TIMELINE

From teratocarcinomas to embryonic stem cells and beyond: a history of embryonic stem cell research

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Abstract | We are currently facing an unprecedented level of public interest in research on embryonic stem cells, an area of biomedical research that until recently was small, highly specialized and of limited interest to anyone but experts in the field. Real and imagined possibilities for the treatment of degenerative and other diseases are of special interest to our rapidly ageing population; real and imagined associations of stem cells to cloning, embryos and reproduction stir deeply held beliefs and prejudices. The conjunction of these factors could explain the recent sudden interest in embryonic stem cells but we ought to remember that this research has a long and convoluted history, and that the findings described today in the scientific and popular press are firmly grounded in research that has been going on for several decades. Here I briefly recapitulate this fascinating history.

The general public has discovered stem cells in the past few years; the science and ethics of this research is now hotly debated by politicians, the media and academics of many disciplines. This is not surprising considering the potential benefits on the one hand and the slew of real and perceived ethical and moral dilemmas on the other. It is, however, surprising that the majority of discussants perceive stem cells, and especially embryonic stem cells, as something that emerged in the past couple of years, not realizing that recent developments are deeply rooted in past research and accomplishments.

The research on teratocarcinomas, embryonal carcinoma cells and embryonic stem cells (ES cells) that has been carried out over the past 50 years has passed through several distinct phases (TIMELINE). These were influenced by the technologies available at the time, contemporary scientific interest (fashion) and societal demands. The relatively modest interest in the subject during the 1950s and 1960s increased significantly in the 1970s, parallel to the increased interest in mammalian developmental biology and cell differentiation. The isolation of mouse ES cells in the early 1980s and the realization that they could be used as a vehicle for introducing targeted genetic modifications into the germ line made ES cells a favourite tool for gene function analysis. Finally, the isolation of human ES cells and their potential applications in regenerative medicine focused the attention of a much broader scientific and general-public community on the subject.

Although work using mouse and human material proceeded along the same lines, advances using human cells usually lagged behind by a decade or so. Only recently have both models been used simultaneously in comparative experiments. It is clear that research using human cells profited substantially from the experience that had accumulated using mouse cells. It is also clear that, despite many obvious similarities, results obtained with mice cannot always be directly extrapolated to the human.

What were the crucial discoveries that transformed the study of teratocarcinoma and embryonic stem cells from an esoteric subject into one that now occupies the centre of attention of the biomedical scientific community?

Teratocarcinomas

Teratomas (benign) and teratocarcinomas (malignant) are tumours that are most commonly found in the gonads (FIG. 1d,e), but also occasionally in extragonadal sites. Their name, which stems from the Greek word 'teratos' (monster), describes their appearance well, as these tumours are composed of a haphazard mixture of adult tissues and misshapen organs. It is no wonder that tumours containing teeth, pieces of bone, muscles, skin and hair have fascinated people since antiquity. The study of teratomas/teratocarcinomas was for a long time restricted to the description of occasional spontaneous human gonadal tumours. Although relatively rare, their bizarre histological appearance attracted attention and interest that was disproportionate to their clinical importance. They are extremely rare in the most common experimental animals — that is, mice and rats — and for this reason they are difficult to study experimentally. This changed with the first description in the 1950s of the mouse strain 129 that showed an incidence of spontaneous testicular teratoma of about 1% (REF. 1). Histologically these tumours were typical teratomas; that is, they were composed of many haphazardly arranged adult tissues. The malignant nature of these tumours was indicated by their ability to grow rapidly when repeatedly transplanted either subcutaneously or intraperitoneally. In the following decade two investigators, Leroy Stevens and Barry Pierce, painstakingly (and for the most part ignored by the wider scientific community) assembled a detailed picture of the biology of spontaneous testicular teratocarcinoma. This work was significantly aided by the discovery that tumour incidence in testes derived from grafted fetal genital ridges is much higher[2]. This discovery allowed Stevens to precisely follow the initial event of teratocarcinogenesis and to describe how a small nest of cells within the fetal testes develops into a teratoma or teratocarcinoma.

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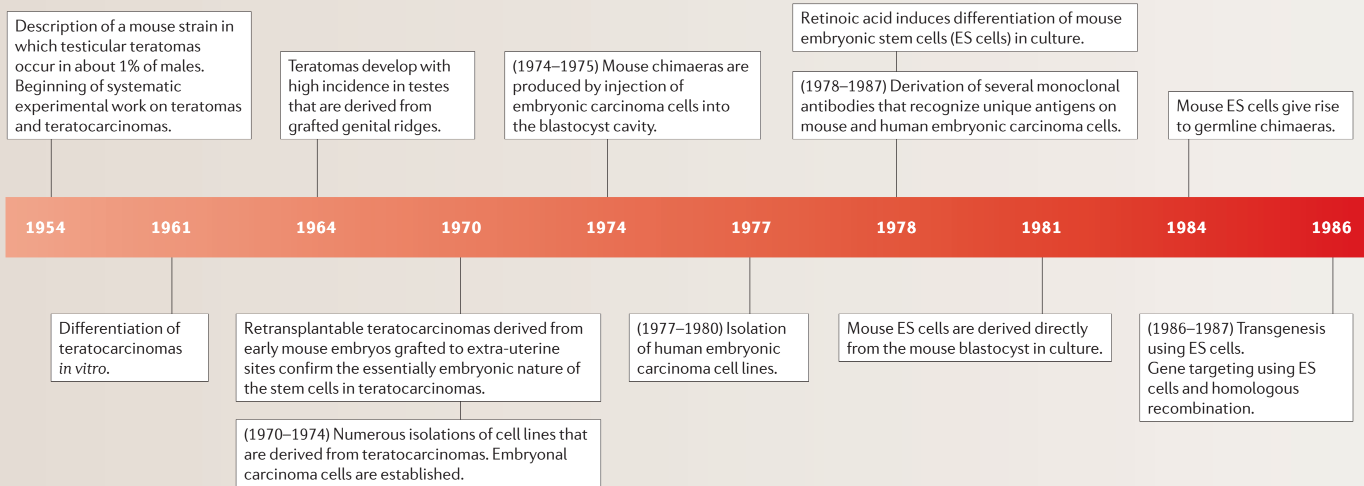

