nature REVIEWS
MOLECULAR CELL BIOLOGY

From teratomas to embryonic stem cells: discovering pluripotency

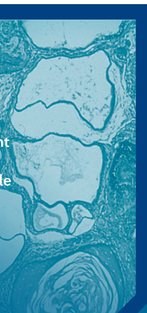
Peter W. Andrews and Paul J. Gokhale

The term 'teratoma' denotes the weird manifestations of benign or malignant tumours that possess the hallmarks of abnormal embryogenesis. They contain an array of tissues found in developing embryos, and malignant teratomas contain the archetypal cancer stem cell, the embryonic carcinoma (EC) cell. The resemblance of these tumours to abnormal embryos, and the discovery that EC cells are pluripotent, like the ICM of the early embryo, suggested an opening to experimental mammalian developmental biology before the tools that we now use became available. The study of EC cells led to the isolation, 30 years ago, of mouse embryonic stem (ES) cells. ES cells now provide tools for experimental embryology and regenerative medicine.

Teratoma or teratocarcinoma?

The term teratoma describes a tumour containing differentiated elements of all three embryonic germ layers. The term teratocarcinoma describes malignant tumours that contain EC cells, the presumed malignant stem cells, in addition to these three layers[38]. It is valuable to distinguish benign teratomas from malignant teratocarcinomas[39].

Characterization of teratomas

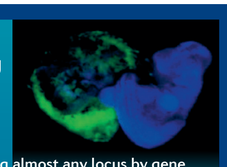


The discovery that 129 strain mice develop testicular teratocarcinomas[2] allowed the experimental study of these cancers, which are GCTs arising from the PG cells in the embryo[40]; an example of teratoma histology is shown (right). A strong genetic component contributes to a predisposition to GCT development. Lewis Kleinsmith and Barry Pierce showed that a single EC cell from a teratocarcinoma can generate a differentiated tumour when transplanted to a host mouse — a demonstration of a cancer stem cell[4]. EC cells in the tumour are malignant and capable of self-renewal and differentiation.

Characterization of EC cells in culture

Beginning with work by Boris Ephrussi and Brenda Finch in 1967 (REF. 5), mouse EC cells cultured *in vitro* were studied through the 1970s[12,41–45]. Their capacity for differentiation and expression of common markers suggested equivalence to the pluripotent ICM cells of the early embryo. Indeed, chimeric mice could develop following blastocyst injection[11,46,47] (above). In one report, the EC cells populated the germline[46]. This work, especially the development of EC culture conditions using feeder cells[12,43] and the discovery of F9 antigen[10] and, later, of the monoclonal antibody-defined antigen SSEA1 (REF. 14) as cell surface antigens of EC cells, paved the way for the isolation of ES cell lines directly from the ICM[17,18].

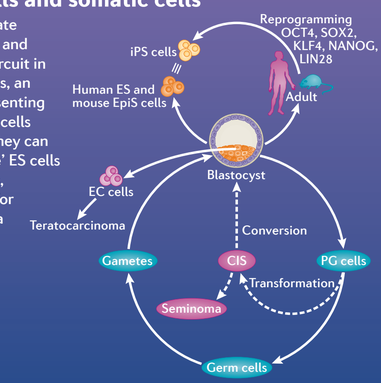
ES cells in culture and their use in gene targeting



ES cells transformed the field of mouse genetics, and they can be used to form germ line chimaeras (right; the embryo derived from ES cells is shown in blue, the host placenta in green) after altering almost any locus by gene targeting[22,27,28]. Analysis of the signalling pathways that regulate ES cell self-renewal and differentiation[48] is providing important insights into early embryonic development, cancer and the potential application of ES cells in regenerative medicine. The International Mouse Knockout Consortium intends to mutate every protein-coding gene in the mouse.

Pluripotent stem cells, germ cells and somatic cells

Pluripotent cells have the capacity to differentiate to all somatic cell types and germ cells. EC cells and teratocarcinomas probably arise from a short-circuit in normal germ cell development (right). In humans, an intermediate stage of CIS and seminoma, representing malignant PG cells, is also found, but mouse PG cells convert directly to a pluripotent state; *in vitro* they can give rise to EG cells[49,50]. EC cells are 'degenerate' ES cells that have adapted to tumour growth. EpiS cells, derived from the epiblast stage[51,52], are primed for differentiation, whereas ES cells correspond to a more primitive, naive state of the ICM[48]. Human ES cells resemble mouse EpiS cells[51,52]. Somatic cells can be reprogrammed to iPS cells by the expression of key pluripotency factors[35–37].



Mouse timeline

600 BC — Monstrous tumours have been a source of fascination since the dawn of time[1].

1879 — Rudolf Virchow coins the term 'teratoma'.

1954 — Barry Pierce reports passaging human teratocarcinomas as xenografts[3].

