Directing Smooth Muscle Cell Fate
A Partial Reprogramming Approach to Engineer Vessels

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Cellular reprogramming has opened new doors for scientists to study human disease and develop methods for diagnosis and therapy. Cellular reprogramming has been around since first achieving somatic nuclear reprogramming in 1962, and identifying a single transcription factor could convert fibroblasts into muscle in 1986. But in 2006, the reprogramming field was suddenly transformed when Shinya Yamanaka discovered that ectopic expression of only 4 transcription factors consisting of OCT4, SOX2, KLF4, and c-MYC (OSKM) could transform skin fibroblasts into an embryonic stem cell–like fate, aka, induced pluripotent stem (iPS) cells that have the potential to become any cell type in the body. The realization that a cell’s fate is reversible through defined and simple genetic manipulation created a wave of new direct reprogramming studies—reprogramming fibroblasts into an alternative mature cell type without passing through a pluripotent state—to search for genetic cocktails to manipulate and direct cell fate. Studies performed by multiple groups elegantly showed that a cocktail of 3 cardiac transcription factors important during cardiac development could directly reprogram cardiac fibroblasts into cardiomyocyte-like cells in vitro. Furthermore, the reprogramming process was more effective in endogenous cardiac fibroblasts after injury in a mouse model. As direct reprogramming of fibroblasts into cardiomyocytes and neurons has been successful, it is thought that other mature cell types can be generated from fibroblasts if given the right cocktail of factors.

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In the meantime, while researchers search for the right cocktail to drive direct differentiation for each cell fate, alternative cell reprogramming approaches have emerged, which provide a faster, safer, and more efficient strategy than the current iPSC technology. These approaches involve shortcuts to reprogram fibroblasts through a process of partial reprogramming. Rather than a cocktail of transcription factors to direct differentiation from fibroblasts, the Yamanaka factors are introduced, and after a few days in culture, the fibroblasts take on a partially induced pluripotent state (PiPS), which gives them sufficient plasticity to differentiate into a particular cell lineage on defined culture conditions. This approach was first reported when Kim et al and Efe et al showed that partial reprogramming of fibroblasts generated a progenitor-like population of cells that had potential to differentiate toward the neuronal and cardiac lineage. The study by Karamariti et al in this issue of Circulation Research used a similar strategy of partial reprogramming. They introduced OSKM into human lung fibroblasts to generate PiPS cells that differentiated into the vascular smooth muscle cell (SMC) phenotype when exposed to SMC culture conditions. This work followed their previous study that showed that PiPS cells could differentiate into functional endothelial cells in response to vascular endothelial growth factor and endothelial culture conditions.

Through this approach, Karamariti et al characterized the PiPS cells after a 4-day exposure to OSKM and noted morphological and gene expression changes indicative of a de-differentiated or a partially reprogrammed cell. Furthermore, they found no sign of teratomas when injecting the PiPS cells into nude mice, which suggests that this strategy may offer an advantage over iPSC cells by lowering the risk of tumor formation. Characterization of the PiPS cells that differentiated into SMCs revealed expression of smooth muscle (SM)–specific genes and proteins, including the mature SM-myosin heavy chain and contractile-specific smoothelin, and downregulation of fibroblast markers. However, the authors did not address the expression of SM-specific microRNAs (ie, miR-143 and miR-145), which are expressed in mature SMCs and promote SMC differentiation from multipotent and embryonic stem cells.

In vessels, mature SMCs contain a highly specialized ultrastructure and they function to regulate vascular tone via contraction. More convincingly, the PiPS-SMCs possessed a contractile response to the depolarizing agent, KCl, suggesting that they are functional SMCs. In addition, SMCs possess the ability to de-differentiate into a proliferative cell on stimulation of different growth signals, and this phenotypic modulation allows vessel remodeling and repair on injury. However, the authors did not test whether the PiPS-SMCs undergo phenotypic modulation. Despite this caveat, the authors did show that the PiPS-SMC growth rate was similar to that of primary human SMCs cultured in similar conditions. Phenotypic modulation of SMCs can also contribute to the pathogenesis of diseases, including atherosclerosis, restenosis, and arterial aneurysms. As we continue to pursue reprogramming strategies, it will be important to incorporate our understanding of the key developmental pathways that regulate SMC maturation, contraction, and dedifferentiation. This further understanding may allow us to reverse the phenotypic modulation of SMCs that occurs during vascular disease.
Isolation and purity of SMCs have been difficult in embryonic stem and iPSC-directed differentiation protocols because of the lack of a cell-surface marker to identify and distinguish them from other similar cell types, such as fibroblasts and activated fibroblasts (myofibroblasts). Researchers have relied on antibiotic selection or negative selection from sorting.\textsuperscript{17,18} Without antibiotic selection, Karamariti et al\textsuperscript{12} obtained \( \approx 40\% \) SMC conversion, and with antibiotic selection, they claim that 100\% of iPSC cells become SMCs. This is a remarkable feat in gaining SMC purity, but the authors do not show quantification achieved with antibiotic selection. Fibroblasts, myofibroblasts, and SMCs share expression of most SM-specific genes, but have very different functions in vivo. Several reports highlight transient expression of contractile SM genes in other cell types besides SMCs,\textsuperscript{19,20} but these authors used multiple criteria to show that they had achieved SMC differentiation.

