Genetically engineered human MSCs as supporting inducers of *in vitro* T cell production

Supervisors: Professor Richard Boyd, Professor Alan Trounson
Co-supervisors: Dr Roland Shu, Dr Nicholas Boyd

Summary:
Genetically modified chimeric antigen receptor T cells (CAR-T cells) represent a new revolution in anti-cancer immunotherapy. A major problem, however, is that the treatment currently relies on using the cancer patients own blood but they invariable have too few T cells available for genetic enhancement. Furthermore, prior treatment with chemotherapy substantially reduces their function. This study aims to develop a new approach to generating CAR-T cells from stem cells.

Aim:
T cells derived *in vitro* from both hematopoietic stem/progenitor cells (HSCs) and induced human pluripotent stem cells (iPSC) offer great potential advantages in generating a self-renewing source of T cells that can be readily genetically modified for immunotherapy [1]. The project aims to generate a genetically modified human stromal cell line from human Mesenchymal Stem Cells (MSC), for supporting the T cell *in vitro* differentiation.

Background:
T lymphocytes play a central role in the adaptive immune system and now serve as therapeutic agents in cancer immunotherapy such as CAR-T cell. Autologous T cells can be isolated and expanded from patients for CAR-T cell production, and they can also be derived *in vitro* from hematopoietic stem cells (HSC) and induced human pluripotent stem cells (iPSC). The current in vitro system for T cells differentiation from HSC or iPSC usually needs a mouse bone marrow (BM)-derived stromal cell line, the OP9 cell, as the feeder cell [1]. However, murine cells are not allowed for human clinical usage. To solve this problem, we are aiming to generate a human feeder cell line for inducing and supporting human T cell differentiation from stem cells *in vitro*.

MSC's are multipotent stromal cells that are present in multiple tissues, including umbilical cord, bone marrow (BM) and fat tissue. MSC's can self-renew by dividing and can differentiate into multiple tissues including bone, cartilage, muscle and fat cells, and connective tissue. Preclinical studies have demonstrated that the biological activities of the biomolecules secreted by MSCs on damaged tissues include: blood vessel function and regeneration, tissue repair and immunomodulation. MSCs are the most commonly used adult stem cells in regenerative medicine, and to date 493 MSC-based clinical trials have been approved for clinical studies in the database of the US National Institutes of Health [2].

Proposal Outline:
In the thymus, complex interactions between stromal cells, cell-surface ligands, cytokines, chemokines, and extracellular matrix create a microenvironment that guides T-lymphocyte differentiation from bone-marrow-derived progenitors [3]. The OP9-DL culture system permits the generation of HSC-derived T cells in vitro, serving both as a means to facilitate the study of T-cell differentiation, as well as the potential to produce large numbers of cells for adoptive transfer. OP9-DL1 cells provide Notch signaling, which is a crucial mediator of T-cell development. The mouse bone marrow (BM)-derived stromal cell line, called OP9, is engineered to overexpress the Notch ligand, Delta-like ligand 1 (Dll-1); hence the line is termed OP9-DL1 [1].

In this project, we will replace the OP9-DL1 cells with genetically enhanced human feeder cells, such as MSCs. To achieve this goal, we will engineer the MSCs by genetic modification to create a thymus-like niche for in vitro T cell production in the following ways:

1. Overexpression of Notch ligands on MSC
OP9 cell is engineered to overexpress the Notch ligands like Dll-1 and Dll-4. Both OP9-DL1 and OP9-DL4 cells yield similar results when driving T-cell differentiation in vitro. Thus, overexpression of Dll-1 and/or Dll-4 on MSC may be essential for T cell in vitro differentiation.

2. Overexpression of VCAM-1 on MSC

Synergistic interactions between Dll4 and vascular cell adhesion molecule 1 (VCAM-1) were leveraged to enhance Notch signalling and progenitor T-cell differentiation rates [3]. As a result, co-expression of VCAM-1 with Dll1 and/or Dll4 on MSC may facilitate the T cell in vitro production.

3. HSC *in vitro* differentiation to T cell

While the OP9-DL system was originally created to support T-cell development in the mouse system, it was successfully adapted for use with human umbilical cord blood derived HSCs [4]. To test the genetic engineered MSCs for T cell production as the feeder, we will differentiate UBC-derived HSC in vitro on these MSCs, and evaluate the efficiency of T cell differentiation as compared to the OP9-DL1 cells.

4. T cell differentiation using defined media

Similar to thymic stromal cells, OP9 cells provide key factors that support T lymphocyte development, including interleukin 7 (IL-7), chemokine (C-X-C motif) ligand 12 (CXCL12), and stem cell factor (SCF). It is also reported that addition of Flt3L, IL-6, IL-3, thrombopoietin and low-density lipoprotein led to many fold expansion of CD34+ cells [5]. To produce T cell in vitro efficiently on the genetic engineered the MSC feeder cells, a T cell differentiation medium with addition of defined supplements needs to be optimized and evaluated.

References:

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