Harnessing Pluripotency from Differentiated Cells: A Regenerative Source for Tissue-Specific Stem Cell Therapies

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Abstract: Processes involving conversion of mature adult cells into undifferentiated cells have tremendous therapeutic potential in treating a variety of malignant and non-malignant disorders, including degenerative diseases. This can be achieved in autologous or allogeneic settings, by replacing either defective cells or regenerating those that are in deficit through reprogramming more commited cells into stem cells. The concept behind reprogramming differentiated cells to a stem cell state is to enable the switching of development towards the required cell lineage that is capable of correcting the underlying cellular dysfunction. The techniques by which differentiated cells can reverse their development, become pluripotent stem cells and transdifferentiate to give rise to new tissue or an entire organism are currently under intense investigation.

Examples of reprogramming differentiation in mature adult cells include nuclear reprogramming of more commited cells using the cytoplasm of empty oocytes obtained from a variety of animal species, or cell surface contact of differentiated cells through receptor ligand interaction. Such ligands include monoclonal antibodies, cytokines or synthetic chemical compounds. Despite controversies surrounding such techniques, the concept behind identification and design/screening of biological or pharmacological compounds to enable re-switching of cell fate *in-vivo* or *ex-vivo* is paramount for current drug therapies to be able to target more specifically cellular dysfunction at the tissue/organ level. Herein, this review discusses current research in cellular reprogramming and its potential application in regenerative medicine.

Keywords: Somatic cell plasticity, Nuclear transfer, Reprogramming, Retrodifferentiation stem cells, Regenerative medicine.

STEM CELL BASICS

Pluripotent stem cells give rise to tissue comprising a variety of differentiated cell types that can cure many haematological disorders [1], transgenic organs for xenotransplantation or synthesis of beneficial medicines [2] and an entire organism for the propagation of an elite livestock [3]. These giant developmental feats are invariably achieved through multiple rounds of self-renewal, subsequent commitment and differentiation into a variety of specific cell lineages [4]. Generally, stem cells have a simple morphology [5] and, only following asymmetric cell division one daughter cell can embark on differentiation while the other remains quiescent [6]. Upon completion of development, stem cells acquire more specific differentiated features that allow them to execute more complex functions. The developmental program of any given stem cell into a certain specialised cell type, known as ontogeny [7], is executed in step-wise fashion, which under normal circumstances exhibits lineage fidelity [8]. During this program stem cells progressively lose stem cell markers prior to displaying maturity or specialisation-associated characteristics [9]. For example, at the genetic and cellular level, pluripotent stem cell step-wise differentiation into primitive and definitive erythromyelopoiesis is preceded by loss of undifferentiated characteristics. This is then followed by the acquisition of mesodermal-endodermal features that can generate endothelium, and form organised yolk sac-like structures that secondarily generate multipotent primitive haematopoietic stem progenitor cells, erythroblasts, and macrophages.

DEFINING POTENCY

The potency of a stem cell is defined by its optional developmental destiny. For example, more commited stem cells, such as monopotent [10] or bipotent [11] have limited developmental potentials and are only capable of executing one or two developmental programs, respectively. The monopotent erythroid stem cell for instance, gives rise to haemoglobin containing enucleated red blood cells, while epithelial liver progenitors exhibit biliary and hepatocytic bipotentiality. In other words, these types of stem cells have determined their developmental destiny that is irreversible to a pluripotent or an embryonic stem cell fate. In stark contrast, pluripotent or embryonic stem cells are, more developmentally versatile born only following fertilization [12] of an oocyte. These totipotent embryonic stem cells [13, 14] exhibit complex developmental potentials. This is achieved through weaving multiple tissue and cell lineages which get molded into a variety of sophisticated organ systems that culminate in the creation of a viable and functional organism [15]. In this sense, apart from embryonic stem cells, all somatic cells in a mammalian body are considered relatively more commited. Though more restricted in development than embryonic stem cells, tissue-specific adult stem cells are hierarchical [16] in their developmental decisions. In other words, those most primitive reconstitute durable and complete tissue such as the lymphohaematopoietic system in surrogate animal models while those more commited are short lived and have

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a narrower repopulation potential, one lineage short of recreating an entire tissue [17].

