Nature Reviews Molecular Cell Biology | AOP, published online 21 January 2015; doi:10.1038/nrm3945

STEM CELLS

Human primordial germ cells in a dish

> SOX17 was essential and sufficient to induce PGC fate

Primordial germ cells (PGCs) are the precursors of sperm and eggs. In mammals, PGCs are induced during grastrulation from epiblast cells in response to several cues. Much of what is known about mammalian germ cell specification is based on studies in mice, in which PGCs are specified at embryonic day 6.5 (E6.5) by bone morphogenetic protein 4 (BMP4) and other signals. Studies on human PGC specification would require E9–E16 human embryos, which is not practicable. Nevertheless, human PGC-like cells can be generated, albeit at very low frequency, by spontaneous differentiation of human embryonic stem cells (ESCs).

In a study published in *Cell*, Surani and colleagues at Cambridge University (UK), jointly with Hanna and colleagues at the Weizmann Institute (Israel), report the development of a robust method for the specification of human PGC-like cells from ESCs and induced pluripotent stem cells (iPSCs). They identify SOX17 as a crucial regulator, and the earliest marker, of PGC fate, revealing a key difference in PGC induction between humans and mice.

The authors generated three independent human ESC lines containing a reporter (mCherry) fused to the highly specific PGC marker NANOS3. These cells were cultured in a four-inhibitor-containing (4i) medium, previously developed by Hanna's group, which maintains cells in a distinct, more 'naive' pluripotent state. The cells were then transferred to a culture medium containing BMP2 or BMP4, leukaemia inhibitory factor (LIF), stem cell factor (SCF), epidermal growth factor (EGF) and RHO-kinase (ROCK) inhibitor, which induced PGC-like cells. Within a few days, a large proportion of cells formed in embryoid bodies expressed NANOS3–mCherry as well as other key PGC genes, indicating that they were probably nascent germ cells. Professor Azim Surani commented: "It's remarkably fast. We can now take any embryonic stem cell line and once we have them in the proper conditions, we can make these primordial cells in five to six days" (*The Guardian*, 24 Dec 2014).

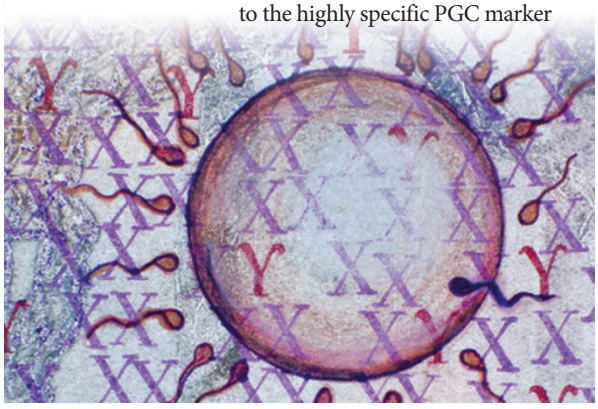
To characterize these induced PGC-like cells and confirm their germ cell identity, the authors performed gene expression profile analyses, comparing the induced cells to human PGCs from 7-week-old male embryos and to a human seminoma originating from the germline *in vivo*. These analyses revealed that induced PGC-like cells shared expression profiles (including core germ cell genes) with early PGCs and seminomas. Moreover, PGC-like cells initiated DNA demethylation, consistent with a germline-specific epigenetic programme.

When studying the mechanisms underlying PGC specification, the authors found that the transcription factor SOX17 was the earliest PGC marker to be upregulated. SOX17 was essential and sufficient to induce PGC fate in a cell-autonomous manner. This finding was surprising, as SOX17 is a known endodermal marker and has no role in the specification of mouse PGCs. Expression of the transcription factor B lymphocyte maturation protein 1 (BLIMP1) followed that of SOX17. BLIMP1 repressed endodermal and other somatic genes, which may allow SOX17 to specify PGCs.

This study reports the development of a robust method to induce human PGC-like cells. As PGC-like cells represent the earliest stage of the human germ cell lineage, they provide a tool for further understanding the mechanisms underlying the specification and maintenance of the human germline, which cannot always be extrapolated from studies in mice. Moreover, as these cells erase their epigenetic marks by undergoing global demethylation, Surani suggested: "This could tell us how to erase [age-related] epigenetic mutations. Epigenetics is used to regulate gene expression, but in age-related diseases, these changes can be aberrant and misregulate genes" (*The Guardian*, 24 Dec 2014). Importantly, the authors could also obtain PGC-like cells from iPSCs, which widens the range of potential applications, such as disease modelling from patient-derived iPSCs.

The possibility of making germ cells in a dish also raises some hope for the treatment of infertility; however, as Professor Azim Surani commented: "It's not impossible that we could take these cells on towards making gametes, but whether we could ever use them is another question for another time" (*The Guardian*, 24 Dec 2014).

Kim Baumann



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ORIGINAL RESEARCH PAPER Irie, N. *et al.* SOX17 is a critical specifier of human primordial germ cell fate. *Cell* **160**, 1–16 (2015)