This is a repository copy of *The origins of human pluripotent stem cells: the road from a cancer to regenerative medicine*.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/210723/

Version: Published Version

Article:

Andrews, P.W. orcid.org/0000-0001-7215-4410 (2024) The origins of human pluripotent stem cells: the road from a cancer to regenerative medicine. In Vitro Cellular & Developmental Biology - Animal, 60 (5). pp. 514-520. ISSN 1071-2690

https://doi.org/10.1007/s11626-024-00865-8

Reuse

This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here: https://creativecommons.org/licenses/

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

In Vitro Cellular & Developmental Biology - Animal
https://doi.org/10.1007/s11626-024-00865-8

INVITED REVIEW

The origins of human pluripotent stem cells: the road from a cancer to regenerative medicine

Peter W. Andrews[1]

Received: 15 January 2024 / Accepted: 31 January 2024 / Editor: Yohei Hayashi
© The Author(s) 2024

Abstract
The notion of using pluripotent stem cells (PSCs) as a source of differentiated cell types for replacement of disease or damaged tissues in regenerative medicine is now an active area of research, with approaches to treating eye diseases such as age-related macular degeneration or Parkinson's disease now on the horizon. But the foundations for this research lie in a quite different area of science, namely the role of genetics of cancer. In this review, we trace the evolution of ideas starting with the discovery that strain 129 mice are particularly subject to develop germ cell tumors, through the identification of embryonal carcinoma (EC) cells as the stem cells of the teratocarcinoma manifestation of these tumors, to the recognition of their relationship to pluripotent cells of the early embryo, and eventually their role in the derivation of embryonic stem cells, first from mouse embryos and then from primates including humans. This is a story that illustrates how science commonly develops through the interests and insights of individual investigators, often with unexpected and unintended outcomes.

Keywords Embryonal carcinoma · Embryonic stem cells · Induced pluripotent stem cells · Pluripotent stem cells · Human · Mouse · Differentiation

Introduction

In 1954, Leroy Stevens was working at the Jackson Laboratory in Bar Harbor Maine, a research center established to use the laboratory mouse to investigate the origins of cancer (https://www.jax.org/news-and-insights/2014/november/85-years-of-discovery). Research there had shown that different cancers seemed to appear more often in some strains of mice than in others, suggesting a genetic link to cancer susceptibility. In that year, Stevens published a paper showing that the males of a mouse strain called 129 had a propensity to develop a testicular cancer known as a teratoma, which was unknown in other strains of mice (Stevens and Little 1954).

Teratomas are peculiar tumors that typically arise in the gonads and contain a wide array of jumbled tissues as if from an embryo that had become disorganized (Mostofi and Price 1973; Scully 1979). Although rare in humans, they had long attracted the attention of pathologists because of their unusual nature (Damjanov and Wewer-Albrechtsen 2013). In women, they are typically benign but they can grow to large sizes so are life threatening if not surgically removed. By contrast, in men, these tumors are almost always highly malignant and so were often designated teratocarcinomas. They tend to occur in young men after puberty when other cancers are rare, so in that age group they are among the most common cancers. The testicular tumors that Stevens found in 129 mice were also often malignant and could be maintained indefinitely by retransplantation to successive male 129 mice. They were therefore seen as a new tool for investigating this type of cancer.

Teratocarcinomas are distinguished from teratomas by the presence of a histologically distinctive, undifferentiated cell type called embryonal carcinoma (EC) (Damjanov and Andrews 2007) and it was thought that these are the cells that are responsible for the malignant properties of these tumors, as well as being able to differentiate into all the somatic cell types that characterize teratomas. A crucial early development of this cancer model was the demonstration by Larry Kleinsmith and Barry Pierce, published in 1964, that a single cell, likely an EC cell, isolated from a teratocarcinoma and transplanted to another mouse was able to generate another teratocarcinoma with a typical wide

✉ Peter W. Andrews
P.W.Andrews@sheffield.ac.uk

[1] The Centre for Stem Cell Biology, The School of Biosciences, The University of Sheffield, Western Bank, Sheffield S10 2TN, UK

Published online: 23 February 2024

array of differentiated cell types (Kleinsmith and Pierce 1964). Work over the succeeding years confirmed the stem cell nature of EC cells. The sensitivity of these cells to new chemotherapeutic agents such as cis-platinum (Oosterhuis *et al*. 1984) also provided the basis for the current successful treatment of a type of cancer that had killed many young men in previous years (Einhorn 1981).

