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Review

Engineering cell fate: Spotlight on cell-activation and signaling-directed lineage conversion

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ABSTRACT

Direct reprogramming of specialized cells into other cell types has revolutionized the fields of stem cell, differentiation, and regenerative medicine. Direct reprogramming technology can convert various differentiated cell types to other fates by the forced expression of lineage-specific transcription factors. In addition to this approach, transdifferentiation can be induced in somatic cells by a method named cell-activation and signaling-directed (CASD) lineage conversion, which uses pluripotency reprogramming factors in combination with specific differentiation signals. This approach is capable of generating tissue-specific progenitors in addition to functional mature cells through a challenging transitory (pluripotent or non-pluripotent) state. Interestingly, the CASD lineage conversion has been accomplished by using small molecules and growth factors in a chemical-only paradigm. This approach will have a substantial positive impact on the field and bring reprogramming technology into the translational pipeline. From the regenerative medicine perspective, in future, new therapies might be designed based on the patient's own cells that are directly reprogrammed into the desired cell types *in vitro* and *in situ*. This review provides an overview of direct reprogramming to different lineages and describes what is known about the cell-activation and signaling-directed transdifferentiation.

Contents

1. Introduction

Generation of specialized cells of various tissues has attracted considerable attention and is seen as a promising hope for medical purposes. Specialized cells can be generated by distinct approaches that differ based on the starting cells and their cellular and molecular mechanisms. "Differentiation" is a developmental process, which allows derivation of various cell types from stem/progenitor cells by appropriate developmental cues. Notably, one potential application of pluripotent reprogramming is that somatic cell-derived induced pluripotent stem cells (iPSCs) can undergo differentiation to produce desired cell types. Interestingly, somatic cells can also be converted into different specialized cells using a

Abbreviations: iPSCs, Induced Pluripotent Stem Cells; MEFs, Mouse Embryonic Fibroblasts; CASD, Cell-Activation and Signaling-Directed; GRNs, Gene Regulatory Networks; TRN, Transcriptional Regulatory Network; PDGF, Platelet-derived growth factor; FLT3LG or Flt-3L, Flt3 ligand; IL3, Interleukin 3; SCF, Stem cell factor; BSA, Bovine Serum Albumin; TPO, Thrombopoietin; EGF, Epidermal Growth Factor; bFGF, Basic Fibroblast Growth Factor; DLPC, Dilauroyl phosphatidylcholine; NaB, Sodium Butyrate; BMP4, Bone morphogenetic protein 4; HGF, Hepatocyte growth factor; OSM, Oncostatin M; C-E, Compound E; LiCl, Lithium chloride; RA, Retinoic Acid; FBS, Fetal Bovine Serum; IGF-1, Insulin-like growth factor 1; LPA, Lysophosphatidic Acid; VEGF, Vascular endothelial growth factor; PEG, Poly ethylene glycol; FCS, Fetal Calf Serum; BDNF, Brain-derived neurotrophic factor; LIF, Leukemia inhibitory factor; Flt3, FMS-like tyrosine kinase 3; ESGRO®, Leukemia Inhibitory Factor (LIF) supplement; NEAA, Non-Essential Amino Acids; KOSR, knockout serum replacement; ITS, insulin-transferrin- selenium; Poly I, C, Polyinosinic-polycytidylic acid.

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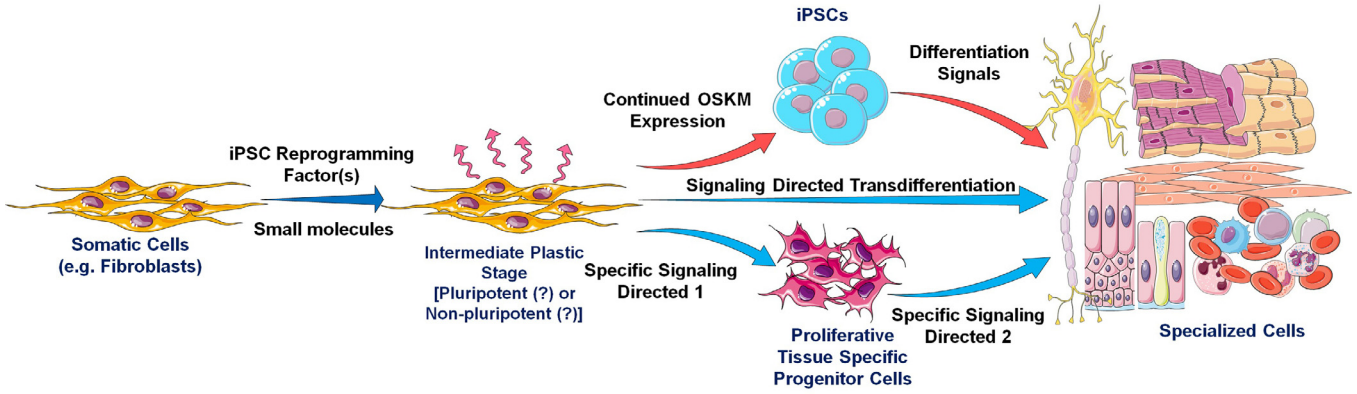

Fig. 1. A schematic representation of the topics discussed in the current paper. The original Yamanaka's method can reprogram somatic cells into iPSCs, and in the subsequent step, the generated iPSCs can undergo differentiation toward distinct lineages. The CASD lineage conversion system allows the generation of both differentiated cells and tissue-specific proliferative progenitors directly from activated cells. In addition, specific soluble signals can differentiate induced progenitors to the desired fates. (OSKM: Oct4, Sox2, Klf4 and c-Myc).

