Best Practice & Research Clinical Obstetrics and Gynaecology 31 (2016) 112–120

Contents lists available at ScienceDirect

Best Practice & Research Clinical Obstetrics and Gynaecology

journal homepage: www.elsevier.com/locate/bpobgyn

8

Induced pluripotent stem (iPS) cells from human fetal stem cells

Pascale V. Guillot, BSc, MSc, PhD *

UCL Institute for Women's Health, University College London, London, UK

Keywords:
reprogramming
small molecules
fetal stem cells
induced pluripotency
transcription factors
translational medicine

Pluripotency defines the ability of stem cells to differentiate into all the lineages of the three germ layers and self-renew indefinitely. Somatic cells can regain the developmental potential of embryonic stem cells following ectopic expression of a set of transcription factors or, in certain circumstances, via modulation of culture conditions and supplementation with small molecule, that is, induced pluripotent stem (iPS) cells. Here, we discuss the use of fetal tissues for reprogramming, focusing in particular on stem cells derived from human amniotic fluid, and the development of chemical reprogramming. We next address the advantages and disadvantages of deriving pluripotent cells from fetal tissues and the potential clinical applications.

Rejuvenating somatic cells to pluripotency

Fetal cell fate is reversible chemically. We previously reprogrammed human fetal stem cells to functional pluripotency using valproic acid (VPA) and without genetic manipulation or ectopic expression of pluripotency factors [1–3]. Somatic cells can be reprogrammed into pluripotent stem cells, that is, induced pluripotent stem (iPS) cells, to regain the developmental potential of embryonic stem (ES) cells, including the capacity to self-renew indefinitely and to differentiate into all the lineages of the three germ layers. Rejuvenated cells are attractive tools in biomedical research to study development and disease, and in regenerative medicine to produce patient-specific cells that can be injected without immunosuppressant. Pioneered by Yamanaka's group in 2006–2007, iPS

* Corresponding author. UCL Institute for Women's Health, University College London, 86-96 Chenies Mews, London WC1E 6HX, UK. Tel.: +44 (0)207 242 2842; Fax: +44 (0)207 404 6181.
E-mail address: p.guillot@ucl.ac.uk.

http://dx.doi.org/10.1016/j.bpobgyn.2015.08.007
1521-6934/

cells were first generated by retroviral transduction of a combination of four transcription factors, that is, OCT4, KLF4, SOX2 with or without c-MYC [4–6] or OCT4, SOX2, NANOG with or without LIN28 [5,6]. Various reprogramming strategies have since been developed to increase reprogramming efficiency and to create iPS cells free of exogenous DNA (footprint-free) with the purpose of avoiding tumourigenic insertional mutations and reactivation of transgene expression during differentiation, which represent a major hurdle for iPS cell applications. Integration-free techniques use, for example, minicircle DNA, transposons, RNA viral vectors, acid-free methods and episomal vectors, with episomal reprogramming being at present the most efficient method and the most likely translatable technology for producing clinical-grade human iPS cells via ectopic expression of transcription factors [7]. Manipulation of the environment, such as biophysical cues, can also modulate cell fate [8]. Similarly, chemicals, in particular small molecules, have been used to modulate reprogramming, either by increasing reprogramming efficiency and/or by replacing one or more transcription factors [9]. Several small-molecule compounds involved in epigenetic regulation have been identified for their role in the reprogramming process via either somatic cell nuclear transfer (SCNT) or ectopic expression of a define set of transcription factors to replace one, or more, of the reprogramming factors. For example, it was shown that hyperacetylation improves reprogramming of somatic nuclei following nuclear transfer [10], and that the histone deacetylase inhibitor trichostatin A (TSA) reduces abnormal DNA hypermethylation following cloning by SCNT, thereby increasing the success rate of mouse cloning via SCNT [11]. Similarly, the histone deacetylase inhibitor VPA improves the reprogramming efficiency of mouse embryonic fibroblasts (MEF) without the introduction of c-Myc [12], and it enables the reprogramming of primary human fibroblasts with only two factors, Oct4 and Sox2, without the need for the oncogenes c-Myc or Klf4 [13]. Generally used for the treatment of epilepsy and bipolar disorder, VPA (2-propyl pentanoic acid) enhances OCT4 promoter activity via activation of the PI3K/Akt/mTOR pathway in mouse cells [14,15]. VPA is involved in several regulatory mechanisms including GSK3b, Akt, extracellular signal-regulated kinase (ERK), phosphoinositol, tricarboxylic acid cycle, gamma-aminobutyric acid (GABA) and OXPHOS pathways [16].

iPS cells, which can be generated from different tissues, have properties that differ according to their tissue of origin, because they maintain a unique residual DNA methylation that influences their differentiation potential, that is, 'epigenetic memory of the donor tissue.' [17] In addition, the tissue of origin can also influence the efficiency and yield of reprogramming. For example, keratinocytes are being reprogrammed more readily than fibroblasts [18,19], and fibroblasts isolated from different tissues vary in their reprogramming efficiency. In that context, stem cells, which are less lineage-committed than terminally differentiated somatic cells, are easier to reprogramme, and they can be reprogrammed with a reduced number of factors, and adult NSCs can revert to functional pluripotency using OCT4 only [20]. In addition to the differentiation status of the tissue of origin, the development age of the parental population also influences the capacity of the cells to revert to an earlier state of pluripotency. For example, embryonic and fetal tissues can be reprogrammed with a higher efficiency than adult tissues, yielding iPS cells that are functionally most comparable to ES cells [17,21,22]. Of importance, the *Ink4/Arf* locus, which encodes p15(INK4b), ARF and p16(INK4a) genes in human chromosome 9p21, has been shown to be a barrier for iPS cell derivation, with the locus being silenced in both murine and human iPS and ES cells [21]. The expression level of the Ink4/Arf locus, which is progressively upregulated in older cells and in cells from late developmental stages, inversely parallels reprogramming efficiency, explaining why older cells are more difficult to reprogramme. Indeed, embryonic and fetal tissues can be rejuvenated using a reduced number of transcription factors. For example, human NSCs, $CD34^{+}$ amniotic fluid cells and keratinocytes, as well as mouse fibroblasts have been reprogrammed with only OCT4, either alone [23,24] or in conjunction with a small-molecule cocktail [25,26]. Small molecules are advantageous because they are not expensive, nonimmunogenic, cell permeable, easily synthesized and target-specific pathways. Complete chemical reprogramming in the absence of genetic manipulation has been overcome in mouse epiblast stem cells, which already express pluripotency markers oct4, sox2 and nanog, but they are incapable of contributing to chimerism, using a combination of small molecules to simultaneously inhibit LSD1, ALK5, methyl ethyl ketone (MEK), fibroblast growth factor receptor (FGFR) and GSK3 [27]. More recently, MEFs have been fully reprogrammed to pluripotency using only small-molecule compounds [28].