News Release

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Kyoto University

Virus-free iPS cells become a reality

October 10, 2008 – Since the initial report of induced pluripotent stem (iPS) cells in 2006, much attention has been focused on their potential applications in drug discovery and regenerative medicine. But the use of retroviral vectors to convey the four genes used in the induction process has remained a major stumbling block to future clinical uses. Of the four genes (*Oct3/4*, *Sox2*, *Klf4*, and *c-Myc*) used in the original combination first described by Kazutoshi Takahashi and Shinya Yamanaka in Kyoto University (now at the Center for iPS Cell Research and Application (CiRA), Institute for Integrated Cell-Materials Sciences, Kyoto University), *c-Myc* is a known oncogene, meaning it carries a risk of tumorigenesis if its retrovirus were to be reactivated in vivo. Experience from the retroviral transgenesis in the early days of gene therapy also indicates that retroviral integration alone may activate or inactivate other genes in the recipient genome, making it an unacceptable risk for use in human patients.

In the short time since that first report of induced pluripotency by the Yamanaka lab, significant progress has been made in the development of alternative methods for generating these versatile cells. But a means of introducing the necessary genes without integration into host chromosomes has remained elusive.

Now, in an article published in *Science*, Keisuke Okita and colleagues in CiRA report for the first time a virus-free method for inducing pluripotency in differentiated cells. By using a pair of plasmid vectors (Fig.1) to introduce the four iPS genes into embryonic mouse fibroblasts, Yamanaka's group found they could obtain colonies of iPS cells showing essentially the same morphological, genetic, proteomic and epigenetic hallmarks of pluripotency as do conventional iPS cells and embryonic stem (ES) cells. The iPS cells also produced various tissues (Fig.2) and chimera mice.

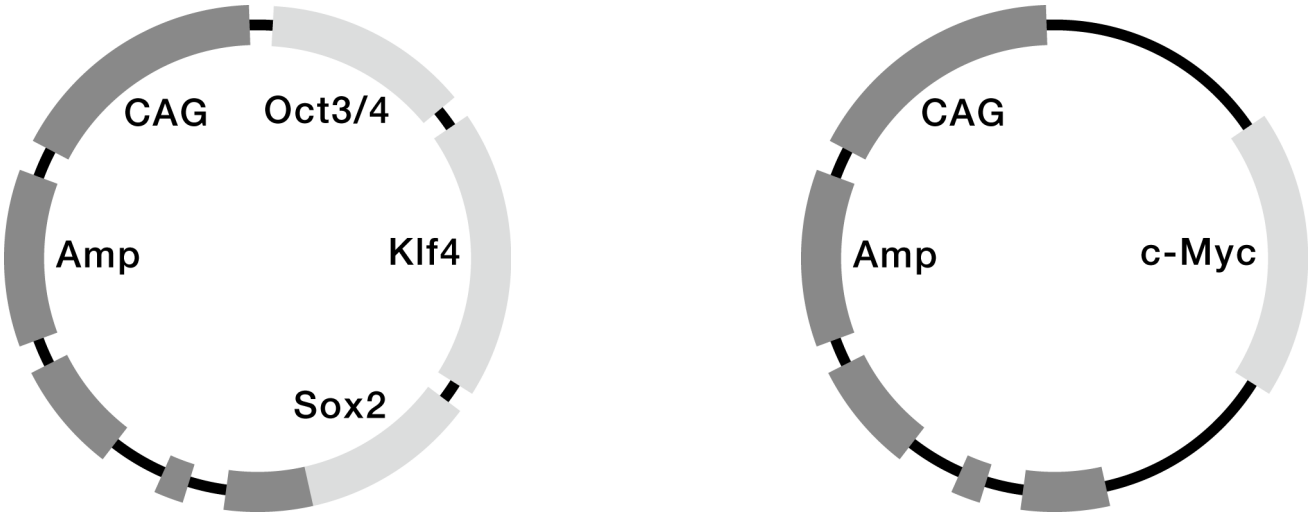

Fig.1: Schematic diagram of 2A plasmid construct.