Embryonal carcinoma cells and embryos The complex histology of teratomas had always suggested a relationship to embryogenesis. Not only do they contain tissues derived from all three germ layers of the embryo—ectoderm, mesoderm, and endoderm—they often also contain structures known as embryoid bodies that morphologically resemble early embryos (Damjanov and Andrews 2016). In his extensive body of work following his 1954 publication, Stevens provided definitive evidence that teratomas in the mouse have a germ cell origin: in males they are initiated in utero from primordial germ cells around the time that they migrate into the genital ridge, and do not form in embryos homozygous for *Sl/Sl*, in which germ cells do not develop (Stevens 1967a, b). In humans, more circumstantial evidence indicated that here too testicular teratocarcinomas are initiated by a defect in germ cell development in utero, albeit that they typically do not manifest until after puberty (Skakkebaek *et al*. 1987). In females, it seems likely that ovarian teratomas may arise later following parthenogenetic activation of oocytes in the ovary (Stevens and Varnum 1974).

The germ cell origin of teratomas and teratocarcinomas helped to support the notion that their development reflects a caricature of embryogenesis. Further evidence was provided by the development of teratomas and teratocarcinomas from mouse embryos that had been transplanted to ectopic sites (Solter *et al*. 1970; Stevens 1970). The establishment and detailed characterization of mouse EC cell lines in vitro also contributed to understanding the relationship of teratomas to embryos. Brenda Finch and Boris Ephrussi were the first to successfully establish cell lines from explanted murine teratocarcinomas, and their cells retained the ability to differentiate when transplanted back into a mouse (Finch and Ephrussi 1967). Subsequently, several groups established such lines and showed that the cells could be cloned while retaining the capacity for differentiation in vitro and in vivo (Rosenthal *et al*. 1970; Martin and Evans 1974, 1975; Nicolas *et al*. 1975). Further, these pluripotent cells, now characterized as EC cells, were found to express markers, notably alkaline phosphatase (Bernstine *et al*. 1973) and a cell surface antigen named 'F9' (Artzt *et al*. 1973), in common with the pluripotent cells of the inner cell mass at the blastocyst stage of embryonic development. However, definitive proof of the embryonic character of mouse EC cells came from the direct demonstration, first by Ralph Brinster (Brinster 1974) and confirmed by others (Mintz and Illmensee 1975; Papaioannou et al. 1975), that EC cells injected into a mouse blastocyst, which was allowed to implant in a pseudo pregnant female, would take part in embryonic development and contribute to normal tissues of the mouse that was born.

These observations together with more detailed characterization of cultured mouse EC cells provided the basis for the discovery, independently by Martin Evans and Matt Kauffman (1981) and by Gail Martin (1981), that inner cell mass cells from mouse embryos, explanted to culture, could be maintained indefinitely in vitro, while maintaining pluripotency. Such embryo-derived cells, termed embryonic stem (ES) cells, proved capable of forming teratomas when transplanted to ectopic sites in adult mice, or taking part in development to form chimeric mice when transferred to blastocysts that were allowed to develop to term. Since the chimerism included the germline (Bradley *et al*. 1984), genetic manipulation of ES cells in culture provided a new route to generating 'transgenic' mice to investigate the function of key genes of interest in development or disease (Robertson *et al*. 1986).

Human embryonal carcinoma cells Building on the success of studies with mouse EC cells, during the 1970s, several researchers began to establish cell lines from human teratocarcinomas, from biopsies of testicular cancers. This work was driven in part by a medical interest in these tumors, but also by the thought that, as in the mouse, these cells might be useful tools for human embryology. Initially, the discovery that two such human teratocarcinoma cell lines contained cells expressing the F9 antigen, which had been used to characterize mouse EC cells, encouraged the view that human EC cells would closely resemble mouse EC cells (Hogan *et al*. 1977; Holden *et al*. 1977). However, in a detailed comparison of eight human teratocarcinoma cell lines, we found several that exhibited the typical morphology of mouse EC cells, and formed, in immunodeficient mice, xenograft tumors that were histologically similar to EC cells in clinical examples of human teratocarcinomas (Andrews *et al*. 1980). These cells did not express another, monoclonal antibody-defined, antigen, Stage-Specific Embryonic Antigen-1 (SSEA-1), which otherwise showed similar expression patterns to the F9 antigen, being also expressed by mouse EC cells and the ICM of mouse embryos (Solter and Knowles 1978).

