The impact of TCR rearrangement on iPSC-derived T cell differentiation for novel cancer therapies

Background:

Allogeneic cell therapies are emerging as promising candidates for the treatment of liquid and solid tumours, with the potential to provide cost-effective, on-demand treatments which are highly compatible to a broad spectrum of patients. A common approach for prospective immunotherapies is the use of induced pluripotent stem cells (iPSCs) as a starting source for differentiation into cytotoxic effector cells (T or NK cells) capable of controlling cancer. This allows for gene editing to be performed at the iPSC stage, such as the insertion of an antigen-specific chimeric antigen receptor (CAR), or knockout of immunomodulatory genes. Accordingly, effector cells differentiated from these gene-edited iPSCs should exhibit enhanced, on-target anti-tumour function.

Several methods for the differentiation T cells from iPSCs (iT cells) have been investigated in recent years. Early studies relied on the use of xenogeneic feeder cells to stimulate Notch pathway signalling through Delta-like ligands (DLL)[1-4]. While these approaches were able to induce differentiation of $CD3^+$ T cells, the risks associated with the use of xenogeneic components in the process have limited their clinical applicability. To address this, a number of feeder-free methods have been developed, centred around the presentation of plate- or bead-bound ligands such as DLL1/4 and VCAM1 to differentiating cells[5-7].

iPSCs reprogrammed from adult T cells (T-iPSCs) may offer several advantages over iPSCs reprogrammed from other (i.e., non-T-cell) sources in the context of T cell differentiation. As adult T cells have already undergone T cell receptor (TCR) rearrangement, the endogenous TCR genes in T-iPSCs will maintain a 'pre-rearranged' state[3, 4, 8-10]. Accordingly, when T-iPSCs undergo differentiation towards T cells, there is no requirement to undergo further TCR rearrangement, potentially improving the survival and yield of resultant iT cells. Furthermore, it is speculated that, when cells are reprogrammed to pluripotency, an 'epigenetic memory' of the parental cell is retained[11-13]. Therefore, re-differentiation into iT cells could be aided by this residual epigenetic memory, allowing cells to reacquire T cell identity more readily.

This PhD project will investigate the impact of TCR rearrangement throughout *in vitro* iT cell differentiation of iPSCs derived from both T and non-T cell parental lines. Furthermore, an understanding of how epigenetic memory contributes to differentiation propensity will be investigated, with the potential to identify mechanisms to promote T cell identity.

Aim 1:

Isolate TCRαβ and TCRγδ T cells from peripheral blood mononuclear cells and $CD34^+$ hematopoietic progenitors from cord blood mononuclear cells. Reprogram isolated cells into iPSCs using the Sendai-vector based method. Assess the pluripotency of derived clones and select top candidates for differentiation.

Aim 2:

Differentiate iPSC clone candidates into T cells using Cartherics' feeder-free differentiation method. Compare the differentiation propensity of T-iPSCs with non-T-iPSCs. Profile the expression of RAG1/2 to attempt to understand if TCR recombination in non-T-iPSCs is associated with a 'bottleneck' in differentiation and cell death.

Aim 3:

Perform gene editing to reduce the immunogenicity (TCR, β2 microglobulin, CD155 knockout) and enhance the cytotoxic function (CAR knock-in) of derived iT cells.

Determine if any enhancement in iT differentiation is retained following knockout of TCR loci in T-iPSCs.

Potential future studies:

To investigate the methylome of iPSCs and resultant iT cells to understand if epigenetic memory plays a role in biasing cell fate decisions.

To investigate new methods for presentation of Notch ligands to differentiating cells using the Culturon method of cell differentiation.

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