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Editorial

From cancer to pluripotent stem cells – a long and winding road

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We are now on the cusp of realizing the promise of Pluripotent Stem Cells (PSC) as powerful tools for exploring disease mechanisms, facilitating the discovery of new drugs, and replacing diseased or damaged tissues, just 26 years since Jamie Thomson first described the long term culture of human embryonic stem (ES) cells [1], and 18 years since Shinya Yamanaka discovered how to reprogram somatic cells to produce induced Pluripotent Stem (iPS) cells with a state equivalent to that of ES cells [2]. But this field has a much longer experimental history, stretching back to 1954 when Leroy Stevens first described the propensity of male laboratory mice of the 129 inbred strain to develop testicular teratomas [3]. In many ways the pluripotent stem cell field provides a striking example of how science develops through a labyrinth of pathways – some successful, some not so successful, sometimes leading in unexpected directions and arriving in places far removed from those originally envisaged.

So much of modern life depends on technology that we often take for granted, and consequently we pay little attention to how the underlying science developed – who did it, and why? A case in point is our recent experience of the Covid pandemic. As that fades in our collective memory, we forget how remarkable it was that within a year of the first cases being identified in China, the virus had been isolated, its genome sequenced, rapid assays based upon PCR developed and innovative vaccines produced. However, the knowledge that made possible that rapid response to Covid came from diverse research stretching back over the past century or more – the identification of DNA and later RNA as carriers of genetic information, the deciphering of the genetic code, and recognition of its universality to all living organisms, the understanding of the mechanisms that protect some bacteria from infection with some viruses, leading to the discovery of restriction enzymes used as tools for DNA

sequencing, or the discovery of heat stable Taq polymerase in bacteria growing in hot volcanic strings, that allowed the development of PCR. Yet none of this research was remotely driven by thoughts of solving the problems of a novel viral pandemic. It was mostly impelled by the curiosity of individual scientists, with funding often provided through peer-reviewed grants focused on increasing basic knowledge, not trying to solve specific practical problems. It was also supported by teamwork and widespread open communication between the different research groups involved, many in different countries, an atmosphere well captured by Horace Judson in his book, "*The Eighth Day of Creation*", about the development of molecular biology [4]. Yet it often seems that we are in danger of ignoring these lessons as governments, funding agencies and companies worldwide seek to exploit the past developments in science for solving current problems. Undoubtedly funding 'translational' research aimed at harnessing existing knowledge is essential. But it brings with it the danger that we neglect adequate sponsorship of new curiosity driven research that will open up new knowledge for the future.

When Stevens described teratomas in 129 male mice, he certainly was not thinking of regenerative medicine. His focus was on the genetics of cancer. Yet his work opened up the experimental study of these strange tumors that contain a haphazard mixture of different tissues and had been a medical curiosity for many hundreds of years. Within a few years, studies of these tumors evolved to studies of the mechanisms of cell differentiation during early embryonic development. That in turn led to the discovery of ES cells, first in the mouse and later in humans. In 2016 a symposium dedicated to Stevens' work was held at the Jackson Laboratory, where he worked throughout his life [5]. At that meeting, several researchers who had contributed to the early work on mouse teratocarcinomas reflected on Stevens' work in those early days and how it provoked others to take the field forward. Elsewhere, Ivan Damjanov and Davor Solter have provided a thorough review of the early studies of teratomas in mice and humans [6], while Davor Solter has provided a detailed overview of how the study of teratomas evolved into the study of pluripotent stem cells [7]

In this collection of essays, we have brought together a number of authors who have contributed to the field as it developed since the early work of Stevens up until the present day. Several give their own personal insights into what drove their curiosity and how and why particular directions of research were followed. They highlight the importance of curiosity driven research in driving the field forward. They also highlight the blind alleys that were sometimes followed and the importance of teamwork and collaboration between researchers with different expertise and interests.

In the first essay Ginny Papaioannou picks up on the field as it became evident that embryonal carcinoma (EC) cells, the cancer stem cells associated with teratomas, closely resemble the pluripotent cells of the early embryo at the blastocyst stage, discussing how she and others

demonstrated that relation by transfer of EC cells to mouse blastocysts that were allowed to develop to term. This provided the evidence that drove Martin Evans and Gail Martin to derive ES cells from mouse blastocysts [8, 9].