method named direct lineage conversion, direct reprogramming or transdifferentiation in the absence of a pluripotent state. This method utilizes forced expression of lineage-instructive transcription factors as the main strategy for the generation of various cell types (Sancho-Martinez et al., 2012; Wagers and Weissman, 2004; Manohar and Lagasse, 2009; Yechoor et al., 2009; Morris and Daley, 2013; Vierbuchen and Wernig, 2012; Takahashi and Yamanaka, 2006; Zhou et al., 2008), bypassing the multiple steps of lineage specification during development (Xu et al., 2015). Direct reprogramming using lineage-instructive transcription factors was first done to convert mouse fibroblasts into myoblasts by overexpression of *Myod* (Davis et al., 1987), and subsequent studies showed the feasibility of this strategy (Heins et al., 2002; Kulessa et al., 1995; Shen et al., 2000; Xie et al., 2004). Generally, there are two routes for direct lineage conversion. The first method utilizes overexpression of lineage-specifying transcription factors to directly reprogram cell type A into cell type B (Ieda et al., 2010; Wada et al., 2013; Fu et al., 2013; Vierbuchen et al., 2010; Xue et al., 2013; Sekiya and Suzuki, 2011; Huang et al., 2011; Huang et al., 2014; Du et al., 2014; Hickey et al., 2013; Kapoor et al., 2012). In this regard, various cells (e.g. cardiomyocytes (Ieda et al., 2010; Wada et al., 2013; Fu et al., 2013; Ifkovits et al., 2014), neural cells (Vierbuchen et al., 2010; Xue et al., 2013; Liu et al., 2013), and hepatocytes (Sekiya and Suzuki, 2011; Huang et al., 2011; Huang et al., 2014; Du et al., 2014; Swenson, 2012)) have been directly generated from somatic cells (mostly fibroblasts) using overexpression of defined sets of transcription factors. Reportedly, there is not an intermediate state (e.g. pluripotent state or prime state) during the direct lineage conversion (Sancho-Martinez et al., 2012). However, this method needs the examination of many transcription factors and a process of elimination for identification of a key set of reprogramming factors. Furthermore, researchers have shown that lower and higher expression levels and stoichiometry of master regulators could result in different fates and qualities (Carey et al., 2011; Papapetrou et al., 2009; Tiemann et al., 2011; Morris Samantha et al., 2014) and that the manufactured cells via direct conversion have less similarity to their *in vivo* correlates than the generated ones through directed differentiation (Morris Samantha et al., 2014; Cahan et al., 2014). The second approach by a different mechanism uses iPSC transcription factors (pluripotency-TFs) in conjunction with appropriate conditions (soluble signals) favoring lineage specification to do cell fate conversion through a transitory pluripotent or non-pluripotent/unstable state. Here, the cell-activation and signaling-directed (CASD) lineage conversion is reviewed as a new transdifferentiation method that utilizes primed cells at the early steps of pluripotent reprogramming to induce somatic cells into defined lineages using appropriate inductive signaling conditions.

2. Cell-activation and signaling-directed lineage conversion

It has been demonstrated that by modifying the iPSC reprogramming process, different cell lineages could be achieved as outcomes (Efe et al., 2011; Szabo et al., 2010). Based on this paradigm, starting cells (e.g. fibroblasts) transiently become "activated" by overexpression of pluripotency-TFs as an early "unstable" intermediate stage and then lineage-specific soluble signals (cytokines and small molecules) redirect them toward diverse specific lineages (Wang et al., 2014) (Fig. 1). Different names are being used for this approach, including cell-activation and signaling-directed (CASD) lineage conversion, CASD transdifferentiation, Oct4, Sox4, Klf4 and c-Myc-mediated transdifferentiation (OSKM-TD) and pluripotency factor-induced transdifferentiation (Wang et al., 2014; Li et al., 2013; Maza et al., 2015).

2.1. The intermediate state: pluripotent or non-pluripotent?

Two different characteristics have been postulated to describe the intermediate state. The primary description of the intermediate state indicates that this is a non-pluripotent, plastic and unstable state, which is induced by a transient expression of pluripotency factors in the presence of differentiation stimulating media and in the absence of pluripotency-supporting conditions (Efe et al., 2011; Mitchell et al., 2014a; Yang et al., 2014; Li et al., 2014). Interestingly, Yang et al. recently defined appropriate markers for a special 'prime' stage as Thy1$^-$/SSEA1$^-$ status during pluripotent reprogramming of mouse embryonic fibroblasts (MEFs) prior to any fate commitment (Yang et al., 2014). In this cell-fate-decisive stage, the subsequent specified signaling pathways can define a specific fate in cells undergoing reprogramming (Yang et al., 2014). Similarly, Polo et al. demonstrated that in the intermediate stage of pluripotent reprogramming, mouse fibroblasts pass through a Thy1$^+$ to a Thy1$^-$ to a SSEA1$^+$ state (Polo et al., 2012). These findings indicate that fibroblasts lose their fibroblast program and become plastic in the intermediate stage of reprogramming, before the establishment of pluripotency. Therefore, it could be hypothesized that this special prime stage may be in concordance with the unstable stage that has been reported during the CASD lineage conversion. According to this possible interpretation, the unstable stage of the CASD transdifferentiation in mouse might be a Thy1$^-$/SSEA1$^-$ cell state. Notably, Ding et al. speculated that transient expression of pluripotency-