FURTHER DEFINING POTENCY

The potency of a given somatic cell can also be measured by the number of times its nucleus can give rise to an immature or mature viable organism [18]. Nuclear transfer technology is one way of measuring cell potency. This involves the transfer of a given cell nucleus into an enucleated cell such as an oocyte [19]. Briefly, this involves the mechanical removal of the genetic material of an oocyte using a micromanipulation technique. Following this enucleation step, the donor nucleus is introduced into the oocyte by microinjection or fusion. The transferred nucleus undergoes disassembly in response to high levels of maturation promoting factor in the metaphase II cytoplasm. Following artificial activation, both by chemical or electrical treatment of the reconstituted oocyte, nuclear reassembly occurs and this enables the ultimate reproduction of a viable embryo. Earlier work on nuclear transfer experiments involving amphibian and mammals clearly demonstrate that the cloning efficiency of a nucleus decreases as cells differentiate. Other means of measuring potency also exist which involve the ability of a cell, usually an embryonic stem cell, to give rise to a chimeric animal when injected into an early blastocyst by contributing to most, if not all, somatic cell types [20]. In addition, the ability of a given cell to form teratomas consisting of differentiated derivatives of all three embryonic germ layers; mesoderm, ectoderm and endoderm following injection into an immunocompromised animal host [21] is the 'gold standard' of measuring implicit pluripotency and the hall mark of embryonic stem cells. In contrast, measuring potency in tissue specific stem cells derived from a more discernable tissue such as the bone marrow, cord or mobilized blood involves the reconstitution of an entire tissue such as the haematopoietic system [22, 23] in a surrogate irradiated immunodeficient animal model. In these assays, selectable pressure such as irradiation [24] is necessary for stem cell engraftment and repopulation. Furthermore, the durability and extent of adult stem cells to engraft an animal host, as well as having secondary repopulation potential upon serial transfer into a second animal, is another measure of potency [25]. Such stem cells are considered more potent or primitive and can be found in minute quantities in the bone marrow, cord blood and fetal liver.

SOMATIC CELL REPROGRAMMING OR DEVELOPMENTAL ANARCHY

Interestingly, by definition, embryonic stem cells are created from the union of two differentiated cells [26], the egg and sperm that are incapable of totipotency on their own. In the past century, the notion of pluripotency being exclusive to embryonic stem cells has been challenged. This is due to the fact that the nuclei of somatic cells, if given a chance, can also exhibit pluripotency. This is invariably achieved by altering the immediate microenvironment of the nucleus of a more commited cell by introducing it into an enucleated oocyte. In these experiments, a variety of animal species were generated from differentiated cells such as gut epithelial cell [27], keratinized skin cell [28], erythroid cells [29] and mammary epithelial cells [30] including lymphocytes [31]. Soon after the birth of Dolly the sheep a viable progeny of a milk producing cell, mice [32], cows [33], goat [34], pigs [35], rabbits [36] and a cat [37] were generated from adult donor nuclei. The efficiency of mammalian nuclear transfer experiments is low and very similar to that obtained in amphibians. Only less than 1% of all nuclear transfers from adult or differentiated cells result in apparently normal offspring [38].

Reprogramming specialisation in more primitive somatic cells was also extended to tissue-specific or adult stem cells. These adult stem cells have been noted to exhibit developmental infidelity or plasticity outside the tissue from which they were originally derived. For example, neuronal stem cells appear to convert to blood [39] and vice versa [40], haematopoietic stem cells into liver [41, 42] and bone marrow cells into heart [43]. This phenomenon has been noted to occur in response to remote environmental cues or selective pressure *in-vivo* in surrogate animal models. In addition, bone marrow cells were shown to give rise to multiple tissue types following culture and expansion [44]. Paradoxically, despite carrying the title 'stem cells', the plasticity of adult stem cells are more intensely debated [45] when compared to those formed from far more differentiated cells following nuclear transfer. For example, some would argue that donor stem cell plasticity and engraftment is due to fusion with host resident stem cells, albeit in some instances with the ability to correct a congenital mutation [46].

