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REVIEWS

The roles of natural compounds in somatic reprogramming

LEI Zili[1], HAO Yanmei[2], YANG Yanhong[3*]

[1]*Guangdong Metabolic Diseases Research Center of Integrated Chinese and Western Medicine, Guangdong Pharmaceutical University, Guangzhou 510006, China;*

[2]*Department of Nuclear Medicine, The First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou 014010, China;*

[3]*The First Affiliated Hospital (School of Clinical Medicine), Guangdong Pharmaceutical University, Guangzhou 510080, China*

[Abstract] Somatic reprogramming is a big breakthrough in stem cell technology. A deep understanding of the mechanisms of reprogramming will promote manipulating the cell fates and is expected to treat various diseases caused by the degeneration of functional cells, tissues, and organs. However, the existing reprogramming problems include low efficiency and unsafe, and many small molecules have been found to improve this state. Here in this review, the important roles of natural compounds in reprogramming are summarized, providing new insights into the cell fate transformation.

[Key words] iPS; Somatic reprogramming; Natural compounds

1 Introduction

Growing from a fertilized egg into a mature life is called development. There is a class of special cell groups at all development stages with the potential for self-renewal and differentiation called stem cells. The proliferation ability and differentiation potential of stem cells are the premises of the diversity and quantity of cells and the basis of development. In 2006, Takahashi and Yamanaka introduced four factors, Oct3/4, Sox2, *c*-Myc, and Klf4, into the mouse adult fibroblasts, and obtained induced pluripotent stem (iPS) cells, exhibiting the morphology and growth properties of embryonic stem (ES) cells and expressing ES cell marker genes[1]. This method of reversing differentiated adult cells to pluripotent cells is called somatic reprogramming. A major question in regenerative medicine is how to obtain individualized functional cell types. Somatic reprogramming has shown great promise for manipulating cell fate that avoids the ethical and immunogenic risks associated with pluripotent stem cells, thus having great clinical values for treating many diseases. Conversely, the induction low efficiency and the security problem caused by the genomic integration of the viral vectors have raised concerns about the potential for clinical applications of this approach. Many methods have been developed in recent years to optimize reprogramming; for example, the adoption of nonintegrated carriers and the alternative to

[*Corresponding author] E-mail: 1764941457@qq.com.

transcription factors using chemical molecules would be advantageous because such a strategy would be easy to manipulate and is reversible.

Natural compounds have been used for centuries for their wide medicinal properties, characterized by high safety, availability, accessibility, and low cost. They represent an invaluable source of chemicals with potential therapeutic effects[2]. In addition, natural compounds also play important roles in epigenetics and epithelial-mesenchymal transition (EMT)[3-6]. In fact, more and more natural compounds have been found to improve the efficiency and security of reprogramming. Here in this review, we will summarize the roles of natural compounds in somatic reprogramming, aiming at applying natural compounds in reprogramming for potential clinical use.

2 Somatic reprogramming

The success of the cloned sheep Dolly using nuclear transfer showed that the differentiated somatic cells could still develop into a whole organism under suitable conditions[7]. Fusing human ES (hES) cells with human fibroblasts could result in hybrid cells that maintain morphology, growth rate, and antigen expression patterns characteristic of hES cells[8]. Epithelial 293T cells treated with an extract of undifferentiated human NCCIT carcinoma cells had the transition from a 293T to a pluripotent cell phenotype[9]. The three methods of reprogramming mentioned above require ES cells or other embryo-originated cells, which limit their application in reproductive medicine because of ethical reasons.

The iPS technology has triggered a sensation in life science. It does not require ES cells and avoids nuclear transplantation and cell fusion, so it has no ethical problem. Meanwhile, iPS cells come from patients themselves and solve the problem of immune rejection. Although iPS has great advantages over traditional reprogramming, it has many defects. The use of protooncogenes of Klf4 and c-Myc, and retrovirus as the carrier, may cause insertion mutations and increase the risk of tumorigenicity. The induction efficiency also needs improvement.

Kim et al reported that exogenous expression of Oct4 was sufficient to generate pluripotent stem cells from adult mouse neural stem cells (NSCs)[10]. iPS cells can be generated with plasmids, nonintegrating episomal vectors, and synthetic modified mRNA instead of viral vectors[11-13]. Recently, HLA-homo iPS cells were developed using blood samples from healthy donors who were homozygous for three loci of human leukocyte antigen (HLA-A, HLA-B, and HLA-DRB1)[14]. Another method was the generation of gene-edited low antigenic iPS cells; for example, scientists removed the human leukocyte antigen gene on one chromosome of HLA-heterozygous donor-derived iPS cells using CRISPR-Cas9 genome-editing technology to generate iPS cells with a low risk of immune rejection [14]. Despite this progress, iPS still could not be obtained high efficiently and safely.

