Embryonic Stem Cells: The Mouse Source – vehicle for Mammalian Genetics

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In this presentation I wish to introduce mouse embryonic stem cells and to tell you

- where the ideas came from
- the story of their isolation and development
- their use as a vehicle for genetic manipulation
- some of our latest work which indicates exactly where in the early mouse embryo these embryonic stem cells come from.
Lineages of cells and stability of differentiated state

- Structure and the function of the body depends upon the autonomous but integrated action of a large number of diversely functioning specialised (that is, differentiated) cells that are organised into specific tissues (e.g., the cornea of the eye, skin, blood) and organs (e.g., liver, kidneys).
Lineages of cells and stability of differentiated state

• These cells have all developed from the single cell of a fertilised egg by cell division. This proliferation and differentiation is accompanied by progressive restriction of the potential fate of the cell’s progeny.
Lineages of cells and stability of differentiated state

- Cells, both during development and in the adult do not, typically, change from one type to another.
Lineages of cells and stability of differentiated state

• At the very early stages of development, therefore, there must be cells from which the entire organism is derived. What is not necessarily self-evident, however, is that a replicating population of such cells may exist. Evidence for such pluripotential stem cell populations came from studies of the biology of mouse teratocarcinomas.

Testicular teratocarcinomas

Spontaneous Testicular Teratomas in an Inbred Strain of Mice
Leroy C. Stevens, Jr. and C. C. Little

• Inbred strain of mice which spontaneously develop Teratomas in testis
• These are from primordial germ cells
• also from ectopic embryos

“Following repeated serial transplantations, these tumors have retained their pleomorphic character. Pluripotent embryonic cells appear to give rise to both rapidly differentiating cells and others which, like themselves, remain undifferentiated.”
Dr G. Barry Pierce

Two models for source of multiplicity of cell types in teratoma

a) Multiple precursor lines
b) Single pluripotential stem cell line

Kleinsmith L J and Pierce GB
MULTIPOTENTIALITY OF SINGLE EMBRYONAL CARCINOMA CELLS.
Cancer Res. 1964 Oct;24:1544-51
The isolation and properties of a clonal tissue culture strain of pluripotent mouse teratoma cells

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SUMMARY

A clonal tissue culture strain of pluripotent cells has been isolated from a transplantable teratoma of inbred strain of mice 129 Sv-Sli CP. This cell strain SIKR when re-inoculated into mice produces teratomas containing at least ten types of tissue. Sub-clones have been isolated and two types distinguished.

(1) ‘C-type’ with a densely-piled in vitro growth. These are tumourigenetic and pluripotent displaying a comparable range of differentiation to the original SIKR.

(2) ‘E-type’ spreading, often epithelioid growth. These grow to a lower density in culture than ‘C-type’. Mostly non-tumourigenetic; in those cases where a tumour has been obtained it did not display multiple differentiations.

The results are interpreted as demonstrating that the culture consists of equivalently pluripotent cells which may become determined and differentiate spontaneously in vitro into slower growing cell types which are continuously overgrown by the culture.
Differentiation of EC cells

1) in vivo in tumour
2) in vivo in chimaeric embryo
3) in vitro in tissue culture

**Differentiation of EC cells**

1) *in vivo* in tumour
2) *in vivo* in chimaeric embryo
3) *in vitro* in tissue culture

- **1)** Clone grows as colony on feeders
  - Feeders die and outer cells differentiate to embryonic endoderm

- **2)** Mass culture allowed to overgrow
  - Clumps float off and form endoderm on outer surface -- Embryoid Body
  - Further growth on a surface gives extensive differentiation
• One of the conceptual breakthroughs on the road to ES cells was the realisation that their differentiation was not abnormal, disorganised, random or stochastic but followed the normal pathways of early embryonic development.
Embryoid body stained for alphafoetoprotein (green) in some of the endoderm cells

Embryonal Carcinoma cells in culture

Electron Microscope section of edge of embryoid body

Embryoid body
• In this review I presented the evidence that EC cells should be able to be isolated into tissue culture directly from normal early embryos.

• I surmised that maybe there were three explanations for failure up until now:

  – **NUMBER** The number of *pluripotential cells in the embryo* at any one time may be *very low*; sufficient in vivo but insufficient in vitro where there is greater cell mortality.

  – **TIME** There may be a *short time window* - in vivo this is extended by growth of the embryo up to this point or regression of some of the cells of a later embryo following damage of transplantation.

  – **TOO GOOD!** EC cells which differentiate readily are more difficult to maintain in tissue culture than those which are more culture adapted and differentiate less well. “..*the genuine embryonic cell counterpart may differentiate and lose its pluripotency and rapid growth characteristics all too readily under culture conditions.* ..”
Matt Kaufman

- Haploid (parthenogenetic) embryos grown to egg cylinder
- I could grow cell lines from ICM’s - e.g. ICME
- Had refined media in particular in growing human teratocarcinoma cells
- Genetic opportunity! Haploid cells in culture
Isolation of Embryonic Stem Cells

“Giant blastocysts from 129 mice put into delay by ovariectomy and depo provera”
3. Isolation and Culture of Embryos

M. H. K. and J. Evans

Although mouse embryos have been isolated at various stages of development, the pattern of karyotypic changes in the cells of the embryonal cells is not known. The embryos were isolated from mouse embryos at the stage of the 10-11 cell stage (Fig. 2), and the cells were cultured in EM medium supplemented with 10% FCS. The cultures were examined daily for the formation of colonies, and colonies were transferred to EM medium supplemented with 1% FCS and 10% FCS just prior to the time of FCS (Fig. 2A).

