RESEARCH REPORT

Generation of Induced Pluripotent Stem Cells (iPSCs) through Cellular Reprogramming

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ABSTRACT

Induced pluripotent stem cells (iPSCs) are primarily derived from completely differentiated somatic cells or non-stem cells following molecular induction and cellular reprogramming. Molecular induction, leading to formation of iPSCs, has successfully been accomplished at multiple molecular hierarchies of biomolecules, including DNA, RNA, and Protein, and even using small organic inducer molecules like non-steroid anti-inflammatory drug, Nabumetone, the anticancer drug, 4-hydroxytamoxifen and so on. Such molecular induction leads to almost complete reversal of cellular machinery from differentiated state to undifferentiated state, restoring the immense biological potential which is considerably similar to Embryonic Stem cells (ESCs). This scientific breakthrough and tremendous accomplishment has given researchers rays of hope and equipped them to carry out various preclinical and clinical trials and translate the trial-based findings to design cure for various dreaded and degenerative diseases. Furthermore, its implication also transcends the various biological boundaries wherein it can be employed to slow down the physiological degeneration, such as aging, occurring owing to the cellular senescence and stem cell exhaustion. Besides, iPSCs can also help in understanding the normal development process as well as underlying mechanism leading to diseases. Pursuing research in this direction, various promising results have already been achieved which need to be consolidated and further extended to the next level of translational research. The uses of iPSCs is not only limited to cell-based therapy but have also been extended to the level of biological manufacturing, therapeutic cloning, and biosynthesis wherein several biomolecules, including growth hormones, chemokines, immunological molecules, such as interferon and interleukin of therapeutic significance are being synthesized by iPSCs-derived cells and organs. Moreover, iPSCs-derived cells and tissues are being used for toxicity testing and drug development.

Keywords: Induced Pluripotent Stem Cells, Molecular Induction, and Cellular reprogramming, Embryonic Stem Cells, Translational Research

INTRODUCTION

Most of the multicellular organisms primarily contain two types of cell populations, namely somatic cell and stem cell. Somatic cell populations are biologically differentiated and functionally diverse, and continue to perform most of the metabolic functions across the different set of organs throughout the life of organisms. On the other hand, Stem cells are biologically undifferentiated and serve as cellular backup in different organs, and undergo functional differentiation into cells of specific types as and when required (David., 2011). However, such cellular repertoire has multiple limitations on account of specificity, numbers, differentiation and trans-differentiation potential, replicative stress and age-based repertoire exhaustion (Ruzankina *et al*., 2008). Similarly, Embryonic Stem Cells (ESCs) has its own limitation in terms of several concerning moral and ethical issues (Henon *et al*., 2003). Therefore, it became very important for researchers and clinicians to find out cells with immense biological and therapeutic potential, and devoid of any limitations, and ethical and moral issues surrounding uses of ESCs and other kind of stem cells. This is where induced Pluripotent Stem Cells (iPSCs) come into picture, and since its invention and discovery over a decade ago (Takahashi and Yamanaka., 2006), scientists across the globe have found solace in it and are quite hopeful that iPSCs would prove to be the therapeutic saviour as the conversion of somatic cell to iPSCs has huge potential in helping us understand the underlying molecular basis of development and disease.

iPSCs derived following Gene/DNA-induced cellular reprogramming

Induced-Pluripotent Stem Cells are derived from somatic or non-stem cell population following molecular induction and cellular/nuclear reprogramming. The spectacular foundational stone, upon which the cellular reprogramming was successfully achieved, can be said to be laid down over decades ago by experimentations leading to animal cloning. Animal cloning empirically demonstrated a battery of uncharacterized potential factors present in oocyte could reprogramme adult somatic cells into that of embryonic cells with concomitant restoration of all the embryonic characteristics. Dr. Gurdon, Dr. Wilmut and Dr. Campbell are among the leading researchers whose immense contributions in the field of animal cloning and related research must be highly appreciated and acknowledged (Gurdon., 1962; Wilmut *et al*., 1997). Following such distinct lead, several researchers embarked upon this journey leading to revelation of multiple mysterious factors involved in nuclear reprogramming, thereby conferring pluripotency to adult somatic cells. Eventually success came to Shinya Yamanaka, a Japanese researcher who, following meticulous planning, study and experimentation find out the involvement of four important transcription factors, namely Oct3/4, Sox2, c-Myc and Klf4, responsible for cellular reprogramming in mouse adult fibroblast (Figure 1). A year later, the same result was successfully achieved in human adult fibroblast cells as well (Takahashi *et al*., 2006). Employing these factors, most of cells can be reprogrammed in to iPSCs, which show most of the characteristics, including pluripotency-associated marker expression, chimerism, teratoma formation, germ-line contribution similar to embryonic cells (Takahashi *et al*., 2006).

