ImmunoTools *special* Award 2014

Margarita Bartish, PhD student

Supervisor: Prof. Dr. Qiang Pan-Hammarström

Div of Clinical Immunology, F79, Dept of Laboratory Medicine,
Karolinska University Hospital Huddinge, SE141 86
Stockholm, Sweden

Directed differentiation of induced pluripotent stem cells to B lymphocytes

In our lab, we wish to harness the advantages of pluripotent stem cells (PSC) to better understand primary immunodeficiencies (PIDs), primarily those manifested through defects in late B cell development. To this end, we are employing the recently established technology of induced pluripotency. This entails reprogramming mature somatic cells to a pluripotent state (resulting in cells known as induced pluripotent stem cells, iPCSs) through forced overexpression of a combination of key transcription factors [1]. By reprogramming adult cells from individuals with defined genetic backgrounds - in our case individuals diagnosed with PIDs - we can use these cells to recapitulate phenotypes of complex disorders. As these cells are immunologically matched to the donor, iPSCs could, in theory, be used for gene therapy and transplantation later on. To date, we have successfully reprogrammed cells from four patients suffering from immunoglobulin A deficiency (a PID characterised by the inability of the B cell to secrete IgA) and one healthy individual to be used as a control.

Despite the many theoretical applications of stem cells in general, and induced pluripotent stem cells in particular, their practical benefits are highly contingent on the availability and quality of protocols established to guide the differentiation of the stem cell into tissue of interest. Hemapoietic *in vitro* differentiation from human umbilical cord-derived progenitors is relatively well-studied; *in vitro*-matured Ig-secreting B cells were described already in 1998 [2]. The degree of success of streamlining human PSC into the hemapoietic lineage is more unresolved. While some progress has been made reaching the CD19-expressing, DJ-recombined pre-B stage [3], directed differentiation of human PSCs towards mature antibody-producing class-switching B lymphocytes is, as yet, unreported in the scientific literature.

Two general differentiation strategies are pursued today. In the first, the one we mainly apply in our experiments, PSCs are allowed to form structures called embryoid bodies that are cultured in particular cocktails of cytokines. In particular, Flt-3L, G-CSF, IL3, IL6, IL7, BMP-4, h-SCF have been found to be instrumental in promoting development into the hemapoietic lineage [4]. As embryoid bodies contain cells from all three embryonic germ layers, the hemapoietic progenitors are selected on the basis of CD34 expression and cultured further, with additional cytokines to guide the progenitors into the lymphoid lineage. In the second strategy, PSCs are co-cultured with the OP9 murine stromal cell line [3]. This requires fewer supplied cytokines, but has the disadvantage of being dependent on animal feeder cells to drive the differentiation, making this strategy less defined than the cytokine-driven embryoid body-mediated protocol. To validate commitment to B cell lineage, we use such early B cell markers as CD45, CD43, CD19, CD20, CD10. To date, we have been successful

in observing a small CD19 population arisen from our iPSC-derived CD34 progenitors, but ultimately, we wish to derive the mature immunoglobulin-secreting, class-switched B cell.

Our research in culturing iPSCs, driving their hemapoietic differentiation and analyzing its degree of success by flow cytometry is dependent on a vast supply of growth factors, cytokines and antibodies. In return, if successful, ImmunoTools will have contributed to a platform of directed cell differentiation from pluripotent stem cell origin that entails unprecedented opportunity to follow the complete developmental spectrum of the B cell *in vitro*. This will allow us to address questions concerning the very early events in PID-specific B cell specialization previously beyond the scope of PID research. We further expect this platform to be applicable to other research questions involving the need for *in vitro* generation of mature B cells from readily available patient material.

[1] Takahashi, K. and S. Yamanaka, *Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.* Cell, 2006. **126**(4): p. 663-76.

[2] Fluckiger, A.C., et al., *In vitro reconstitution of human B-cell ontogeny: from CD34(+) multipotent progenitors to Ig-secreting cells.* Blood, 1998. **92**(12): p. 4509-20.

[3] Carpenter, L., et al., *Human induced pluripotent stem cells are capable of B-cell lymphopoiesis.* Blood, 2011. **117**(15): p. 4008-11

[4] Chadwick, K., et al., *Cytokines and BMP-4 promote hematopoietic differentiation of human embryonic stem cells.* Blood, 2003. **102**(3): p. 906-15