Beyond obtaining functional SMCs, Karamariti et al\textsuperscript{12} elucidated the mechanism of PiPS-SMC differentiation. They provide evidence that a member of the Dickkopf family, DKK3, enhances SM gene expression by activating the SM-specific gene, SM22, by positively modulating the canonical Wnt pathway. This observation contrasts with findings that show that DKK family members negatively regulate canonical Wnt signaling.\textsuperscript{21,22} In addition, they showed that DKK3 acts as a cytokine by interacting with the transmembrane protein Kremen1 to induce \( \beta \)-catenin translocation, which, in turn, activates SM22 transcription. This study reveals a novel role for \( \beta \)-catenin translocation, which, in turn, activates fibroblasts (myofibroblasts). Researchers have relied on antibiotic selection or negative selection from sorting.\textsuperscript{17,18} Without antibiotic selection, Karamariti et al\textsuperscript{12} obtained \( \approx 40\% \) SMC conversion, and with antibiotic selection, they claim that 100\% of PiPS cells become SMCs. This is a remarkable feat in gaining SMC purity, but the authors do not show quantification achieved with antibiotic selection. Fibroblasts, myofibroblasts, and SMCs share expression of most SM-specific genes, but have very different functions in vivo. Several reports highlight transient expression of contractile SM genes in other cell types besides SMCs,\textsuperscript{19,20} but these authors used multiple criteria to show that they had achieved SMC differentiation.

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Importantly, Karamariti et al\textsuperscript{12} reported that they seeded decellularized vessels with PiPS-SMCs and PiPS-endothelial cells, thus creating an engineered vessel from sources that were once lung fibroblast cells. The architecture of the ex vivo double-seeded vessels resembled that of native mouse arteries after culture for 5 days. In addition, engraftment of the PiPS-SMC and PiPS-endothelial cell seeded vessels in mice led to the generation of mostly patent vessels mimicking healthy native vessels. Further evidence supported that the cells contract in decellularized vessels ex vivo, but better tools to measure and assess contractility of engineered vessels are required for future in vivo studies. Some issues arise from this study. For example, the fibroblasts were derived from human embryonic lung. However, will lung biopsies to generate SMCs prove too invasive for modeling patient-specific disease or designing patient-specific therapies? The efficiency to differentiate human dermal fibroblasts into SMCs was quite low. Can the protocol be optimized to find the right cocktail of factors to generate SMCs from skin? In addition, is a better definition of the type of functional SMC that is generated through reprogramming approaches necessary to help model a particular disease or engineer vessels for areas with increased blood pressure?

Advances in the field of regenerative medicine will continue to unravel and shed light on the best approaches to achieve a particular cell type. The approach described above, combined with other techniques of generating SMCs, will undoubtedly provide clues to generate the most efficient, functionally relevant SMCs for tissue engineering, modeling vascular disease in a dish, and screening drugs for vascular therapies. It will be necessary to define SMCs more accurately to understand the different properties of aortc and intestinal SMCs, among other types, for example. Thanks to recent advances in high-throughput genome sequencing and epigenetic mapping, this type of analysis may reveal the subtle differences between SMC lineages and help us to reconstruct the proper vascular structure in vitro and in vivo.

We have entered an exciting and revolutionary era in regenerative medicine that has begun to transform our approach to treatment and prevention of human disease. Although many challenges lie ahead, the prospect of regenerating damaged vessels or tissues through cell reprogramming approaches and vascular engraftment holds promise to heal previously irreparable tissues.

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References


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