In a subsequent study, we cloned and characterized in detail one particular human testicular teratocarcinoma cell line, 2102Ep (Andrews *et al*. 1982). These cells, which formed xenograft tumors that were recognizable by clinical histopathologists as pure embryonal carcinoma, likewise did not express SSEA-1 in culture provided that they were continually maintained at a high cell density. However, they did express another antigen, SSEA-3, that is expressed on cleavage stage mouse embryos but not on their inner cell mass

cells, or mouse EC cells (Shevinsky *et al*. 1982). SSEA-3 had also been shown to mark EC cells in clinical testicular tumors (Damjanov *et al*. 1982). Nevertheless, if 2102Ep cells were cultured at low cell densities, they appeared to differentiate morphologically, apparently towards a trophoblastic lineage (Damjanov and Andrews 1983), when they did begin to express SSEA-1 while downregulating SSEA-3. Thus, we concluded that human EC cells differ from their murine counterparts, at least with regard to expression of these cell surface antigens, and that in humans, in contrast to mice, EC cells, expression of SSEA-1 is an indicator of differentiation.

Unfortunately, 2102Ep cells and several of the other human teratocarcinoma cell lines initially available showed little sign of further differentiation into clearly identifiable somatic cells. Subsequently, we and others were able to identify human EC cell lines that did show somatic differentiation and did exhibit the antigen phenotype that we first characterized in 2102Ep cells (Andrews *et al*. 1984b; Thompson *et al*. 1984; Pera et al. 1989). In particular, we studied in more detail a pluripotent human EC cell line, NTERA2, which formed well-differentiated xenograft teratocarcinomas in immunosuppressed mice, and differentiated extensively in vitro, generating neurons as well as other cell types, in response to retinoic acid (Andrews *et al*. 1984b; Andrews 1984). Using these cells, additional developmentally regulated cell surface antigens of human EC cells were characterized, including SSEA-4, TRA-1–60 and TRA-1–81, and GCTM2 and differences from mouse EC cells were confirmed (Kannagi *et al*. 1983; Andrews *et al*. 1984a; Fenderson *et al*. 1987; Pera *et al*. 1988). What was unclear at this stage was whether the differences reflected species differences or differences in embryonic stage to which the cells correspond.

Human embryonic stem cells Unlike mouse EC cells, which are typically diploid though occasionally with some limited chromosomal rearrangements, human EC cells are generally highly aneuploidy, typically with an approximately triploid chromosome number with many rearrangements. Further, even the best human EC cells seemed limited with respect to the differentiated cells they would form. Consequently, from the early days following the description of mouse ES cells, there was an interest in whether it would be possible to derive corresponding cells from human embryos. In principle, human embryos could be obtained after the successful development of in vitro fertilization, but progress was hampered not only by the logistical problems of accessing embryos, but also by ethical concerns about the use of human embryos in research.

A significant step forward came from the derivation of ES cells first from rhesus monkey and then marmoset embryos by Jamie Thomson, working at the Wisconsin Primate Centre (Thomson *et al*. 1995, 1996). Strikingly, these monkey ES cells more closely resembled human rather mouse EC cells with respect to their surface antigen phenotype. Nevertheless, they were capable of extensive differentiation in vitro and formed well-differentiated teratomas in xenogeneic hosts. Importantly, they provided the experience for Thomson to derive ES cell lines from human embryos some 3 yr later (Thomson *et al*. 1998). Again, these human cells also closely resembled human EC cells, but with normal karyotypes and a capacity for extensive differentiation.

Opportunities and challenges Following the first publication describing human ES cells, the notion that these cells could provide a source of differentiated cells to replace diseased or damaged tissues, a field now often encapsulated by the term 'regenerative medicine,' gained rapid traction (Gearhart 1998; Pedersen 1999; Daley 2002). In fact, the idea had already been floated by a group working with neuronal derivatives of NTERA2 EC cells for the treatment of stroke (Borlongan *et al*. 1998; Kondziolka *et al*. 2000), but ES cells with their apparently normal karyotype and extensive capacity for differentiation were immediately seen as better candidates for the approach. Also, reversing the extensive damage caused by stroke presents enormous challenges making it a poor candidate for early trials. Interest then coalesced around medical conditions that were confined to the well-characterized loss of particular cell types, notably diabetes, age-related macular degeneration (AMD), and Parkinson's disease. Despite the many challenges of preparing cells to standards that permit regulatory approval for clinical applications, trials of transplanting retinal pigment cells derived from ES cells for treating AMD begun within 20 yr of the first description of human ES cells (Schwartz *et al*. 2012; Vitillo *et al*. 2019; da Cruz et al. 2018), and trials for Parkinson's disease have also been planned (Barker *et al*. 2017). These two conditions have the further advantages for first in man trials because they affect cells in confined organs, the eye and the brain, that may represent immune privileged sites, they required only small numbers of cells, and there were already prior studies that provide a proof of concept that the approach could potentially effect a cure.

