Cellular reprogramming of somatic cells

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The process of ‘cell reprogramming’ can be achieved by somatic cell nuclear transfer, cell fusion with embryonic stem cells, exposure to stem cell extracts, or by inducing pluripotency mediated by defined factors giving rise to what are termed induced pluripotent stem cells. More recently, the fate of a somatic cell can be directly induced to uptake other cell fates, termed lineage-specific reprogramming, without the need to de-differentiate the cells to a pluripotent state. In this review we will describe the different methods of reprogramming somatic cells.

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The process of de-differentiating somatic cells to a pluripotent state whereby they adopt characteristics of embryonic stem cells is referred to as ‘cellular reprogramming’. This can be achieved through somatic cell nuclear transfer (SCNT), cell fusion with embryonic stem (ES) cells, exposure to stem cell extracts, or by inducing pluripotency mediated by defined factors giving rise to what are termed induced pluripotent stem (iPS) cells. The process of reprogramming and resulting pluripotency in the resulting cells vary considerably, and the interrogation of different approaches has aided in elucidation of reprogramming process. In this review we will describe the different methods of reprogramming somatic cells to a pluripotent state and the knowledge gained from each.

Somatic cell nuclear transfer—Nuclear transfer typically involves the removal maternal chromosomes from an oocyte resulting in an enucleated oocyte this is followed by insertion of the donor cell nucleus (Fig. 1). Embryonic development is then artificially triggered by inducing an increase in intracellular calcium. In mammals the reconstituted embryo can recapitulate embryogenesis and on transfer into a recipient animal can resulting in full term development giving rise to a cloned animal. This process is known as reproductive cloning. Alternatively, embryonic stem (ES) cells can be isolated from the cloned blastocysts, and this is accordingly termed therapeutic cloning. The resulting NT-ES cells can be used as a tool for biomedical research or as a source of cells for transplantation back to the somatic cell donor without the fear of immune rejection, as the cells are genetically identical to the donor.

The process of nuclear transfer was pioneered in frogs. In 1952, Briggs and King have shown that replacing the nucleus of an enucleated frog (Rana pipiens) oocyte with the embryonic nuclei of blastula cells do not hinder embryo development. The process of somatic cell nuclear transfer (SCNT) has been first demonstrated by Gurdon and colleagues in 1958 using enucleated oocytes from the African clawed frog (Xenopus laevis) and differentiated adult intestinal cells as the somatic cell donor. They have shown that a somatic cell can be reprogrammed to a totipotent state which results in the generation of adult clones. Ability of an adult mammalian cell to be reprogrammed has been demonstrated almost half a century later with the birth of Dolly the sheep as a result of SCNT using a mammary epithelial cell. Collectively these results demonstrate that adult cells which are programmed to express a subset of specific genes to the differentiated cell, can be reprogrammed or de-differentiated to give rise to an organism genetically identical to the donor cell. Definitive evidence that terminally differentiated cells can be reprogrammed has been reported when mice were cloned from mature lymphocytes. The resulting cloned mice and ES cell lines harbor receptor rearrangements which only occur in...
terminally differentiated lymphocytes identical to that of the donor cell line. The biggest impact of SCNT to date has been its ability to be translated to the agricultural industry. The process of reproductive cloning has been applied to cattle, goat, sheep, pig, and horse. This technique can be used to create multiple copies of animals with highly valued or desirable traits, such as cows with high milk production or bulls with high quality meat. It has been shown that there are no significant differences in biological properties and the nutritional value of milk and meat obtained from clones compared with non-cloned animals, suggesting that products from cloned animals and their offspring are safe for human consumption and do not seem to pose any risks to human health as determined by the United States Food and Drugs Administration.

Therapeutic cloning is the most applicable form of SCNT for humans. Ability to generate pluripotent stem cells from a patient with a disease in combination with gene therapy has the potential to treat diseases through autologous cellular transplantation. The first proof of principle study on

![Diagram of Somatic Cell Nuclear Transfer (SCNT)](image)

**Fig. 1—Different methods of reprogramming somatic cells.**
the therapeutic application of SCNT derived cell lines has been reported in 2002 whereby ES cells were derived from SCNT blastocysts using donor cells from immune-deficient Rag2/- mice. Following targeted homologous recombination to correct the Rag2 recombinase gene in the SCNT derived ES cells, the cells were differentiated in vitro into hematopoietic stem cells (HSCs) for transplantation. However, there are a number of technical hurdles that need to be addressed before SCNT can be a viable source of pluripotent cells for human cell therapy. SCNT is a very resource intensive procedure requiring large number of oocytes. In humans there have only been three reports of human SCNT embryos reaching the blastocyst stage, with no reports of ES cell derivation.

