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Review on Cell Reprogramming: Methods and Applications

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Abstract
Cellular differentiation and development appears as a unidirectional process to specific cell fates irreversibly. Once differentiated, mature cells seems permanently locked into the differentiated state and unable to return to pluripotent stem cell state. However, using cell reprogramming methods it is possible to do reversal cell fate from a mature differentiated state to an undifferentiated state or directly to that of progenitors or mature cells of a different cell type. This is due to the resetting of the somatic cell specific epigenotype to the pluripotential cell specific epigenotype. Different methods are used to reprogram somatic cells into pluripotent cells. Among which are somatic cell nuclear transfer, cell fusion, genetic integration of cells extracts into chromatin and direct reprogramming using transcription factor integration. These methods result in morphological and molecular changes because of modification in chromatin and gene expression. The integration of the genome can be performed by the help of viral and non-viral vectors which have great variability the integration efficiency. Reprogrammed induced Pluripotent stem cells (iPS) and the recent induced endodermal cells are a few cell types to mention. Though these cells have numerous limitation in cell transplantation therapy but are promising cell for diseases modeling, drug discovery and bio-artificial organ synthesis. The major problems observed is retaining somatic cell genetic memory. Generally it is possible to reprogram personalized cells using different methods so that it can be patient specific.
Keywords: pluripotent, reprogramming, stem cell, transcription factors, vectors.

INTRODUCTION

Stem cells are the special primordial structures in the body that retain two distinctive properties: the ability to self-renew and differentiate into a specific cell type (Pera *et al*., 2000). Based on their differentiation potential stem cells can be categorized as totipotent, pluripotent, multipotent, oligopotent and unipotent cells. The commitment is more specified as it goes from multipotent cells down to oligopotent and unipotent cells (Odorico *et al*., 2001).

Cellular differentiation and development was described as a "one way process" like a ball rolling towards different one-way ramified valleys to specific cell fates irreversibly (Waddington, 1957). But in-vitro studies shows the presence of plasticity to erase epigenetic barriers using different technical approaches. As a result cells can re-acquire their pluripotency through a process, known as "reprogramming" (Campbell, 1996; Wilmut *et al*., 1997).In reprogramming it is possible to create pluripotent cell using two different ways: pluripotent reprogramming and lineage reprogramming. In the former case the entire developmental process is reversed, and a differentiated cell is returned to a pluripotent state (Yu, 2007) whereas in the later, conversion happens directly to another mature cell (Orkin and Zon, 2008). In contrary a normal development process, a pluripotent cell (e.g: embryonic stem cell) goes to all possible developmental paths and differentiates into a mature cell (Waddington, 1957).

Transcription factors and culture medium are the two most important cell fate determinants. This was shown to reprogram adult fibroblasts to pluripotent state using TF based technology. It was done by using mouse embryonic cells and adult fibroblasts, transduced with retroviral vectors encoding for Oct4, Sox2, Klf4 and cMyc. This phenomenon erased epigenetic state and re-established the pluripotent state, named as induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006).This induced pluripotent stem cells (iPS) are identical to ES cells and have the ability to become every cell type in the mammalian body (Shevchenko *et al.,* 2009). Another recent study showed also using the idea of linage reprogramming it is possible to create a transdifferentaited personalized pluripotent cells called induced Endodermal progenitor cells (iENDO) cells from human multipotent adult progenitor cells (Fanos *et al*., 2015). Therefore, the objective of this review paper is to highlight the different techniques of cell reprogramming as well as the application of reprogrammed cells.

METHODS OF CELL REPROGRAMMING

Cell reprogramming is a forced method by which morphological and molecular changes that happen as a result of modification in chromatin and gene expression. This is due to the resetting of the somatic cell specific epigenotype to the pluripotential cell epigenotype. In epigenetic landscape the normal development of cell is a one way process from the totipotent cells down to the determined cell population where as in the reprogramming (lineage and pluripotent) process somatic cells erases the epigenetic memory and shifted back to the pluripotent state(Waddington, 1957) (A), (Yu *et al.,* 2007; Orkin and Zon, 2008) (Figure 1).