Another crucial experiment was the demonstration that a single cell derived from a tumour and injected intraperitoneally can produce all cell types that are encountered in a teratocarcinoma[3]. This was an important finding as it demonstrated that teratocarcinomas possess a unique type of stem cell, a single one of which has the capacity to grow indefinitely, ensuring the malignant nature of the tumour but at the same time being able to differentiate into multiple adult cell types (FIG. 1a,b).

The existence of such pluripotent stem cells (see BOX 1 for a discussion of nomenclature) and the observation of specific structures in the tumours that are called embryoid bodies (FIG. 1c) — because of their similarity to early embryos — indicated the embryonic origin and nature of teratocarcinomas. The demonstration that retransplantable teratocarcinomas can be directly derived from embryos grafted to extra-uterine sites[4,5] confirmed this hypothesis and initiated the concept that pluripotent stem cells of early mouse embryos and teratocarcinomas might be highly similar, if not identical.

The incidence of spontaneous teratocarcinoma is genetically controlled to a significant degree. Stevens identified a mouse strain, 129/terSv, in which the incidence of testicular teratoma was about 30% (REF. 6). It took more than 30 years to clone the gene that, when mutated, causes this high incidence[7]. The gene dead-end homologue 1 (*Dnd1*) belongs to a class of genes that code for RNA-binding proteins with a possible RNA-editing function. It is so far unclear why aberrant nucleic-acid editing would specifically cause testicular teratocarcinomas.

Another mouse strain, LT, shows a high incidence of ovarian teratoma[8] that is due to the spontaneous parthenogenetic activation of ovarian oocytes. Oocytes probably start developing owing to a failure of the meiotic check-point. The resulting embryo turns into a teratoma, again emphasizing the close relationship between embryos and tumours.

Although intensive analysis of teratomas in mice was conducted in the 1950s and 1960s, little work was carried out on similar tumours in humans, except for an attempt to study them following xenotransplantation into the hamster cheek pouch[9]. For further details and a comprehensive bibliography of this early period the reader should consult REF. 10.

Embryonal carcinoma cells

Virtually from the beginning of experimental work using *in vivo* models, attempts were also made to culture teratocarcinoma fragments and to dissect the process of differentiation from pluripotent stem cells to adult cell types. Clonal cell lines were derived that, on injection into a suitable host, recapitulated the broad differentiation pattern of the original tumours. In culture the same clones produced various cell types, some of which were morphologically identifiable as cartilage, neural tissue, myocardium and so on. It was also possible to isolate subclones that proliferated poorly *in vitro* and were unable to produce tumours *in vivo*. The overall conclusion from these early studies further emphasized the existence of pluripotent stem cells that possess the ability to differentiate into multiple cell types that are apparently identical to the cells in adult tissues. The observed differentiation was, however, haphazard and unpredictable[11–16]. A refinement of culture techniques, most notably the introduction of the cell feeder layer, allowed Martin and Evans to reliably subclone mass cultures of pluripotent teratocarcinoma cell lines[17–20]. They identified a unique cell type that grew in the form of small, tight colonies of cells that had large, clear nuclei containing prominent nucleoli and sparse, dark cytoplasm (FIG. 2a). These cells could proliferate indefinitely and produced teratocarcinomas on subcutaneous injection. When cultured in bacteriological dishes, and therefore unable to attach to plastic, the cells formed clumps that developed into simple embryoid bodies containing a core of stem cells surrounded by epithelial cells. Because these simple embryoid bodies developed extensive cavities and various intermixed cell types after further culture, they were termed cystic embryoid bodies[18]. Morphological similarities between embryoid bodies and early post-implantation mouse embryos were noticeable[20] (FIG. 1c). Cells that formed the typical colonies described above, which can differentiate and proliferate, were obviously the stem cells of the tumours and so became known as embryonal carcinoma cells.

The initial analysis of human teratocarcinomas *in vitro* was similar to the work done with mice; fragments of human tumours were placed in culture and different cell types were observed and tentatively characterized[21,22] (FIG. 2c). Subsequently, cloned cell lines derived from human teratocarcinomas were established and their properties and ability to differentiate were analysed[23–26]. This analysis included cell surface antigen expression and the ability to differentiate

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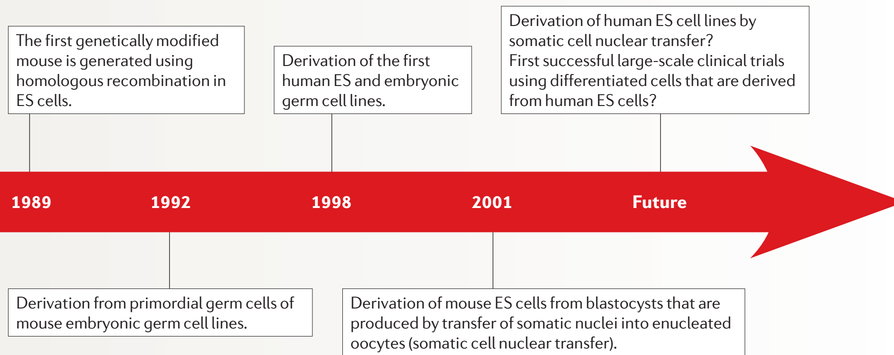

in vivo and *in vitro* (for details and further references see REFS 27,28).

