., 2010). We found that SNSs from iPS cells have therapeutic potential upon transplantation into injured spinal cord, by increasing number of 5 HT-positive fibers and myelinated fibers, and by some trophic effects. Intriguingly, we conclude that pre-evaluated safe iPS clone-derived neural stem/progenitor cells may be a promising cell source for transplantation therapy for SCI.

References

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