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BRIEF COMMUNICATION

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Morphological and electrophysiological features of mature neurons in differentiated skin-derived precursor cells

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In vitro modelling of neuronal pathologies is, in particular, demanding and a lot of efforts have been undertaken to differentiate skin derived precursor cells into neuronal cells. However, so far all attempts did not result in cells with functional features of neurons like the ability to generate action potentials or synaptic activity. Here, we report that simple modifications of the protocols result in neuronal cells that display action potentials and synaptic activity. We think that our observation is an important step to model individual neuronal pathologies *in vitro*.

Neurons are postmitotic cells that cannot be easily replaced in the adult nervous system and are consequently not available for studying cellular pathologies in patients with neurodegenerative disorders. Considerable effort has been made to reprogram patient-derived fibroblasts into induced pluripotent stem cells (iPSCs) and to differentiate these into neurons as an *in vitro* model for e.g. pharmacological intervention or a cellular source for therapeutic replacement strategies.[1] As an alternative approach, protocols have been developed to differentiate autologous adult stem cells into neuronal cells.[2] For this purpose, multipotent skin-derived precursor cells (SKPs) are isolated and propagated as self-renewing floating spheres.[3] Upon exposure to neurotrophic factors, these cells start to express early neuronal markers like nestin or beta-tubulin.[3-6] As adult astrocytes from the hippocampus are capable of regulating neurogenesis by instructing stem cells to adopt a neuronal fate, [2] SKPs have also been cultured with hippocampal astrocyte-conditioned medium.[4] However, the functional features of neurons, such as action potentials or synaptic activity, have never been reported for SKP-derived neuronal cells.

After cultivation and propagation of SKPs as floating spheres (Fig. 1A, B), we differentiated human SKPs in co-culture with primary murine glia cells, which increased the amount of neuronal cells as defined by expression of beta III-tubulin (Fig. 1C) and microtubule-associated protein 2 (MAP2abc) (Fig. 1D). We tested whether these cells also display functional features of neurons and detected voltage-gated K+ and Na+ channels in most of the cells at the electrophysiological level (Fig. 1G). In addition, the majority of the cells were able to generate action potentials (Fig. 1H) as a basic requirement for neuronal activity. Moreover, the cells expressed the synaptic marker synaptophysin (Fig. 1E) and displayed synaptic activity as shown by the appearance of spontaneous miniature excitatory postsynaptic currents (Fig. 1I). Although it is thought that SKPs are derived from

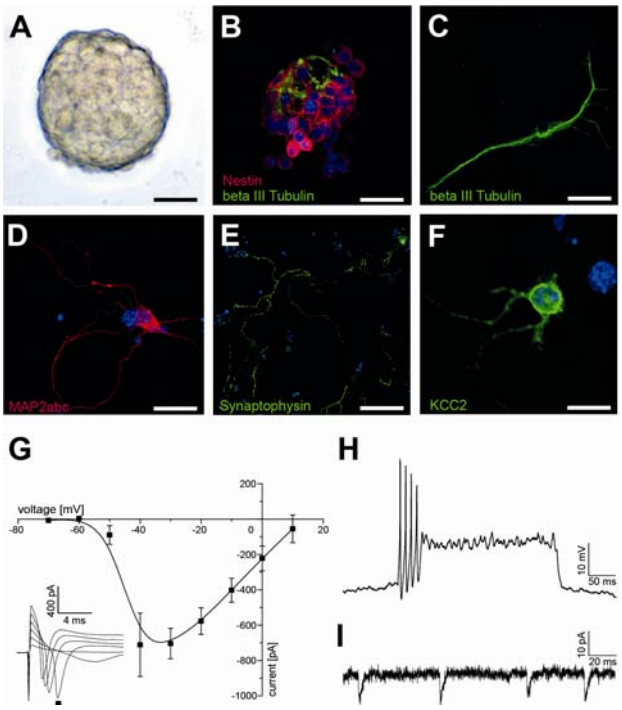

Figure 1. Differentiated skin-derived precursor cells exhibit a mature neuronal phenotype. A, Spheres consist of approximately 50–100 round, morphologically undifferentiated cells. B, These cells express the intermediate VI filament nestin (red), a marker of neuronal stem cells. Scale bars in A and B 50 µm. C, D Upon neuronal differentiation, the majority of cells express beta III-tubulin (green) and MAP2abc (red). Scale bar 10 µm. E, Approximately 50% of the cells express the presynaptic marker synaptophysin (green). Scale bar 40 µm. F, Some cells also express KCC2 (green), a CNS-specific neuronal marker of mature neurons. DAPI (blue) was used to counterstain cell nuclei. Scale bar 10 µm. G, More than 50% of the cells with a neuronal morphology display voltage-gated Na+ channels. The I-V relationship of the Na+ conductance was recorded in the voltage clamp mode; n=3. Inset: original voltage clamp traces. H, These cells also generate action potentials, which were recorded at resting membrane potential under current clamp conditions. I, Cells also display glutamatergic synaptic activity as exemplified by a typical trace of miniature excitatory postsynaptic currents.

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the neural crest, [6] some cells even expressed the K-Cl cotransporter KCC2 (Fig. 1F), a marker of mature central neurons,[7] which lowers the intraneuronal chloride concentration and thereby mediates the GABA switch from depolarizing to hyperpolarizing during maturation of neuronal circuits.

Our identification of previously unreported key features of mature neurons in differentiated SKPs highlights a large potential of this cellular model to study pathologies in neurodegenerative disorders in a patient-individual manner. Moreover, our findings suggest that SKP-based neurons may be worth consideration for therapeutical replacement strategies given the potential risks associated with viral gene transfer in iPSCs.

References

1. Pang ZP, Yang N, Vierbuchen T, Ostermeier A, Fuentes DR, Yang TQ, Citri A, Sebastiano V, Marro S, Südhof TC, Wernig M. Induction of human neuronal cells by defined transcription factors. Nature. 2011; 476: 220-223.
2. Song H, Stevens CF, Gage FH. Astroglia induce neurogenesis from adult neural stem cells. Nature. 2002; 417: 39-44.
3. Fernandes KJ, McKenzie IA, Mill P, Smith KM, Akhavan M, Barnabé-Heider F, Biernaskie J, Junek A, Kobayashi NR, Toma JG, Kaplan DR, Labosky PA, Rafuse V, Hui CC, Miller FD. A dermal niche for multipotent adult skin-derived precursor cells. Nat Cell Biol. 2004; 6: 1082-1093.
4. Joannides A, Gaughwin P, Schwiening C, Majed H, Sterling J, Compston A, Chandran S.. Efficient generation of neural precursors from adult human skin: astrocytes promote neurogenesis from skin-derived stem cells. Lancet. 2004; 364: 172-178.
5. Toma JG, Akhavan M, Fernandes KJ, Barnabé-Heider F, Sadikot A, Kaplan DR, Miller FD. Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat Cell Biol. 2001; 9: 778-784.
6. Wong CE, Paratore C, Dours-Zimmermann MT, et al. Neural crest-derived cells with stem cell features can be traced back to multiple lineages in the adult skin. J Cell Biol. 2006; 175:1005-1015.
7. Hübner CA, Stein V, Hermans-Borgmeyer I, Meyer T, Ballanyi K, Jentsch TJ. Disruption of KCC2 reveals an essential role of K-Cl cotransport already in early synaptic inhibition. Neuron. 2001; 30: 515-524.

Supplementary Information

Supplementary materials and methods are linked to the online version of the article.

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Competing interests

The authors declare no competing financial interests.

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