Commentary for Journal of Neuroscience Methods

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Unmasking the true identity in neuron differentiation

The recently published protocol by Hu et al. (2016), describing the differentiation of basal forebrain cholinergic neurons (BFCN) from stem cells, contains three key shortcomings in regards to the type and efficiency of the differentiated cells. These three limitations can be addressed to improve the generating of reproducible and relevant cultures.

Hu et al. use induced pluripotent stem cells (iPSCs) to generate spheres of neuroprogenitor cells (NPCs) for neural differentiation. The NPCs are plated onto iPSC-derived astrocytes and driven towards a BFCN fate by supplementation with nerve growth factor (NGF) and sonic hedgehog (SHH). After 17 days in the co-culture environment, 40% of the β-III-Tubulin expressing cells are positive for choline acetyltransferase (ChAT). While Hu et al. make the valid argument that genetically modifying cells for fluorescent activated cell sorting can potentially compromise their integrity, existing protocols produce cultures with up to 90% of ChAT expressing neurons at the end of their differentiation without using selecting methods (Bissonnette et al., 2011; Crompton et al., 2013; Nilbratt et al., 2010).

The main shortcoming of the protocol, however, is the insufficient evidence for the identity of the created cells. The key finding of 40% ChAT-positive β-III-Tubulin expressing cells is not sufficient evidence for the creation of BFCNs, since ChAT and β-III-Tubulin are expressed in NPCs and astrocytes (Consonni et al., 2009; Dráberová et al., 2008; Wessler et al., 1997). Considering that NPCs are plated onto astrocytes, it is very likely that the astrocyte layer strongly contributes to this finding. Existing BFCN protocols have therefore begun to include electrophysiological confirmation of the created cells to address this issue and use more specific markers such as P75 and TrkA (Bissonnette et al., 2011; Crompton et al., 2013; Engel et al., 2016).

Co-culturing with astrocytes presents a further limitation to users of this protocol. The selected astrocyte differentiation protocol requires 120 days of differentiation to achieve the high efficiency of 90% S100β and GFAP expressing cells (Krencik et al., 2011), while protocols exist with equal efficiency after 50 days (Engel et al., 2016; Serio et al., 2013). Furthermore, by differentiating two different cell types from the same iPSC source, variations in the differentiation efficiency between different stem cell lines can make the process more complicated as indicated by Brennand et al. (2014). Critically, the

differentiated BFCNs by Hu et al. cannot be studied in isolation from the astrocytes, making this approach unsuitable for studying BFCN-specific mechanisms.

The reliable and efficient generation of BFCNs from iPSCs has the potential to be of valuable assistance in identifying disease mechanisms and evaluate novel treatments for disorders such as Alzheimer's disease and Down Syndrome. Hu et al. provide interesting data towards this goal, including the substitution of SHH with Purmorphamine and a possible co-culture system. Three key limitations remain, which are best addressed through confirmation of a robust panel of cell-specific markers, coupled with functional assays to reliably identify the end product of the protocol as discussed in our recent review (Engel et al., 2016). These steps can provide the evidence and foundation needed for a reliable and reproducible methodology with relevance to clinical and pre-clinical research.

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