From the beginning, the derivation and characterization of mouse and human embryonal carcinoma cell lines depended on the existence of suitable markers. An ideal marker had to be able to identify the live cell and had to be absolutely unique to the embryonal carcinoma cell; it also had to be lost on differentiation. Although few markers approached this standard, all of them were useful and were applied as research in this field progressed. The first marker was expression of the enzyme alkaline phosphatase, which was highly expressed in mouse and human embryonal carcinoma cells, in the cells within the inner cell mass of the mouse blastocyst, and in ectoderm and primordial germ cells[29,30]. This pattern of expression was to be repeated with most of the subsequently identified markers.

With the development of monoclonal antibodies in the mid-1970s several useful and specific reagents were soon identified. The first two monoclonal antibodies to react specifically with mouse embryonal carcinoma cells reacted with the Forssman antigen[31] and stage-specific embryonic antigen 1 (SSEA1) (REF. 32). Anti-SSEA1 monoclonal antibody, which reacts to the Le^x-like (α1–3 fucolylated N-acetyllactosamine) carbohydrate antigenic determinant[33], proved to be especially versatile and effective in monitoring embryonal carcinoma cell differentiation and isolation of primordial germ cells and is widely used today. Interestingly, although it reacts with mouse embryonal carcinoma cells, it does not react with human embryonal carcinoma cells but only with their differentiated derivatives. Two further monoclonal antibodies, one raised against mouse embryos[34] and another against human embryonal carcinoma cell lines[35], proved to be highly specific for human embryonal carcinoma cells. They both recognize cell surface carbohydrate molecules that belong to a unique globo-series ganglioside[35,36] and are known as stage-specific embryonic antigen 3 (SSEA3) and 4 (SSEA4). Mouse embryonal carcinoma cells (and also ES cells) express SSEA1 and turn on SSEA3 and SSEA4 on differentiation (see below). By contrast, human embryonal carcinoma cells (and ES cells) express SSEA3 and SSEA4 and turn on SSEA1 on differentiation[37]. Therefore, the use of this panel of monoclonal antibodies (and others isolated later) provided an easy way of monitoring both the isolation and early differentiation of mouse and human embryonal carcinoma cells (and ES cells). Early mouse embryos express SSEA1, as do mouse embryonal carcinoma cells and ES cells, and early human embryos express SSEA3 and SSEA4, as do human embryonal carcinoma cells and ES cells[38]. Apart from the SSEA series of monoclonals, another set that has proved to be equally valuable are derived against human embryonal carcinoma cells — TRA1-60 and TRA1-81 — and have been shown to react with human embryonal carcinoma cells, ES cells and early human embryos[28,37,38].

Two further aspects of the early work with mouse embryonal carcinoma cells had a unique effect on subsequent advances. The differentiation of mouse or human embryonal carcinoma cells in culture is, as mentioned previously, haphazard and unpredictable. However, Strickland and Mahdavi[39] showe d for the first time that a specific chemical compound, retinoic acid, either alone or in combination with dibutyryl cAMP, can induce the nullipotent embryonal carcinoma cell line F9 to differentiate into cells that resemble the

Glossary

Blastocyst
A mammalian embryo that is at the end of cleavage and is ready for implantation into the uterine epithelium. Depending on the species, it contains a hundred or more cells and is composed of: a continuous outside layer called the trophectoderm, which gives rise to the placenta; an inner cell mass, which gives rise to the embryo proper; and some extra-embryonic membrane. The cells of the inner cell mass can give rise to embryonic stem cells in culture.

Cell feeder layer
Cells, usually fibroblasts, that are incapable of division but provide physical support and soluble factors for the cells growing on them. The feeder layer was essential for the early derivation of embryonic stem cells.

Inner cell mass
A small clump of apparently undifferentiated cells in the blastocyst, which gives rise to the entire fetus plus some of its extra-embryonic membranes.

Intraperitoneal
Refers to injection or insertion between the viscera and the abdominal wall.

Karyotype
The chromosomal complement of a given cell.

Matrix material
Solid support surrounding and secreted by cells. Known components of extracellular matrix (for example, collagen) can be used as support for *in vitro* cell culture.

Meiotic check-point
An event during meiosis that can only proceed if some earlier event has been completed. The fully grown mammalian oocyte is arrested in the prophase of the first meiotic division (first meiotic check-point). Following hormonal stimulation the oocyte undergoes maturation by completing first meiotic division and arresting in the metaphase of the second meiotic division (second meiotic check-point). The oocyte is released from this check-point on fertilization and can complete the second meiotic division.

Monolayer culture
Growth of cells *in vitro* as a single cell layer that is attached to the bottom of a culture dish.

Parietal endoderm
One of the extra-embryonic membranes. It participates in the formation of the maternal–fetal barrier.

Primordial germ cells
Cells that are localized in a specific part of the early post-implantation embryo that will eventually migrate into gonads and give rise to germ cells (eggs and sperm). They are also probable precursors of embryonic germ cells.

