ID 72

Stem Cell Manipulation with Plasmid-Based Transcription Factor Over-Expression Systems to Successfully Generate Pre-Specified Cells Fates *in-vitro*; Proof of Concept of *in-vitro* Stem Cell Fate Reprogramming Using cDNA Vectors

PM Athauda-arachchi[1,2#] and S Chandran[2]

[1]*General Sir John Kotelawala Defence University, Ratmalana, Colombo, Sri Lanka*
[2]*University of Cambridge-BRC, Forvie Site, Addenbrookes Hospital, Cambridge, University of Cambridge, UK*

[#]athaudarachchipm@kdu.ac.lk

The differentiation of stem cells in a controlled fashion is essential to achieve a predefined daughter cell types required for research or regenerative therapies. Transcription factors play a key role in switching cellular differentiation fate in-vivo, at the initiation of the neuro or glial cell fate specification phase in rodents and humans. The study aimed to assess if stem cell differentiation can experimentally be manipulated using expression of cDNA of regulatory homeodomain transcription factors Olig2, Nkx2,2, or Ngn2 in-vitro mirroring the in-vivo development. Mouse embryonic stem (ES) cells and human foetal neural stem (FNS) cells were cultured according to standard protocol. pIRES plasmid vector system with Olig2 transcription factor expression, with or without the co-transcription factor Nkx2.2 (or Ngn 2), were created using molecular biological techniques and introduced into differentiating stem cells. Using biomarkers, final cell fates were compared with one another, including a placebo version. Both mouse embryonic and human neural precursor cells can be made to prematurely differentiate towards neuroglial fate with forced expression of Olig2 transcription factor, whereas co-expression of Olig2 and Nkx2.2 leads to premature oligodendroglial fate specification, compared to placebo. The quantitative effect of fate switching was marked with embryonic stem cell differentiation. Forced expression of key transcription factors as illustrated, may be an attractive method to control stem cell fate modification in in-vitro, and this may successfully be used to generate rare live human cells (such as Oligodendroglia or other specialized cells) for further experiments.

Keywords: *stem cell re-programming, fate modulation, transcription factors*