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Research Highlight

Setting a highway for converting skin into neurons

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Direct conversion of human skin fibroblasts into induced neuronal (iN) cells has been recently achieved by using different combinations of transcription factors eventually associated with microRNAs. These findings lay the ground for a straightforward and efficient generation of human neurons *in vitro* with elaborated functional properties instrumental for disease modeling and cell-based approaches of brain repair.

Six groups have independently succeeded in generating functional human neurons by direct conversion of skin fibroblasts *in vitro* (Ambasudhan et al., 2011; Caiazzo et al., 2011; Pang et al., 2011; Pfisterer et al., 2011; Qiang et al., 2011; Yoo et al., 2011). Their work is based on a recent research breakthrough which enables mouse fibroblasts to be directly reprogrammed into neurons by the expression of only the three transcription factors (TFs), ASCL1/MASH1, BRN2, and MYT1L (ABM) (Vierbuchen et al., 2010). However, the same combination is not as proficient to elicit a similar outcome in post-natal human fibroblasts generating neurons with limited functional properties (Pang et al., 2011). From this standpoint, these groups succeeded in promoting neurogenesis in human fibroblasts by adding to the original cocktail new TFs and eventually microRNAs (miRNAs). Indeed, supplementing the ABM combination with ZIC1 or NEUROD1/2 enables the generation of functional neurons from post-natal human fibroblasts (Pang et al., 2011; Qiang et al., 2011; Yoo et al., 2011). In particular, NEUROD1/2 are bHLH factors that have been shown to exert a strong proneuronal activity and are expressed in overlapping areas with the ABM factors during central nervous system development and adult neurogenesis (Hevner et al., 2006). Thus, it is not surprising from a biological standpoint that NEUROD1/2 are able to synergize with the induced neuronal (iN) cells inducing factors. It would be appealing to understand whether these TFs physically interact with each other forming multiproteic complexes and binding to common targets in order to promote cell conversion. Through a hypothesis-driven approach, two of these groups (Ambasudhan et al., 2011; Yoo et al., 2011) showed that miRNAs can synergize with the reprogramming factors for sustaining the conversion of human fibroblasts into neurons. Ambasudhan et al. (2011) found that miR-124 induced neurogenesis by complementing with the BRN2 and MYT1L factors. Surprisingly, here for the first time ASCL1/MASH1 was found dispensable for direct neuronal reprogramming. Yoo et al. (2011) further elaborated the cocktail describing a strong cooperative effect in neurogenesis when miR-124 was combined with miR-9/9*. Indeed, both miR-124 and miR-9/9* are co-expressed in post-mitotic neurons and promote neuronal differentiation (Shibata et al., 2011). How can their activity complement the action of TFs? In a general prospective, the reprogramming factors are known as transcriptional activators; on the other side, miRNAs exert their function through a direct repression of multiple genes. Thus, it is conceivable that miRNAs provide a direct and effective repressive action targeting a specific set of genes not directly bound by the TFs that need to be inhibited for improving and accelerating cell reprogramming. A further possibility is that miR-124 and miR-9/9* regulate gene expression promoting a pervasive chromatin remodeling process. Indeed, Yoo et al. (2009) previously showed that both miR-124 and miR-9/9* control the expression of the subunits composing the BAF–chromatin complexes, which act globally in the genome to control polycomb repressive complex 2 and the H3K27me3 repressive marks. Interestingly, these miRNAs when expressed in neural cells facilitate the formation of BAF complexes that promote neuronal differentiation, thus suggesting that they can exert a similar role during fibroblast neuronal conversion as well. Thus, miRNAs might synergize with TFs at least in a dual manner by repressing a distinct set of genes which complement with those re-activated by the exogenous factors as well as modifying the epigenetic chromatin landscape of the target cells. Noteworthy, only when miRNAs were added to the reprogramming cocktail, the induced neurons developed spontaneous synapse activity without the need of co-cultures with 'exogenous' mouse neuronal cells (Ambasudhan et al., 2011; Yoo et al., 2011). This might suggest that miRNA supplementation enhances neuronal maturation, an assumption that is also corroborated by a pronounced dendritic arborization of the reprogrammed neurons (Yoo et al., 2011). However, more solid evidences need to be collected to confirm this hypothesis. Independently by the reprogramming cocktail used by these authors, the majority of the reprogrammed neurons obtained were glutamatergic (vGLUT1^{+}) with only a residual amount of GABAergic neurons. Nonetheless, understanding how this small fraction of GABAergic neurons is generated might offer a robust way to derive