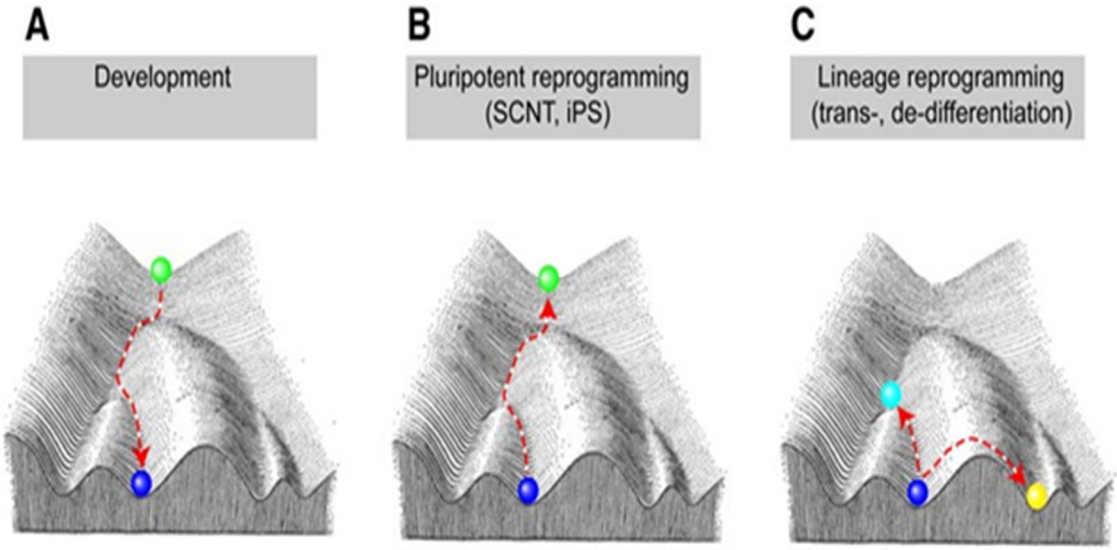

Figure 1. Schematic diagrams of development and reprogramming of cells in the epigenetic landscape. Adapted from: (Waddington, 1957) (A), (Yu *et al.,* 2007) (B), (Orkin and Zon, 2008) (C).

Traditionally there are different methods by which somatic cells could be reprogrammed into pluripotent cells. Among which are somatic cell nuclear transfer, cell fusion, genetic integration of various factors into chromatin or direct reprogramming (Do and Scholer, 2006; Hochedlinger and Jaenisch, 2006).

Somatic Cell Nuclear Transfer

Generation of reprogrammed cells through nuclear transfer has been documented in mouse models (Agarwal, 2006; Beyhan *et al.*, 2007; Campbell *et al*., 2007). The nuclear DNA of somatic cells is transplanted into the enucleated oocyte leading to union of both components and then initiated for embryonic development. After maintaining the pre-implanted embryo in a culture media finally the developed embryo is transferred to a foster mother (Fulka *et al.*, 2004). The aim of method is to generate stem and progenitor cells that are not committed to a specific lineage (Jaenisch *et al.,* 2004).

As a result of inefficient number of available unfertilized oocytes and ethical concern, this technique has not been demonstrated in humans. Apart from these, it is also dependent upon voluntary donation of oocytes and the efficiency of this technique is also low (Byrne *et al.*, 2007). In general, nuclear transfer (Figure 2) involves two steps: The first one is de-differentiation of a somatic donor cell to an embryonic state and the *in vitro* maturation till the blastocyst stage (also known as therapeutic cloning); The other one is further development of the cloned blastocyst, after the implantation in the maternal uterus (reproductive cloning) **(**Egli, 2007).

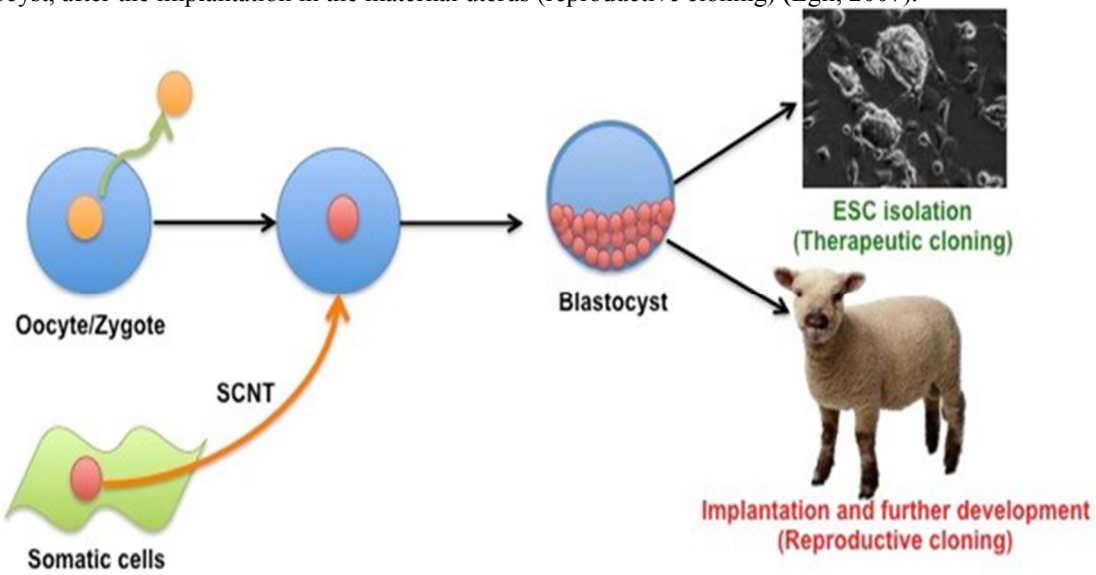

Figure 2. SCNT, Therapeutic and Reproductive cloning. Adapted from: **(**Egli, 2007)

Cell Fusion

Somatic cells and pluripotent embryonic stem cells go through cell fusion to generate reprogrammed cells (Figure 3). The cytoplasm of embryonic stem cells contains reprogramming factors which can alter the epigenetic state of a somatic cell into a pluripotent cell being fused with a somatic cell (Flasza *et al*., 2003; Tada *et al*., 1997).

In cell fusion, two cells can be fused together by using polyethylene glycol (PEG) to generate a single pluripotent cell. The more dividing or the larger cell type is the "dominant" one and the "recessive" cell will convert its gene expression profile to the one imposed by the dominant cell type (Yamanaka, 2010). Hybrid cells generated have exhibited properties similar to embryonic stem cells. These cells expressed reactivated pluripotent markers like Oct4, Sox2 and Nanog (Cowan *et al*., 2005; Do and Scholer, 2004) (Figure 3).

