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Transdifferentiation by defined factors as a powerful research tool to address basic biological questions

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Ectopic expression of master regulators can change the fate of a given cell and convert it to a different cell type. This has been demonstrated by the conversion of fibroblasts into several cell types, such as embryonic stem cells (ESCs), neurons, hepatocytes and cardiomyocytes, that could function properly in their native environment.[1] Recently, we demonstrated the conversion of fibroblasts into embryonic Sertoli-like cells (ieSCs) by ectopic expression of five factors.[2] These cells harbor many of the characteristics of the endogenous cells and could incorporate into testicular cords and support the survival of germ cells and neurons in culture. It is clear today that transdifferentiated cells provide a new impetus in the field of regenerative medicine. Here we will discuss whether transdifferentiation could also be used as a research tool to illuminate basic developmental processes in animals.

There are several traditional approaches to identify regulators of biological processes. These include analysis of candidate genes in transgenic/knockout mice, computational analyses followed by in vitro loss/gain-of-function experiments and high-throughput screen using random mutagenic agent or shRNA libraries. However, not all cell types can be sustained in culture, and there are many genes that cause early lethality and therefore are difficult to be explored in vivo. Moreover, a systematic approach to uncover the most pivotal regulators of a given process is still missing.

During reprogramming, each factor either alone or in combination with the other factors contributes to the conversion by initiating a unique process that controls the transformation. The ability to dissect the key factor(s) responsible for the initiation of a particular cellular alteration can shed light on the regulation of a given process. For example, during embryonic development, the mesenchymal-to-epithelial transition (MET) process is vital and contributes to the formation of many tissues.[3] Li and co-authors found that early in the conversion of fibroblasts into induced pluripotent stem cells (iPSCs), the Oct4/Sox2 complex downregulates the key epithelial-to-mesenchymal transition (EMT) gene *Snail*, the expression of *Tgfb1* and *TgfbR2* is reduced by c-Myc and Klf4 upregulates the expression level of the epithelial marker *E-cadherin*.[4] In the conversion of fibroblasts to ieSCs, we showed that MET is an essential early event as well, and out of nine examined factors, we identified Nr5a1, Wt1 and Dmrt1 as key MET regulators that downregulate EMT master regulators like, *Twist1*, *Snail*, *Slug* and *Foxc2*.[2] The factors that regulate MET in vivo during embryonic Sertoli cell differentiation are unknown to date. Thus, the in vitro transdifferentiation approach could shed light on the endogenous process and suggests novel candidates. Attempting to address such a question with conventional strategies would require the production of mice carrying multiple mutant alleles and is time consuming and difficult to execute. By performing transdifferentiation experiments, a large number of factors can be screened in an easy and rapid manner, and the most pivotal factor(s) in inducing a given process are highlighted.

Another example of how transdifferentiation can be utilized to address basic biological questions is that of gene redundancy. In an attempt to identify genes with redundant activity during the formation of iPSCs, Nakagawa and co-authors substituted Sox2, Klf4 and c-Myc with their corresponding homolog, and found that Klf2 and Klf5 can substitute for Klf4, Sox1 for Sox2 and N-Myc for c-Myc.[5] In agreement with that, Jiang and co-authors demonstrated that mouse ESCs depleted for Klf4 or Klf2 or Klf5 retain normal self-renewal.[6] They could not observe any obvious effect, even when two of the three factors were depleted simultaneously. However, depletion of all the three genes led to a robust differentiation. They showed that Klf2, Klf4 and Klf5 share many common targets of Nanog, and in the absence of one of them the other two could compensate for its loss.

Similarly, we observed that substitution of Gata4 by Gata1 could mimic many of the phenotypes exerted by Gata4 during the conversion of fibroblasts to ieSCs. For example, when we monitored the levels of *Amh*, we found that MEFs transduced with Nr5a1, Wt1, Dmrt1, Sox9 and Gata1 were capable of activating the *Amh* gene to a high level that was a mere 2-fold less than the expression of *Amh* in ieSCs (data not published). This is an important finding, because the Amh hormone plays a pivotal role in suppressing the development of the Mullerian duct in the male embryo and thereby inducing testis formation.[7] Gata4 has been shown to be a potent inducer of *Amh*;[8] however, our data indicate that other Gata proteins might compensate for its loss and contribute to the induction of *Amh*.

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Table 1. Advantages and limitations of different approaches to uncover developmental processes

Approach	Advantage	Limitation
Computational analyses and in vitro cell culture manipulations	*Screen of the entire genome *The results are obtained fast *based on a large set of databases	*Only a prediction of a gene function *Not all cell types can be maintained in culture *The effect can be masked by redundant genes
Transgenic and knockout animals	*Demonstrate an actual function of a gene candidate in vivo	*Time consuming *The effect can be masked by redundant genes * Genes may cause early lethality
Transdifferentiation	*Rapid identification of master regulators and of factors with redundant activity *May facilitate in vitro growth of cell types that are difficult to culture *Provides an accessible supply of cells with reliable and predictable characteristics	*In vitro observation – may not recapitulate the full in vivo function of a candidate gene *Hard to get mature functional cells *The transgene expression levels must be optimized

This observation might also explain why knockout of Gata4 in gonadal somatic cells at 9.5 dpc express normal levels of *Amh*.[9] Such questions are complicated to pursue in explanted primary cells, because endogenous Sertoli cells degenerate very fast in culture, and *Amh* expression decreases significantly after only one day of culturing.[2] In addition, addressing this question using alternative approaches, such as a luciferase reporter fused to the *Amh* promoter, suffer from undefined promoter sequence and the absence of a relevant enhancer. Thus, the robust generation of ieSCs from fibroblasts may facilitate gain/loss-of-function experiments that would be difficult to execute using endogenous Sertoli cells.

In conclusion, transdifferentiation of different cell types in vitro may enable the identification of key regulators of developmental processes more readily than complex and cumbersome in vitro/vivo approaches (summarized in **Table 1**). Understanding the control of transdifferentiation of one somatic cell type into another may not only be utilized for isolation of cell types that are difficult to obtain and culture and that can be used for therapeutic applications, but may also be informative for the study of basic developmental processes.

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