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Direct Reprogramming to Vascular Cells: A Mini-Review

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ABSTRACT

Recent advances in the field of direct reprogramming have changed the way we see and study the plasticity of somatic cells. Many research groups worldwide are developing techniques with which one cell type is directly converted into another without passing through an intermediate multipotent stem cell-like state. Ectopic overexpression of transcription factors, microRNAs, epigenetic and metabolic regulators, even exosomal particles, have been proven sufficient in yielding a variety of cell types from fibroblasts, with neurons, hepatocytes, cardiomyocytes, endothelial cells and hematopoietic progenitor cells being amongst them. These studies have been an inspiration for creating new approaches in regenerative medicine, especially in the field of cardiovascular biology. Developing novel methods of regenerating the damaged myocardium and endothelium are crucial to millions of patients worldwide suffering from diabetes and cardiovascular disease. In the present study, we aim to review the progress of direct reprogramming and discuss the possible applications of this technology in regenerative therapy, disease modelling and drug discovery.

FROM INDUCED PLURIPOTENT STEM CELLS TO DIRECT REPROGRAMMING

In 2006, Takahashi and Yamanaka [1] reported the existence of the first successful line of induced pluripotent stem cells (iPSCs) derived from mouse fibroblasts by forcing the overexpression of 4 transcription factors, *Oct4*, *Sox2*, *Klf4* and *c-Myc* (also known as OSKM or Yamanaka factors). Shortly after, iPSCs were successfully derived from other species including humans [2], rats [3] and rhesus monkeys [4]. Since then, multiple groups have developed protocols modifying the OSKM cocktail to improve efficiency and address safety challenges, resulting in the generation of well-characterised iPSCs from a variety of sources. While iPSCs are a valuable tool in regenerative medicine, the risk of tumour development makes their clinical application in humans challenging. Arguably, using iPSCs-derived cells for disease modelling, drug development and study in the lab is invaluable; for example, there are multiple protocols that efficiently produce functional cardiomyocytes and endothelial cell (EC) types. However, the safety of these cells is questioned due to the integration of foreign DNA in the human cells.

HISTORY OF CELLULAR REPROGRAMMING

The road to pluripotency has inspired the hypothesis that, during reprogramming, there is a short window when the cell is epigenetically "fluid". Slowly but steadily, the idea of manipulating the cell fate during this time frame excited the scientific community and, thus, attempts to directly reprogram a somatic cell line into another commenced. In 1987, Davis et al. [5] introduced the idea of transdifferentiation from fibroblasts into myoblasts via epigenetic modulation. Even though it did not receive the same amount of attention as the reprogramming attempts to pluripotency that took place almost 20 years later, it did serve as inspiration for scientists. At this point, it is very important to note that during these years the idea was not forgotten or ostracised. There are multiple publications - some of them now retracted - claiming that during embryogenesis blood cells turn into neurons or hepatic cells but these claims were never reproduced so the idea of direct reprogramming was very hard to be accepted [6]. The OSKM reprogramming was the catalyst of a revolution in the field that resulted in hundreds of publications tweaking

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and twisting every little aspect of the original idea so that the generation of therapeutic cells becomes a reality.

PARTIAL REPROGRAMMING

Moving forward, in 2011 reports of successful conversion of mouse fibroblasts into neural progenitors [7] revived the interest and in 2012 the idea of a short partial reprogramming was introduced, in which the cells bypassed the intermediate multipotent state and were directed towards fully functional ECs [8] that had high angiogenic ability both in vivo and in vitro. A year later, human smooth muscle cells (SMCs) were generated through the partially-induced pluripotent stem (PiPS) cells technology [9]. The combination of PiPS-ECs and PiPS-SMCs in vascular grafts was reported to increase the survival of SCID mice [9] in vivo and create a very promising mode of studying cardiovascular disease in vitro [10-12].

The protocols kept evolving and shortly only two or even one, out of the four OSKM factors was enough to reprogram fibroblasts towards an EC fate. Li et al. [13] generated functional human ECs in a month using only Oct4 and Klf4 while murine fibroblasts were directed into an EC fate using only Oct4 and small molecules [14].