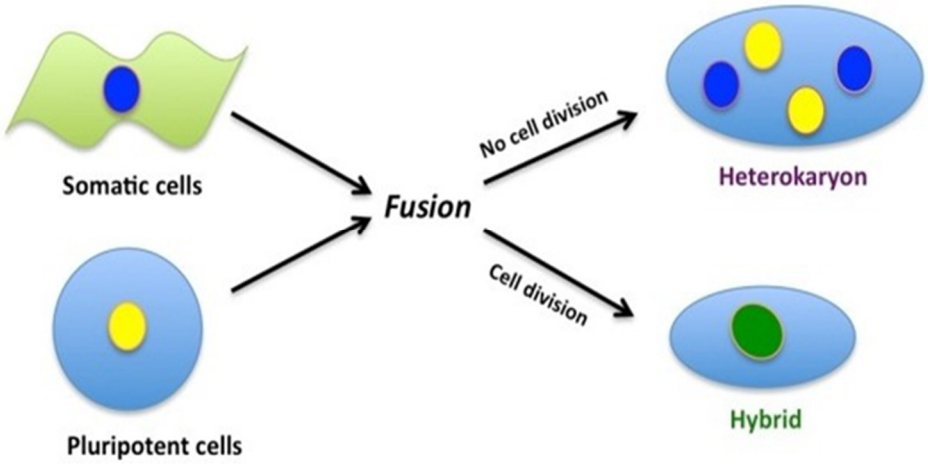

Figure 3. Reprogramming by Cell fusion. Adapted from: (Ying *et al*., 2002)

Reprogramming Through Cell Extracts

The process of reprogramming somatic cells by this method involves the exposure of differentiated cells or nuclei to cell- extracts made from mammalian embryonal carcinoma or ES cells (Han *et al*., 2010; Neri *et al*., 2007).It involves the reversible permeabilisation of differentiated cells using the chemical streptolysin- O (SLO), followed by exposure to cell extracts and has been shown to partially reprogram the treated cells towards an embryonic state, mainly in transformed and immortalized cell lines (Walev *et al*., 2001).

Direct Reprogramming

By using viral or non-viral vector transcription factors are introduced into somatic cells to generate pluripotent stem cells. The field jumpstarted in 2006 when Takahashi and Yamanaka demonstrated that the overexpression of pluripotency-related transcription factors (TFs) into induced pluripotent stem cells (iPSCs) from adult fibroblasts which is strongly resemble ESCs. The use of different "cocktails" of transcription factors (TFs) allows not only to redirect fibroblasts to an ESC-fate but also to a lineage-specific cell types/precursors, from a different tissue (Asuelime, 2012). Creation of iPS cells has paved a way to reprogram a cell in its somatic state back to its pluripotent state which might be used for disease modeling, drug screening and patient-specific cell therapy (Amabile and Meissner, 2009) (Figure 4).Mouse embryonic and adult fibroblasts, transduced with retroviral vectors encoding for Oct4, Sox2, Klf4 and c-Myc and cultured in ESC-medium, erased their differentiated epigenetic state and re-established the pluripotent state; these cells were named induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006).

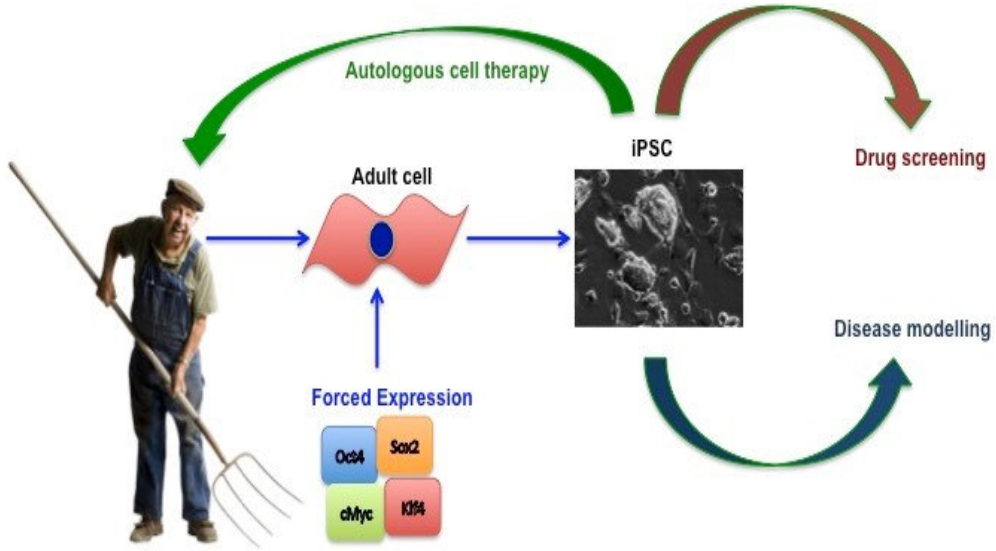

Figure 4. iPSC technology and applications. Adapted from: (Takahashi and Yamanaka, 2006)

VECTORS IN CELL REPROGRAMMING

There are a number of different approaches to transduce reprogramming factors into somatic cells that can affect the efficiency of reprogramming and the quality of resultant cells (Wernig *et al*., 2007). Retroviral vectors were used in reprogramming of somatic cells, but nowadays different methods allows the generation of cells like iPSCs. These vectors are generally categorized into two major groups as: integrative and non-integrative methods. Integrative methods are more efficient than non-integrative methods, but are less safe than non-integrative methods (Gonzalez *et al*., 2011; Okita *et al*., 2011).