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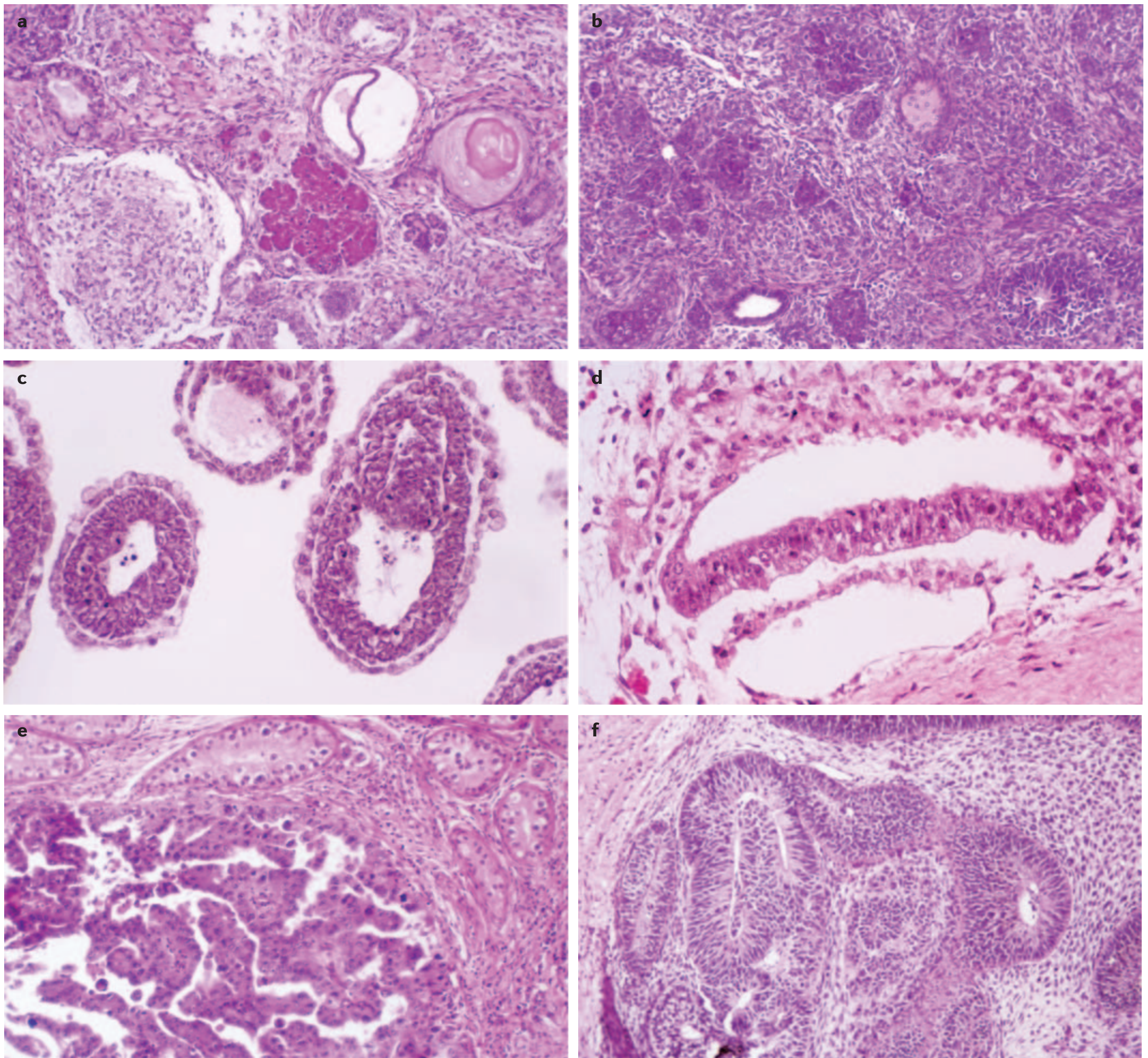

Figure 1 | **Mouse and human teratomas and teratocarcinomas.** **a** | Mouse teratomas derived from an embryo placed under the kidney capsule[4]; a haphazard mixture of mature tissues including secretory glands, muscle, keratin pearls and neural tissue. **b** | A mouse teratocarcinoma; numerous nests of embryonal carcinoma cells in highly cellular mesenchyme. **c** | Mouse embryoid bodies, which were formed after the intraperitoneal injection of mouse embryonal carcinoma cells; the structures contain two layers and are similar to early post-implantation mouse embryos. **d** | A human embryoid body in a spontaneous human testicular teratocarcinoma; it is highly similar to an early human embryo. **e** | Human testicular embryonal carcinoma adjacent to several seminiferous tubules. **f** | A human teratocarcinoma, which was formed after the injection of human embryonic stem cells into a nude mouse (that is, one that lacks T cells). Several immature neural tubes are surrounded by mesenchyme.

parietal endoderm. Similar results were obtained using another compound, hexamethylbisacetamid[40]. These observations provided the basis for all subsequent work attempting to direct and control the differentiation of embryonal carcinoma cells and ES cells *in vitro*. Similar results were also obtained using human embryonal carcinoma cells[41,42].