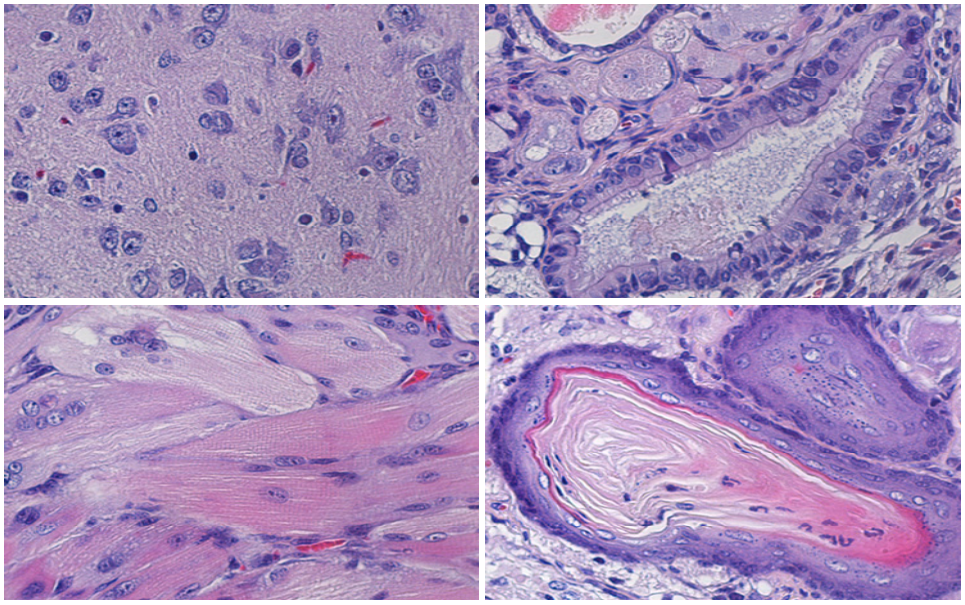

Fig.2: Virus-free iPS Cells differentiate into various tissues, such as neurological tissue (Upper left), striated muscle (Lower left), intestinal epithelial tissue (Upper right) and epidermal tissue (Lower right).

Motivated by the desire to identify a workaround for inducing pluripotency without relying on the controversial retroviral vectors, Okita et al. first looked to a different viral delivery system, using adenoviruses, which unlike retroviruses, do not persist in infected cells. Testing different combinations of the four iPS genes in adeno- and retroviral vectors, they found that they could generate ES-like cells when either Sox2 or Klf4 was piggybacked onto an adenovirus, but, importantly, that the critical pluripotency gene *Oct3/4* was only effective when introduced via retrovirus.

Thinking the problem might be one of insufficient transgene copy numbers, they tried loading the essential factors known to be necessary and sufficient for the iPS transformation (*Oct3/4*, *Sox2*, *Klf4*) in different orders onto a single retroviral vector to test for the optimal linear sequence, and found that the combination *Oct3/4-Klf4-Sox2* yielded the best results.

Armed with this knowledge, they created plasmid vectors (a means of introducing genes into cells without integration into the host genome) carrying the three key genes in this optimal order, as well as a second plasmid bearing *c-Myc*. After looking exhaustively at various possible transfection protocols, they found that transfection of the two plasmid vectors concomitantly on days 1, 3, 5 and 7 of the 12-day induction period allowed them to isolate multiple colonies of cells with all the characteristics of iPS cells. All except one, that is; in 9 of 11 of the plasmid-induced colonies, no foreign DNA was detected.

This achievement of virus-free iPS cells marks an important step closer to clinically compatible, patient-specific pluripotent cells, but a number of issues still must be addressed. The efficiency of the new method is significantly lower than that seen in the use of retroviruses to introduce the iPS factors. And it has yet to be shown whether the plasmid approach will work in human adult somatic cells, as the mouse embryonic fibroblasts used in this study may be more amenable to reprogramming. This latest report from the Yamanaka group nonetheless represents an exciting advance for the field and source of encouragement for those who look to future applications of iPS cells in drug discovery and regenerative medicine.

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