Integrative Vectors

Retrovirus is a DNA based integrative vector and is the original method of reprogramming by transduction of TFs (Oct4, Sox2, Klf4, and c-myc) (Takahashi and Yamanaka, 2006). This vector system, allows the cloning of large number of DNA fragments more efficiently and are generally silenced in pluripotent cells, but only infect dividing cells (Hotta and Ellis, 2008). *Lentivirus* is another integrative DNA based vector which is more preferable than *retrovirus* vector as it can infect both non-dividing and proliferative cells but, it is less efficient than retrovirus (Papapetrou *et al.,* 2009).

Non-Integrative Vectors

The major weakness of reprogramming strategies by *retrovirus* and *lentivirus* is the integration of viral vectors into host chromosomes. Integration can cause insertional mutagenesis, interference with gene transcription, genome instability and induce malignant transformation (Nakagawa *et al.,* 2008).

None DNA based, replication-incompetent vectors are *Adenoviruses* are used to allow the transient expression of exogenous genes without integration into the host genome, but less efficient than integrating vectors. Although this is less efficient, when compared to integrative vectors it appears to be an excellent expression vehicle for generating reprogrammed cells, because it is a non-integrating virus (Stadtfeld *et al.,* 2008; Zhou and Freed, 2009).

Sendaivirus has been widely studied as an efficient expression vector and is known to effectively express transgene without integration (Armeanu, 2003; Bernloehr *et al*., 2004). A *Sendaivirus* vector carrying the four TFs (Oct4, Sox2, Klf4 and c-myc) is sufficient to successfully generate iPS colonies. Even though it does not integrate into the genome, the persistence of a viral genome within iPS clones and requirement of many passages (18-20) to degrade induced pluripotent stem cells remains a concern for downstream applications (Fusaki, 2009).

Episomal factors, allow for the introduction of genetic factors without integration into the host genome. A lack of host genome integration inherently removes the risk of random transgene reactivation associated with viral vectors (Okita *et al*., 2008). As plasmids are a well-developed technology and are very easy to generate them in a large quantity in the laboratory and have a relatively low cost-of-use compared to viral vectors (Yu, 2009). Although plasmids are desirable vectors for reprogramming, their efficacy remains well below that of viral integration, limiting the potential for large scale cell reprogramming using plasmids. This reduced efficacy be due to the temporary nature of plasmids and the speculated ongoing nature of the reprogramming process transcription factor expression may be reduced before the iPS reprogramming process is complete. This resulted in altering the stoichiometric balance of factors and ending reprogramming (Okita and Yamanaka, 2011). For liposomal

magnetofection (LMF), cationic lipids mediate the self-assembly of complexes containing plasmids and nanoparticles of super paramagnetic iron. These "CombiMag-DNA" ternary complexes can be concentrated at cell surfaces using a strong magnetic field to transfect vectors into the targeted cells (Park *et al*., 2012).

Modified mRNAs transcribing for the four TFs (Oct4, Sox2, Klf4 and cMyc) are capable of reprogramming when passed into the cytosol of various human cell types with a catatonic delivery vehicle (Warren, 2010). Initially, cytotoxicity of transfected mRNAs inhibited effective reprogramming, requiring modifications to the mRNA. This was modified the ribonucleotide bases of the vector mRNAs by substituting 5-methylcytidine for cytidine and pseudouridine for uridine resulting in reducing the immunogenicity of the mRNAs (Rosa and Brivanlou, 2010) (Figure 5).

