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Current Techniques in Reprogramming Cell Potency

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ABSTRACT

The first successful attempt to reprogram somatic cell into embryonic-like stem cell was achieved on 2006. Since then, it had sparked a race against time to bring this wonderful invention from bench to bedside but it is not easily achieved due to severe problems in term of epigenetic and genomic. With each problem arise, new technique and protocol will be constructed to try to overcome it. This review addresses the various techniques made available to create iPSC with problems hogging down the technique. J. Cell. Biochem. 114: 1230–1237, 2013.

KEY WORDS: REPROGRAMMING; STEM CELL; IPSC

Stem cell is a concept that has been around ever since 1868. Haeckel [1892] coined the term *stamzelle* which refers to a group of undifferentiated cell that would give rise to a huge range of cell types that functioned to repair the body, but with no proof to back up his supposition, the concept remained dormant. Around the 1960s, new insight was introduced into this subject when a research team led by James Till and Ernest McCulloch discovered the capability of bone marrow cells to differentiate into characteristically different cells such as erythroid cells and myeloid cells in the spleen of mice [Becker et al., 1963]. Isolation of such interesting cell was only made possible after the invention of flow cytometry by utilizing the mechanism of antibody directed at antigen or marker present on the surface of the cell. Twenty years after James Till's discovery, the first embryonic stem cell was isolated from mice and by 1998, human embryonic stem cell (hESC) was successfully derived from the inner cell mass (ICM) of blastocysts [Thomson et al., 1998]. Nowadays, researchers have categorised stem cells into two broad and distinctive types, which are embryonic stem cells and adult stem cells. Most cells will fall into either category but not both at the same time and the classifications are made based on their location and potency or fate of the cell. Embryonic stem cell is isolated from the ICM of blastocyst and they are pluripotent which means they are capable of differentiating into a wide variety of cell types except extraembryonic cell which contribute to the placenta. Apart from that, its potential to unlimited self-renewal is highly intriguing to be manipulated. On the other hand, adult stem cell is derived from postnatal tissue and is a part of tissue-specific cells. They are typically found in organs that are capable to regenerate such as bone marrow, skin, and intestinal epithelium. Their ability to differentiate is limited to their tissue of origin, thus they are termed as multipotent or unipotent.

Ever since hESC is isolated, countless researches have been carried out in order to tap into its potential and convert it into some form of therapeutic treatment for diverse range of diseases. Comprehensive protocols have been established to guide the process of in vitro differentiation of hESC into different germ layers and formed tissues such as neurons, cardiomyocytes, and hematopoietic cells [Kaufman et al., 2001] thus, enhancing the understanding of early embryogenic development. Through implanting the respective differentiated hESC in a patient, diseases that act by destroying patients' cells such as diabetes [Kroon et al., 2008] Alzheimer's and Parkinson's diseases [Bjorklund et al., 2002] could possibly be treated. In the United States, clinical trials are being carried out to utilize hESC in cellular therapy to treat spinal

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cord injury and macular degeneration while pharmaceutical companies could test their products directly on human cells for faster and more accurate results [Aboody et al., 2011]. Notwithstanding all the benefits hypothesized by scientists, great debates have been going on for years between proponents and opponents of hESC that greatly impaired its progress in the scientific world. The opponents ranging from scientific, social, legal, and the most highly debated prospect, ethics, have raised different reasoning and objections. The main reason why certain segments of society oppose the idea of hESC is the destruction of blastocyst that act as the primary source for embryonic stem cells. They argue that once the conception begins, the resulted blastocyst is considered as a living human being [Doerflinger, 2010] much to the dismay of the embryonic research advocates who proclaim that such cells do not yet have human features [Romeo-Casabona, 2008]. Arguments on medical grounds revolve around the accumulation of chromosomal anomalies due to long storage of embryonic stem cell [Cowan et al., 2004] and immune incompatibility between the donor and recipient that require prolong immunosuppressive therapy and the possibility of Graf versus Host disease. With such opposition burdened on hESC, new and more suitable alternatives that should function similarly like hESC but devoid of all the shortcomings need to be devised.