In parallel with the interest in regenerative medicine, it also became apparent that the differentiation of ES cells could offer opportunities to obtain large numbers of differentiated cell types that could be used for testing the safety and efficacy of potential new drugs, or for exploring the mechanisms of many medical conditions. Indeed, in the pharmaceutical industry, many candidate drugs fail in late stages of development because of liver or cardiac toxicity. Consequently, much effort has been put into generating hepatocytes and cardiomyocytes from ES cells for this purpose (Lu and Yang 2011; Meseguer-Ripolles *et al*. 2018). Likewise, recent studies have developed ways to produce

'embryoids' from ES cells to provide tools for investigating early embryogenesis and causes of abnormal development (Amadei *et al*. 2022).

However, notwithstanding the great excitement about the potential uses of ES cells, exploiting their potential has also faced many challenges. Of these, perhaps the biggest has been the ethical issues of research involving human embryos (https://www.eurostemcell.org/embryonic-stem-cell-research-ethical-dilemma). To some, any experimental work with human embryos is an anathema, and this is reflected in the laws and regulations preventing work with ES cells in some jurisdictions. To others, for example in the UK, a more pragmatic approach is acceptable, so that work with early embryos, typically up to 14 d post fertilization, is legally permissible for particular purposes such as the production of ES cells. However, the discovery by Shinya Yamanaka and others (Takahashi and Yamanaka 2006; Takahashi *et al*. 2007; Yu *et al*. 2007) that somatic cells can be reprogrammed to a state similar, if not identical to that of embryo-derived ES cells, has provided a solution to this problem. These so-called induced pluripotent stem (iPS) cells typically express all the characteristics of ES cells including their capacity for differentiation, though present their own challenges such as the need to avoid genetic changes resulting from retention of the genes transfected into the cells to achieve reprogramming. Nevertheless, iPS cells are now a widely used alternative to ES cells in many studies, and trials of iPS cell–derived derivatives for AMD and Parkinson's disease have been initiated (Takahashi 2021; Akiba et al. 2023).

Of the other practical difficulties in exploiting the opportunities of ES and iPS cells, perhaps the most tricky is their propensity to acquire genetic changes after prolonged passage (Halliwell *et al*. 2020a). Although human ES and iPS cells are typically euploid when first derived, they have a propensity to acquire non-random karyotypic changes, particularly gains of the long arm of chromosomes 1, 17, and 20, and the short arm of chromosome 12. Intriguingly, these changes are also common among the many other deviations from diploidy in human EC cells. This propensity for non-random karyotypic change has been since widely confirmed (The International Stem Cell Initiative *et al*. 2011; Andrews *et al*. 2017). In addition to karyotypic changes, smaller genomic changes including single base changes also occur and these may also be non-random such as variants affect *TP53* (Merkle *et al*. 2017). These non-random variants can appear in cultures very rapidly and almost certainly reflect selective growth advantages that they confer on the cells.

In contrast to somatic cells, ES and iPS cells are particularly susceptible to DNA replication stress and the formation of double strand breaks (Halliwell *et al*. 2020b). Surprisingly, however, the overall mutation rate in ES cells is very low, comparable to that of somatic cells and much lower than many cancer cells (Thompson *et al*. 2020). These seemingly contradictory observations can be reconciled by a further observation that in response to DNA replication stress of ES and iPS cells tend to die through apoptosis, in contrast to somatic cells (Desmarais *et al*. 2012, 2016). Tellingly, many of the non-random changes seen in ES and iPS cells appear to control apoptosis; e.g., gains of the long arm of chromosome 20 appear to be driven by increased expression of *BCL2L1* located on that chromosome (Avery *et al*. 2013). Cells with such mutations appear able to escape apoptosis in response to DNA damage (Halliwell *et al*. 2020a).

Although progress is being made in understanding the mechanisms by which genetic variants arise, assessing the consequences of particular genetic variants for different applications remains a substantial problem. A recent report on 'Standards for Research with Human Stem Cells' by the International Society for Stem Cell Research (https://www.isscr.org/standards) highlighted this point, and strongly recommended that careful attention is paid to reporting in full the nature of any genetic variants present in cells used for particular experiments, so that retrospective analysis may provide important clues in the future. The biggest concern is the possibility of cancer developing in patients arising from derivative cells used for regenerative medicine applications. Although animal models of tumorigenicity can be useful, they are expensive to carry out and certainly cannot provide a definitive conclusion as the tumorigenic potential of particular variant cells in specific human situations. It is likely that new approaches, perhaps based on extensive bioinformatics data, will be needed.

