From fibroblasts to neurons – how fluid is cell identity?

Subtitle: Meaning, impact and perspective of Vierbuchen et al.paper, 2010

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The function of our body relies on specialized cell types. Brain cells need to compute information, red blood cells need to bind oxygen and liver cells need to deal with metabolic demands and toxins – otherwise we are in trouble. Since DNA is identical in all cells of an organism, besides very specific cases (e.g. immune cells, germ cells, or somatic mutations), cells have to express different genes and proteins to implement the cell type-specific tasks, which, in turn, define distinct cell types. The selection and maintenance of specific cascades is particularly relevant for cells that need to guard their identity for many decades as they persist life-long, such as neurons or β-cells in the pancreas. This task was never seen as a problem, as cell identity was thought to be irreversibly fixed after development. However, this concept has been overthrown entirely in the last decades, and the work by Vierbuchen et al. [1], published exactly 10 years ago, was essential for changing this concept as it showed that even fibroblasts can be converted into functional neurons by expressing just 3 transcription factors.

This great achievement was a big step forward built on a century of visionary experiments on cell identity manipulation. Changing cell identity during development dates back about 100 years, when Hans Spemann could change entire body axis upon transplanting an organizer region [2]. Beyond this, nuclear cloning experiments pioneered in 1962 by John Gurdon showed that development can also be reverted, and a nucleus of an adult cell can re-acquire a pluripotent state capable of generating a new frog when transplanted into an oocyte [3]. Surprisingly, this gigantic task can be

achieved by the co-expression of few transcription factors (Oct4, Sox2, Klf4, Myc) [4], which are sufficient to convert fibroblasts into induced pluripotent stem cells (iPSC), capable of generating the entire multicellular organism or specialized cells from different germ layers under appropriate culture conditions. This work, awarded with the Nobel Prize in 2012, has not only revolutionized our concepts of cell identities, but provided us also with an amazing toolbox for disease modelling and cell-based therapies when applied to human cells [5].

Strikingly, pioneering work of the Weinberg group revealed that cells can be directly converted from one differentiated cell type to another. MyoD was the first transcription factor sufficient to directly reprogram fibroblasts into muscle cells [6]. Some years later, the neurogenic fate determinant Pax6 [7] was used to turn glial cells into neurons *in vitro* [8]. This approach was then translated to the *in vivo* situation [9], thus opening a new branch of cell-based replacement therapy, based on converting reactive glia after brain insults or neurodegeneration into new neurons [10]. *In vitro*, B cells were converted into macrophages [11], exocrine cells into islet β-cells [12] and fibroblasts into cardiomyocytes [13]. These remarkable fate conversion paradigms have an important common hallmark, namely that they all occurred within the same germ layer. Germ layers are early developmental separations of cells – endoderm, ectoderm and mesoderm – that then generate different organs. Therefore, it was assumed that cells may be converted to other cell types only within the same germ layer, based on a closely related developmental origin.

This dogma was shattered by the work of the Wernig lab 10 years ago, when fibroblasts from embryos or newborn mice were converted into functional neurons by the co-expression of Brn2, Ascl1 and Myt1l (now known as BAM factors, Figure 1). This was the first time that a mesoderm-derived cell type was directly reprogrammed into a cell type of the ectodermal origin. By showing that developmental barriers do not represent an unsurmountable hurdle, the paper had tremendous impact on several levels. First, it sparked the interest in direct neuronal reprogramming, as all of a sudden a relatively accessible cell type, easy to prepare and cheap to grow, could be converted into a cell type of great therapeutic interest, neurons. Second, this was followed by the direct conversion of fibroblasts of human origin into neurons [14]; within few years, transcription factor “cocktails” were defined to instruct diverse neuronal subtypes (e.g. somatic motor neurons, dopaminergic neurons, spiny neurons [15-17]). The third fundamental step forward in direct neuronal reprogramming was the

discovery that 'induced neurons', as coined by Vierbuchen et al., retain their cellular age – i.e. fibroblasts derived from a 60 year old donor and the reprogrammed neurons showed an according cellular age, very different from IPSCs reprogramming, where aging hallmarks are reset and cells rejuvenatesd [18]. Thus, direct neuronal reprogramming is well suited to obtain neurons for studying age-related neurodegenerative diseases [19].

Beyond the high impact on translational aspects, this work raised profound conceptual questions on the role of developmental origin and cell identity maintenance. If common germ layer origin eases the direct reprogramming (Figure 1), keratinocytes, derived from the ectoderm, should be readily converted into neurons, but they are not [20]. Even more strikingly, the conversion of one neuronal subtype into another is restricted to development [21]. This calls to re-think of models that account for more fluid switch across different cellular identities, such as Cook's islands model [22,23]. However, no systematic studies on exploring the reprogramming potential of the same starter cell into different target cells have yet been performed (Figure 1), thus preventing the identification of common rules in the conversion process so far. This includes the identification of programs of fate erasure and, conversely, of fate maintenance. As the mechanisms of fate maintenance have crumbled under the ease of direct reprogramming, we need to understand what keeps cells stable over decades. Which are the mechanisms (passive and active) that regulate the expression of transcription factors that can readily change fate? Are long-lived cells indeed more difficult to convert due to more elaborated fate maintenance mechanisms? Could this be the reason why human cells are much harder to convert into other cell types than murine cells? Clearly, the identification of these mechanisms may not only be a conceptual breakthrough, but would also help to overcome conditions with slight fate erasures as part of functional deterioration during aging.

