Induced Pluripotency with Endogenous and Inducible Genes

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Abstract

The recent discovery that two partly overlapping sets of four genes induce nuclear reprogramming of mouse and human has opened up new possibilities for cell replacement therapies. Although the combination of genes that induce pluripotency to some extent, Oct4 and Sox2 appear to be a prerequisite. The introduction of four genes, several of which have been linked with cancer, using retroviral approaches is however unlikely to be suitable for future clinical applications. Towards developing a safer reprogramming protocol, we investigated whether cell types that express one of the most critical reprogramming genes endogenously are predisposed to reprogramming. We show here that three of the original four pluripotency transcription factors (Oct4, Klf4 and c-Myc or its inducible version MYCER^{TAM}) induced reprogramming of mouse neural stem (NS) cells exploiting endogenous SoxB1 protein levels. The reprogrammed NS cells differentiated into cells of each germ layer *in vitro* and *in vivo*, and contributed to mouse development.

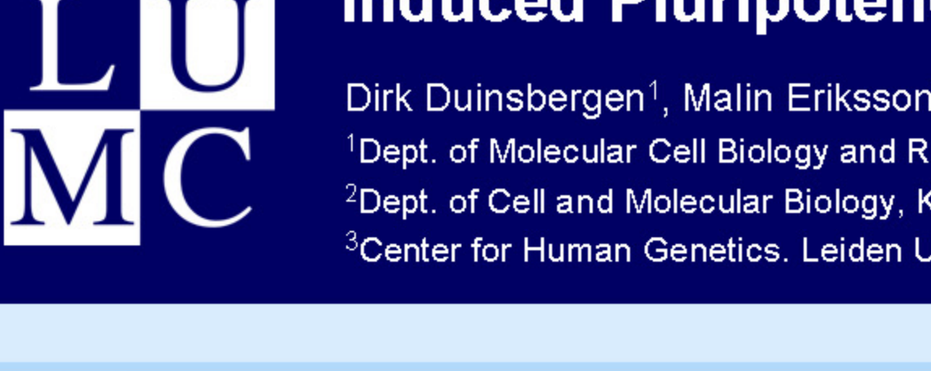

Figure 1. Generation and characterization of a pluripotency selectable NS cell line.

a) Oct4PurΔTK transgene. b) Southern blot analysis identifying the transgene copy number. c) Positive (left) and negative (right) selection of Oct4PurDTK ES cell line. d) Generation of NS cell line from selected ES cell clone. Neuronal (middle) and glial (right) differentiation. e) fusion of Oct4PurΔTK NS cells with wildtype ES cells yielded puromycin resistent colonies.

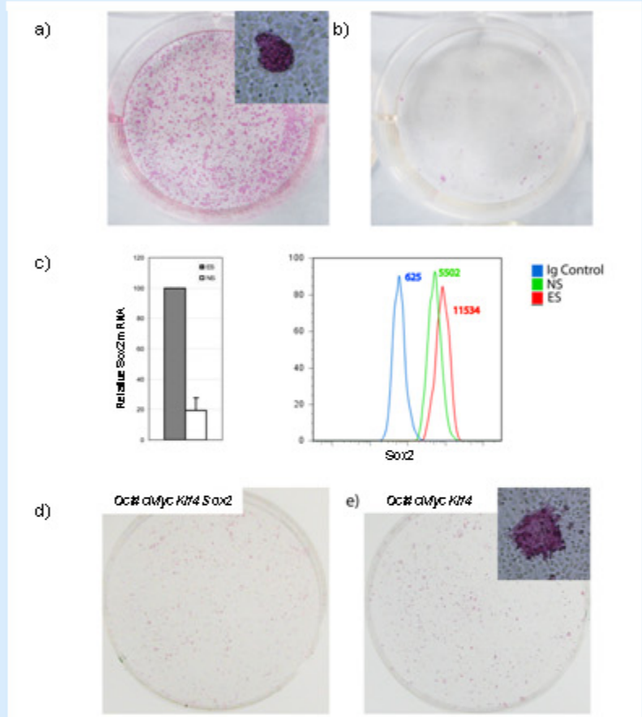

Figure 2. Nuclear reprogramming of NS cells

a) Alkaline phophatase staining of Oct4,Sox2, Klf4 and cMyc reprogrammed NS cells (14 days after transduction) or b) 11 days. c) Sox2 expression in NS cells determined by Q RT-PCR and flow cytometry. d) Reprogramming of NS cells with extra and e) only endogenous Sox2.