In 2006, a research team led by Dr. Shinya Yamanaka proved that cell differentiation is actually reversible by reprogramming mouse embryonic and tail-tip fibroblast using four transcription factors, Oct4, Sox2, Klf4, and c-Myc, back into pluripotency state that exhibited similar developmental potential to ESC [Takahashi and Yamanaka, 2006]. They termed such cells as induced pluripotent stem cell (iPSC). In a year time, similar cell could be derived from human fibroblast using the same transcription factors [Takahashi et al., 2007]. Three hallmarks set up by ESC that must be achieved for any iPSC to be deemed on par with ESC are in vitro differentiation into germ layer, chimeric formation, and in vivo germline transmission for animal study. Currently, there are several limitations and problems associated with the generation of iPSC. It was shown that due to the reprogramming process, iPSC could undergo genetic modification at two stages, somatic differentiation and reprogramming [Mira and Puri, 2012]. This would lead to genomic instability and abnormalities of iPSC [Mayshar et al., 2010] and formation of early genomic copy number variations [Hussein et al., 2011]. Apart from that, iPSC retain the gene expression profile of cell origin even after reprogramming took place [Ghosh et al., 2010]. This memory retention might have an effect on the frequency of teratoma formation since mouse iPSC derived from different tissues showed different frequencies of teratoma formation in the brain with residual presence of undifferentiated cell [Miura et al., 2009]. Ever since the successful reprogramming, various labs across the world have strived to replicate the research with different types of cells using different methods with varying degrees of success. Various methods were invented to carry out the process of reprogramming such as viral integration that utilized retrovirus and lentivirus [Takahashi and Yamanaka, 2006], polycystronic vector [Okita et al., 2008; Carey et al., 2009; Sommer et al., 2009], non-integrating viral vector such as adenovirus [Stadtfeld, 2008], RNA virus [Fusaki et al., 2009], small molecules [Huangfu et al., 2008], and purified protein [Kim et al., 2009; Zhou et al., 2009; Hyun-Jai et al., 2010]. This review intends to discuss different techniques that had been conceived in various perimeters such as design, mechanism, efficiency, and quality of the iPSC produced. Somatic cell nuclear transfer is included in this review even though it was not part of the techniques to create iPSC since it also leads to cell reprogramming.

SOMATIC CELL NUCLEAR TRANSFER

Somatic cell nuclear transfer (SCNT) is a technique developed decades ago where it was first tested on amphibians that led to formation of viable tadpoles from keratinized skin cell of adult *Xenopus laevis*. It is achieved through the introduction of donor nuclei into enucleated egg (cell with its own nucleus removed) which is subjected to shock to induce it into mitotic division stage and resulted in the formation of blastocyst. By implanting the blastocyst into a surrogate mother, it is possible, theoretically, to produce an exact clone to the donor of the nuclei since it has the same DNA make-up with the donor nuclei. The protocols to carry out SCNT could be summarized into four major steps which are preparation of recipient cytoplasts, preparation, and transfer of the donor nucleus and resumption of embryonic development by parthenogenetic activation [Lewis et al., 2001]. The first step involves the selection of suitable oocyte and removal of recipient nuclei. Previously, researchers utilized zygote [Wakayama et al., 2000], 2-cell blastomere and pronuclear [Robl et al., 1987] with very low success rates either for formation of blastocyst or survivability of offspring. It took them awhile before it is possible to exploit metaphase-II oocyte in mammalian cell experiment even though it had been used extensively in amphibian study. Through the advance in enucleating protocol, several new techniques has been designed which composed of bisecting the oocyte [Peura et al., 1998], extraction of oocyte nuclei by microsurgery, or expelled chemically [Costa-Borges et al., 2011]. Two other techniques that could be selected from are eradication of maternal chromosome by UV irradiation or laser and centrifugation [Hua et al., 2007]. The preparation of donor nuclei follows similar methods with a few exceptions where harsh procedures such as chemical isolation and UV irradiation are excluded to avoid destruction and disruption of donor nuclei [Yu et al., 2008]. Once both donor nuclei and recipient oocyte are prepared, the karyoplast would be fused with the recipient cytoplast by injecting it into the perivitelline space and subjected to certain stimulants such as inactivated Sendai virus [Song et al., 2011], electrical current or chemicals [Cervera et al., 2010].

