Opening up the black box of human cell plasticity

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Cell plasticity represents the ability of cells to acquire new characteristics. Cell plasticity can be reset in somatic cells via cellular reprogramming, a process that occurs in regeneration. However, cell plasticity occurs rarely in humans. Recently, Guan et al. recreated cell plasticity in mature human cells, which they found to be essential for reprogramming human somatic cells to pluripotent stem cells using only small molecules. As the first study demonstrates that chemical compounds can completely reprogram human somatic cells to a pluripotent state, it is a paradigm shift in developing pharmacological or therapeutic reprogramming approaches to control human cells.

Research on nuclear reprogramming dates back to the 1950s when British biologist John Gurdon cloned a frog by experiments with nuclear transplantation. His work in *Xenopus*, including the nuclear transfer technique, rapidly attracted attention from the research community to explore nuclear and cell plasticity in development. Similar observations were reported in other species—the birth of Dolly the sheep marked the first-ever cloned mammal.1 In distinction to the use of oocyte cytoplasm, Japanese scientist Shinya Yamanaka identified four transcription factors that together reprogrammed mouse somatic cells to pluripotent stem cells in 2006; the approach was applied to human cells 1 year later (Figure 1).2 These cells are called induced pluripotent stem cells (iPSCs).

These breakthroughs suggested that cell plasticity could be controlled using cellular or genetic materials, which led to the question of whether we could manipulate the cell plasticity using only defined chemical compounds that could mimic external stimuli on cells by modulating signal and transcription networks. In 2013, the Deng lab succeeded in using only chemical compounds to reprogram mouse somatic cells to iPSCs, referred to as CiPSCs,3 which prompted the entire field to generate human CiPSCs. Over the following years, the Deng lab further analyzed the detailed molecular roadmaps of the mouse CiPSC induction process.4 A major surprise was that chemical reprogramming did not induce the activation of

Figure 1. Historical view of the development of reprogramming and schematic of chemical reprogramming from somatic cells to human CiPSCs. Left: selected advances in the development of chemical reprogramming are highlighted. Right: schematic diagram for key molecular events during the chemical reprogramming process including epithelial-like cells, intermediate plastic state, XEN-like colonies, and a primary pluripotency network. These human CiPSCs are able to be differentiated into HPCs, T cell lineages, hepatocytes, and neural stem cells. SCNT, somatic cell nuclear transfer; iPSCs, induced pluripotent stem cells; PSCs, pluripotent stem cells; CiPSCs, chemical induced pluripotent stem cells; XEN-like, extraembryonic endoderm-like.
Yamanaka factors directly, suggesting a fundamentally different way to pluripotency induction by external chemical stimulation. Despite multiple generations of mouse chemical reprogramming protocols, little progress has been made in the generation of human CiPSCs. These failures suggested that simply providing chemical stimuli in cell culture media may not be able to reprogram human somatic cells because of their restricted cell plasticity.

It has been thought that somatic cells of different organisms have distinct degrees of ability to respond to environmental stimulations. Following environmental stimulations, plants and animals, including invertebrates, amphibians, and reptiles, show remarkable reprogramming activity that can lead to regeneration, which has not been observed in humans. For example, axolotl somatic cells have a remarkable capability to regenerate almost any body part, including whole organs such as limbs. Additionally, human somatic cells are suggested to be more resistant to reprogramming, and, thus, no study has been able to induce pluripotency in human cells using chemical stimulation. Therefore, the key question is how to liberate human cell pluripotency by external stimulation.

Guan and colleagues have now devised a strategy to emulate the stepwise process during the regeneration by chemical-induced reprogramming. Somatic cells in invertebrates and some vertebrates often create an intermediate stage via cell dedifferentiation. In particular, cell proliferation is an important characteristic of this stage—highly proliferative cells in this transient state have unchained chromatin accessibility, providing unique plasticity for rewiring the whole gene circuitry toward pluripotency. By screening chemical libraries, Guan and colleagues identified the combination of small molecules that could reprogram human somatic cells to CiPSCs in a sequential manner including four stages (Figure 1). The resultant human CiPSCs rival embryonic stem cells for similar characteristics, including transcriptomic and epigenomic profiles, genomic stability, and developmental potentials to generate three germ layers and functional cell types including hematopoietic cells, neural progenitor cells, and hepatocytes.