Given the perceived similarities between mouse embryonal carcinoma cells and the cells of early embryos, and the observation that teratocarcinomas can be derived from embryos, it was tempting to investigate whether embryonal carcinoma cells retain their embryonic nature. The most stringent test would be to determine whether they could contribute to the development of chimaeras following injection into the mouse blastocyst cavity. The first success of such an attempt was reported by Brinster in 1974 (REF. 43) and, although the contribution of embryonal carcinoma cells was modest, it was sufficient to encourage further experiments. The subsequent progress of experiments involving embryonal carcinoma cell-embryo chimaeras is puzzling. One group described

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an extensive contribution of embryonal carcinoma-derived cells in all tissue, no appearance of tumours, and even germline chimerism[44–47]. These results implied that the malignant phenotype of embryonal carcinoma cells is completely reversible and that they are in essence identical to early embryonic cells. However, other groups using different embryonal carcinoma cell lines reported limited chimerism, recurrent development of tumours derived from embryonal carcinoma cells and the absence of germline chimerism[48–50]. This would in turn indicate that embryonal carcinoma cells do retain a limited ability to differentiate but that they undergo irrevocable changes towards malignancy. The fact that different cell lines were used could provide an explanation for this discrepancy, although it is difficult to understand how one specific embryonal carcinoma cell line could produce germline chimerism in the light of recent observations that this line has an abnormal karyotype[51]. However, the issue of normalcy of embryonal carcinoma cells became irrelevant once ES cells had been isolated, after which time the extensive use of embryonal carcinoma cells became a thing of the past. In fact, the entire golden age of teratocarcinoma research lasted less than 10 years, more or less bracketed by two symposia volumes[10,52]. Although teratocarcinomas and embryonal carcinoma cells are relatively rarely used today, the information obtained from research on them was crucial for the development of ES cells.

Box 1 | **Nomenclature for stem cell differentiation potential**

The terms totipotent, pluripotent and multipotent tend to be used interchangeably, which at times causes confusion. Although it is difficult to enforce a general consensus on the precise meaning of these terms, some agreement seems to be emerging in the stem cell community. A multipotent cell can give rise to multiple cell types, but these would nevertheless be restricted to derivatives of a single germ layer (for example, a mesenchymal cell that is able to differentiate into fibroblasts, adipocytes, chondrocytes, muscle cells and so on) or to a specific sublineage (for example, haematopoietic stem cells that give rise to erythrocytes, leucocytes and lymphocytes). A pluripotent cell should be able to give rise to derivatives of all three germ layers; that is, essentially all cell types that are found in the adult organism. An embryonic stem cell would be a typical example of a pluripotent cell, although considering several recent descriptions of cells that have a similar potency but are derived from various fetal and adult tissues and organs, embryonic stem cells (ES cells) might not be the only pluripotent cell type. The term totipotent should be reserved for a cell that can produce an entire organism. In the mouse, only a zygote and a blastomere from a 2-cell stage embryo would be considered totipotent. Although it is possible to derive an entire newborn and adult mouse from ES cells, these would not be considered totipotent as the extra-embryonic membranes and placenta would be derived from a tetraploid embryo[67]. It might one day be possible to manipulate ES cells so that they and their derivatives form a complete conceptus without any contribution from an embryo. At that point ES cells could be called totipotent, but until then this term is best avoided when describing ES cells. Biological concepts often form a continuum and attempts to define precise and sharp distinctions will eventually fail. Mouse embryonal carcinoma cells are usually able to differentiate into several cell types. However, some embryonal carcinoma cell lines lose the capacity for differentiation on prolonged *in vitro* culture and are then termed nullipotent.

Embryonic stem cells

Embryonal carcinoma cells can be derived from embryos, albeit indirectly — an embryo grafted to an extra-uterine site gives rise to a teratocarcinoma from which embryonal carcinoma cells are subsequently isolated. In view of this, the next obvious step was to find out if similar cells could be obtained directly from embryos. To provide easily accessible and abundant material to study mammalian development, attempts to grow cells from early mammalian embryos were first made more than 50 years ago and many cell lines with various morphologies and characteristics have since been derived (see REFS 53,54). Although these cell lines have interesting phenotypes, none of them fulfils the criteria by which we define ES cells today. An ES cell should be capable of essentially complete differentiation, that is, it should give rise to many, if not all, tissues found in an adult animal. This differentiation should take place *in vitro*, *in vivo* in tumours derived from injected ES cells and in chimaeras. Most stringently, an ES cell should be able to give rise to germ cells in chimaeras and these germ cells should in turn be able to develop into normal, fertile adults. So far only mouse ES cells fulfil all these criteria (see below for further discussion).

The first mouse ES cell lines were derived independently by two groups from mouse blastocysts grown on a feeder layer of division-incompetent mouse fibroblasts[55,56] (FIG. 2b). Irrespective of the different methods used, the presence of the feeder layer (which had previously been used to study the differentiation of embryonal carcinoma cells) was crucial. The mouse ES cells expressed all markers of mouse embryonal carcinoma cells and were capable of remarkably extensive differentiation *in vivo* and *in vitro*. The pattern of *in vitro* differentiation was essentially the same as for embryonal carcinoma cells, going through simple and cystic embryoid bodies in which numerous adult tissues could be observed[57]. Although a feeder was necessary for the isolation of ES cells from blastocysts, it was subsequently shown that for maintenance of an established culture in its undifferentiated state (ES cells are prone to spontaneous differentiation *in vitro*) a soluble factor identified as leukaemia inhibitory factor (LIF) was required, which then became the standard ingredient of ES cell cultures[58,59]. Withdrawal of LIF and growth of ES cells in suspension results in the formation of embryoid bodies and differentiation.