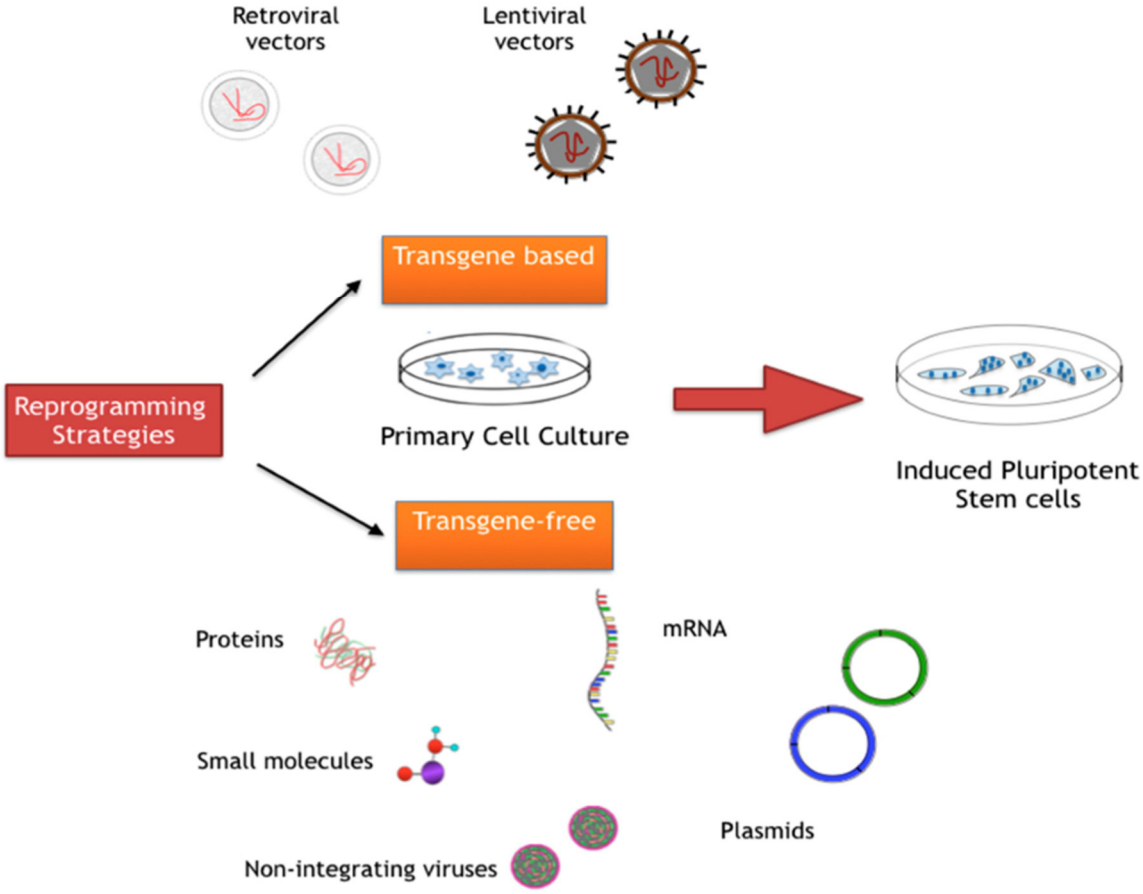

Figure 5. Schematic displaying of the various reprogramming strategies used in generation of reprogrammed cells (iPSCs). Adapted from: (Anokye-Danso *et al.,* 2011).

APPLICATIONS OF REPROGRAMMED CELLS

The development of induced pluripotent stem cell (iPSC) has created new avenues in basic research for disease modeling, drug discovery, screening, bio-artificial organ synthesis and cell transplantation therapy (Takahashi and Yamanaka, 2006). This is discussed as follows.

Disease Modeling

Accurate disease modeling is a biotechnological problem of fundamental importance. Most current disease models rely upon murine model organisms, which are capable of providing insight, but are less than ideal due to interspecies differences. iPSC technology could allow for in vitro disease modeling, using cultures isolated from those suffering with a given condition. If widely applied, patient specific iPS cultures could potentially be created to analyze the nuances of a disease in a particular patient, determining which course of treatment would be best (Saha and Jaenisch, 2009).

An attractive feature of iPS cells is the ability to derive them from adult patients to study the cellular basis of disease as it provides pathophysiological course of the disease. Since iPS cells are self-renewing and pluripotent, they represent unlimited source of patient-derived cells which can be turned into any type of cell in the body. iPS cells have been generated for a wide variety of human genetic diseases (Park *et al*., 2008; Freedman *et al*., 2013). In many instances, the patient-derived iPS cells exhibit cellular defects not observed in iPS cells from healthy patients, providing insight into the pathophysiology of the disease (Grskovic *et al.,* 2011).

Drug Discovery and Screening

Reprogrammed cells (iPSCs) also has a promising research application for drug discovery. Developing new drugs is exceedingly expensive and many drug candidates are rejected in the final human trial stage due to toxicology concerns (Nsair and MacLellan, 2011; Rubin, 2008). If drugs could have been screened for toxicology earlier in the development cycle and eliminated earlier, allowing for increased funding to more promising drugs (figure 6). For example; using iPS cell it is possible to harvest cells from patients afflicted with certain diseases in culture for drug testing without risking potentially life-threatening side effects in the patient (Wu and Hochedlinger, 2011). In addition, using iPS cells for drug screening should save a lot of money because the costs associated with raising and caring for laboratory research animals would be greatly reduced or eliminated (Wu and Hochedlinger, 2011).

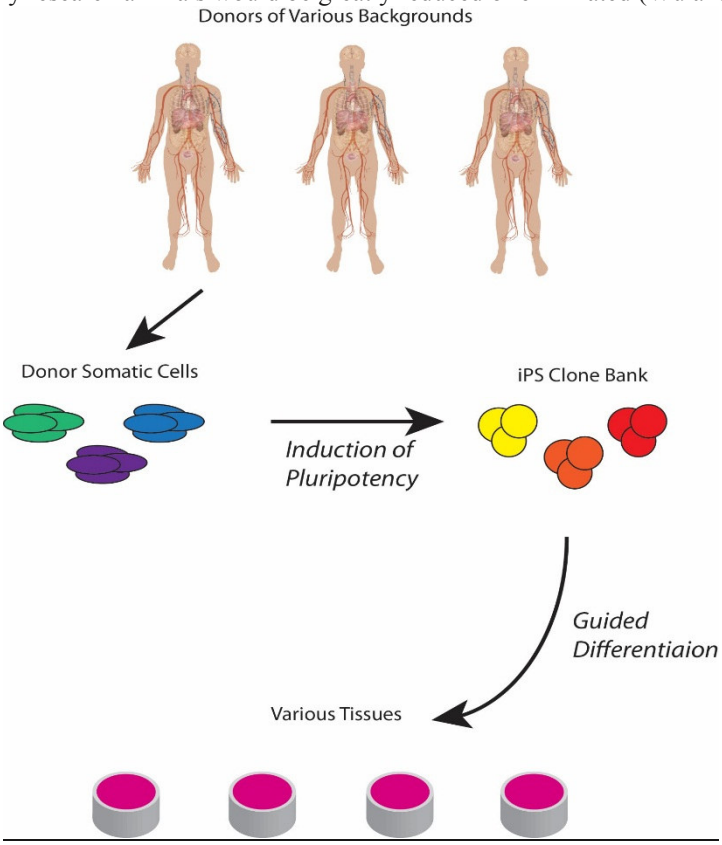

Figure 6. Drug discovery and toxicology workflow. Somatic cells are isolated from a broad cross section of donors, reprogrammed, and differentiated into relevant tissues for toxicology screening and drug testing. Adapted from: (Rubin, 2008).