1957 — Leroy Stevens discovers that testicular teratomas occur frequently in the 129 mouse strain[2].

1964 — Richard Gardner demonstrates that chimeric mice can be produced following blastocyst injection of ICM cells[6].

1967 — Pluripotent mouse EC cells are cultured *in vitro*[5].

1968 — Leroy Stevens[7], and Davor Solter, Nikola Skreb and Ivan Damjanov[8], find that teratomas can be derived from ectopic embryos.

1970 — Niels Skakkebaek identifies abnormal intratubular germ cells (CIS) as the likely precursor of invasive testicular teratocarcinomas[9].

1972 — Ralph Brinster reports that EC cells are able to chimerise the developing embryo, indicating their functional equivalence to ICM cells[11].

1973 — Karen Artzt and colleagues show that the F9 cell surface antigen links EC cells and ICM cells of the early embryo[10].

1974 — Gail Martin and Martin Evans show differentiation of EC cells in embryoid bodies *in vitro*[12].

1975 — Barbara Knowles and Davor Solter define the EC cell surface marker SSEA1, a counterpart of the F9 antigen, using a monoclonal antibody[14].

1978 — Sid Strickland shows that differentiation of EC cells in culture can be induced by retinoic acid[15].

1979 — TERA1 and TERA2 cell lines are established from human teratocarcinomas[13].

1980 — Martin Evans and Gail Martin isolate mouse ES cells[17,18].

1981 — SSEA3 is found on cleavage stage mouse embryos but absent from mouse EC cells[19].

1982 — It is demonstrated that mouse ES cells can form germ line chimaeras[22].

1984 — Peter Andrews and colleagues suggest that human EC cells differ from mouse EC cells and do not express SSEA1 (REF 16).

1987 — SSEA3 is found to be expressed by human EC cells[20,21].

1989 — Human EC TERA2 cells are found to be pluripotent[23,24,25].

1990 — TRA-1-60 and TRA-1-81 surface antigens of human EC cells are defined[26].

1995 — Homologous recombination is used to alter the genotype of mouse ES cells[27,28].

1998 — OCT4 (POU5F1) is identified as a pluripotency factor[29].

2003 — SOX2 is identified as a pluripotency factor[30].

2006 — NANOG is identified as a pluripotency factor[33,34].

2007 — The first iPS cells are derived from mouse somatic cells by Shinya Yamanaka and colleagues[35].

2011 — The first clinical trial of human ES cell-derived cells in regenerative medicine is started, for spinal cord injury.

Jamie Thomson and colleagues isolate rhesus monkey ES cells[31].

Jamie Thomson and colleagues isolate human ES cells[32].

The groups of Shinya Yamanaka and Jamie Thomson separately report that iPS cells can also be derived from human somatic cells[36,37].

Human

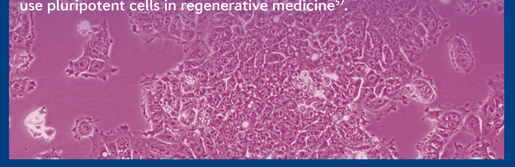
Clinical pathology of teratomas

Teratomas occur most commonly as benign ovarian tumours, dermoid cysts and, rarely, as tumours of newborns. The description on the clay cuneiform tablet with omens from Nineveh (left) is almost certainly of a newborn's sacrococcygeal teratoma[1]. Teratomas also occur in the testis, where they are always malignant and are described as teratocarcinomas. They belong to a group of GCTs that includes seminomas, which resemble PG cells. As in the mouse, testicular GCTs are most likely to originate from defects in germ cell development *in utero*[9]. They are the most common, but most curable, cancers in young men.

"When a woman gives birth to an infant that has three feet, two in their normal positions attached to the body, and the third between them, there will be great prosperity in the land."

Development of human EC cell lines

The relationship of mouse EC cells to the early embryo suggested a use for human EC cells in studying human development[53,54]. However, developmental differences and differences in surface antigen expression indicated that human and mouse EC cells correspond to different embryonic cells, or that equivalent cells differ between species[16]. Nevertheless, pluripotent human EC cells[23,26,55] (below) contributed to our understanding of development, including the realization that retinoic acid acts through HOX genes in the embryo[56]. The transplantation of NTERA2 EC cell-derived neurons into stroke patients was the first attempt to use pluripotent cells in regenerative medicine[57].