Embryonal carcinoma and ES cells, early embryos and primordial germ cells all belong, at least theoretically, to the class of totipotent or pluripotent cells, explaining the extensive similarity in the expression of various markers between these cell types. These considerations, as well as the germ-cell origin of spontaneous teratocarcinomas, led to attempts to derive ES-like cells from primordial germ cells, and these attempts resulted in the isolation of embryonic germ cells[60,61]. In most aspects embryonic germ cells are identical to ES cells, although their biology has not yet been studied extensively. Interestingly, a capacity for pluripotency seems to persist among cells that give rise to gametes, and pluripotent cell lines that are similar to ES cell lines have recently been isolated from the neonatal mouse testis[62]. We now know that ES cells can be derived from primordial germ cells and possibly later stages of germ-cell differentiation. It was therefore logical to ask whether ES cells that can differentiate into many adult tissues can also differentiate into germ cells. One could visualize many practical applications if this were true. Some recent reports have suggested that ES cells can differentiate into cells that are similar to male and female germ cells[63–65]. However, so far there is no indication that these are also completely functional gametes that can support normal embryonic development.

Relatively soon after their isolation, mouse ES cells were tested for their ability to form germline chimaeras and proved to be much superior to embryonic carcinoma cells[66]. Today, properly handled mouse ES cells are extremely efficient in contributing to

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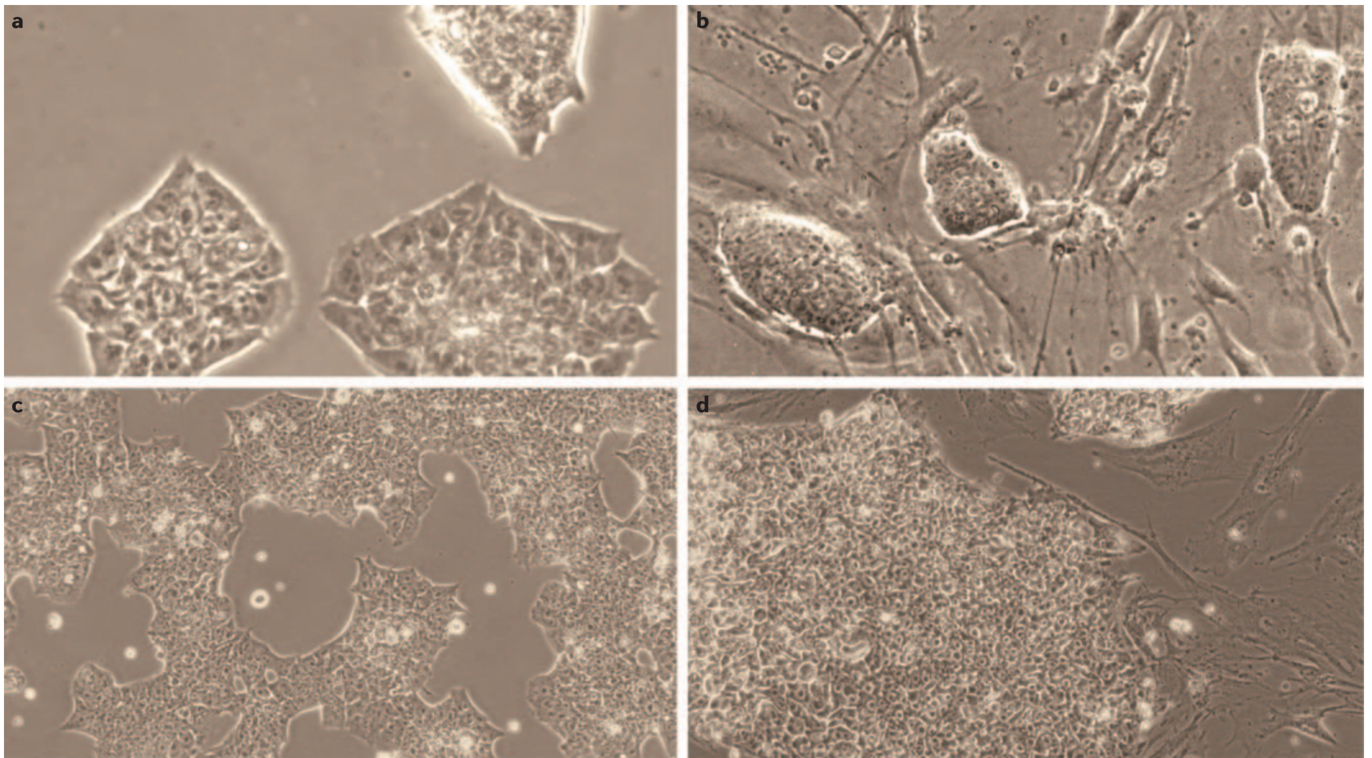

Figure 2 | **Mouse and human embryonal carcinoma and embryonic stem cells *in vitro*.** **a** | Typical nests of mouse embryonal carcinoma cells, from a culture of an F9 cell line[29]. **b** | Mouse embryonic stem cells on a fibroblast feeder layer, line D3 (REF. 57). **c** | Human embyonal carcinoma cells, line 2102Ep (REF. 23). **d** | Human embyonic stem cells on a fibroblast feeder layer, line H7 (REF. 86).

germline chimaeras, moreover they are able to form an entire adult when injected into tetraploid blastocysts, the contribution of which is restricted to extra-embryonic membranes and placenta[67]. Germline chimerism initially enabled transgenesis[68,69] and, in combination with another remarkable development — gene targeting by homologous recombination — turned mouse ES cells into a primary tool for the study of gene function[70–77]. The success of this particular line of research is such that we now have several thousands of mutations and can expect that a mutation for every mouse gene will soon be available.