Bio-Artificial Organ Synthesis and Cell Therapy

Human 'liver buds' (iPSC-LBs) were grown from a mixture of three different kinds of stem cells: hepatocytes (for liver function) coaxed from iPSCs; endothelial stem cells (to form lining of blood vessels) from umbilical cord blood; and mesenchymal stem cells (to form connective tissue). This allowed different cell types to self-organize into a complex organ, mimicking the process in fetal development. After growing in vitro for a few days, the liver buds were transplanted into mice where the 'liver' quickly connected with the host blood vessels and continued to grow. Most importantly, it performed regular liver functions including metabolizing drugs and producing liver-specific proteins. Further studies will monitor the longevity of the transplanted organ in the host body (ability to integrate or avoid rejection) and whether it will transform into tumors (Baker and Monya, 2013; Takebe *et al*., 2013

Diseases causing destruction of specific cell type present needs use of cell therapy. In the future, treatments or cures for these diseases could involve differentiating a patient's iPS cells into the specific cell types and reintroducing them into the patient to restore the original function of the missing cells (Wu and Hochedlinger, 2011).

LIMITATIONS OF REPROGRAMMED PLURIPOTENT CELLS

Although recent research has made clear regarding the potential application of iPSCs in translational medicine, there are certain limitations associated with the reprogramming of adult somatic cells. The lack of efficient differentiation (Hu *et al.,* 2010) and the risk of genetic instability (Laurent *et al.,* 2011) were among the challenges.

Reprogrammed cells by SCNT has also limitations; it involves manipulation of pre-implanted embryos and therefore raise serious legal and ethical issues (Camporesi, 2007). The scarcity of fresh donated mature human oocytes of high quality available for research is another significant obstacle of SCNT (Hall *et al*., 2007). The efficiency of the overall cloning process is quite low as the majority of embryos derived from animal cloning do not survive after implantation (Solter, 2000).

CONCLUSION AND RECOMMENDATIONS

The concept of reprogramming mature cells in to a pluripotent stem cell state is a paradigm shifting discovery. Understanding of reprogramming pathways helps the development of autologous cellular therapies for the treatment of numerous diseases and the improved efficiency of animal-based biotechnology. Strategies such as nuclear transfer and induced reprogramming have been used to induce somatic cells into an embryonic-like pluripotent state. The discovery of iPSC and its technology boosted the stem cell field in that it allows to obtain pluripotent stem cells for autologous therapy, drug discovery, disease modeling and bio-artificial organ synthesis. Problems of immune rejection as well as the ethical issues related to the use of human embryo for scientific purposes was avoided in these cell category. At present, there still remains a risk of teratoma formation in the event that a subpopulation of reprogrammed (iPS) cells. Efficiency of reprogrammed (iPS) cell generation remains an issue, especially with regards to non-integrated vector technologies. Additionally somatic cell genetic instability by retaining genetic memory of their parent cell. Generally it is possible to reprogram personalized cells using different methods so that the cell harvested can be patient specific and more stable.

Based on the above conclusions the following recommendations are forwarded:

- Detailed studies on cellular and molecular mechanisms that mediate reprogramming, should need further investigation to develop practical applications in veterinary and human medicine.
- For patient-specific autologous treatment using reprogrammed cells, methods should be developed and optimized so that cells can be generated in sufficient quantity, reliably and in a time frame appropriate for the targeted disease.
- Pharmaceutical companies should practice stem cells for the toxicological analysis of newly discoverd drugs, this can avoid the use of laboratory animal targeted drug discovery.

REFERENCES

Agarwal, S. (2006): Cellular reprogramming. *Methods Enzymology*, 420: 265–283.

Amabile, G., and Meissner, A. (2009): Induced pluripotent stem cells: current progress and potential for regenerative medicine. *Trends of Molecular Medicine*, 15(2): 59–68.

Anokye-Danso, F., Trivedi, C.M., Juhr, D., Gupta, M., Cui, Z., Tian, Y., Zhang, Y., Yang, W., Gruber, P.J., Epstein, J.A. (2011): Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. *Stem Cell,* 8: 376–388.

Armeanu, S. (2003): Cell cycle independent infection and gene transfer by recombinant Sendai viruses. *Journal of Virological Methods,* 108(2): 229-233.

Asuelime, G.E. (2012): A case of cellular alchemy: lineage reprogramming and its potential in regenerative medicine. *Journal of molecular cell biology*, 4(4): 190-196.

Baker, Monya (2013): "Miniature human liver grown in mice". Nature.com. Retrieved 19 July 2013.

Bernloehr, C., Bossow, S., Ungerechts, G., Armeanu, S., Neubert, W.J., Lauer, U.M., Bitzer, M. (2004): Efficient propagation of single gene deleted recombinant Sendai virus vectors. *Virus Research,* 99: 193–197.

Beyhan, Z., Iager, A.E., Cibelli, J.B. (2007): Interspecies nuclear transfer: implications for embryonic stem cell biology. *Stem Cell,* 1(5): 502–512.