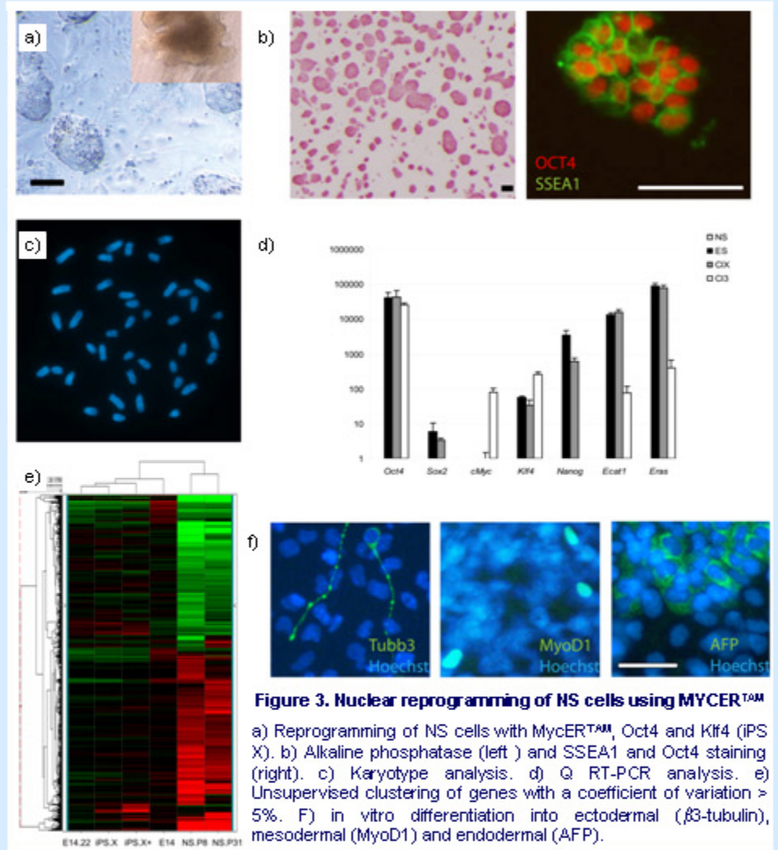

Figure 3. Nuclear reprogramming of NS cells using MYCER^{TAM}

a) Reprogramming of NS cells with MycER^{TAM}, Oct4 and Klf4 (iPS X). b) Alkaline phosphatase (left) and SSEA1 and Oct4 staining (right). c) Karyotype analysis. d) Q RT-PCR analysis. e) Unsupervised clustering of genes with a coefficient of variation > 5%. F) in vitro differentiation into ectodermal (β3-tubulin), mesodermal (MyoD1) and endodermal (AFP).

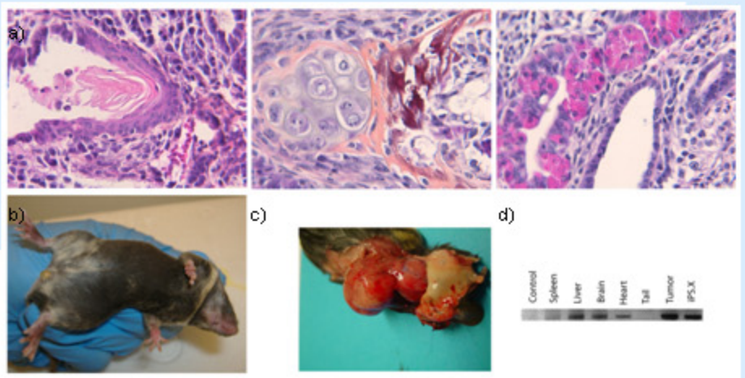

Figure 4. In vivo differentiation and tumor development

a) Ectodermal (skin epithelium), Mesodermal (cartilage) and endodermal (salivary gland-like tissue) in teratomas derived from MycER^{TAM}, Oct4 and Klf4 reprogrammed NS cells (clone X). b) 8 out of 12 born animals contain cells derived from the injected iPS cells. c) Tumor formation (derived from iPS cells) in female mice 1 month after delivery. d) Chimerism is detected in tissue derived from all three germlayers.

Summary

- NS cells were reprogrammed using endogenous Sox2 and an inducible cMyc protein.
- Partially reprogrammed cells have not silenced the proviruses.
- Reprogrammed NS cells were similar to pluripotent ES cells.
- NS derived iPS cells were tumorigenic.

NWO Dutch Organization for Scientific Research