Surface antigens and other markers

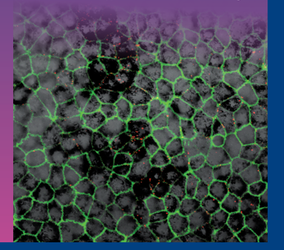
Surface antigens have played a key part in dissecting complex developmental systems since they were first used in the immune system by Edward Boyse and Lloyd Old in the 1960s[58]. Subsequently, the identification of F9 antigen by a syngeneic anti-EC cell serum[10] and, later, of its counterpart SSEA1 by a monoclonal antibody[14] were central to relating mouse EC and ICM cells. SSEA1, SSEA3 and SSEA4 are glycolipids with differential expression in mouse and human embryos and EC cells[19,59,60]. An extensive panel of surface antigen markers of human EC cells has subsequently been defined[61,62] (right) and is in use in human ES cell and iPS cell studies. Other markers, such as ALP[63,64] and, notably, the transcription factors OCT4, SOX2 and NANOG, which have a key role in maintaining pluripotency, are commonly expressed between mouse and human EC and ES cells[29,30,33,34].

EC and ES cell phenotypes

Human	Mouse
SSEA1⁻	SSEA1⁺
SSEA3⁺	SSEA3⁻
SSEA4⁺	SSEA4⁻
TRA-1-60⁺	TRA-1-60⁻
GCTM2⁺	GCTM2⁻
THY1⁺	THY1⁻
MHC⁺	MHC⁻
ALP⁺	ALP⁺
OCT4⁺	OCT4⁺
NANOG⁺	NANOG⁺

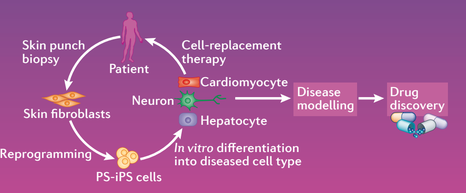
Human ES cells and regenerative medicine

The surgical replacement of diseased or damaged tissues with cells differentiated from ES cells (for example, RPE cells (below, stained for the tight junction protein ZO1)) is a real possibility. The first clinical trial of ES cell-derived oligodendrocytes for spinal cord injury is underway, and trials for other diseases are near, including those for eye diseases, such as macular degeneration. Many other opportunities remain for using ES cells in drug discovery and toxicology.



Pluripotent stem cells and disease models

The production of human iPS cells[36,37], now a rapidly developing field, offers the possibility of circumventing the problems of immune rejection in regenerative medicine applications (below). The production of iPS cells from patients with a specific genetic disease presents a new opportunity to create disease models with which to search for new treatments[65–68].



Abbreviations
ALP, alkaline phosphatase; CIS, carcinoma *in situ*; EG, embryonic germ; EpiS, epiblast stem; GCT, germ cell tumour; ICM, inner cell mass; iPS, induced pluripotent stem; KLF4, Krüppel-like factor 4; MHC, major histocompatibility complex; PG, primordial germ; PS-iPS, patient-specific iPS; RPE, retinal pigment epithelium; SSEA, stage-specific embryonic antigen; THY1, thymocyte differentiation antigen 1; ZO1, zonula occludens 1.

Image credits
Photograph of L. Stevens courtesy of The Jackson Laboratory, USA | Photographs of B. Pierce and of teratoma histology courtesy of I. Damjanov, University of Kansas, USA | Image of chimeric mouse is reproduced, with permission, from REF. 9 © (1974) The Rockefeller University Press | Photograph of M. Evans courtesy of M. Evans, Cardiff University, UK | Photograph of G. Martin courtesy of G. Martin, University of California, San Francisco, USA | Photograph of J. Thompson courtesy of J. Thompson, Morgridge Institute for Research, USA | Image of gene-targeted embryo is reproduced, with permission, from REF. 69 © (2002) BioMed Central | Photograph of S. Yamanaka courtesy of the Center for iPS cell Research and Application, Kyoto University, Japan | Image of Cuneiform tablet with omens © The Trustees of the British Museum. All rights reserved | Photograph of RPE cells derived from human ES cells courtesy of P. Coffey, University College London, UK | Disease models schematic is modified, with permission, from REF. 70 © (2010) American Society for Clinical Investigation.

Further reading
Damjanov, I. & Solter, D. Experimental teratoma. *Curr. Top. Pathol.* **59**, 69–130 (1974) | Stevens, L. C. The biology of teratomas. *Adv. Morphog.* **6**, 1–31 (1967) | Andrews, P. W. From teratocarcinomas to embryonic stem cells. *Phil. Trans. R. Soc. B* **357**, 405–417 (2002) | Solter D. From teratocarcinomas to embryonic stem cells and beyond: a history of embryonic stem cell research. *Nature Rev Genet.* **7**, 319–327 (2006) | Evans, M. Discovering pluripotency: 30 years of mouse embryonic stem cells. *Nature Rev. Mol. Cell Biol.* http://dx.doi.org/10.1039/nrm3190 (2011).

For the reference list please see:
http://www.nature.com/nrm/posters/discoveringpluripotency

Contact information
p.w.andrews@sheffield.ac.uk
p.gokhale@sheffield.ac.uk
Edited by Katharine H. Wrighton; copy-edited by Antony Bickenson; designed by Vicky Summersby.

http://www.nature.com/nrm/posters/discoveringpluripotency