Byrne, J.A., Pedersen, D.A., Clepper, L.L., Nelson, M., Sanger, W.G., Gokhale, S. (2007): Producing primate embryonic stem cells by somatic cell nuclear transfer. *Nature,* 450(7169): 497–502.

Campbell, K.H. (1996): Sheep cloned by nuclear transfer from a cultured cell line. *Nature,* 380(6569): 64-66.

Campbell, K.H., Fisher, P., Chen, W.C., Choi, I., Kelly, R.D., Lee, J.H. (2007): Somatic cell nuclear transfer: Past, present and future perspectives. *Theriogenology,* 68: 214–231.

Cowan, C.A., Atienza, J., Melton, D.A., Eggan, K. (2005): Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science*, 309(5739): 1369–1373.

Do, J.T., Scholer, H.R. (2004): Nuclei of embryonic stem cells reprogram somatic cells. *Stem Cells*, 22(6): 941–949.

Do, J.T., Scholer, H.R. (2006): Cell-cell fusion as a means to establish pluripotency. *Ernst Schering Res Found Workshop*, 60: 35–45.

Egli, D. (2007): Developmental reprogramming after chromosome transfer into mitotic mouse zygotes. *Nature*, 447(7145): 679-685.

Fanos, T.W., Rangarajan, S., Ana Rita, M.R., Rob, V.R., Manoj, K., Philip, R., Catherine, V. (2015): Generation of hepatocyte and biliary epithelial-like cells from human Multipotent Adult Progenitor Cells (hMAPC)

by transdifferentiation into foregut endodermal progenitor-like cells. Inter university stem cell meeting, April 20, 2015, Luven, Belgium (Unpublished MSc Thesis).

Flasza, M., Shering, A.F., Smith, K., Andrews, P.W., Talley, P., Johnson, P.A. (2003): Reprogramming in inter-species embryonal carcinoma-somatic cell hybrids induces expression of pluripotency and differentiation markers. *Cloning Stem Cells,* 5(4): 339–354.

Freedman, B.S., Lam, A.Q., Sundsbak, J.L., Iatrino, R., Su, X., Koon, S.J., Wu, M., Daheron, L., Harris, P.C., Zhou, J., Bonventre, J.V. (2013): Reduced ciliary polycystin-2 in induced pluripotent stem cells from polycystic kidney disease patients with PKD1 mutations.*Journal of the American Society of Nephrology*, 24(10): 1571–1586.

Fulka, J.r., Loi, P., Fulka, H., Ptak, G., Nagai, T. (2004): Nucleus transfer in mammals: noninvasive approaches for the preparation of cytoplasts. *Trends Biotechnology*, 22(6): 279–283.

Fusaki, N. (2009): Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. *Proceedings of the Japan Academy, Series B. Physical and Biological Sciences,* 8(8): 348-362.

Gonzalez, F., Boue, S., Izpisua-Belmonte, J.C. (2011): Methods for making induced pluripotent stem cells: reprogramming a la carte. *Nature reviews Genetics,* 12(4): 231–242.

Grskovic, M., Javaherian, A., Strulovici, B., Daley, G.Q. (2011): Induced pluripotent stem cells opportunities for disease modelling and drug discovery. *Nature reviews. Drug discovery,* 10 (12): 915–929.

Hall, V.J., Compton, D., Stojkovic, P. (2007): Developmental competence of human in vitro aged oocytes as host cells for nuclear transfer. *Human Reproduction,* 22(1): 52–62.

Han, J., Sachdev, P.S. and Sidhu, K.S. (2010): Combined epigenetic and non-genetic approach for reprogramming human somatic cells, *PLoS. One,* 5(8).

Hochedlinger, K., Jaenisch, R. (2006): Nuclear reprogramming and pluripotency. *Nature*, 441(7097): 1061–1067.

Hotta, A., Ellis, J. (2008): Retroviral vector silencing during iPS cell induction: an epigenetic beacon that signals distinct pluripotent states. *Journal of cellular biochemistry,* 105: 940–948.

Hu, B.Y., Weick, J.P., Yu, J., Ma, L.X., Zhang, X.Q., Thomson, J.A., Zhang, S.C. (2010): Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc. Natl. Acad. Sci. USA.*, 107: 4335–4340.

Jaenisch, R., Hochedlinger, K., Blelloch, R., Yamada, Y., Baldwin, K., Eggan, K. (2004): Nuclear cloning, epigenetic reprogramming, and cellular differentiation. *Biology*, 69: 19–27.

Laurent, L.C., Ulitsky, I., Slavin, I., Tran, H., Schork, A., Morey, R., Lynch, C., Harness, J.V., Lee S., Barrero, M.J. (2011): Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. *Stem Cell*, 8: 106–118.

Nakagawa, M., Koyanagi, M., Tanabe, K., Takahashi, K., Ichisaka, T., Aoi, T., Okita, K.,Mochiduki, Y., Takizawa, N., Yamanaka, S. (2008): Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nature, Biotechnolology,* 26: 101–106.

Neri, T., Monti, M., Rebuzzini, P., Merico, V., Garagna, S., Redi, C.A. and Zuccotti, M. (2007): Mouse fibroblasts are reprogrammed to Oct-4 and Rex-1 gene expression and alkaline phosphatase activity by embryonic stem cell extracts, *Cloning Stem Cell,* 9(3): 394.