Cell fusion—Reprogramming of somatic cells by cell fusion involves the hybridization of a somatic cell with a pluripotent cell resulting in a tetraploid cell hybrid (Fig. 1). Cell fusion can be induced experimentally in a number of ways including using chemicals such as PEG, electrical currents and virally. In most of the cell hybrids, the phenotype of the less-differentiated fusion partner dominates the phenotype of the more-differentiated partner. The first study to show that the phenotype of the pluripotent cell dominates in cell fusion has been performed by Miller and Ruddle who have found that cell hybrids made between mouse EC and thymocytes take on the pluripotent characteristics of ECC and can form tumors containing derivatives of the three germ layers in the teratoma assay. Furthermore, other pluripotent stem cells including embryonic germ cells and embryonic stem cells have been shown to reprogram somatic cells by cell fusion with the resulting hybrids having adopting a number of pluripotency properties.

Although ES-somatic cell hybrids display most properties of pluripotent stem cells their tetraploid state renders them incapable of significantly contributing to the late gestation epiblast and chimeras. Therefore, in order for cell fusion based reprogramming of somatic cells to be used as an alternative method of generating autologous pluripotent cells the removal of the ES cell derived DNA would be required following reprogramming. One approach that has been proposed involves the successful reprogramming of a somatic cell in a heterokaryon before nuclear fusion, followed by subsequent enucleation of the ES nucleus, which can potentially lead to the generation of autologous pluripotent cells. This approach results in only partial reprogramming of the somatic nucleus. An alternative approach involves the removal of the specific ES cell derived chromosomes responsible for self-recognition in the ES-somatic cell hybrid resulting in a hybrid aneuploid cell that is immune matched to the somatic cell. Despite these technical advances more advances in the cell fusion field are required before it can be considered a viable alternate method for generating autologous pluripotent stem cells for clinical applications.

Cell extracts—The third process of reprogramming somatic cells involves the exposure of differentiated cells or nuclei to cell extracts made from amphibian oocytes, or more recently to cell extracts made from mammalian embryonal carcinoma and ES cells. This technique involves the reversible permeabilisation of differentiated cells using the chemical streptolysin-O (SLO) a member of the family of cholesterol-dependent cytolysins, followed by exposure to cell extracts (Fig. 1). The lesions induced in the plasma membrane by SLO are repaired/resealed when Ca²⁺ is added to the media. Such cell extract exposure has been shown to partially reprogram the treated cells toward an embryonic state, predominantly in transformed and immortalized cell lines.

The first reprogramming studies using cell extracts have shown that incubation of somatic cells in Xenopus egg extracts results in remodeling of chromatin and changes to gene expression. Reprogramming of differentiated cells by exposure to mammalian cell extracts was first demonstrated by Hakelian et al. who have shown that HEK293T (human embryonic kidney) cells exposed to stimulated T cell extracts results in direct reprogramming toward a lymphoid-specific phenotype. Furthermore, it has been demonstrated that cell extract based reprogramming involves ATP-dependent chromatin remodeling.

The first reprogramming studies using pluripotent stem cell extracts show that both HEK293T cells and the immortalized NIH/3T3 mouse fibroblast cells acquire characteristics of pluripotent stem cells when exposed to extracts made from a pluripotent human carcinoma cell line (NCCIT). The resulting partially reprogrammed cells formed colonies (alkaline phosphatase positive) express pluripotency markers and switch off differentiation markers, and have undergone epigenetic changes at the promoters of a
number of pluripotent gene loci. More recently, human fetal fibroblasts have been shown to form hES cell-like colonies when treated with a combination of chromatin inhibitors and hES cell extracts. In this study, it has been shown that it is essential to pre-treat somatic cells with the epigenetic modifiers 5-aza-2′-deoxycytosine and trichostatin A, histone deacetylase and DNA methyltransferase inhibitors respectively, prior to exposure to hES cell extracts. The resulting reprogrammed cells have up-regulated a number of pluripotency genes and although reprogramming to a complete pluripotent state cannot achieved, the cells may be trans-differentiated into neurons under differentiation conditions. However, an important point of note on the cell extract technique is that it has also been demonstrated that pluripotent stem cells that are the source of the cell extracts can in fact survive the extract isolation technique/treatment and constitute a potential source of contamination in subsequent analysis.

