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Previews

And Then There Were None: No Need for Pluripotency Factors to Induce Reprogramming

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While most factors used as reprogramming transgenes can be replaced by other means, Oct4 has remained essential until now. Three recent papers have now broken this barrier through the use of opposing lineage specifying transgenes and chemical modulation, thus signifying a milestone in advancing our understanding of pluripotency induction.

Seven years ago, Takahashi and Yamanaka (2006) provided a method to reprogram somatic cells to induced pluripotent stem cells (iPSCs) by using a small group of transcription factors, OCT4, SOX2, KLF4, and c-MYC, that are highly expressed in mouse and human embryonic stem cells (ESCs). Other core pluripotency genes such as NANOG and LIN28 that are also enriched in ESCs and iPSCs were later added to achieve reprogramming of human fibroblasts or enhance reprogramming efficiency of refractory somatic cell types. A prevailing model that effectively explains the existing data is the following: once overexpressed in somatic cells, these core pluripotency genes switch on the core transcriptional circuitry associated with pluripotency, change or reverse expression of genes controlling the somatic cell fate, and gradually establish a pluripotent state that is maintained by endogenous core genes. This model also predicts and explains the success of using other transgenes (including microRNAs) or small molecules to replace several of these core pluripotency factors as reprogramming transgenes. However, as a transgene, *Oct4* has remained indispensable in somatic cell reprogramming to iPSCs, underscoring its master role in pluripotency regulation. Moreover, recent investigation has revealed that core pluripotency genes (such as *Oct4* and *Sox2*) also function in lineage specification and their overexpression drives stem cells toward a specific embryonic germ layer (ectoderm, mesoderm, or endoderm) while blocking other lineages (Loh and Lim, 2011; Thomson et al., 2011; Wang et al., 2012). Based on these insights, a new model has been formulated that describes the pluripotent state as a precarious balancing condition that results from continuous competition between rival lineage specifications (Loh and Lim, 2011).

This new model, as opposed to the classic "ground state" model of ESCs and PSC induction by pluripotency factors, has now been substantiated by a recent study in *Cell* (Shu et al., 2013). In this elegant study, Shu et al. conducted a large-scale search for genes that can replace *Oct4* as a transgene in the reprogramming of mouse fibroblasts. Their systematic analysis demonstrated that *Gata3*, as well as other lineage-specifying genes (such as *Gata6*) that are known to positively regulate meso-endodermal commitment, can replace *Oct4* as a reprogramming factor. This substitution ability likely operates by counteracting the elevation of ectodermal genes induced by *Sox2* present the reprogramming gene cocktail. Reciprocally, they demonstrated that Gmnn, a known ectodermal specifying factor, can replace *Sox2* in reprogramming, because it hampers the elevation of meso-endodermal genes induced by *Oct4* present in the reprogramming cocktail. Most strikingly, they showed that concurrent coexpression of *Gata3* and *Gmnn* can substitute for *Oct4* and *Sox2* as transgenes for reprogramming mouse fibroblasts to iPSCs (in the presence of *Klf4* and *c-Myc* transgenes), thus eliminating the need for any core pluripotency genes as reprogramming factors. Their data suggest a "seesaw" model wherein achieving a precarious balance of the two counteracting forces, commitment to ectoderm and meso-endoderm lineages, results in induction of pluripotency (Shu et al., 2013).

Now, in this issue of *Cell Stem Cell*, Montserrat et al. (2013) show that lineage-specifying factors can also be used to replace *OCT4* and *SOX2* as transgenes in reprogramming human fibroblasts to iPSCs. Montserrat et al. (2013) first observed that in addition to core pluripotency markers (such as OCT4 and SOX2), human PSCs express several genes that were previously known for roles in lineage specification. They found that *GATA3* plays a similarly powerful role in lineage specification to that of *OCT4*, and they successfully replaced *OCT4* with *GATA3* in human fibroblast reprogramming. Molecular analyses revealed that *GATA3* and *OCT4* can upregulate each other in the presence of *SOX2*, *KLF4*, and *c-MYC* transgenes. Surprisingly, *GATA3* knockdown in human iPSCs attenuated *SOX2* and *NANOG* expression, but not *OCT4*, and resulted in iPSC death, indicating that appropriate *GATA3* expression is crucial for human iPSC or ESC maintenance. Using similar logic, the authors identified factors that are related to ectoderm commitment and demonstrated that *ZNF521* can replace *SOX2*. Lastly, they showed that *GATA3* with a combination of three ectoderm specifiers, *ZNF521*, *OTX2*, and *PAX6*, can replace *OCT4* and *SOX2* for human iPSC induction in the presence of *KLF4* and *c-MYC*.

Taken together, data generated by Montserrat et al. support the seesaw model in which lineage-specifying factors can replace OCT4 and SOX2 for iPSC induction of human fibroblasts, as previously proposed in the mouse

fibroblast reprogramming study (Shu et al., 2013).

It has been known for 20 years that nullipotent embryonic germ cells could be induced to pluripotency without any pluripotency genes or any exogenous genes (Matsui et al., 1992; Resnick et al., 1992). As of recently, many pluripotency factors, such as SOX2, have been successfully replaced by small-molecule compounds that alter cell signaling or epigenetic landscapes of somatic cells in reprogramming. However, the question remained as to whether somatic cell reprogramming could be achieved with minimal or even no exogenous genes, mRNAs, or proteins. A recent study led by Deng's group now appears to have an answer. Hou et al. (2013) have now demonstrated that mouse fibroblasts can be induced to iPSCs solely by chemical manipulation, or more precisely, by seven small molecule compounds. The preliminary investigation of the underlying mechanisms of this process, called chemical reprogramming, suggested that the chemical combination activated upstream genes of *Oct4*, like *Sall4* and *Sox2*, and also promoted Oct4 expression by epigenetic modulation. It remains to be determined whether reprogramming of human somatic cells to iPSCs can be achieved solely by chemical means. It is of importance to investigate whether the chemical reprogramming of human somatic cells by a cocktail of chemical compounds (if feasible and efficient enough) would be a better option than that accomplished by either an RNA cocktail or a nonintegrating method with a single DNA vector that can be easily manufactured under current good manufacture practices (cGMP) conditions. Time will tell which method is preferred by most practitioners for generation of cGMP-compliant iPSC lines, which is also an economic, practical, and regulatory matter. The chemical reprogramming approach, however, will no doubt provide new insights into the minimal or alternate requirements for pluripotency induction.

In the exciting journey toward reducing genes and developing better methods for somatic cell reprogramming, these recent studies have shown that the *Oct4* transgene can be replaced finally by the lineage-specifying genes or a cocktail of small molecules. Therefore, none of the original four Yamanaka factors, in addition to Nanog, Lin28, and many other pluripotency genes, are indispensable. This recent unexpected development in the journey to understand and solve the pluripotency mystery reminds us of the nursery rhyme that mirrors the plot in the detective novel "And Then There Were None" authored by Agatha Christie. These findings do not mean that core pluripotency genes such as *Oct4* and *Sox2* are not important. These recent advances signify that we are now entering a new phase of reprogramming technology that focuses on the means to better decipher the molecular mechanisms and networks of pluripotency induction, and more broadly of cell fate determination in a nonlinear fashion. It seems fitting to describe this milestone with what Winston Churchill once said: "Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning."

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