Germ lineage specification from a pluripotent primitive ectoderm-like substrate: a role for cell-cell contacts

A thesis submitted to the University of Adelaide for the degree of Doctor of Philosophy

By

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Summary

During mammalian development a small number of pluripotent cells proliferate and differentiate to give rise to all the mature cell types of the organism. Among the earliest differentiation events is the process of gastrulation, in which pluripotent primitive ectoderm cells form the three germ lineages, mesoderm, ectoderm and endoderm under the control of complex signalling and environmental cues. This process can be modelled using embryonic stem cells, which have proven to respond to embryologically relevant signals during *in vitro* differentiation and promise to uncover additional insights into the process of germ lineage specification. This thesis describes the differentiation of mouse ES cells to committed cell types via a second intermediate population of pluripotent cells termed Early Primitive Ectoderm-Like (EPL) cells. The similarity of EPL cells to primitive ectoderm and the rapid acquisition of lineage specific markers and loss of pluripotent characteristics upon differentiation of EPL cells suggest they are an excellent model for the cells in the embryo that undergo germ lineage commitment.

EPL cells can be differentiated as EPLEBs, which are highly enriched in mesodermal cell types and contain essentially no ectodermal derivatives and no visceral endoderm. Here it is shown that EPLEBs can be generated from EPL cells grown either adherently or in suspension culture provided the cells are reduced to a single cell suspension before reaggregation as EPLEBs. Since EPLEBs are a rich source of mesoderm and contain less non-mesodermal cell types than traditional ESEBs, they were assayed for definitive blood formation, however none was detected.

Alternately, EPL cells can be differentiated in the presence of MEDII in aggregates termed EBMs, which are restricted to ectodermal cell fates. Here it is demonstrated that the switch from mesodermal to ectodermal differentiation observed in ELPEBs and EBMs relies on two variables; a mesoderm suppressing activity within MEDII and the pro-mesodermal activity of cell dissociation as undertaken during EPLEB formation.

Evidence has been presented that interventions that modulate the epithelial identity of EPL cells are capable of influencing subsequent differentiation such that protection of the epithelial cell state favours ectoderm while disruption favours mesoderm. Staurosporine (SSP) is a kinase inhibitor that has been shown to induce an epithelial to mesenchymal transition in chick neural tube. Here it was added to EPL cells with the result that mesodermal differentiation was enhanced at the expense of ectoderm. DAPT is a potent inhibitor of γ-secretase, which cleaves a number of protein targets including the adherens junction component E-cadherin. Addition of DAPT to differentiating EPL cells has the opposite effect to SSP, with an increase in ectodermal differentiation at the expense of medoderm. It is proposed that DAPT is acting by preventing E-cadherin cleavage and thus stabilising the epithelial state.

Modulation of epithelial contacts between pluripotent cells represents a novel way to control lineage induction and as such the incorporation of these findings into methodologies for directed differentiation in defined culture conditions is likely to provide improved outcomes in the production of desired cell types.

Statement

This work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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Amendments

Chapter 1
P16 line 6 should read ...mice to die...
P21 3rd line from bottom should read ....studied are cardiomyocytes...
P24 line 15 should read ...in vivo and in vitro suggest that at the loss of pluripotence...
P25 6th line from bottom should read ...HepG2 cells...
P26 line 5 should read ...EBM[4] (see Table 2.1)...

Chapter 2
P37 section 2.4 should include - Typically outgrowths were scored on days 4, 8 and 12 post seeding and the maximum recorded score for each cell type was plotted. Typically visible red blood cells had a maximum score on day 4, beating muscle on day 8 and neural projections on day 12.

Chapter 3
P47 paragraph 1 should include the reference;

Rathjen, J., P. D. Rathjen (2001). "Mouse ES cells: experimental exploitation of pluripotent differentiation potential." Curr Opin Gen Dev **11**: 587-594.