With such a huge array of techniques and protocols at disposal, it is no surprise that the efficiency of the transformed cell to reach blastocyst stage will vary accordingly. It could reach as low as 1.2% while on the other end, it floats around 23–26%. As stated previously, it is possible to clone an organism by transferring the activated blastocyst into a surrogate mother. Such feat was accomplished in amphibians through the formation of fertile *X. laevis* that was grown from nuclei isolated from intestinal epithelium though the fertility varies greatly between 50% and 86%

that might be due to artificial induction and unnatural conditions imposed on the frog in the laboratory setting [Gurdon and Uehlinger, 1966]. For mammalian study, the first ever cloned mammal was conceived in 1981 by Illmensee where 3 out of 16 transplanted embryos developed into fertile mice [Illmensee and Hoppe, 1981]. Although the efficiency was pretty high (18.8%), it was conceived from inner cell mass nuclei while attempt to develop embryo using trophectoderm nuclei was to no avail; hence this work cannot be considered as SCNT. In 1996, Dolly was cloned successfully from somatic cell nuclei of a Finn-Dorset ewe [Wilmut et al., 1997]. Ever since then, successful cloning has been carried out on different mammals such as pig [Li et al., 2010], ferret, and rabbit [Tian et al., 2012] though the result was daunting. As the transformed embryo is lacking in viability, roughly only 6% of the transplanted embryo develops into a fetus and subsequently into a viable offspring. Due to strong opposition from ethical boards, none of the transformed human oocyte was developed into offsprings but rather the experiment were terminated once it reached the blastocyst stage though most oocytes will die before morula stage [Egli, 2011].

One of the biggest hurdle that is preventing advancement in SCNT research is the financial constraint placed upon it by ethical boards around the world. For example, in the United States, federal fund for research purpose is only allowed to be spent on research that involves cell lines derived from excess embryo procured from IVF while none will be given to any research that involves creation of embryo specifically for research as is the case for SCNT [Kington, 2009]. Moreover, different countries have different policies outlining their preference of SCNT research. Hence, this makes any international collaboration difficult to be executed. In 2006, there had been reports of a variation to SCNT where the *Cdx2* gene in murine fibroblasts had been inactivated before being transferred into enucleated ocyte which lead to formation of blastocyst that only produced cells of the inner cell mass (ICM) but lacked the capability to develop into a complete human being [Meissner and Jaenisch, 2006]. Even though such technique promised a great advancement in SCNT, it is not easy to be carried out as uncertainties arise from manipulation of the donor cell obscure the safety assessment of the transformed cell and to date invited fierce debate. SCNT requires complex procedures with in-depth knowledge of the protocol for the process to be a success. Even if it is a success, the same protocols might not produce the same result due to differences in the species. That is why it took awhile before the protocols to carry out SCNT in primates were established [Byrne et al., 2007]. When the protocols are tested with human somatic cells, it led to several complications such as oocyte lysis, impaired development of blastocyst and incomplete reprogramming of somatic nuclei. With such intensive glitches that need to be overcome in order to bring SCNT from bench to bed, it will take a long journey before it can be fully utilized to replace embryonic stem cell.

VIRAL INTEGRATION

There are two main viral vectors that are extensively used for integration into host genome which are retroviral vector and lentiviral vector. Both vectors would be discussed separately as there are certain issues that need to be highlighted.