The recent interest in ES cells as a possible means of developing cell and tissue therapies in humans (see below) prompted a renewed interest in mouse ES cells, how to culture them and how to control their *in vitro* differentiation. It is now possible to derive and culture mouse ES cells entirely without feeder cells, in the presence of just LIF and bone morphogenetic proteins (BMPs); these cells will retain their capacity for differentiation and their ability to produce germline chimaeras[78].

From early attempts to manipulate the differentiation of mouse embryonic carcinoma cells by chemicals[79] we have now moved to the stage at which we are almost able to direct the differentiation of mouse ES cells along predetermined pathways using a combination of chemicals, growth factors and matrices (see for example REFS 80–83). Advances along these lines have been made possible by a much-improved understanding of the factors and mechanisms that regulate the differentiation of cells and tissues during normal development[84]. In many examples differentiation required the preliminary step of morphogenesis into embryoid bodies, but it is now apparent that by applying the correct growth factors, direct transition of ES cells into neuronal stem cells can be achieved in monolayer culture[85]; such a development should have a tremendous effect on work with human ES cells.

Human embryonic stem cells

The derivation of the first human ES cell lines[86] (FIGS 1f,2d) and human embryonic germ-cell lines[87] lagged significantly behind their mouse counterparts. Considering that isolation techniques were comparable and the necessary markers to identify human ES and embryonal carcinoma cells were all available[86,87], the reasons for this delay are probably the difficulties involved in obtaining suitable human embryonic material and an understandable reluctance of most investigators to work in a field that is fraught with potential legal problems and political and moral dilemmas. Several factors were probably crucial for these early successes: experience in isolating and working with primate ES cells[88], a reliable source of high-quality human embryos from IVF clinics and the foresight of certain private companies to provide funding for research that, at least in the United States, could not be supported by Federal funds. However, once the first cell lines were isolated and scientists and the public realized that such cells could have an enormous effect on medical practice, the work progressed at a remarkable speed, although much remains to be done in terms of defining optimal culture conditions and designing precise differentiation protocols. The isolation and maintenance of human ES cells, the derivatives of which are intended for clinical use, will require special safety precautions and therefore the absence of any foreign, especially animal, cells and proteins. Although the derivation of human ES cells in feeder-free, serum-free conditions has not yet been achieved, the continuous simplification and optimization of culture conditions indicate that this goal is not far away[89–92]. Similarly, experience with inducing differentiation of mouse cells is continuously

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being applied to human ES cells[93–95]. We should nevertheless bear in mind that human ES cells, the derivatives of which are to be used therapeutically, must be flawless, and the recent observation that significant genomic alterations accumulate in human ES cells in culture is certainly a cause for concern[96].

Other embryonic stem cells

Considering the possible uses of pluripotent cells, it is no wonder that many attempts to isolate ES cells from other mammals have been made. Taking the ability to form germline chimaeras as a mandatory criterion (obviously, this cannot be tested for human ES cells for which we have to rely on the ability to differentiate and on the expression of suitable markers), it would seem that the only true ES cell lines in existence today are mouse lines. Numerous reports described the isolation from various mammalian embryos of cells that are reminiscent of mouse ES cells (for a review see REF. 97) but none of them was capable of germline chimerism. Even when initial investigations led scientists to believe that, for example, rat ES cells can produce chimaeras[98], subsequent analysis demonstrated that these dysmorphic chimaeras were actually derived from mouse ES cells that had contaminated the culture of the presumed rat ES cells[99]. Non-human primate ES cell lines have been isolated[88] and these would be valuable as another model for the clinical application of human cells[100–102]; however, there is as yet no evidence that these cells are able to form chimaeras or germline chimaeras.

Alternatives for and the future of ES cells

Once it was realized that differentiated derivatives of human ES cells could be used in the therapy of many degenerative diseases and injuries, it also became apparent that it would be of clear advantage if the cells transplanted into patients were genetically identical to the recipients, therefore obviating the need for life-long immunosuppression[103]. The most obvious way to achieve this is to produce ES cells from the patient by a procedure now known as somatic cell nuclear transfer (SCNT), which is also called therapeutic cloning (FIG. 3). Mouse ES cells can be produced from blastocysts derived from enucleated oocytes into which somatic cell nuclei have been transferred[104–106]. Similar results were recently reported for human ES cells[107,108], although it is now clear that these reports were fraudulent and that, as yet, no human ES cell line has been derived from cloned human embryos. Although it awaits experimental confirmation, there is no *a priori* reason why this should not be achieved and, considering the number of laboratories working towards this goal, we should know soon. The faked reports are perhaps the most egregious examples of questionable behaviour, which were prompted by the visions of fame and lucre that are associated with stem cells. We have witnessed the publication of poorly supported and vastly exaggerated claims, not to mention dissemination of information by press releases, news conferences and other means, that are not quite compatible with accepted scientific practice. One is inevitably reminded of Joseph Conrad's writing in *Under Western Eyes*, admittedly in another context: "The scrupulous and the just, the noble, humane, and devoted natures: the unselfish and the intelligent may begin a movement — but it passes away from them … Afterwards comes the turn of all the pretentious intellectual failures of the time."