Conclusion

It is now 70 yr since Leroy Stevens described the susceptibility of strain 129 mice to testicular teratomas. In that period, experimental research with these tumors and cell lines derived from them has laid the foundations for the development of ES cells, and eventually iPS cells in both mouse and humans. ES cells from the laboratory mouse continue to provide the chief means for genetically manipulating mice to provide tools to address the mechanisms of embryonic development and the causes of abnormal fetal development, as well as the causes of aging and disease in the adult. Despite the continuing challenges of controlling their differentiation to specific cell types and addressing the problems of culture-induced genetic variation, human ES and iPS cells now offer important opportunities for regenerative medicine as well as for optimizing drug discovery and understanding disease mechanisms in humans. It is striking, though, that these opportunities were not obvious when the first mouse teratocarcinoma lines were isolated.

Funding The author's work has been supported by the UK Regenerative Medicine Platform, MRC reference grant no. MR/L012537/1, the Medical Research Council grant MR/V002163/1, and the European Union's Horizon 2020 Research and Innovation program H2020-FET-PROACT-2018–01 under grant agreement no. 824070.

Declarations

Conflict of interest PWA receives Royalties from the Wistar Institute from sales of the TRA series of antibodies and is a member of the SAB of TreeFrog Therapeutics.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Akiba R, Takahashi M, Baba T, Mandai M (2023) Progress of iPS cell-based transplantation therapy for retinal diseases. Jpn J Ophthalmol 67:119–128

Amadei G, Handford CE, Qiu C, De Jonghe J, Greenfeld H, Tran M, Martin BK, Chen D-Y, Aguilera-Castrejon A, Hanna JH, Elowitz MB, Hollfelder F, Shendure J, Glover DM, Zernicka-Goetz M (2022) Embryo model completes gastrulation to neurulation and organogenesis. Nature 610:143–153

Andrews PW (1984) Retinoic acid induces neuronal differentiation of a cloned human embryonal carcinoma cell line in vitro. Dev Biol 103:285–293

Andrews PW, Banting G, Damjanov I, Arnaud D, Avner P (1984a) Three monoclonal antibodies defining distinct differentiation antigens associated with different high molecular weight polypeptides on the surface of human embryonal carcinoma cells. Hybridoma 3:347–361

Andrews PW, Ben-David U, Benvenisty N, Coffey P, Eggan K, Knowles BB, Nagy A, Pera M, Reubinoff B, Rugg-Gunn PJ, Stacey GN (2017) Assessing the safety of human pluripotent stem cells (PSCs) and their derivatives for clinical applications. Stem Cell Reports 9:1–4

Andrews PW, Bronson DL, Benham F, Strickland S, Knowles BB (1980) A comparative study of eight cell lines derived from human testicular teratocarcinoma. Int J Cancer 26:269–280

Andrews PW, Damjanov I, Simon D, Banting G, Carlin C, Dracopoli NC, Fogh J (1984b) Pluripotent embryonal carcinoma clones derived from the human teratocarcinoma cell line Tera-2: differentiation in vivo and in vitro. Lab Invest 50:147–162

Andrews PW, Goodfellow PN, Shevinsky L, Bronson DL, Knowles BB (1982) Cell surface antigens of a clonal human embryonal carcinoma cell line: morphological and antigenic differentiation in culture. Int J Cancer 29:523–531

Artzt K, Dubois P, Bennett D, Condamine H, Babinet C, Jacob F (1973) Surface antigens common to mouse cleavage embryos and primitive teratocarcinoma cells in culture. Proc Natl Acad Sci USA 70:2988–2992

Avery S, Hirst AJ, Baker D, Lim C-Y, Alagaratnam A, Skotheim RI, Lothe RA, Pera MF, Colman A, Robson P, Andrews PW, Knowles BK (2013) Bcl-xL mediates the strong selective advantage of a 20q1121 amplification commonly found in human embryonic stem cell cultures. Stem Cell Reports 1:379–386

Barker RA, Parmar M, Studer L, Takahashi J (2017) Human trials of stem cell-derived dopamine neurons for Parkinson's disease: dawn of a new era. Cell Stem Cell 21:569–573

Bernstine EG, Hooper ML, Grandchamp S, Ephrussi B (1973) Alkaline phosphatase activity in mouse teratoma. Proc Natl Acad Sci USA 70:3899–3903