With the development of iPS technology, various small molecular compounds have been reported to be involved in the generation of iPS, improving the efficiency, and safety of iPS. Huangfu et al reported that valproic acid (VPA), a histone deacetylase (HDAC) inhibitor, could significantly improve the reprogramming efficiency, and VPA also enabled efficient induction of pluripotent stem cells without the introduction of the oncogene *c*-Myc[15]. Since then, dozens of small molecules have been found to promote iPS induction. These small molecules act on epigenetic, reprogramming-related signaling pathways, metabolic regulation, and maintenance of self-renewal and play important roles in reprogramming.

3 The roles of natural compounds in reprogramming

In recent years, the research ideas and related techniques of iPS have been gradually applied in

traditional medicine and natural compounds. Natural compounds have continuous and long-term effects, regulating multi targets and pathways, so offering a new angle and cut-in point to reprogramming and applying natural compounds in reprogramming is becoming increasingly a research hotspot.

Vitamin C, a natural compound obtained from many vegetables and fruits, has been reported to enhance iPSC generation from mouse and human somatic cells. It can alleviate cell senescence, a recently identified roadblock to reprogramming, and promotes the transition of pre-iPSC colonies to a fully reprogrammed state[16]. Further research showed that the histone demethylases Jhdm1a/1b were key effectors in somatic cell reprogramming downstream of vitamin C, establishing a link between histone demethylases and vitamin-C-induced reprogramming[17]. Vitamin C could also increase the expression of the histone demethylase JARID1A, essential for promoting pluripotency and reducing differentiation downstream of vitamin C[18]. TET hydroxylases are dioxygenases implicated in DNA demethylation. TET1 deficiency enhances reprogramming, and its overexpression impairs reprogramming in the context of vitamin C by modulating mesenchymal-to-epithelial transition (MET)[19]. Therefore, vitamin C shows important functions in regulating the epigenetic modification of both histones and DNA in somatic reprogramming. Since it is common in food and beneficial to human health, vitamin C should be investigated further for its role in epigenetic regulation and reprogramming.

E-cadherin expression is upregulated in the early stage of reprogramming, and E-cadherin mainly mediates the cell-cell contact in iPS cells. Overexpression of E-cadherin can enhance iPS cell generation, whereas knockdown of E-cadherin or abrogation of cell-cell contact by E-cadherin inhibition results in reduced reprogramming efficiency. Through screening a chemical library, two natural compounds, Apigenin and Luteolin, were found to considerably increase the reprogramming efficiency by upregulating E-cadherin[20].

Salvia miltiorrhiza is a Chinese medicine herb commonly used to treat cerebrovascular and coronary artery diseases. The main active components of *S. miltiorrhiza* contain salvianolic acid B and tanshinone IIA[21]. Salvianolic acid B can promote the differentiation of bone marrow-derived mesenchymal stem cells into alveolar epithelial cells type I (ATI) through the activation of the Wnt signaling pathway[22]. Another report demonstrated that salvianolic acid B possesses the ability to promote bone marrow derived-neural stem cells (BM-NSCs) proliferation in a dose-dependent manner and may act as a potential drug to upgrade the therapeutic efficiency of BM-NSCs in CNS diseases[23].

A hypoxic environment and its effector, HIF-1α activation, involved in glycolytic metabolism, improve reprogramming efficiency for both mouse and human cells[24]. Quercetin, rich in red onions and grapes and acts as a hypoxia-inducible factor (HIF) activator, has enhanced reprogramming effects by modulation of metabolism[25-26].

Resveratrol could increase the efficiency of somatic cell reprogramming into iPSCs by activating the AMPK/Ulk1 pathway and suppressing the mTORC1 signaling cascade, which correlates with the enhanced expression pluripotency markers Oct3/4, Sox2, Nanog, and Klf4 [27]. Therefore, the mESC pluripotency was retained by resveratrol regulation of the mTOR/Ulk1/AMPK-autophagy network[27].

Recently, Yoo J et al reported that Porphyra 334 (P334), a secondary metabolite of diverse marine and terrestrial organisms, could promote the efficiency of the cell reprogramming process of mouse tail-tip fibroblasts and human dermal papilla cells by activating the trimethylation of histone 3 lysine 4 (H3K4 me3) which controlled MET during the reprogramming process[28].

The above studies demonstrated that, like synthetic chemical reagents, natural compounds also could increase the efficiency of somatic reprogramming via regulating multiple signaling pathways, metabolism, epigenetics, cell-cell contact, etc. Because of the low toxicity of the natural compounds, they are more useful than other chemical compounds in reprogramming. However, the detailed mechanisms of the natural compounds in improving somatic reprogramming remain to be explored, and the potential efficacies of increasing natural compounds will be reviewed in somatic reprogramming.

4 Perspectives

Somatic reprogramming is a good system for the study of cell fate transformation.