RETROVIRAL VECTOR

Viral integration through retroviral vector is the first method used to generate iPSC. Yamanaka and his colleague utilized gammaretrovirus isolated from Moloney murine leukemia virus to introduce those transcription factors and successfully created the first iPSC [Takahashi and Yamanaka, 2006]. These kinds of viruses operate by intruding into the cell's nucleus and initiate random integration with the help of integrase enzyme. By manipulating this mechanism, it enables scientist to introduce the transcription factor that act to reprogram the cell into the cell genome, thus they could make use of the host machinery to further transcribe and translate the factors. Nowadays, several companies such as Invitrogen and Addgene provide retroviral vector equipped with Oct 4, Sox2, Klf4, and c-Myc due to its efficiency and widespread usage. Some hypothesized that it was due to selection of retrovirus as the vector that permitted Yamanaka to reprogram mouse fibroblast into iPSC and proved its pluripotency since retrovirus is susceptible to epigenetic silencing, thus it is possible to probe the iPSC to differentiate and form germline once the introduced transcription factors were silenced [Matsui et al., 2010]. Although iPSC is hailed as the best solution to replace ESC and SCNT, the results produced do not bode well. In term of the efficiency of the reprogramming and the time for reprogramming to occur, it took approximately 11–25 days to complete the reprogramming with the efficiency hovering around 0.001–0.01% [Takahashi et al., 2007]. With such low efficiency and long period taken, it is not feasible to depend on viral integration alone and certain modification needs to be done in order to magnify the speed and efficiency. One of the factors that affect these two variables is the efficiency of the transduction. Retroviral vector invades cell through binding to the receptor on the cell surface [Valsesia Wittmann, 1997]. Although it is possible for the vector to enter the cell through different channels such as endocytosis or phagocytosis, receptor-mediated endocytosis will propel the efficiency of the transduction up a notch. When a mouse receptor for retrovirus, Slc7a1, was introduced into an adult human dermal fibroblast (HDF), the efficiency of the retroviral transduction increased by threefold from 20% to 60% [Takahashi et al., 2007]. Despite the low efficiency, the quality of the iPSC produced is substantial in terms of differentiation capability, presence of ES markers, and contribution to germline transmission. Expression of ES surface antigens such as stage-specific embryonic antigen (SSEA)-3, SSEA-4, NANOG protein, tumor-related antigen (TRA)-1-60, TRA-1-81, and TRA-2-49/6E could be detected on those iPSC [Takahashi and Yamanaka, 2006; Takahashi et al., 2007]. Apart from that, as retroviral vector is utilized with the introduction of foreign genes into the genome, it is expected that a certain degree of silencing of exogenous gene and re-activation of endogenous gene would be observed and it was. Silencing occurred around passage 14 to 17 gradually while the re-activation took place from passage 12 where Oct4 and Sox2 were activated first followed by NANOG protein [Takahashi et al., 2007; Okada and Yoneda, 2011; Liu et al., 2012]. According to Okada, the timing of the retroviral silencing affects the quality of the iPSC. iPSC that underwent earlier retroviral

silencing exhibited stable morphology and normal karyotype compared to iPSC that had the silencing occur 16 days late [Okada and Yoneda, 2011]. Hence, we need to find a mechanism to control the timing of the silencing so that we can establish a balance between allowing sufficient time for reprogramming to occur while producing the best quality iPSC. Apart from that, the transformed cell differentiated into three different germ layers; ectoderm, endoderm, and mesoderm, when they were removed from the feeder layer [Liu et al., 2012]. One of the major issues that hoard this technique, excluding the efficiency, is that the incorporation of the exogenous gene into the host genome could lead to insertational mutagenesis, thus compromising the safety and the integrity of the genome. One possible way to overcome this hurdle is by designing the vector so that after certain passage or when the reprogramming is completed, the exogenous genes that were introduced into the genome would be excised, returning back the genome into its original state.

LENTIVIRAL VECTOR

As both lentivirus and gammaretrovirus come from the same family, the mechanism is strikingly similar except one difference, lentivirus is able to replicate in non-dividing cell such as neuronal cell, hence it is a suitable candidate for gene delivery. Moreover, in order to circumvent the problem of multiple transfer of viral vector into cell, a single polycistronic vector with all four transcription factors available could be used. It is possible to produce such vector with the aid of internal ribosomal entry sites (IRES) that allow translation of protein in the middle of the messenger RNA rather than just at the beginning of the 5′ cap but it is not recommended as IRES trigger inconsistent expression whereby cistron located downstream of the sequence would be translated less [Pelletier and Sonenberg, 1988]. Another better mechanism that has been manipulated to enable construction of single polycystronic vector is "self-cleaving" 2A peptides [Ryan and Drew, 1994]. 2A peptides prevent the linkage of glycine and the last proline in the sequence that causes the ribosome to skip to the next codon. There has been report of successful co-translational cleavage of two different reporter genes located in the same polypeptides by inserting the 2A peptide between the coding sequences of the reporter genes while Szymczak et al. [2004] proved the capability to express four different proteins using 2A peptide in vivo.

