Embryonic Stem Cells

Key properties of ES cells

Embryonic stem (ES) cells are karyotypically normal continuous cell lines isolated directly from the inner cell mass of the blastocyst embryo. ES cells are unique stem cells as they retain the developmental potency of foetal founder cells even after extended propagation and manipulation in culture. When transferred to a preimplantation mouse embryo, ES cells incorporate to the inner cell mass and generate mice that are chimeric both in somatic and germ tissues1 (Fig 1).

ES cells were initially isolated and maintained by co-culture on feeder layers of mitotically inactivated mouse fibroblasts.2,3 It was identified later that the fibroblast feeders express the stem cell regulator leukaemia inhibitory factor (LIF) which actively suppress differentiation.4,5 LIF is able to completely replace feeder layers, not only in the maintenance of previously established ES cell lines, but also in the de novo establishment of karyotypically normal and germ line competent ES cell lines.6 This property has enabled the culture of homogeneous population of pluripotent ES cells in the absence of contaminating fibroblasts.

Recently, it was discovered that bone morphogenetic proteins (BMPs) act in combination with LIF to sustain self-renewal and preserve multilineage differentiation of ES cells in serum-free condition.7 This fully defined culture paradigm also supports the generation of germ line competent ES cell lines. In the absence of LIF, however, BMP stimulates differentiation, suggesting that a very delicate balance of different signalling pathways regulate ES cell self-renewal versus differentiation.

Creation of designer mouse models

The capacity for germ-line colonisation means that ES cells can be exploited as vehicles for transgenic manipulation of the mouse genome, via introduction of new genetic information or the alteration of the host gene sequences. Indeed, the major use of ES cells to date is as a cellular tool for the production of mice carrying predetermined genetic modification generated by homologous recombination or gene targeting. The planned alteration of a gene is first generated in genome of ES cells in tissue culture. Genetically modified ES cells can then be injected into recipient blastocysts, where they contribute differentiated progeny to their host, resulting in the birth of genetically modified chimeric pups. Following germline transmission, mice that carry a defined mutation of a gene are generated. The genetic modification can now be designed in a sophisticated manner such that the mutation can be temporally and spatially regulated (conditional knock-out), by exploiting the Cre-loxP system and cell/tissue specific regulatory elements.

The past decade, gene targeting by homologous recombination has revolutionised the field of mouse genetics and allowed the analysis of diverse aspects of gene function in vivo.

Cellular model for developmental studies

The integration of ES cells into normal embryonic development demonstrates their capacity to respond to a repertoire of developmental regulatory signals. Therefore, ES cells provide an invaluable in vitro system for the experimental identification and characterisation of factors that control early embryonic growth and differentiation. In particular the system is well suited to investigating the capacity of genes through ‘gain of function’ studies, since they permit the study of specific gene overexpression in a given differentiation lineage without having to worry about the effects of such an overexpression on the overall embryonic development. We have successfully applied this approach in determining Sox B transcription factors in neural fate choice from pluripotent stem cells, and in a functional screen of candidate neural genes following a microarray transcriptome analysis.8,9 Conversely, ES cells also provide an ideal system for ‘loss of function’ studies either via siRNA technology (knock-down) or gene targeting of specific gene.

Therapeutic potential of ES cells

The establishment of human ES cells has sparked much interest in both the scientific and general community regarding their potential in regenerative therapies.10,11 Cell transplantation can in principle be applied to many

Fig 1: Diagram summarises the propagation and use of ES cells. Photograph of self-renewing mouse ES cells cultured in the presence of LIF in serum on gelatine coated plastics. Upper panel depicts the inter relationship between ES cells, the inner cell mass (ICM) cells of the blastocyst embryos, and chimeric mice. In the absence of LIF under suitable culture condition, ES cells can give rise to many differentiated somatic cell types. Photograph of the ES cells provided courtesy of QL Ying.
human diseases such as leukaemia, diabetes and some neural degenerative diseases. Many protocols have been
developed to differentiate ES cells, so far mostly of mice,
into a variety of cell types which includes neurons, adipocytes, skeletal myocytes and hematopoietic cells and
cardiomycytes. Transplantation studies have demonstrated a certain degree of functional repair in ani-
mal models of multiple sclerosis and spinal cord injury.

However, our abilities to direct ES cells into specific
pathways and then to support the viability and matura-
tion of individual differentiated phenotypes in vitro
remains limited. Consequently, the differentiating ES cell
cultures constitute heterogeneous types of differentiated
ES cell progeny with unknown phenotypes. In the absence
of knowledge to instruct ES cells into a specific fate,
strategies have been developed to enrich or isolate pheno-
types of interest from the mix cell population. This can be
achieved through selective culture condition or fluores-
cence-activated cell sorting (FACS). In addition, drug
resistance or cell sorting capacity conferred by genetic
manipulation in ES cells provides another way of isolating
a particular cell lineage or cell types. We have success-
fully applied this approach in purifying ES cell derived
neural stem/progenitors based on specific expression of
Sox1 and Sox2 in developing neuroepithelium (Fig 2).

Recently, we have investigated the ability of Sox5 protein
influencing ES cell lineage choice. We found that
forced expression of Sox1 or Sox2 does not impair propa-
gation of undifferentiated ES cells, but upon release from
self-renewal by LIF withdrawal promoted differentiation into
neuroectoderm at the expense of mesoderm and endoderm.
The efficient specification of a primary lineage by
transcription factor manipulation or their downstream signalling cascade may provide a general paradigm
for instructing differentiation of ES cells for biopharma-
ceutical screening and cell therapy applications.

Conclusions

The mouse ES cells have provided us with an unprece-
dented opportunity to understand mammalian develop-
ment and molecular mechanisms that lead to pathological
situations. The development of human ES cell now offers
the foundation of applying ES cell technology to the treat-
ment of human diseases. However, much remains to be
accomplished with regards to human ES cell technology
before they can be used as a new form of human medicine.

References


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