Through the in-depth study of somatic cell reprogramming, we can better understand the transcription factor regulation network, epigenetic modification, and cell fate maintenance mechanisms. The establishment of the somatic cell reprogramming technique provides good opportunities to treat some special diseases, such as organ transplantation. It is a long and complex process to induce somatic cells into pluripotent states and differentiate them into special cell types. More and more natural compounds have been found to play important roles in this process, providing more effective methods of changing cell fate in the future (Fig. 1).

Recently, it has been reported that somatic reprogramming can be chemically induced using only small-molecule compounds and the four essential small molecules are forskolin, CHIR99021, 3-deazaneplanocin A, and 616452[29-30]. Subsequently, chemically induced neurons (CiNs) from fibroblasts of patients with familial Alzheimer's disease patients[31] and human adult astrocytes[32], chemically induced cardiomyocytes (CiCs) from human fibroblasts[33], chemically induced neural progenitor cells (CiNPCs) from mouse embryonic fibroblasts[34] and chemically induced neural stem cells (CiNSCs) from mouse

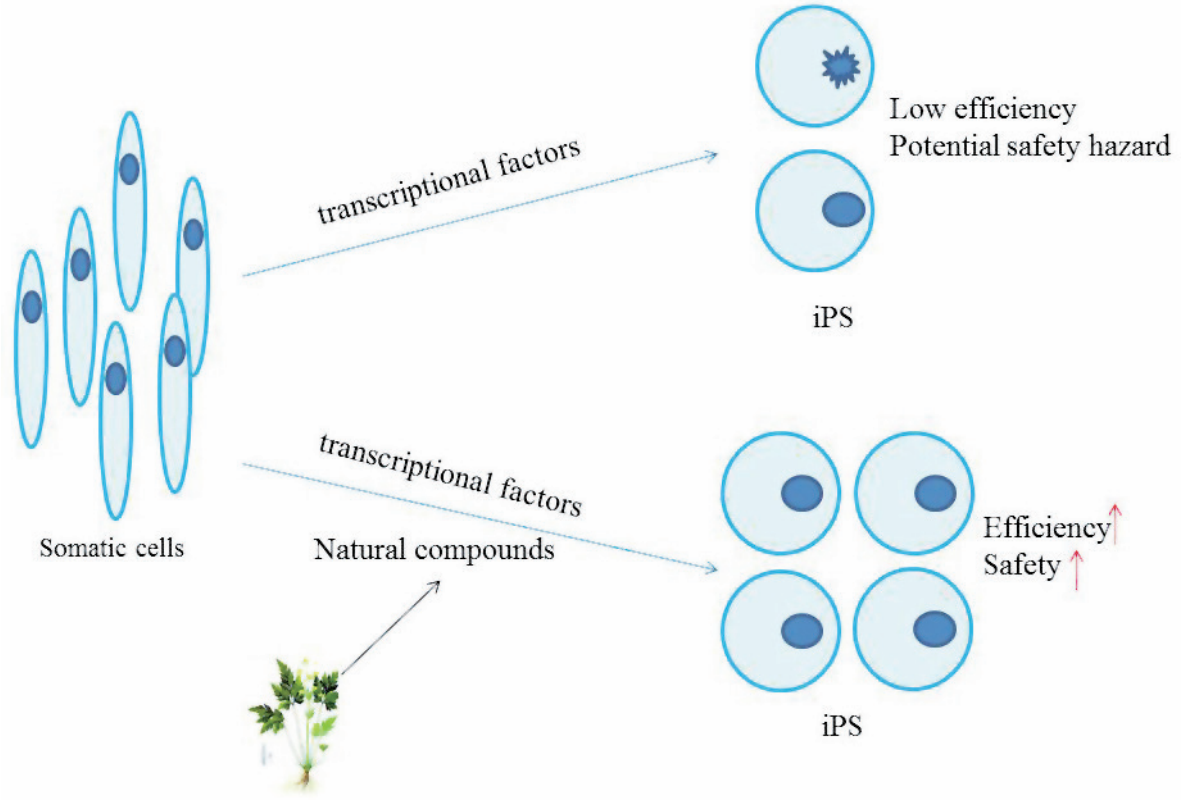

Fig. 1 Natural compounds play important roles in somatic reprogramming

fibroblasts[35] have been successfully generated. Chemically induced somatic reprogramming without genetic manipulation is the safer and more promising technology for regenerative medicine. Small molecules used in chemically induced somatic reprogramming can regulate signaling pathways and factors essential to the fate and functions of cells[36-37]. For example, forskolin can raise the cAMP levels in cells[38], and CHIR99021 is a very selective inhibitor of glycogen synthase kinase-3 (GSK3)[39]. Many natural compounds target signaling pathways or factors that regulate cell fates *in vivo* or *in vitro*[40-42]. Therefore, natural compounds will contribute to chemically induced somatic reprogramming in future research.

5 Conflicts of Interest

The authors have no conflict of interest to declare.

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