Borlongan CV, Tajima Y, Trojanowski JQ, Lee VM, Sanberg PR (1998) Transplantation of cryopreserved human embryonal carcinoma-derived neurons (NT2N cells) promotes functional recovery in ischemic rats. Exp Neurol 149:310–321

Bradley A, Evans M, Kaufman MH, Robertson E (1984) Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. Nature 309:255–256

Brinster RL (1974) The effect of cells transferred into the mouse blastocyst on subsequent development. J Exp Med 140:1049–1056

da Cruz L, Fynes K, Georgiadis O, Kerby J, Luo YH, Ahmado A, Vernon A, Daniels JT, Nommiste B, Hasan SM, Gooljar SB, Carr A-JF, Vugler A, Ramsden CM, Bictash M, Fenster M, Steer J, Harbinson T, Wilbrey A, Tufail A, Feng G, Whitlock M, Robson AG, Holder GE, Sagoo MS, Loudon PT, Whiting P, Coffey PJ (2018) Phase 1 clinical study of an embryonic stem cell-derived retinal pigment epithelium patch in age-related macular degeneration. Nat Biotech 36:328–337

Daley GQ (2002) Prospects for stem cell therapeutics: myths and medicines. Curr Opin Genet Dev 12:607–613

Damjanov I, Andrews PW (1983) Ultrastructural differentiation of a clonal human embryonal carcinoma cell line in vitro. Cancer Res 43:2190–2198

Damjanov I, Andrews PW (2007) Correspondence regarding: The terminology of teratocarcinomas and teratomas. Nat Biotech 25:1212

Damjanov I, Andrews PW (2016) Teratomas produced from human pluripotent stem cells xenografted into immunodeficient mice - a histopathology atlas. Int J Dev Biol 60:337–419

Damjanov I, Fox N, Knowles BB, Solter D, Lange PH, Fraley EE (1982) Immunohistochemical localization of murine stage-specific embryonic antigens in human testicular germ cell tumors. Am J Pathol 108:225–230

Damjanov I, Wewer-Albrechtsen N (2013) Testicular germ cell tumors and related research from a historical point of view. Int J Dev Biol 57:197–200

Desmarais JA, Hoffmann MJ, Bingham G, Gagou ME, Meuth M, Andrews PW (2012) Human embryonic stem cells fail to activate CHK1 and commit to apoptosis in response to DNA replication stress. Stem Cells 30:1385–1393

Desmarais JA, Unger C, Damjanov I, Meuth M, Andrews P (2016) Apoptosis and failure of checkpoint kinase 1 activation in human induced pluripotent stem cells under replication stress. Stem Cell Res Ther 7:17–23

Einhorn LH (1981) Testicular cancer as a model for a curable neoplasm: the Richard and Hinda Rosenthal Foundation Award Lecture. Cancer Res 41:3275–3280

Evans MJ, Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. Nature 292:154–156

Fenderson BA, Andrews PW, Nudelman E, Clausen H, Hakomori S (1987) Glycolipid core structure switching from globo- to lacto- and ganglio-series during retinoic acid-induced differentiation of TERA-2-derived human embryonal carcinoma cells. Dev Biol 122:21–34

Finch BW and Ephrussi B (1967) Retention of multiple developmental potentialities by cells of a mouse testicular teratocarcinoma during prolonged culture in vitro and their extinction upon