About that same time, one of the key groups studying pluripotent EC cell lines was that of François Jacob who, at the Institut Pasteur in Paris, had decided to move from bacterial genetics, where he had discovered the fundamental principles of gene regulation, to addressing the genetic control of mammalian development [10]. In the next essay Bob Erickson discusses how the Pasteur group became caught up by ideas then prevalent about the T-locus in the laboratory mouse, and the notion that it included a set of master genes that encoded a series of cell surface antigens that in turn controlled early embryonic development. Unfortunately, this proved to be a blind alley, illustrating the dangers inherent when established researchers move into new unfamiliar territory, and of stretching techniques beyond the capacity of the then available technology, in that case serology before the advent of monoclonal antibodies

Nevertheless, that work was important in highlighting ideas about the use of cell surface antigens as markers for monitoring the phenotypes of stem cells and following their differentiation. In the next essay, I describe how, after moving from the group of François Jacob to that of Barbara Knowles and Davor Solter at the Wistar Institute in Philadelphia, we were able to use monoclonal antibody technology, which had not been available to the Pasteur group, to define new cell surface markers that enabled us to characterize pluripotent human EC cells and demonstrate how they differ from those of the mouse.

At that time, in the 1980's, the development of mouse ES cells and the means for their genetic manipulation by homologous recombination techniques, for which Martin Evans, Mario Capecchi and Oliver Smithies received the Nobel Prize, had revolutionized the study of embryonic development in the laboratory mouse. However, the existence of well characterized human EC cell lines stimulated the idea that human ES cells might also be useful for understanding human embryology. I the next essay Martin Pera discusses the road that led to the production of human ES cell lines and then the ideas that these could be used, not only for human embryology but also in developing new approaches for human healthcare, including regenerative medicine.

The original derivation of mouse ES cells, and later human ES cells depended on the use of mitotically inactivated fibroblast feeder cells and complex media including fetal calf serum. In these undefined conditions it was impossible to decipher the molecular mechanisms that maintained the capacity for self-renewal of these cells, that is their ability to retain pluripotency while proliferating indefinitely, and understand why ES cells could be readily derived from the

embryos of some mouse strains but not others, and not from some species such as the rat. In the next essay by Qi-Long Ying and Jenny Nichols discuss the relationship of ES cells to the early embryo and the signaling pathways that control differentiation, allowing the development of defined media for mouse ES cells. Among other consequences, this facilitated the derivation of ES cells from many strains of mice and the rat. In his essay, Austin Smith picks up on this theme, discussing how the genetic mechanisms that regulate pluripotency and changes in the pluripotent state during early development help to explain why this state is transient in the embryo but can be maintained indefinitely in vitro, and why there are substantial differences between the ES cells derived from mouse and human embryos.

Following the derivation of ES cells by Jamie Thomson and the discovery of ways to reprogram somatic cells to iPS cells by Shinya Yamanaka, the opportunities for applying these cells for addressing problems related to human health have become apparent, though so have the potential problems. In her essay, Christine Mummery discusses how her career evolved from early studies of mouse ES cells through to developing tools for using human PSC to generate cardiomyocytes that, in turn, could be used for exploring the interaction of different drugs with cardiomyocytes under a range of pathological conditions. Importantly it highlights how early experience with the mouse system enabled her to then exploit the human system. David Turner and Alfonso Martinez-Arias discuss the finding that groups of cells corresponding to the different cell types in the early embryo, often produced by differentiation of PSC, can undergo self-organization to generate structures that closely resemble the early embryo. These embryoids and gastroloids offer important new tools for addressing clinical problems that arise for defects in early human development particularly around the time of gastrulation. Along the same lines, Madeline Lancaster, discusses how the self-organization of later cell types that may be derived from PSC can give rise to organoids – structures that resemble the histological organization of different tissues and can be used to address issues of pathology in those tissues.

Perhaps most prominent of all the ideas that have emerged for applications of human PSC is their use to produce new tissues that can be used to replace diseased or damaged tissues in patients. In their essay Roger Barker and his colleagues discuss the long development of one of the most prominent applications currently on the horizon, namely the treatment of Parkinson's disease. But all is not straight forward. In their essay John Vales and Ivana Barbaric discuss how PSC can acquire genetic changes on prolonged culture. While many of these might be harmless in particular applications, they do need to be considered in safety assessments of regenerative medicine applications of human PSC.

There is no doubt that the development of human PSC, after a long and often convoluted road from mouse teratocarcinomas, do offer many important opportunities for human health care.

In the final essay, Kevin Hui with Shinya Yamanaka, who was the first to produce iPS cells, look to the future and discuss how this technology may develop further.

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CONFLICT OF INTEREST

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