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Abstract

Directed differentiation of HESCs

Our laboratory focuses on the directed differentiation of embryonic stem cells (ESCs) to mesodermal and endodermal cell types with a potential use in cell transplantation therapies.
The current research directions of our laboratory include the generation of haematopoietic cells, endothelium, cardiomyocytes and pancreatic beta cells from ESCs. The underlying strategy is to guide ESC differentiation along the same developmental pathways traversed by cells during the ontogeny of these cell types or organs during embryogenesis. To accomplish this, we have developed algorithms for the maintenance and expansion of HESCs that provide sufficient cells for experiments whilst maintaining stem cell phenotype and a normal karyotype. We have also developed a technique for the differentiation of HESCs (the spin embryoid body [EB]) which results in the reproducible formation of EBs of uniform size that differentiate predictably in response to exogenously added growth factors.
Integral to the spin EB system is the utilisation of a serum free differentiation medium that permits the growth factor directed differentiation of HESCs in the spin EBs. Over the last two years we have developed and validated a novel, animal product free, wholly recombinant protein based version (denoted APEL medium) that not only minimises batch to batch variability caused by the medium components, but also provides a safe platform relevant for future clinical applications of HESC-derived differentiated cells.
To assist in the analysis of the HESC differentiation process, we have used homologous recombination in HESCs to insert DNA sequences encoding reporters such as green or red fluorescent proteins (GFP or dsRed) into gene loci whose expression marks obligate intermediates in the genesis of the end cell types of interest.
Our laboratory has developed technology for genetic modification of HESCs by homologous recombination that does not rely on expression of the target gene. Therefore, modification of a desired locus is not dependent on promoter trapping or other metabolic selection strategies. We have generated multiple targeted alleles for many different genes in which we have inserted sequences for reporter genes into the coding sequence, thus generating cell lines in which the reporter gene expression can be used as a surrogate for expression of the endogenous gene. Our laboratory is the first to have published this technique in HESCs. Our current work is focused on utilizing these tagged cell lines to refine the growth factor requirements for directed differentiation of HESCs.

Biography

I trained as a physician in medical oncology and completed a PhD in leukaemogenesis under the supervision of Prof Suzanne Cory at the Walter and Eliza Hall Institute of Medical Research in 1992. In 1993, I took up a position at the National Institute for Medical Research in London in the laboratory of Professor Frank Grosveld, studying the haematopoietic transcription factor, GATA-1. I returned to the Hall Institute in 1995 and, in collaboration with Professor Glenn Begley, I generated ESC and mice in which lacZ was targeted to the locus of the key haematopoietic transcription factor, SCL. In order to study the events antedating blood cell formation, I cloned mouse and human homologues of a Xenopus laevis homeobox gene, Mix.1, a key protein patterning mesoderm in the frog. The Mixl1 deficient mouse embryos that we generated displayed major defects in mesoderm and endoderm. Our laboratory, headed jointly head by myself and Dr Ed Stanley since 2002, has maintained this focus on ESC differentiation along mesodermal (blood) and endodermal (pancreas) lineages. We moved from The Walter and Eliza Hall Institute of Medical Research to Monash University in 2002 in order to expand our scientific endeavours into human (H)ESC. We have generated genetically modified human ESC lines in which fluorescent reporters have been introduced into key gene loci (such as MIXL1) that allow us to objectively monitor in vitro differentiation of ESC in a logical, step-wise fashion. We have also developed a robust system for the efficient differentiation of HESC (spin EBs), complemented by the development of a recombinant protein, animal product free medium (denoted APEL) in which HESC differentiation can be reproducibly directed to different lineages by the inclusion of specific growth factors.