hybridisation with cells of permanent lines. Proc Natl Acad Sci USA 57:615–621
Gearhart J (1998) New potential for human embryonic stem cells. Science 282:1061–1062
Halliwell J, Barbaric I, Andrews PW (2020a) Acquired genetic changes in pluripotent stem cells: origins and consequences. Nat Rev Mol Cell Biol 21:715–728
Halliwell JA, Frith TJR, Laing O, Price CJ, Bower OJ, Stavish D, Gokhale PJ, Hewitt Z, El-Khamisy SF, Barbaric I, Andrews PW (2020b) Nucleosides rescue replication-mediated genome instability of human pluripotent stem cells. Stem Cell Rep 14:1009–1017
Hogan B, Fellous M, Avner P, Jacob F (1977) Isolation of a human teratoma cell line which expresses F9 antigen. Nature 270:515–518
Holden S, Bernard O, Artzt K, Whitmore WF, Bennett D (1977) Human and mouse embryonal carcinoma cells in culture share an embryonic antigen (F9). Nature 270:518–520
Kannagi R, Cochran NA, Ishigami F, Hakomori S-I, Andrews PW, Knowles BB, Solter D (1983) Stage specific embryonic antigens (SSEA-3 and -4) are epitopes of a unique globo-series ganglioside isolated from human teratocarcinoma cells. EMBO J 2:2355–2361
Kleinsmith LJ, Pierce GB (1964) Multipotentiality of single embryonal carcinoma cells. Cancer Res 24:1544–1551
Kondziolka D, Wechsler L, Goldstein S, Meltzer C, Thulborn KR, Gebel J, Jannetta P, DeCesare S, Elder EM, McGrogan M, Reitman MA, Bynum L (2000) Transplantation of cultured human neuronal cells for patients with stroke. Neurology 55:565–569
Lu TY, Yang L (2011) Uses of cardiomyocytes generated from induced pluripotent stem cells. Stem Cell Res Ther 2:44
Martin GR (1981) Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci USA 78:7634–7636
Martin GR, Evans MJ (1974) The morphology and growth of a pluripotent teratocarcinoma cell line and its derivatives in tissue culture. Cell 2:163–172
Martin GR, Evans MJ (1975) Differentiation of clonal lines of teratocarcinoma cells: formation of embryoid bodies in vitro. Proc Natl Acad Sci USA 72:1441–1445
Merkle F, Ghosh S, Kamitaki N, Mitchell J, Avior Y, Mello C, Kashin S, Mekhoubad S, Ilic D, Charlton M, Saphier G, Handsaker RE, Genovese G, Bar S, Benvenisty N, McCarroll SA, Eggan K (2017) Human pluripotent stem cells recurrently acquire and expand dominant negative P53 mutations. Nature 545:229–233
Meseguer-Ripolles J, Khetani SR, Blanco JG, Iredale M, Hay DC (2018) Pluripotent stem cell-derived human tissue: platforms to evaluate drug metabolism and safety. AAPS J 20:20
Mintz B and Illmensee K (1975) Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc Natl Acad Sci USA 72:3585–3589
Mostofi FK and Price EB (1973) Tumors of the male genital system: atlas of tumor pathology second series Armed Forces Institute of Pathology Washington
Nicolas J-F, Dubois P, Jakob H, Gaillard J, Jacob F (1975) Tératocarcinome de la souris: différenciation en culture d'une lignée de cellules primitives à potentialités multiples. Ann Microbiol Inst Pasteur A 126:3–22
Oosterhuis JW, Andrews PW, Knowles BB, Damjanov I (1984) Effects of cisplatinum on embryonal carcinoma cell lines in vitro. Int J Cancer 34:133–139
Papaioannou VE, McBurney MW, Gardner RL, Evans MJ (1975) Fate of teratocarcinoma cells injected into early mouse embryos. Nature 258:70–73
Pedersen RA (1999) Embryonic stem cells for medicine. Sci Am 280:68–73
Pera MF, Blasco-Lafita MJ, Cooper S, Mason M, Mills J, Monaghan P (1988) Analysis of cell-differentiation lineage in human teratomas using new monoclonal antibodies to cytostructural antigens of embryonal carcinoma cells. Differentiation 39:139–149
Pera MF, Cooper S, Mills J, Parrington JM (1989) Isolation and characterization of a multipotent clone of human embryonal carcinoma cells. Differentiation 42:10–23
Robertson E, Bradley A, Kuehn M, Evans M (1986) Germ-line transmission of genes introduced into cultured pluripotential cells by retroviral vector. Nature 323:445–448
Rosenthal MD, Wishnow RM, Sato GH (1970) In vitro growth and differentiation of clonal populations of multipotential mouse cells derived from a transplantable testicular teratocarcinoma. J Natl Cancer Inst 44:1001–1014
Schwartz SD, Hubschman JP, Heilwell G, Franco-Cardenas V, Pan CK, Ostrick RM, Mickunas E, Gay R, Klimanskaya I, Lanza R (2012) Embryonic stem cell trials for macular degeneration: a preliminary report. Lancet 379:713–720
Scully RE (1979) Tumors of the ovary and maldeveloped gonads: atlas of tumor pathology second series Armed Forces Institute of Pathology Washington
Shevinsky L, Knowles BB, Damjanov I, Solter D (1982) Monoclonal antibody to murine embryos defines a stage specific embryonic antigen expressed on mouse embryos and human teratocarcinoma cells. Cell 30:697–705
Skakkebaek NE, Berthelsen JG, Giwercman A, Müller J (1987) Carcinoma-in-situ of the testis: possible origin from gonocytes and precursor of all types of germ cell tumours except spermatocytoma. Int J Androl 10:19–28
Solter D, Knowles BB (1978) Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). Proc Natl Acad Sci USA 75:5565–5569
Solter D, Skreb N, Damjanov I (1970) Extrauterine growth of mouse egg-cylinders results in malignant teratoma. Nature 227:503–504
Stevens LC (1967a) The biology of teratomas. Adv Morphol 6:1–31
Stevens LC (1967b) Origin of testicular teratomas from primordial germ cells in mice. J Natl Cancer Inst 38:549–552
Stevens LC (1970) The development of transplantable teratocarcinomas from intratesticular grafts of pre- and postimplantation mouse embryos. Dev Biol 21:364–382
Stevens LC and Little CC (1954) Spontaneous testicular teratomas in an inbred strain of mice. Proc Natl Acad Sci USA 40:1080–1087
Stevens LC, Varnum DS (1974) The development of teratomas from parthenogenetically activated ovarian mouse eggs. Dev Biol 37:369–380
Takahashi J (2021) Clinical trial for Parkinson's disease gets a green light in the US. Cell Stem Cell 28:182–183
Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861–872
Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676
The International Stem Cell Initiative - Amps K, Andrews PW, Anyfantis G, Armstrong L, Avery S, Baharvand H, Baker J, Baker D, Munoz MB, Beil S, Benvenisty N, Ben-Yosef D, Biancotti JC, Bosman A, Brena RM, Brison D, Caisander G, Camarasa MV, Chen J, Chiao E, Choi YM, Choo AB, Collins D, Colman A, Crook JM, Daley GQ, Dalton A, De Sousa PA, Denning C, Downie J, Dvorak P, Montgomery KD, Feki A, Ford A, Fox V, Fraga AM, Frumkin T, Ge L, Gokhale PJ, Golan-Lev T, Gourabi H, Gropp M, Guangxiu L, Hampl A, Harron K, Healy L, Herath W, Holm F, Hovatta O, Hyllner J, Inamdar MS, Irwanto AK, Ishii T, Jaconi M, Jin Y, Kimber S, Kiselev S, Knowles BB, Kopper O, Kukharenko V, Kuliev A, Lagarkova MA, Laird PW, Lako M, Laslett AL, Lavon N, Lee DR, Lee JE, Li C, Lim LS, Ludwig TE, Ma