**Induced pluripotent stem cells**—The most robust and exciting advance in the reprogramming field has been reported by Takahashi and Yamanaka who have accurately hypothesized that factors that are intrinsically involved in the maintenance of pluripotency of ES cells also play an important role in the reprogramming process. Following a screen of 24 genes associated with pluripotency, they have identified that the ectopic retroviral expression of four exogenous transcription factors, Oct3/4, Sox2, Klf4 and c-Myc, can reprogram mouse somatic fibroblast cells to a pluripotent state and have been termed these cells ‘induced Pluripotent Stem’ (iPS) cells (Fig. 1). Mouse and human iPS cells have been shown to be similar to their ES cell counter parts in terms of gene expression profile, epigenetic state, differentiation potential both in vitro and in vivo, can be used to generate autologous cells for transplantation studies, can reprogram other somatic cell by cell fusion, and the ability to give rise to germ line competent chimeras in mouse. iPS cells can be generated both from primary somatic cell cultures as well as late passage cells, as well as from a variety of cell types at varying efficiencies. Furthermore, the same 4 factors or the combination of Oct4, Sox2, Nanog and Lin28, have been shown to reprogram human somatic cells, as well as, monkey, rat, and porcine. Use of viruses and random integration of these transgenes into the host genome often result in variable reprogramming and due to the risk off associated insertional mutagenesis iPS cells generated in this way may have limited use in a clinical setting. To circumvent this has been a number of reports demonstrating that iPS cells can be generated by reducing the number of viral constructs used and/or minimize viral integration through substituting combinations of key reprogramming factors with either chemical compounds or utilizing specific populations of somatic cells endogenously expressing one of more of the key pluripotency factors. Furthermore, iPS cells can be generated without genetically modifying the somatic genome by the expression of the pluripotency factors from non-integrating viruses, transposons that can be excised following successful reprogramming, episomal transfection of plasmids, delivery of reprogramming proteins, delivery of microRNA or mRNA. Interestingly, iPS cells appear to have an ‘epigenetic memory’ of the somatic cell type from which they are derived. When the differentiation potential into the cells of hematopoietic or osteogenic lineages are compared for ES cells, blood-derived iPSCs, fibroblast-derived iPSCs and fibroblast cells reprogrammed by SCNT, a number of differences have been observed. Blood-derived iPSCs are more efficient than fibroblast-derived iPSCs at differentiation toward hematopoietic lineages. However, fibroblast-derived NT-ES cells are even more efficient at differentiation towards hematopoietic lineages suggesting that SCNT may result in more complete reprogramming than transcription factor based reprogramming. Similarly, transcriptional profiling of iPS cells derived from tail tip-derived fibroblasts, splenic B cells, bone marrow-derived granulocytes and skeletal muscle precursors have been shown to continue to express transcripts from their cell type of origin, and the blood derived iPS cells are more efficient at in vitro differentiation hematopoietic lineages. Furthermore, the epigenetic and functional differences of iPS cells derived from different lineages can be abrogated when the iPS cells are cultured in the presence of chromatin modifying compounds or after continuous passaging.

The process of transcription factor based reprogramming has been further optimized to directly induce somatic cells to uptake other cell fates, termed lineage-specific reprogramming, without complete de-differentiation to a pluripotent state (Fig. 1). Using a similar candidate gene approach to Yamanaka, a screen of 19 genes that encode transcription factors involve in neuronal development or function...
identified that a minimum of the three genes Ascl1, Brn2 and either Myl11 or Zic1, can convert mouse fibroblasts directly into neurons. Similarly, a screen of 14 candidate genes involved in cardiomyocyte development and function identified that the three transcription factors Gata4, MeF2c, and Tbx5 can convert mouse fibroblasts into cardiomyocytes. Interestingly, the trans-differentiation of fibroblasts into other somatic cell fates is remarkably high when compared to de-differentiation to a pluripotent state. Efficiency of transcription factor induced conversion of mouse fibroblasts into neurons or cardiomyocytes is around 20% (Refs 68, 69), in contrast to the inefficiency of generating iPS cells, which is around 0.01-0.5% (Refs 44, 70).

Summary

The field of reprogramming is rapidly evolving and the various techniques have aided in the understanding of both the differentiation and de-differentiation process. SCNT remains the benchmark for reprogramming somatic cells to a pluripotent state, however, the ability to directly program somatic cells to other cell fates by activating distinct combinations of lineage-specific factors may negate the need to de-differentiate and then re-differentiate cells for therapeutic applications. The added advantage of direct programming approach is that it reduces the risk of tumor formation associated with transplantation of pluripotent cell derived therapeutics. Nevertheless all approaches to programming and reprogramming of somatic cells need to be explored to increase our understanding of this incredible phenomenon before meaningful therapeutic outcomes can be achieved.

References

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