Several different vectors have been constructed that utilize 2A peptide with all transcription factors (Fig. 1). The first design is construction of two pCX plasmids, one with cDNA of Oct4, Klf4, and Sox2 while the other plasmid contained c-Myc [Okita et al., 2008]. Okita et al. [2008] found that the order of the genes on the vector affects the reprogramming efficiency and the optimized order was Oct4, Klf4, and Sox2. The efficiency rate for this design was 0.0015%, higher than adenoviral transduction. Several plasmid integration sites were detected when the two plasmids were introduced at different time points. When the protocol was modified where both plasmid transfected at the same time, no exogenous DNA was detected through Southern Blot and PCR analysis [Okita et al., 2008]. The second design is a DOX-inducible vector with different 2A peptides (P2A, T2A, E2A) partitioning different factors that were ligated to a FUW lentivirus backbone. The efficiency was lower

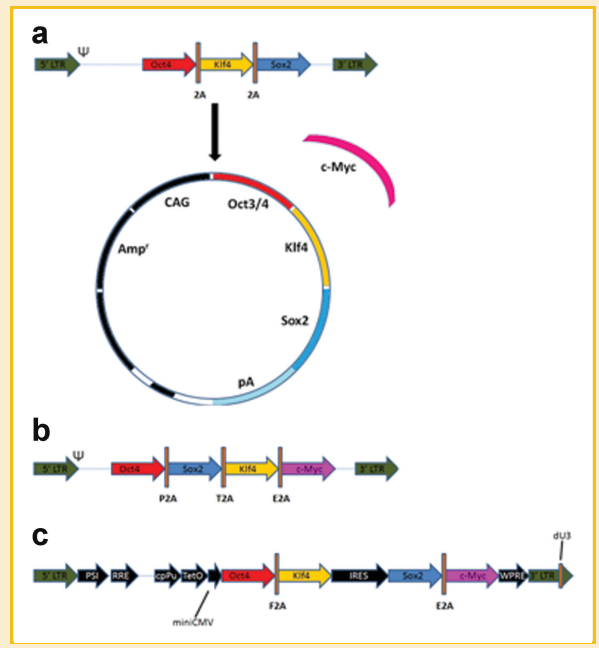

Fig. 1. Three different designs of polycistronic vectors; (a) cDNA of TF in pCX plasmids. b: Dox-inducible TF on FUW lentiviral backbone. c: Sequence of Oct4-klf4 and Sox2-c-Mycseparated by IRES on pHAGE lentiviral vector.

compared to the first design 0.0001%, and took approximately 25 days for the reprogramming to be completed after induction by DOX albeit high efficiency of infection. The final design was based on the pHAGE lentiviral vector [Sommer et al., 2009]. The difference between this design and the previous two designs is that it combines an IRES sequence and 2A peptide in the same lentiviral vector where the IRES element separated the cistrons, Oct4/Klf4, and Sox2/c-myc. Two different types of vector were constructed in which the expression is either constitutively expressed through EF1α promoter or controlled through DOX-inducible TetO-miniCMV promoter [Sommer et al., 2009]. At Day 16 of DOX induction, even with the transduction rate at 10%, 15% of the cell population had been reprogrammed into iPSC. This gave this design the efficiency of 0.5%, the highest between these three designs. iPSC formed by all three designs were able to generate chimeric mice and developed distinct germ layers in vitro. The level of each factors played critical roles in reprogramming and maintaining pluripotency of the cell [Kopp et al., 2008]. As all factors were introduced into the cell in one single polycystronic vector, it is difficult for the cell machinery to achieve optimal stochiometry of the factors expression which will lead to lower efficiency observed in the first two designs.

NON-INTEGRATING VIRAL VECTOR

The use of viruses that integrate into genome for the reprogramming of somatic cells to iPSC is a major obstacle. A more efficient and safer method without any viral integration to introduce the transcription factors had to be established. The suitable candidate for such a task is Adenoviral system. Adenovirus will carry the required factors into the cell nucleus and initiates transient, high-

