Perspective

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Practices on Somatic Cell Information to Predict Intramammary Infection with Major Pathogens

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Description

During mammalian development, the genomic potential of cells is being progressively restricted, with only the earliest stages containing cells of a totipotent or pluripotent phenotype. However, the restricted potential of differentiated cells can be reversed. Mammalian cloning experiments have shown us that the program of differentiated cells can be reset to that of totipotent cells. The exchange of nuclear factors between the donor cell nucleus and the enucleated egg cytoplasm is considered to be important for this process. Somatic cells can be dedifferentiated in vitro by fusion with pluripotent cells, activating genes that are not expressed in adult stem cells. For example, the fusion of a thymic lymphocyte with an embryonic germ cell or an Embryonic Stem Cell (ESC) has led to the activation of the Oct4 gene in the somatic cells suggested that coculture of neurosphere cells (NSCs) and ESCs in embryoid bodies induced the NSCs to transdifferentiate into myocytes due to signals from ESCs. Moreover, the differentiated state of somatic cells could also be altered by fusion with another type of somatic cell, suggesting that the dynamic interaction of proteins between the fused cells might be responsible for the plasticity of nuclear function. To date, however, little is known about how somatic cells are actually reprogrammed. The ooplasm of an enucleated mammalian oocyte has the capacity to recondition or reset the genetic program of a fully differentiated somatic cell nucleus to the point of producing a fully developed organism. However, the experimental difficulties inherent in handling oocytes render them unfeasible for conducting analyses on the underlying mechanisms of genetic reprogramming. In contrast, ESC lines are more amenable to experimental manipulation and present an equally valid tool with which to address the molecular basis of genetic reprogramming. In previous studies, we analyzed the reactivation of Green Fluorescent Protein (GFP) transgene—markers of pluripotency—after transfer of somatic cell nuclei into oocytes. In the present study, we examined whether enucleated cytoplasts of ESCs can also activate the Oct4-GFP transgene in somatic cells or whether nuclear components are required

Mechanisms of Genetic Reprogramming

The first step in the resumption of meiosis is the dissolution of the nuclear membrane or Germinal Vesicle Breakdown (GVB). Fully grown mammalian oocytes, when isolated from antral follicles and placed in culture, undergo a spontaneous GVB, but growing oocytes, isolated from preantral follicles, do not. Thus, oocytes become competent to undergo GVB around the time of the com- pletion of their growth. Even though oocyte growth is strictly dependent on intercellular communication with the surrounding granulosa cells, it appears that the acquisition of meiotic competence is independent of both oocyte growth and gap-junctional association with granulosa cells, suggesting that this might be an autonomous oocyte program. Although they are competent to do so, oocytes nearing the completion of growth in vivo do not undergo GVB until they receive the appropriate hormonal stimulus [3]. This suggests that factors within the follicle maintain the oocyte in meiotic arrest. In fact, the meiosis-arresting effect of the follicular environment is probably mediated by the passage of substances from the granulosa cells that comprise the follicle wall to the oocyte through gap junctions. Cyclic Adenosine Monophos Phate (CAMP) and other purines such as hypoxanthine, guanosine, and adenosine, cyclic guanosine monophosphate, a low molecular weight peptide known as oocyte maturation inhibitor or OMI,3-endorphin, and Mullerian Inhibiting Substance (MIS) have been implicated in the maintenance of meiotic arrest.

In addition to cardiac cells, other lineage-specific precursor cells, such as neural and definitive endoderm precursor cells, have also been generated from mouse and human fibroblasts by the same paradigm with different signaling molecules. In these studies, transient expression (4–6 days) of iPSC TFs in fibroblasts was the initial step shared by all. Another shared step was treatment with a JAK inhibitor to suppress the leukemia inhibitory factor-STAT3 pathway during iPSC reprogramming, which would prevent establishment of pluripotency in reprogrammed cells and facilitate the generation of developmentally plastic intermediate cells. The epigenetically activated cells were treated with FGF4 to generate neural precursor cells or with Activin-A to generate definitive endoderm precursor cells. Importantly, the efficiently and fast converted neural precursor cells could be expanded for serial

passages and then differentiated into mature and subtype-specific neuronal cells and glial cells.95 In addition, human fibroblasts have been directly converted to multipotent blood progenitors by prolonged ectopic expression of Oct4 and treatment with IGFII, bFGF, Flt3L, and SCF.96 In summary, cardiovascular cells and other lineage-specific precursor cells can be directly converted from iPSC TFinduced trans differentiation, which implies that such strategy would provide a general platform to induce a broad range of cells for various applications.

The stem cell field has embarked on exciting discoveries that both iPSCs and lineage-specific cells can be reprogrammed from somatic cells by ectopic expression of iPSC TFs. Because of the close relationship between these 2 types of reprogramming, they share some technical challenges and safety considerations that need to be addressed before their clinical applications. To date, several strategies have overcome these hurdles with respect to iPSC reprogramming, and some may also work well in iPSC TF–based trans differentiation. As reviewed above, regarding iPSC reprogramming, small molecules are valuable not only to significantly promote cellular reprogramming and functionally substitute ectopic expression of TFs but also to provide insights into molecular mechanism underlying this process.

Persisting Genetic Modification

iPSCs represent a widely available, non-controversial and practically infinite source of pluripotent cells. Unlike human ESCs, their use is not restricted for ethical reasons, allowing most laboratories to develop research programmes involving this source of human pluripotent stem-cell lines. Since the first published demonstration from Yamanaka's laboratory that fibroblasts can be reprogrammed merely by retroviral delivery of four factors (OSKM), many alternative approaches have been developed in order to induce pluripotency starting from adult somatic cells. Integrative strategies based on retrovirus or transposons mediated gene transfer are most efficient and can be used for prominent current applications such as disease modelling and therapeutic screens, since the absence of persisting genetic modification is not an absolute prerequisite. In contrast, the generation of clinically relevant iPSCs intended for future cell therapy prospects requires technological approaches which do not leave genetic traces behind the cell conversion phase. Although methods based on proteins delivery are relatively inefficient, strategies involving RNAs, directly or via Sendai virus, and their potential improvement seem promising owing to the high efficiency of cell reprogramming.