Y, Maltby E, Mateizel I, Mayshar Y, Mileikovsky M, Minger SL, Miyazaki T, Moon SY, Moore H, Mummery C, Nagy A, Nakatsuji N, Narwani K, Oh SKW, Oh SK, Olson C, Otonkoski T, Pan F, Park IH, Pells S, Pera MF, Pereira LV, Qi O, Raj GS, Reubinoff B, Robins A, Robson P, Rossant J, Salekdeh GH, Schulz TC, Sermon K, Mohamed JS, Shen H, Sherrer E, Sidhu K, Sivarajah S, Skottman H, Spits C, Stacey GN, Strehl R, Strelchenko N, Suemori H, Sun B, Suuronen R, Takahashi K, Tuuri T, Venu P, Verlinsky Y, van Oostwaard DW, Weisenberger DJ, Wu Y, Yamanaka S, Young L, Zhou Q (2011) Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. Nat Biotechnol 29:1132–1144

Thompson O, von Meyenn F, Hewitt Z, Alexander J, Wood A, Weightman R, Gregory S, Krueger S, Andrews S, Barbaric I, Gokhale PJ, Moore HD, Reik W, Milo M, Nik-Zainal S, Yusa K, Andrews PW (2020) Low rates of acquisition of de novo mutations in human pluripotent stem cells under different culture conditions. Nat Commun 11:1528

Thompson S, Stern PL, Webb M, Walsh FS, Engstrom W, Evans EP, Shi WK, Hopkins B, Graham CF (1984) Cloned human teratoma cells differentiate into neuronlike cells and other cell types in retinoic acid. J Cell Sci 72:37–64

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282:1145–1147

Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Becker RA, Hearn JP (1995) Isolation of a primate embryonic stem cell line. Proc Natl Acad Sci USA 92:7844–7848

Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Hearn JP (1996) Pluripotent cell lines derived from common marmoset (Callithrix jacchus) blastocysts. Biol Reprod 55:688–690

Vitillo L, Victoria E, Tovell VE, Coffey P (2019) Treatment of age-related macular degeneration with pluripotent stem cell-derived retinal pigment epithelium. Curr Eye Res 45:361–371

Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318:1917–1920