SCNT, although straightforward, is technically demanding; moreover, it requires a substantial supply of human oocytes. In addition, the destruction of a human blastocyst that is necessitated by the procedure raises significant opposition in many countries on moral and religious grounds. It is therefore no wonder that several attempts have been

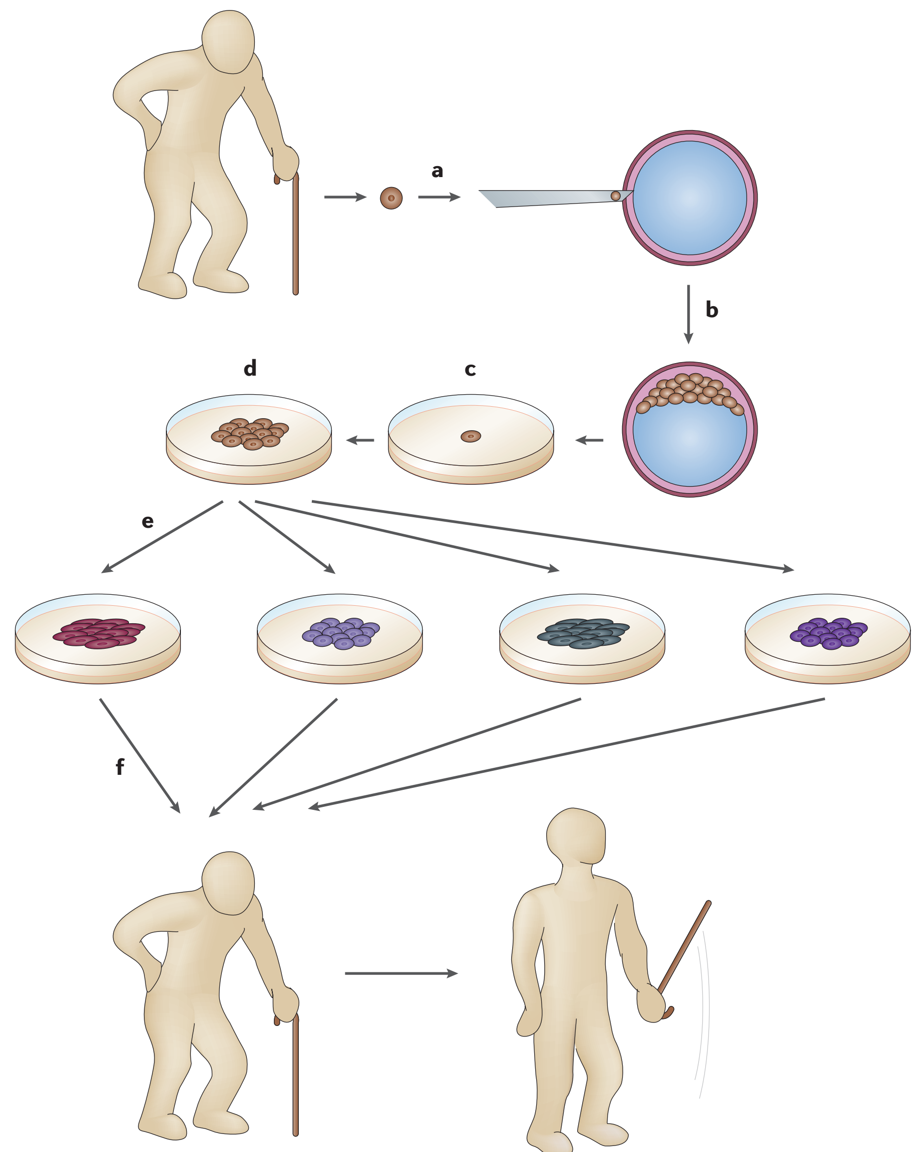

Figure 3 | **Somatic cell nuclear transfer procedure.** Somatic cell nuclear transfer is a procedure that is used to create embryonic stem cells that are genetically identical to a specific individual. A nucleus from a patient's cell is placed into an enucleated oocyte (**a**), which develops into a blastocyst (**b**). The blastocyst is placed in culture (**c**), and embryonic stem cells are then derived (**d**). After directed differentiation, desired cell types are isolated (**e**) and injected into the nuclear donor (**f**), to whom the cells are genetically identical and therefore immunocompatible.

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made to design methods that would enable the isolation of ES cells without destroying an entity that is capable of normal development. These include the isolation of mouse ES cells from a single blastomere of an 8-cell stage mouse embryo[109] or from embryos that have been produced by nuclear transfer and are incapable of development owing to an induced mutation in the nuclear donor cell line[110]. Although technically impressive and scientifically sound, these methods do not in fact satisfy the primary ethical criterion; that is, they cannot ensure with absolute certainty that an entity that is capable of development is not destroyed[111]. It would be ideal if reprogramming a patient's own cells into ES cells could be achieved directly by manipulation in culture; that is, exposure to suitable reprogramming factors and conditions. Although the first small indications that this might be possible have been published[112,113], it is obvious that much more work will have to be done before the technique becomes a reality. In fact, a systemic approach to this problem might be most promising. The generation of random libraries of small molecules or artificial transcription factors would enable the initiation of a large screen for the reprogramming capacity of adult cells into ES cells [114–116]. A similar screen could be designed to test for the role of matrix materials in reprogramming[117]. Initial screens could be designed to test for the maintenance of ES cells and the results of such screens applied to the derivation of ES-like cells directly from adult cells. A better understanding of transcriptional circuitry of ES cells[118] should be of considerable help in designing such experiments. It is to be hoped that these and similar approaches will enable us to fully realize the tremendous scientific and clinical potential of ES cells.

Contemplating the history that has been briefly delineated here, it is impressive to see how early experiments and observations that dealt with the biology of rare tumours and their stem cells led to a much better understanding of developmental processes and also provided hope for the treatment and even cure of many otherwise incurable diseases. This case history again reinforces the old truism that unfettered basic research driven only by scientific curiosity is usually the best way to discover things of enormous practical value.

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