Nsair, A. and MacLellan, W.R. (2011): Induced pluripotent stem cells for regenerative cardiovascular therapies and biomedical discovery. *Advanced Drug Delivery Reviews*, 63 (4-5): 324-330.

Odorico, J.S., Kaufman, D.S., Thomson, J.A. (2001): Multilineage differentiation from human embryonic stem cell lines. *Stem Cells*, 19(3): 193–204.

Okita, K. and Yamanaka, S. (2011): Induced pluripotent stem cells: opportunities and challenges. *London Biological Science,* 366(1575): 2198-207.

Okita, K., Nakagawa, M., Hyenjong, H., Ichisaka, T., Yamanaka, S. (2008): Generation of mouse induced pluripotent stem cells without viral vectors. *Science*, 322(5903): 949–953.

Orkin, SH. and Zon, LI. (2008): *Cell*, 132: 631–644.

Papapetrou, E.P., Tomishima, M.J., Chambers, S.M., Mica, Y., Reed, E., Menon, J., Tabar, V., Mo Q., Studer, L., Sadelain, M. (2009): Stoichiometric and temporal requirements of Oct4, Sox2, Klf4, and c-Myc expression for efficient human iPSC induction and differentiation. *Proc Natl Acad Sci USA.*, 106: 12759–12764.

Park, H.Y., Noh, E.H., Chung, H.M., Kang, M.J., Kim, E.Y., Park, S.P. (2012): Efficient generation of virus-free iPS cells using liposomal magnetofection. *PLoS One,* 7: 45812

Park, I.H., Arora, N., Huo, H., Maherali, N., Ahfeldt, T., Shimamura, A., Lensch, M.W., Cowan, C., Hochedlinger, K., Daley, G.Q. (2008): Disease-specific induced pluripotent stem cells. *Cell,* 134(5): 877–86.

Pera, M.F., Reubinoff, B., Trounson, A. (2000): Human embryonic stem cells. *Journal of Cell Science*, 113(1): 5–10.

Rosa, A. and Brivanlou, A.H. (2010): Synthetic mRNAs: Powerful tools for reprogramming and differentiation of

human cells. *Stem Cell,* 7(5): 549-550.
Rubin, L.L. (2008): Stem cells and drug discovery: The beginning of a new era. *Cell*, 132(4): 549-552.
Saha, K. and Jaenisch, R. (2009): Technical challenges in using human induced pluripotent stem cells to model disease. *Stem Cell,* 5(6): 584-595.
Shevchenko, A.I., Medvedev, S.P., Mazurok, N.A., Zakiian, S.M. (2009): Induced pluripotent stem cells. *Genetika*, 45(2): 160–168.
Sidhu and Ryan (2010): Derivation of a new human embryonic stem cell line, endeavour-2, and its characterization. *In Vitro Cellular & Developmental Biology–Animal,* 46(3): 269-275.
Solter, D. (2000): Mammalian cloning: advances and limitations, *Nature Reviews Genetics*, 1(3): 199–207.
Stadtfeld, M., Nagaya, M., Utikal, J., Weir, G., Hochedlinger, K. (2008): Induced pluripotent stem cells generated without viral integration. *Science*, 322: 945–949.
Tada, M., Tada, T., Lefebvre, L., Barton, S.C., Surani, M.A. (1997): Embryonic germ cells induce epigenetic reprogramming of somatic nucleus in hybrid cells. *EMBO J*, 16(21): 6510–6520.
Takahashi, K., Yamanaka, S. (2006): Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, 126: 663–676.
Takebe, Takanori, Sekine, Keisuke, Enomura, Masahiro, Koike, Hiroyuki, Kimura, Masaki, Ogaeri, Takurnori, Zhang, Ran-Ran, Ueno, Yasuharu, Zheng, Yun-Wen (2013): Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature*, 499: 481–484.
Waddington, C.H. (1957): The strategy of the genes; a discussion of some aspects of theoretical biology. *London, Allen & Unwin*.
Walev, I., Bhakdi, S.C., Hofmann, F., Djonder, N., Valeva, A., Aktories, K., Bhakdi, S. (2001): Delivery of proteins into living cells by reversible membrane permeabilization with streptolysin O, *Proc Natl Acad Sci USA.,* 98(6): 31-85
Warren, L. (2010): Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Stem Cell*, 7(5): 618-630.
Wernig, M., Meissner, A., Foreman, R., Brambrink, T., Ku, M., Hochedlinger, K., Bernstein, B.E., Jaenisch, R. (2007): In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature,* 448: 318–324.
Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J. and Campbell, K.H. (1997): Viable offspring derived from fetal and adult mammalian cells. *Nature*, 385: 810–813.
Wu, S. and Hochedlinger, K. (2011): Harnessing the potential of induced pluripotent stem cells for regenerative medicine. *Nature Cell Biology,* 13: 497-505.
Yamanaka, S. (2010): Nuclear reprogramming to a pluripotent state by three approaches. *Nature*, 465(7299): 704-712.
Ying, Q.L., Nichols, J., Evans, E.P., and Smith, A.G. (2002): Changing potency by spontaneous fusion. *Nature*, 416(6880): 545–548.
Yu, J. (2009): Human induced pluripotent stem cells free of vector and transgene sequences. *Science*, 324(5928): 797-801.
Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz, B.J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R. (2007): *Science,* 318: 1917–1920.
Zhou, W., Freed, C.R. (2009): Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. *Stem Cells,* 27: 2667–2674