Beyond the generation of human CiPSCs, the authors also generated a human chemical reprogramming cell atlas, providing insights into the regulation of cell plasticity in human somatic cells. Single-cell RNA sequencing and assay for transposase-accessible chromatin with high-throughput sequencing revealed a potential reprogramming mechanism by which chemical stimulations induced dedifferentiation that subsequently removed epigenetic barriers, reduced global DNA methylation, and generated an intermediate plastic state. At the intermediate plastic state, cells developed a regeneration-like program and expressed genes whose ontology pertained to limb development and regeneration. As such, Guan and colleagues compared their plastic intermediates with the blastema cells in axolotl’s limb regeneration as well as with frog and mouse cells that were incompetent for limb regeneration. The results showed that the intermediates were reprogrammed to obtain a human embryonic limb-bud-like gene program, a situation that is similar to the early blastema of the axolotl that also develop an embryonic limb-bud gene program during dedifferentiation. However, dedifferentiation was not captured in frog and mouse, which did not show significant activation of an embryonic gene-expression program following injury. This observation suggests that human somatic cells are able to reacquire cell plasticity following chemical stimulation, similar to the process occurring in axolotl limb regeneration, which is of interest in regeneration research across species.

Additionally, Guan and his colleagues identified an extraembryonic endoderm-like (XEN-like) state, which bridges the induction of pluripotency during the late stage. Interestingly, the intermediate XEN-like state is conserved between mouse and human chemical reprogramming and mediates the generation of CiPSCs. It is possible to find other small molecules to booster the reprogramming process by manipulation of the XEN state to facilitate establishing a more robust system. Additionally, previous study showed that mouse XEN-like cells are plastic in cell fates and can be induced into alternative cell fates, including neurons, hepatocytes, and other functional cells, instead of the pluripotent cells. Consequently, it is important to further test whether human XEN-like cells also have such capacity.

Guan et al. also determined key genes governing the plastic intermediates, including the homeobox (HOX) genes MSX1, MSX2, and HOXB9, as well as reprogramming-associated genes SALL4 and LIN28A. Knockdown of these gene products arrested reprogramming at the plastic intermediate stage and prohibited the generation of CiPSCs. Knockdown of SALL4 and LIN28A also downregulated the expression of MSX1, MSX2, and HOXB9 in the plastic intermediates, suggesting that MSX1, MSX2, and HOXB9 are downstream targets of SALL4 and LIN28A. In addition, the author identified the JNK signaling pathway as a reprogramming barrier, inhibition of which is essential for SALL4 reactivation. Moreover, without JNK inhibition, tumor necrosis factor α and interleukin-1β pro-inflammation pathways were significantly enriched in cells. Indeed, adding tumor necrosis factor α or interleukin-1β to the culture impeded the generation of CiPSCs, highlighting pro-inflammation pathways associated with the JNK pathway as a major barrier to the recreation of plasticity in human somatic cells.

Generating human CiPSCs using only small molecules provides a unique route to manufacture pluripotent stem cells that meet clinical requirements. In principle, small molecules do not integrate into the genome, and their effects are more controllable than biological materials, which will make CiPSC manufacturing protocols easier to be standardized and, thus, provide an attractive pathway for producing patient-specific stem cells for clinical applications. Recently, another research from Deng’s lab showed that pancreatic islets can be efficiently generated from human CiPSCs and that these islets could effectively restore insulin secretion and improve glycemic control after transplanting into diabetic non-human primates. These results strongly demonstrated that human CiPSCs hold great potential as “seed cells” to generate functional cells for clinical application. However, the currently published protocol uses serum-sourced products, including fetal bovine serum, that could entail safety and reproducibility concerns. Additionally, considering the complexity and the amount of time that the chemical reprogramming process takes, it is important to fine-tune the small molecules and culture conditions to develop a faster and more robust chemical reprogramming system with high efficiency. Optimizing the reprogramming method to be serum free, and ideally the entire production process of human CiPSCs, will be a primary focus of future efforts.

In summary, the chemical reprogramming of human somatic cells to CiPSCs underscores the capacity of external chemical stimulations to unlock the restricted epigenetic landscape of human cells into a plastic state. The chemical approach provides a new platform for generating and applying human pluripotent stem cells in biomedicine. This study also lays new foundations for developing regenerative therapeutic strategies that use well-defined chemicals to change human cell fates.

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DECLARATION OF INTERESTS
The authors declare no competing interests.