Screening techniques to identify genomic instability of pluripotent stem cells in ensuring the safety of applications in regenerative medicine

Several years have passed from discovery of stem cells by Drs. James A. Till and Ernest A. in 1961, human embryonic stem cells (hESCs) in 1998 by James Thomson and Induced PSCs (iPSCs) in 2006 by Dr. Shinya Yamanaka, but there are still a number of safety, quality, functionality, and efficacy issues with hPSC-derived cells/tissues that need to be resolved before they can be brought into routine clinical applications. The first safety study using human embryonic stem cell (hESC)-derived oligodendrocyte progenitors for the treatment of subacute spinal cord injury was launched in 2007 and approved in 2009. This study was followed by the first hESC-based clinical studies for the treatment of macular degeneration diabetes mellitus and ischemic heart disease with some early positive indications after 2-3 years of follow-up. The first autologous hiPSC-based clinical study was started in 2014 for age related macular degeneration. Subsequently, a large number of further clinical trials using hiPSC-derived cells were either announced or started. By the end of 2019, at least 54 hPSC-based clinical studies for 22 different disorders have been reported [1,2].

A meta-analysis of published genetic abnormalities found in hPSCs reported that majority of these genetic defects are recurrent. For instance, using normal cytogenetic techniques (G-banding), gains of chromosomes 12 (most usually 12p), 17, (especially 17q), 20, or X have frequently been found. Recurrence is also possible for sub-chromosomal abnormalities such 20q11.21 amplification [2]. Screening for the genetic abnormalities is crucial to guarantee the quality, safety and traceability of the cells. Actually, it has been identified that up to 20% of hPSC lines had 20q11.21 amplification, which confers a survival advantage for the cells [2,3]. For more than 15 years in hESCs and longer in pancreatic cancer, recurrent amplification in the 20q region has been noted, and it is linked to an increase in the rate of proliferation [3]. In the current issue of JSRM, Pridgeon et al [4] report the effects of engraftment of hESCs and hESC-derived hepatocyte-like cells (HLCs) with and without amplification of the 20q11.21 minimal amplicon and isochromosome 20q (i20q) in SCID-beige mice. Their study provides proof of concept on how differences in in vivo tumorigenicity related to such abnormalities can be assessed. This in vivo technique is a step further than in vitro assessment alone which is very critical for excluding the cells containing the abnormalities from therapeutic applications. Gain of this aberration 20q11.21 provides pluripotent stem cells with a considerable growth advantage over their non-CNV counterparts through protection against apoptosis [5]. This aberration has been found to be mediated by BCL-XL and observed to be not from the donor but rather acquired during culture [6,7]. Therefore, culture components also are to be screened and assessed to identify optimal conditions wherein such aberrations are not acquired. BCL-XL, NK cells

In terms of cell culture techniques, enzymatic passaging and mechanical passaging of colonies, have been associated with promoting genome aberrations. As opposed to 2D culture platforms, 3D culture platforms may be able to support the cellular densities required to produce higher quantities of hPSCs [8]. Additionally, it has been shown that 3D microenvironmental characteristics, such as pertinent material properties, biochemical cues, and cell-adhesion motifs, significantly improve stem cell expansion, boost differentiation effectiveness, and generate more mature and functional cell types compared to 2D culture. Though synthetic and natural ECM-derived hydrogels are potential 3D platforms, covalent crosslinking, which can limit cell proliferation and differentiation apart from cellular recovery necessitating severe enzymatic and mechanical destruction, which restricts bioprocessing and impairs the health and integrity of cells are disadvantages. 3D thermostressive hydrogels are one type of material that has a lot of potential to meet the manufacturing requirements of hPSC-derived therapeutics. Especially a PEG-PNIPAAm block copolymer based thermoresversible gelation polymer (TGP) has been used in the culture of hPSC expansion without the need for enzymes for cell harvest as lowering the temperature below the sol-gel transition temperature releases the cells. These TGPs are chemically defined, synthetic polymers and have been employed for the culture of different types of stem cells other than hPSCs [9]. This TGP has been shown to stably maintain the genetic expression profile of mesenchymal stem cells in culture which could be advantageous in preventing the chromosomal aberrations [10]. Another approach to tackle the cells in vitro and in vivo would be to use immunotherapy [11] in which Natural Killer (NK) cells have been shown to synergise with BH3 mimetics which are compounds that compete for the hydrophobic groove in anti-apoptotic proteins to antagonize the function of anti-apoptotic proteins such as BCL-2, BCL-XL, MCL-1, or BFL-1, in reducing tumour burden and improving survival [12].

Apart from focussing on various approaches to assess and prevent chromosomal aberrations, similar to meta-analysis of chromosomal aberrations in PSCs, meta-analysis of culture conditions should be performed to arrive at scalable and optimal production of hPSCs without chromosomal aberrations wherein the studies as that of Pridgeon et al [4] could contribute to techniques for assessing the culture conditions that lead to aberrations and for identifying solutions to prevent them.
References