Cell culture analysis has revealed that the nuclei of the embryonal cells are called XX and XY (Fig. 2B) cells. The formation of colonies from these cells is a pluripotential...
A record book page from July 1980 setting out some of the characterisation needed to show that these cells really were equivalent (but better) than the embryonal carcinoma cells derived from tumours. In addition to the needs listed here was it was known already already that these cells had the in vitro morphology, cell-surface and histochemical markers expected.

- Produced teratomas with a full diversity of differentiated tissues.
- Normal male 40XY and female 40XX often rapidly becoming 39XO.
- Made excellent chimaeric mice which were normal and didn't produce tumours.

Absolutely!

Splendid!

QuickTime™ and a Planar RGB decompressor are needed to see this picture.

And didn't produce tumours.

- Single
- In vitro

As the B series separately - HG
B1, B2, B3, B4

I could inject B & B7 and

I still have some
ES Cells expressing a green fluorescent marker (GFP) when inserted into a blastocyst are traced to the Embryonic Epiblast. Showing that ES cells can become embryo cells.
Experimental Mammalian Genetics
ES cells are a vector to the whole animal genome
ES cells are a vector to the whole animal genome

- Test function of gene
- Illuminate understanding of genetic disease process
- Allow experimental approaches to therapy

- Mutate, Trap, Target, Manipulate
Germ-line transmission of genes introduced into cultured pluripotential cells by retroviral vector.

Robertson E, Bradley A, Kuehn M, Evans M.

Embryonic stem cells isolated directly from mouse embryos can be cultured for long periods in vitro and subsequently repopulate the germ line in chimaeric mice. During the culture period these embryonic cells are accessible for experimental genetic manipulation. Here we report the use of retroviral vectors to introduce exogenous DNA sequences into a stem-cell line and show that these modified cells contribute extensively to the somatic and germ-cell lineages in chimaeric mice. Compared with current methods for manipulation of the mouse genome, this approach has the advantage that powerful somatic-cell genetic techniques can be used to modify and to select cells with germ-line potential, allowing the derivation of transgenic strains with pre-determined genetic changes. We have by this means inserted many proviral vector sequences that provide new chromosomal molecular markers for linkage studies in the mouse and that also may cause insertional mutations.

| Table 1. Identification of a homozygous lethal mutation in pedigrees derived from male 413 |
|----------------------------------|----------------|---------|-----------|-------------|
| Proviral band tested | Number of progeny genotyped | Wild type | Heterozygous | Homozygous |
| 413.a | 27 | 6 | 12 | 9 |
| 413.b | 42 | 12 | 18 | 12 |
| 413.c | 44 | 7 | 21 | 16 |
| 413.d | 79 | 26 | 53 | – |

A total of 106 F₂ progeny were genotyped. F₁ parents shared 1 or 2 bands.

413d Conlon, Barth & Robertson
Development 111 969 (1991)
A potential animal model for Lesch–Nyhan syndrome through introduction of HPRT mutations into mice

Michael R. Kuehn, Allan Bradley, Elizabeth J. Robertson & Martin J. Evans

A retroviral gene trap insertion into the histone 3.3A gene causes partial neonatal lethality, stunted growth, neuromuscular deficits and male sub-fertility in transgenic mice.

Three oncogenes

• brca2
• c-mos
• hox11
“The known sequence of 2,329 amino acids encoded by the BRCA2 gene does not show strong homology to sequences in the publicly available DNA or protein databases, and therefore we have no clues to its functions.”
What are they?

• Are mouse ES cells a cell type normally found in the early embryo or are they effectively an artefact of culture?

• Lines of evidence
  – 2d protein separations
  – Microarray expressionomics
Stages used

Thieler stages from EMAP

88hpc (3.6d)

6.5 dpc

5.5dpc

105 hpc (4.35d)

Delayed

5.6d and 7.5d
ES microarray phenotyping

- 20 ICM’s (~500 cells)
- Two rounds T7 amplification
- Amino-allyl labelling
- NIA 15k probes

Stepped aside or from normal pathway?

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.
Where ES cells come from!
Two platform technologies

• Use of germ line chimaerism
  – vector to whole animal genetics and animal models of disease (mouse) understanding and drug discovery

• Wide range of developmental studies; *in vitro* differentiation
  – fundamental understanding of cell developmental biology
  – therapeutic scenario of damaged tissue being repaired by appropriate tissue specific stem and precursor cells possibly derived by specific differentiation of human ES cells. Moreover the possibility of using histcompatible cells either from a large pre-prepared bank or by dedifferentiation of other cells self-donated by the patient has done much to power interest in the field.
Future

• Whole animal genetics
• Analysis of differentiation
• Embryo surrogate and source of specific cells
• Understanding control of mammalian developmental cell biology & genetic readout in differentiation
• Practical medical applications
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Nobel Lecture in Physiology or Medicine