iPSCs derived following RNA-induced cellular reprogramming

Following aforementioned huge success in the field of cellular conversion, researchers started using some other logic and see whether similar results could be obtained. One of the main ideas was to use *in vitro*-produced mRNA encoding for Oct4, Lin28, Sox2 and Nanog. The iPSCs produced so was named as RiPS (RNA-produced iPS) (Figure 1). This idea gained momentum over the gene-mediated cellular reprogramming as it involves RNA, which unlike DNA, does not need genomic integration and hence minimizes DNA damage and associated potential tumorogenicity. Besides, no extra caution such as cleaning is required. Furthermore, modulation of transcription factors can be easily accomplished by appropriately adjusting the concentration and amount of various transcripts added in culture medium. RNA-produced iPS was found to be similar on account of spatio-temporal protein expression, colony formation, and expression of alkaline phosphatase, an early pluripotency marker and ESC-associated markers (Yakubov *et al*., 2010; Warren *et al*., 2012).

iPSCs derived following protein-induced cellular reprogramming

Further pursuit led to the revelation of phenomenon of protein-mediated cellular conversion/nuclear reprogramming and accomplishment of pluripotency,

and iPSCs so obtained named as PiPS (Protein-produced iPS) or piPSCs (protein-induced pluripotent stem cells). Protein-mediated piPSCs involves production of recombinant proteins with across the plasma-membrane-penetrating ability. To confer such ability, the C-terminal of Oct3/4, Sox2, c-Myc and Klf4 proteins were fused with polyarginine (11 R residues) protein transduction domain. These fusion proteins, once put in the culture medium, easily enter in cells within 6 hr and then eventually translocate into nucleus and remain stable for quite some time. Cells to be reprogrammed were treated with multiple consecutive payload of reprogramming proteins (i.e., Oct4-11R, Sox2-11R, Klf4- 11R, and c-Myc-11R) (Figure 1). Following such treatment of reprogramming factors, cell colonies stained positive with ALP (Alkaline phosphatase), an early pluripotency marker among others. Besides, these colonies were expanded and bear striking morphological resemblance with murine Embryonic Stem Cells (mESCs). Moreover they showed expression of pluripotency-defining markers like Oct4, Nanog, Sox2, and SSEA1, suggesting successful reprogramming (Zhou *et al*., 2009).