level expression of the exogenous gene without integrating those into the host genome. By modifying it into replication-deficient, the virus would not be able to proliferate and cause damage to the cell [Stadtfeld, 2008]. However, such modification proved to be detrimental to the efficiency to reprogram somatic cell into iPSC. The efficiency was extremely low compared with the efficiency obtained from integrating virus with the time taken, which is approximately 24–30 days [Stadtfeld, 2008]. Since the virus had been engineered to be replication-deficient, the concentration of the virus in the cell will not remain constant while the cells kept dividing. As the cells keep dividing, each cell will have a lower virus concentration. Thus, most are not able to sustain the expression of the exogenous gene long enough to activate the endogenous factors again [Okita et al., 2008; Stadtfeld, 2008]. Due to this constraint, this method works well for non-dividing cells but not for rapidly dividing cells. Forcing high concentration of virus into the cell or secondary transfection of the virus may help to increase the efficiency by maintaining the exogenous gene expression. Albeit the low efficiency, those iPSC fulfilled all hallmarks that need to be achieved by any iPSC. For instance, formation of teratoma with three different germ layers was observed when it was injected into the flank of NOD–SCID mice and development of viable chimera when it was injected into blastocysts. There was no tumor development observed in any of the chimeric mice even after 20 weeks of age [Stadtfeld, 2008]. Tumor development in chimeric mice is linked to the reactivation of transgene transcription factors, especially c-Myc [Aoi et al., 2008]. As the transgenes in iPSC formed with adenoviral vector were diluted out as the cells divide, there would not be sufficient transgenes available to be activated and induce tumor formation. Although, in theory, adenovirus does not integrate into the host genome, it does happen at a very low frequency at the efficiency of 10^{-3} to 10^{-5} per cell [Harui et al., 1999]. Using PCR and Southern blot analysis, no traces of adenoviral vector was detected in the iPSC although it could be due to limitation of the Southern blot analysis [Stadtfeld, 2008]. These results obtained indicate that reprogramming is not due to insertational mutagenesis as speculated by some [Yamanaka, 2007]. Small portion of the mouse iPSC population but not human induced by adenoviral vector is reported to be tetraploid [Stadtfeld, 2008]. Stadtfeld [2008] speculated that this rare polyploidy occur due to induction of cell fusion by adenoviral vector or adenovirus selectively prefer tetraploid cell from the starting cell population. As the ratio of tetraploidy in iPSC correlated with the ratio in liver cells [Gupta, 2000], it is likely due to the latter than the former.

PROTEIN-BASED REPROGRAMMING

Notwithstanding the choice of vector used or the presence of oncogene, c-myc, it cannot be denied that there exists a small chance of genomic disruption as long as genomic material is being used to reprogram cell to iPSC. Such possibility could be eliminated through direct delivery of the reprogramming protein into the cell nucleus. Since protein is a large macromolecule with various R′ group, it has to be modified in order to enable it to transverse the cellular and nuclear membrane. It has been reported that a small, basic sequence in HIV-transactivator of transcription (TAT) protein is the main perpetrator in aiding HIV to penetrate the cell membrane during the infection process. Known as cell penetrating peptide (CPP), this peptide and several other penetrating peptides have a high percentage of arginine or lysine in its sequence make-up [El-Sayed et al., 2009]. Apart from conjugating CPP to the transcription factors, several mechanical means such as electroporation and microinjection can also be utilized to transfer the required proteins. The transcription proteins that will be used in the delivery are extracted from several sources (Fig. 2). Kim et al. [2009] engineered a stable mammalian cell line, HEK293, that would synthesize the required proteins coupled with nine arginine that acted as the CPP while another group led by Zhou manipulated an *E. coli* system [LaFevre Bernt, 2008] for the same purpose with a small modification where instead of nine arginine, they used 11 arginine as the CPP [Zhou et al., 2009]. The proteins constructed by the *E. coli*