Direct reprogramming has revolutionized our concepts in many regards and allows exploring fascinating basic questions beyond achieving a revolution in disease modelling – one decade after the breakthrough discovery published by Thomas Vierbuchen, Marius Wernig and co-workers.

1 Vierbuchen, T. *et al.* Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **463**, 1035-1041, doi:10.1038/nature08797 (2010).

2 Mangold, O. & Spemann, H. *Wilhelm Roux Arch Entwickl Mech Org* **111**, 341-422, doi:10.1007/BF02080953 (1927).
3 Gurdon, J. B. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J Embryol Exp Morphol* **10**, 622-640 (1962).
4 Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-676, doi:10.1016/j.cell.2006.07.024 (2006).
5 Takahashi, K. *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861-872, doi:10.1016/j.cell.2007.11.019 (2007).
6 Davis, R. L., Weintraub, H. & Lassar, A. B. Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* **51**, 987-1000 (1987).
7 Quiring, R., Walldorf, U., Kloter, U. & Gehring, W. J. Homology of the eyeless gene of Drosophila to the Small eye gene in mice and Aniridia in humans. *Science* **265**, 785-789, doi:10.1126/science.7914031 (1994).
8 Heins, N. *et al.* Glial cells generate neurons: the role of the transcription factor Pax6. *Nature neuroscience* **5**, 308-315, doi:10.1038/nn828 (2002).
9 Buffo, A. *et al.* Expression pattern of the transcription factor Olig2 in response to brain injuries: implications for neuronal repair. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 18183-18188, doi:10.1073/pnas.0506535102 (2005).
10 Barker, R. A., Gotz, M. & Parmar, M. New approaches for brain repair-from rescue to reprogramming. *Nature* **557**, 329-334, doi:10.1038/s41586-018-0087-1 (2018).
11 Xie, H., Ye, M., Feng, R. & Graf, T. Stepwise reprogramming of B cells into macrophages. *Cell* **117**, 663-676 (2004).
12 Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J. & Melton, D. A. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* **455**, 627-632, doi:10.1038/nature07314 (2008).
13 Ieda, M. *et al.* Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* **142**, 375-386, doi:10.1016/j.cell.2010.07.002 (2010).
14 Pang, Z. P. *et al.* Induction of human neuronal cells by defined transcription factors. *Nature* **476**, 220-223, doi:10.1038/nature10202 (2011).
15 Caiazzo, M. *et al.* Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature* **476**, 224-227, doi:10.1038/nature10284 (2011).
16 Son, E. Y. *et al.* Conversion of mouse and human fibroblasts into functional spinal motor neurons. *Cell Stem Cell* **9**, 205-218, doi:10.1016/j.stem.2011.07.014 (2011).
17 Victor, M. B. *et al.* Generation of human striatal neurons by microRNA-dependent direct conversion of fibroblasts. *Neuron* **84**, 311-323, doi:10.1016/j.neuron.2014.10.016 (2014).
18 Mertens, J. *et al.* Directly Reprogrammed Human Neurons Retain Aging-Associated Transcriptomic Signatures and Reveal Age-Related Nucleocytoplasmic Defects. *Cell Stem Cell*, doi:10.1016/j.stem.2015.09.001 (2015).
19 Liu, M. L., Zang, T. & Zhang, C. L. Direct Lineage Reprogramming Reveals Disease-Specific Phenotypes of Motor Neurons from Human ALS Patients. *Cell Rep* **14**, 115-128, doi:10.1016/j.celrep.2015.12.018 (2016).
20 Wapinski, O. L. *et al.* Hierarchical mechanisms for direct reprogramming of fibroblasts to neurons. *Cell* **155**, 621-635, doi:10.1016/j.cell.2013.09.028 (2013).

21 Rouaux, C. & Arlotta, P. Direct lineage reprogramming of post-mitotic callosal neurons into corticofugal neurons in vivo. *Nat Cell Biol* **15**, 214-221, doi:10.1038/ncb2660 (2013).
22 Gascon, S., Masserdotti, G., Russo, G. L. & Gotz, M. Direct Neuronal Reprogramming: Achievements, Hurdles, and New Roads to Success. *Cell Stem Cell* **21**, 18-34, doi:10.1016/j.stem.2017.06.011 (2017).
23 Sieweke, M. H. Waddington's valleys and Captain Cook's islands. *Cell Stem Cell* **16**, 7-8, doi:10.1016/j.stem.2014.12.009 (2015).