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Does Retroviral Insertional Mutagenesis Play a Role in the Generation of Induced Pluripotent Stem Cells?

Robert G. Hawley[1]

1*Department of Anatomy and Regenerative Biology, The George Washington University Medical Center, 2300 I Street NW, Washington, DC 20037, USA.*

In their recent report, Aoi *et al.*[1] described the establishment of induced pluripotent stem (iPS) cells from adult mouse hepatocytes and gastric epithelial cells (designated iPS-Hep and iPS-Stm cells, respectively) by retroviral transduction of cDNAs encoding the Oct 3/4, Sox2, Klf4 and c-Myc transcription factors. The authors noted that no common retroviral integration sites (CISs) were detected among four iPS cells analyzed and therefore concluded that retroviral integration into specific sites was not required for iPS cell generation.

Examination of their data (Figures S7-S11 in Aoi *et al.*[1]) indicates that the iPS cell clones contain integrations into CISs previously identified by retroviral-mediated tumor induction in mice, as listed in the Mouse Retrovirus Tagged Cancer Gene Database (RTCGD) (summarized in Supplementary Table S1).[2] This is particularly noteworthy in the cases of iPS-Stm cell clones 99-1 and 99-3. In the iPS-Stm cell clone 99-1, the c-Myc retroviral vector was found to have integrated downstream of the *Zfp36l2* gene (originally designated the Evi134 CIS) associated with oncogenesis in a number of tumor models, and required for the progression of mouse embryos beyond the two-cell stage of development.[3] Among the retroviral integration sites in the iPS-Stm cell clone 99-3, the c-Myc retroviral vector was demonstrated to have integrated into the Sgms1 locus, a CIS implicated in several B lymphoid and myeloid tumor models, whereas the Klf4 retroviral vector was shown to have integrated downstream of the *Snai1* gene (originally designated the Evi154 CIS) that encodes a zinc finger transcriptional repressor associated with B and T cell lymphomagenesis, and which plays a critical role in the establishment of left-right asymmetry during early embryonic development.[4]

It is of interest that the iPS-Hep and iPS-Stm cells were observed to have fewer vector integrations on average than iPS cells induced from mouse fibroblasts (Figure 3 in Aoi *et al.*[1]). Two other recent publications have reported the reprogramming of mouse fibroblasts using a strategy in which the same four transcription factor genes were expressed from doxycycline-inducible self-inactivating (SIN) lentiviral vectors.[5,6] Because lentiviral vectors tend to preferentially integrate into transcriptional units,[7] in contrast to the gene-activating tendency exhibited by the gammaretroviral vectors used by Aoi *et al.*[1], they have generally been assumed to be more likely to inactivate endogenous gene expression upon integration. However, another recent study showed that an uninsulated SIN lentiviral vector with an internal promoter caused significant insertional dysregulation of genes within 600-kb regions surrounding the vector integration sites, with instances of both increases as well as decreases in gene expression documented.[8] No integration site analyses were performed in either of the fibroblast reprogramming reports that used the SIN lentiviral systems,[5,6] but previous investigations involving various cell types have revealed that lentiviral gene delivery frequently results in high copy numbers of vector integrations per transduced target cell.[9,10] Of relevance in this

Phone: 202-994-3511; Fax: 202-994-8885; E-mail: rghawley@gwu.edu

context, we[11] and others[12] have recently described growth stimulatory effects on mouse hematopoietic stem/progenitor cells associated with integration of SIN lentiviral vector backbones, albeit at lower frequencies than conventional LTR-driven gammaretroviral vectors.

For iPS cell reprogramming to become clinically relevant, approaches that can replace high-copy retroviral transduction are clearly desirable.[13] In the interim, in view of the intriguing coincidences between the vector integration sites elaborated in the iPS-Stm cell clones characterized by Aoi *et al.*[1] and the above-mentioned RTCGD CIS genes, and until detailed analyses of other iPS cells are carried out, it would seem premature to rule out a contribution of retroviral insertional mutagenesis to iPS cell generation. Indeed, to rule out a contribution of vector insertion events to the generation or biology of iPS cells, one would first have to demonstrate that all integration sites had been identified. A number of PCR-based protocols have been developed for integration site recovery, but a limitation of all widely used methods is a bias introduced by the choice of restriction enzyme for genomic DNA cleavage which may result in a failure to recover a significant proportion of insertions.[14] On the other hand, taking lessons from the gene therapy field, it is not unreasonable to imagine that comprehensive elaboration of retroviral integration sites in iPS cells (notwithstanding the caveat regarding restriction enzyme bias) may provide new insights into the mechanisms of somatic cell reprogramming.[15]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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