this class of neurons as well. Glutamatergic neurons induced in these studies displayed an impressive array of functional properties including evoked and spontaneous action potentials, Na^+ and K^+ currents, functional neurotransmitter receptors, and synaptic activity with detectable miniature excitatory and inhibitory post-synaptic currents (mEPSCs and mIPSCs). Thus, human iN cells represent an outstanding system where to investigate cellular and molecular mechanisms of neuronal activity in functional human neuronal cells. However, an obvious question is how iN cells resemble primary neurons in terms of gene expression dynamics, chromatin landscape, and physiology. To approach this question, Qiang et al. (2011) performed global expression profiling of iN cells and fibroblasts of origin and compared them with those of human neurons and other cells available in public databases. This comparison illustrated that iN cells clustered together with neurons rather than with fibroblasts and expressed crucial neuronal cell fate factors, synaptic proteins, neurotransmitter receptors, neuronal signaling molecules, and axon-guidance molecules. Although this is informative, it has not yet been evaluated whether some fibroblast-specific expression signature remnants are still detectable in iN cells and to what extent. Conversely, it remains unaddressed whether reprogrammed cells lack or aberrantly express some components of the molecular neuronal machinery. Thus, more studies are needed to investigate the degree of relationship existing between iN cells and primary neurons. A bottleneck in future investigations is whether or not iN cells are endowed with any specific regional identity. Indeed, conclusive evidences have been not presented as to whether iN cells exhibit a specific regionalized pattern. Some reports emphasized the expression of Tbr1 by iN cells, a well-known marker of deep cortical layers. However, this finding by itself does not guarantee a reliable cell identity, since Tbr1 is expressed by other neurons in the olfactory bulbs, eminentia thalami, and superficial cortical layers. Further analysis is required to better substantiate this important issue. If iN cells lack any regional specification, as a consequence, it will be difficult to compare them with any specific primary neuron in a meaningful way, leaving open the question how to impose a specific regional patterning in iN cells. However, it is plausible that iN cells might acquire a regional identity only after transplantation into a specific neuronal district. This hypothesis is particularly intriguing and provides an elegant experimental setting to assess how the nature of a reprogrammed neuron might be influenced by the overall brain environment.

Efficiency of neuronal reprogramming is $\leq$5% at average among those reported studies, which is a rather low ratio but still compatible with many of the next applications of this technology. However, Yoo et al. (2011) reported that the efficiency was even further reduced when starting from adult human cells and neuronal maturation is delayed as well. Thus, it is reasonable that reprogramming procedures need to be further improved in order to allow an extensive and straightforward use of adult cells. Another issue might be regarding reprogramming efficiency at different *in vitro* passages of the fibroblasts. Indeed, Ambasudhan et al. (2011) wrote that the cells employed for reprogramming were exclusively from early passages (P2–P5). Thus, it would be informative to know whether iN neuronal production remains stable over passages or, alternatively, if *in vitro* culturing restrains their conversion potential. Fibroblasts are known to become senescent after a limited number of passages *in vitro* and this might limit the amount of neurons which can be generated from each skin biopsy. Although these numbers might not be limiting for *in vitro* studies, they might not represent enough cells for *in vivo* cell-replacement approaches. Thus, it would be relevant to explore new cellular sources with a higher renewable potential and, maybe, also with an easier accessibility than skin fibroblasts.

Despite those possible improvements, these new studies offer for the first time a reliable technology to generate high numbers of human neurons with sophisticated neuronal activities that can be employed for many different applications as in particular in modeling infantile neurodevelopmental as well as neurodegenerative diseases. On the other end, assessing whether iN cells will be a source of transplantable neurons with regenerative potential represents the next big challenge.

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