REFINING THE DIRECT REPROGRAMMING APPROACH TO IMPROVE EFFICIENCY

Additional approaches towards more effective reprogramming have also been examined, with the OSKM factors left out in favour of transcription factors that are specific to the endpoint cell line [15]. For example, Ieda et al. [16] showcased the conversion of human fibroblasts into cardiomyocytes with Gata4, Mef2c and Tbx5 (GMT) and Han et al. [17] into endothelial cells with Foxo1, Er71, Klf2, Tal1 and Lmo2 [17]. However, in both cases the efficiency was quite low (5%). This led to trying out even more combinations where, for example, the addition of Hand2 and NK2 homeobox 5 (Nkx2.5) achieved a 50-fold increase in efficiency compared to GMT alone [18]. Other combinational strategies evaluated specific miRNAs (miR-1, miR-133, miR-208 and miR-499) for the in vitro induction of direct cell reprogramming of fibroblasts to cardiomyocytes [19]. Furthermore, reprogramming efficiency was also shown to be improved by modulators of epigenetic-related enzymes, such as DNA methyltransferases [20].

Despite all these advances, screenings for genes that regulate the cell fate towards a cardiovascular cell fate continued and eventually introduced the idea of a "master regulator" that plays a key role in the early development of the vessels and the heart [21,22]. In ECs, for example, Etv2 was shown to be quite promising, with several groups using it to convert human amniotic cells or fibroblasts into ECs [23-25]. At the same time, it is long known that inhibiting or activating certain pathways will change the cell fate. Harding et al. [26] proved that the MAPK and the PI3K pathways are crucial to

EC differentiation from iPSCs with high purities (94%-97% CD31+ and 78%-83% VE-cadherin+) in 8 days without cell sorting. Cases like this showcase the importance of epigenetic modulation, with subsequent stabilisation of this state, so that it can produce functional cells.

STABILISING THE CELL FATE

Attempting to stabilise the cell fate in the reprogramming cells led to the use of small molecules either during or after differentiation. The use of recombinant proteins to enhance the activity of certain pathways is widely used, with the VEGF pathway being one of the most important ones due to its significant role in ECs; even a small amount of VEGFA supplement in the culture media can lead to the significant enhancement of EC function and morphology [27-29]. Currently, reprogramming with small molecules is investigated with breakthroughs in neuron [30] and cardiac [31] generation. Cases like this are distinctive and their integration into the reprogramming technology will give rise to protocols that have higher efficiency and, in some cases, are shorter in duration.

CHALLENGES AND A LOOK INTO THE FUTURE

In animal embryos, cells transition from a multi potential state, with the capacity to adopt multiple fates, into an irreversible, committed state of differentiation [32]. This is a phrase that was accepted as a universal truth for many years. Being able to rewind the clock on a committed cell line by manipulating the microenvironment and its genetic makeup, contradicts its finality and allows us to look past that: 1. Investigating the molecular mechanisms during differentiation is the key to unlocking the epigenetic networks at work so that a usable vascular model can be created. 2. Further studies, including optimization of vascular reprogramming in human fibroblasts, are needed. As the demand is high for new regenerative therapies, the opportunities and the potential benefits of this direct reprogramming approach are significant. With this final thought two questions remain; which approach is better? What is the difference between the mechanisms involved in the two approaches? Maybe we are not any closer to answering these than we were 30 years ago but studying how the epigenetic and metabolic profile of the cells change during differentiation from iPSCs to a committed cell type is certainly the key to discovering the missing piece of the puzzle of direct somatic reprogramming. The epigenetic changes are crucial in directing a different phenotype and mimicking the microenvironment of the target-cell will facilitate the transition. On this basis, the idea of in vivo reprogramming – an interesting sum of techniques, albeit still not proven safe [33-35] and its possible combination with in vitro re-modelling would create a model to study vascular regeneration universally.

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