Stem Cells Research

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Research Area: Biotechnology, Stem Cell Research

Objective

To create novel bioreactors and elucidate factors required for the self-renewal of embryonic and primitive haematopoietic stem cells.

In these frontier days of regenerative medicine, BTI has assembled a team of scientists who are exploring the factors and conditions necessary for the growth and self-renewal of embryonic and primitive haematopoietic stem cells. Such studies will be crucial in developing scaleable methods for future cellular therapies.

Current projects include:

Human embryonic stem cell culture

Human embryonic stem (hES) cells hold great potential for regenerative medicine because of its ability to differentiate to any cell type in the body. However, a major bottleneck is the ability to generate large numbers of hES cells for therapeutic purposes. Our group has identified several key areas to focus our research in Fig 1:

Fig. 1: Schematic representation of stem cell research areas

Development of alternative strategies to passage hES cells

The hES cell line used was obtained from ES Cell International and has been cultured on mouse embryonic fibroblast (MEF) and passaged using mechanical dissection. The advantage of this technique is that it allows for the selection and passaging of regions within the hES colony that maintain an undifferentiated morphology, however, this method is highly laborious and requires skilled operators to dissect the colonies. We have established an alternative approach by passaging the hES cells as clumps using enzymatic
treatment. Morphologically, the cells remained round and small, with a high ratio of nucleus to cytoplasm and have been passaged for 25 passages (~131 population doublings) without any loss of markers unique to undifferentiated hES cells(Fig 2).

**Fig. 2: Human ES cell clumps in culture (a) 4x and (b) 90x magnification. (c) Positive staining for alkaline phosphatase**

**Evaluation of different mouse and human feeders for undifferentiated expansion of hES cells**

Feeders are crucial for maintaining the undifferentiated proliferation of hES cell cultures. Most groups currently use MEF and these are primary cells derived from mouse embryos with limited proliferative potential. Variability has also been observed between mouse strains and within batches of MEF from the same strain. In order to maintain a reproducible and consistent source of MEF, we have evaluated different strategies to immortalize supporting MEF. Experiments are now on-going to optimize transfection into MEF and to determine the ability to immortalise MEF and subsequently their support of hES cells. We have also established co-cultures on several commercially available human feeder lines. hES cells have been cultured for at least 10 passages and have maintained the markers for pluripotency. SCID mouse assays have been initiated to determine the ability of these cells to form teratomas *in vivo*.

**Identifying secreted and membrane factors from feeders that selectively enhance hES cell growth**

To identify factors which are differentially expressed between supporting feeders (MEF) and non-supporting feeders (*delta*MEF), proteomics and microarray approaches were utilised. Preliminary analysis using 2-D electrophoresis of secreted proteins in the culture supernatant revealed proteins that have been down-regulated in *delta*MEF compared with MEF, some of which are implicated in cell proliferation, migration, B-cell growth stimulation. This work is currently on-going to identify other secreted proteins that are down regulated in *delta*MEF. We have also recently initiated a project investigating the difference in membrane protein expression because proteins such as extracellular matrices on feeder cells may be crucial in aiding cell adhesion and support for the hES cells. From the microarray aspect, we found that there were 129 genes down regulated and 289 genes up regulated in *delta*MEF as compared to MEF (Fig 3). We are currently validating our microarray results using quantitative real-time PCR.

**Fig. 3: % Breakdown of genes up regulated and down regulated in *delta*MEF**
**Novel Bioreactor Systems**

Human ES (HES) cells have been cultured with different concentrations of conditioned media made from MEF cells. Initial results show that feeding with 1x and 2x conditioned media provides 30% better growth than feeding with unconditioned media. hES cells also continue to express Oct-4 an important marker of pluripotency after 1 week of culture. These results suggest that secreted 'factors' in condition media is important for hES cell growth. Experiments are ongoing to determine if perfusion with conditioned media will further increase cell densities.

**Mouse embryonic stem cell perfusion culture**

Using mouse embryonic stem (mES) cells as a model, we have developed a perfusion system that enhances proliferation of these cells by two-fold compared to petri dish cultures. Expanded mES cells have been determined to be of a normal phenotype expressing pluripotent markers such as Oct-4, SSEA-1, alkaline phosphatase, and are able to differentiate into embryoid bodies and have a normal karyotype. Work on the scale-up of mES cells is completed and findings will be consolidated for patent and publication.

**Exploring the roles of growth factors on hematopoietic stem cells**

Microarrays were used to study the expression profiles of stromal cells representing different supporting activities towards hematopoietic stem cells (HSC). Several secreted and membrane proteins associated with good supporting stromal cells were identified. One collection of these proteins lies within the prolactin growth factor family. The role of 4 proteins, namely, proliferin-2, osteopontin, LIX and KC in the *in vitro* expansion of HSC were investigated and results obtained were submitted for publication.

**Selected Publications:**

Wuang SC, Choo ABH and Oh SKW. Assessment of stem cell markers during long term culture of embryonic stem cells. 2003 Cytotechnology.


Choong ML, Luo B, Lodish HF. Microenvironment driven changes in expression profile of haematopoietic cobblestone area forming cells. *Annals of Haematology* 2003 (Accepted for publication).

Choong ML, Yong YP, Tan ACL, Luo B, Lodish HF. LIX: A chemokine with a role in hematopoietic stem cells maintenance. 2003, *Cytokine*.