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COMMENTARY

Transgene-free induced pluripotent dental stem cells for neurogenic differentiation

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See related research by Zou *et al.*, http://stemcellres.com/content/3/5/43

Abstract

A stem-cell-based therapy could be the ultimate strategy for the regeneration of degenerated nervous tissues. While neural progenitor cells are limited, the generation of functional nervous tissue cells from non-neural somatic cells (for example, dental stem cells) is highly desired. The recent publication in *Stem Cell Research and Therapy* by Huang and colleagues is an interesting contribution to this topic. The present commentary puts this paper in context with contemporary reports about (transgene-free) induced pluripotent stem cells and neurogenic differentiation.

The generation of neural tissue cells is the ultimate goal for stem-cell-based therapies of currently untreatable neurodegenerative diseases such as Parkinson's disease or multiple sclerosis. To date the production of sufficient numbers of functional neurons or glial cells from non-neural stem/progenitor cells is difficult to achieve. A recent study by George Huang and colleagues published in *Stem Cell Research and Therapy* used stem cells from the dental apical papilla (SCAP) [1]. These neural-crest-derived dental cells express typical neural cell markers such as β-III-tubulin and are closely related to the neural-crest-derived cells of the peripheral nervous system. SCAP, like other types of dental stem cells, are therefore a favorable cell source for therapies of degenerated nervous tissues. Although neurogenic differentiation occurs, protocols for dental stem cells are sophisticated and the neurogenic differentiation is less complete than that of neuroectodermal stem/progenitor cells [2–4]. Moreover, the use of SCAP for the regeneration of nervous tissues is also problematic, because numbers of stem cells are limited in dental apical papillae. Huang and colleagues' new publication has tackled this problem with transgene-free (TF) induced pluripotent stem cells (iPSCs) from SCAP [1].

An appropriate strategy to improve the proliferation and the differentiation potential of somatic (stem) cells is the establishment of iPSCs with similar functional and molecular phenotypic characteristics to embryonic stem cells (ESCs) [5]. The generation of iPSCs from somatic cells was a decisive step for the direction of stem cell research, and it is no coincidence that the father of iPSCs, Shinya Yamanaka, received the Nobel Prize for this achievement [6]. In an earlier study by Huang and colleagues, three types of dental stem cells – SCAP, dental pulp stem cells and stem cells from human exfoliated deciduous teeth – were easily reprogrammed into iPSCs at a higher reprogramming rate than dermal fibroblasts [7]. Although these dental iPSCs had typical characteristics of ESCs, they did also have unfavorable features. For example, most of the frozen-down dental iPSCs did not survive after thawing or the dental iPSCs underwent massive cell death after differentiation toward mesenchymal cell lineages with an ESC standard differentiation protocol [1,7].

In the present study Huang and coworkers speculated that a permanent integration of viral vectors in iPSCs with a constitutive transgene expression may contribute to the unfavorable features of dental iPSCs [1]. They therefore generated TF iPSCs with a single lentiviral stem cell cassette flanked by a loxP site (hSTEMCCA-LoxP) vector [1,8]. Two years ago, Sommer and colleagues established this cre-recombinase excisable lentiviral stem cell cassette with an efficiency to obtain hundreds of iPSCs from a single starting 35-mm plate of human dermal fibroblasts [8]. The efficiency for the generation of TF iPSCs with this method is much higher than that of other protocols; for example, strategies with recombinant proteins [9]. Although a low theoretical risk for insertional mutagenesis remains after cre-recombinase excision, the article by Huang and colleagues showed that the use of the hSTEMCCA-LoxP vector is an appropriate

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strategy for the reprogramming of dental stem cells [1]. The TF SCAP iPSCs, for example, were able to recover better after freezing/thawing, they were able to differentiate into mesenchymal cell lineages without cell death and, most importantly, the embryonic body-mediated neurogenic differentiation was successful [1].

Surprisingly, TF SCAP iPSCs expressed neural cell markers even without the induction of neurogenic differentiation [1]. The expression of neural cell markers suggests that TF SCAP iPSCs could have retained residual features of their neural crest cell origin. General variations of iPSCs from different donor cells are known that are probably caused by an incomplete reset of the tissue-specific epigenetic memory [10]. Although the actual induction of pluripotency is successful, residual retained imprinting variations of donor cells may also have an impact on the quality of iPSC differentiation potentials. For example, keratinocyte-derived iPSCs showed an enhanced keratinocyte potential relative to cord blood-derived iPSCs [11]. Moreover, the neurogenic differentiation of iPSCs is variable in comparison with that of ESCs [12]. The preferential neurogenic differentiation potential of TF SCAP iPSCs has not been established, but the absence of the glial cell marker glial fibrillary acidic protein in both differentiated and undifferentiated TF SCAP iPSCs may favor a differentiation similar to that of ordinary dental stem cells [2,3].

The induction of neural cell markers may also consider a relation of TF SCAP iPSCs to the recently established induced neural progenitor cells (iNPCs) [13]. A transient expression of the reprogramming factors, which were also the reprogramming factors for TF SCAP iPSCs, could efficiently transdifferentiate fibroblasts into functional iNPCs. Here, fibroblasts were cultivated in two different cell culture media including a specific serum-free neural stem cell reprogramming medium [13]. In contrast, in the recent study by Huang and colleagues the reprogramming and the subsequent neural differentiation were achieved by the induction of a reprogrammed pluripotent state. TF SCAP iPSCs expressed Oct4, which is one of the most specific factors of pluripotency [1]. This initial differentiation was followed by differentiation into embryonic bodies and later into neural-like cells with a specific neurogenic differentiation medium for pluripotent stem cells. However, undifferentiated and neurogenic differentiated TF SCAP iPSCs are possibly related to iNPCs. Further elaborating experiments are required to evaluate the nature of TF SCAP iPSCs and their relation to iNPCs.

In conclusion, although the results of this work are promising, we cannot foresee whether a strategy employing iPSCs will be the optimal strategy for cellular therapies with dental stem cells. Dental stem cells (SCAP, dental pulp stem cells or dental follicle cells) are neural crest derived and as such they have a reasonable neurogenic differentiation potential [2–4]. Nevertheless, to determine the optimal strategy, the neurogenic differentiation potential of dental stem cells needs further evaluation. TF SCAP iPSCs from this work should be involved in these new investigations.

Abbreviations

ESC, embryonic stem cell; hSTEMCCA-loxP, single lentiviral stem cell cassette flanked by loxP site; iNPC, induced neural progenitor cell; iPSC, induced pluripotent stem cell; SCAP, stem cells from the dental apical papilla; TF, transgene free.

Competing interests

The author declares that he has no competing interests.

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