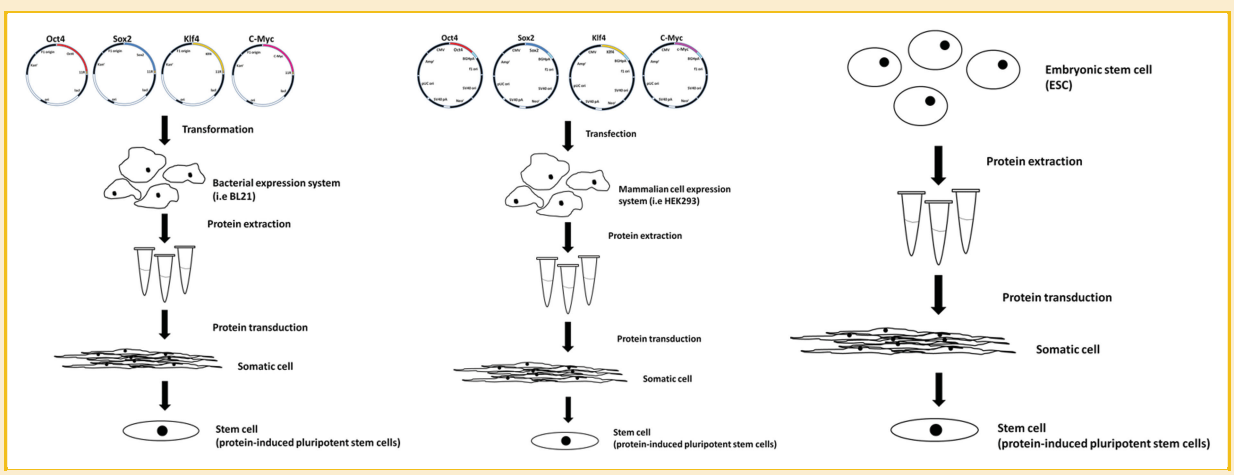

Fig. 2. Induction of pluripotent stem cells from somatic cells by protein-based reprogramming. a: 11 arginine-tagged reprogramming protein. b: 9 arginine-tagged reprogramming protein. c: ESC-derived protein.

system was not able to reprogram fibroblast cells and required further help from small molecule, valproic acid. The transferred protein was found to be stable in the cell for up to 48 h [Zhou et al., 2009]. Since the cell was not able to produce the proteins by itself during the earlier phase of the reprogramming process, repeated transfer of the protein is necessary every 24–48 h. The time taken to reprogram the cells with these proteins was double the time taken by vector transduction methods, approximately 8 weeks with efficiency as low as 0.001% [Kim et al., 2009; Zhou et al., 2009]. Another method that could be exploited to prepare the proteins is by harvesting it from actively proliferating ES cells [Hyun-Jai et al., 2010]. Using this extract, both permissive and nonpermissive strain were successfully reprogrammed. This method fared better in term of the time taken as compared to the first two extracts. It only required a single transfer of the extract with distinct colonies appearing at day 7. It was not possible to compare the efficiency as it was not provided though it is speculated to be higher due to earlier colony formation [Hyun-Jai et al., 2010]. Cho also found that neither cytosolic nor nuclear fraction of the proteins alone was sufficient to generate iPSC. This shows that there are certain proteins in cytosolic part of the cell that is necessary in inducing and maintaining pluripotency of the cell [Hyun-Jai et al., 2010]. Even though this approach sounds promising to replace all other methods, the need to destroy the embryo in order to retrieve the protein extract cannot be overlooked as this is the main reason why iPSC was been created in the first place. Even without making use of viral vector, the resulted iPSC had similar properties with iPSC produced with viral vector [Kim et al., 2009; Zhou et al., 2009; Hyun-Jai et al., 2010].

CURRENT PROSPECTIVE

Although clear guidelines are established on how to produce iPSC, it took the science community awhile to determine the mechanism that make it possible for reprogramming to occur. The discovery of mir-302, a small non-coding RNA, by Lin et al. [2011] has paved the way in understanding the mechanism as the introduction of mir-302 alone was sufficient to induce formation of iPSC. Presence of mir-302 leads to global DNA demethylation by silencing the gene transcript of AOF1/2 and MECP1/2. The function of AOF1/2 is to promote DNA methylation during oogenesis and prevent embryonic lethality. Silencing of these genes constructs leads to DNA demethylation that is further amplified when MECP1/2 are also silenced. As DNA demethylation elevates expression of Oct4, Nanog, and Sox2, there is no longer any need to express those transcription factors exogenously [Bhutani et al., 2010]. The same delivery systems used to deliver the transcription factors could also be utilized to transfer mir-302 into the cell nucleus. Moreover, as it only requires the transfer of only one sequence, the efficiency is expected to be higher compared with other conventional methods. Further research needs to be done to enable us to replace Yamanaka factors with mir-302 in carrying out reprogramming.

CONCLUSION

At its current state, it is not feasible to bring iPSC from bench to bedside with many uncertainties and problems need to be addressed beforehand. New techniques are being invented every once in a while that deals with problems presented in this review. The two main problems that require quick action are tumorigenicity and immunogenicity. Both setbacks are brought upon due to incompetency of the techniques used that lead to memory retention, genome instability, and abnormalities. The best technique would be one that needs short period of time for complete reprogramming with high efficiency and low to no chance for mutation. Once we are able to generate iPSC with optimal efficiency with minimal glitch, tissue replacement therapy will no longer be just a dream.

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