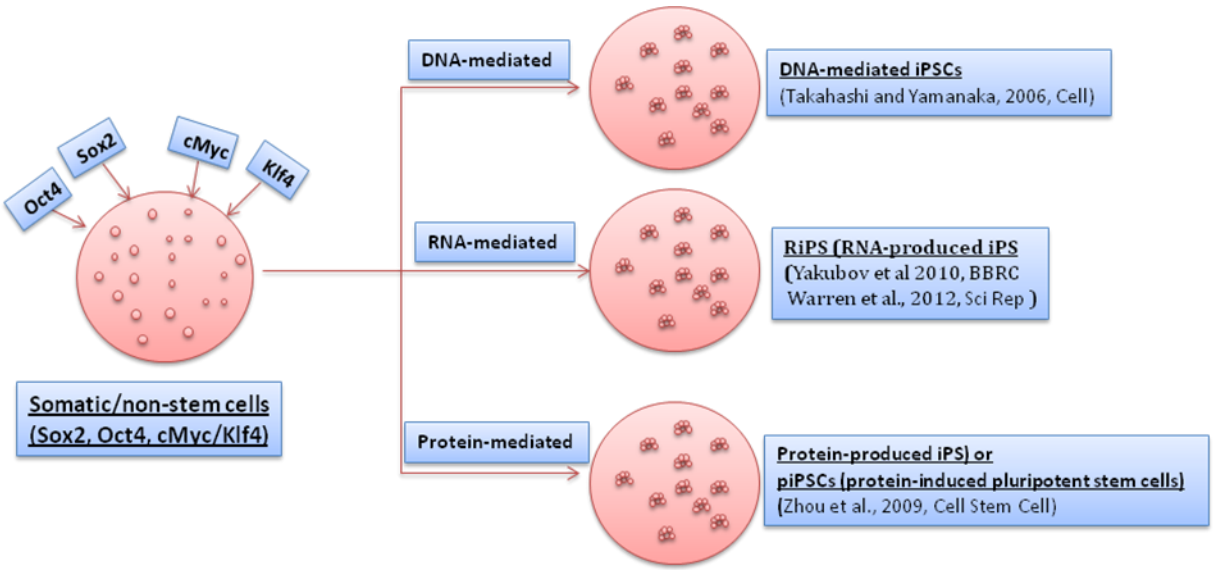

Figure 1: Steps involved in cellular/nuclear reprogramming

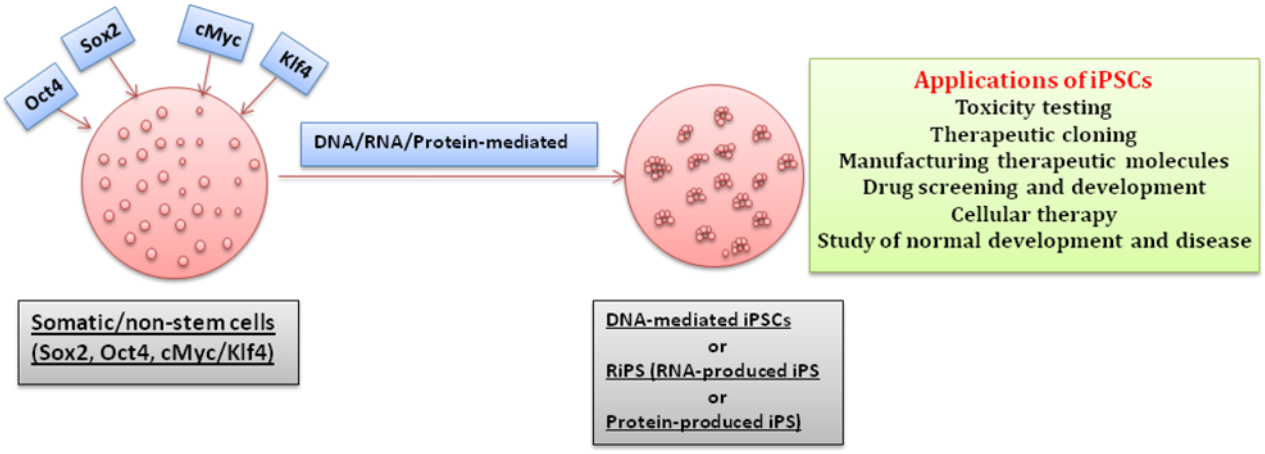

Figure 2: Potential applications of iPSCs

CONCLUSION

Stem cells of multipotent or pluripotent nature can either be derived/harvested from different organs/tissues, including bone marrow, liver, heart, placenta, embryo or be made *in vitro* employing a decade-old molecular induction technique called cell/nuclear reprogramming. Such groundbreaking molecular method was discovered and successfully implemented by Takahashi and Yamanaka, 2006 on murine somatic cells to induce pluripotency (Henon *et al*., 2003). A year later, scientists also succeeded in replicating the same on human somatic cells (Yakubov *et al*., 2010). Ectopic expression of several transcription factors, including Oct3/4, Sox2, c-Myc and Klf4 in somatic cells drive them towards pluripotency through a dynamic and paradigm shift in molecular machinery which is characterized by expression of pluripotency-associated markers and embryonic stage-specific markers, formation of alkaline-phosphatase positive colony, teratoma formation etc. So far achieving pluripotency is concerned, different methods and ways have been designed which include gene-mediated, RNA-mediated, Protein-mediated and small molecule-induced iPSC generation such as anticancer drug, 4-hydroxytamoxifen, non-steroid anti-inflammatory drug, Nabumetone. The results obtained employing aforementioned methods are quite comparable on several accounts. Besides, use of payload of RNA, Protein and small molecules also help in avoiding genome instability as they do not need genomic integration unlike DNA-mediated method and hence least chances of tumorogenicity. The uses and applications of iPSCs are immense, including study of development and disease, toxicity testing, therapeutic cloning, drug screening and development, cellular therapy and so on (Figure 2). With the arrival of iPSCs, there has been awakening of new era of cell-based therapy for various dreaded and degenerative diseases which remains mysterious and difficult, in terms of treatment, till date.

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