Nevertheless, at face value, these findings regarding developmental infidelity of somatic cells are already cementing new and bizarre concepts in our current understanding of developmental biology. For example, three notions that come to mind: is developmental plasticity universal to all somatic cells? Relative to which stem cell type can one assess the potency of a given cell type? If both are capable of pluripotency then what is the difference between a differentiated or stem cell state? Alternatively, is pluripotency an event in space, dictated by the microenvironment in which the cell is positioned in, or artificially transferable?

These fascinating notions, though for now indeterminate, already changing the way we perceive somatic cells and even our very own selves. Regardless of our beliefs or preferences, nuclear reprogramming technologies though exciting and beneficial have suddenly posed some conceptually circular ethical arguments [47]. For example, if all cells are equal in their potency then all somatic cells, if permitted, have the potential to give rise to viable organisms. What is all the fuss then? Does not realising the potentiality of our very own somatic cell make any one a better judge of the ethics of stem cell research or therapy? In this regard, may be it is pertinent not to pass judgment based on scientific grounds as determined by potency, but rather on more basic humane and transparent [48] principles. In fact, a stem cell state can not be measured directly with absolute certainty [49, 50]. Only following development can one determine the potency of a given cell.

All stem cell assays [51] are not impartial. They do not measure the absolute stemness state but rather exhibit the

impact of a set of conditions on a putative stem cell candidate. For example, prior to defining potency, most of the assays mentioned above first involve the isolation of stem cells using toxic chemical [52] dyes, digestive enzymes [53] and cross-linking of cells using antibody coated magnetic beads [54]. This is followed subsequently by exposing the cells to free ligands such as growth factors [55] and cytokines [56]. Both steps may impact the phenotype of the starting population and, therefore, purely reflect the properties of such assays rather than the true identity of the cells under investigation. Furthermore, we are continually improving the efficiency of these assays, such as the various protocols used to kick start the process of embryogenesis in nuclear transfer technology [57]. For example, the type of recipient cell, source of recipient cell, method of reconstruction, activation, embryo culture, donor cell type, and donor and recipient cell cycle stages are all important factors influencing the efficiency of cloning techniques. Presumably, this is why we are now unraveling more readily the phenomenon of somatic cell plasticity in mature adult cells, in response to improved scientific methods.

May be the actual phenotype of a stem cell or a more differentiated cell state is not a permanent characteristic. For example, may be the more or less commited cell state is defined by a set of impending or extant environmental conditions which when met tip the balance either towards hierarchical cell fate determination [58] or indetermination [59]. This kind of oscillatory developmental tilting may conversely impact the number of cells that are occupying a more or less commited cell state by means of shifting development more towards juvenile or adult ontogeny. This kind of seesaw-like developmental potential may be the reason behind the plasticity of adult stem cells and their subsequent transdetermination [60]. For instance, a variety of markers have been associated with a variety of stem cell types, namely CD34 [61], nestin [62] and Oct 4 [63]. But do we really know the exact distribution of such antigens in the living tissue of an adult or developing human? After all, not all haematopoietic stem cells expressing the CD34 marker engraft an animal host [64] or proliferate and differentiate *in vitro* in semi solid medium [65]. Furthermore, OCT-4, an embryonic stem cell marker can be re-expressed by highly specialised cells such as lymphocytes [66], while nestin [67] or CD34 [68] antigens are not exclusive markers of neuronal or haematopoietic progenitor stem cells, respectively. Furthermore, disregarding the phenomenon of adult stem cell plasticity on the basis of a somewhat rare cell fusion event [69] may turn out to be one of cell biology's greatest follies. It may turn out that the cell fusion hypothesis is a way of explaining the very phenomenon under investigation. For example, the reversibility of the differentiated state does occur in response to the formation of a heterokaryon [70], a form of primitive cellular abduction which may be a rare transitory physiological phase in reprogramming the differentiated state. Alternatively, the rare cell fusion hypothesis may simply reflect an inherent inadequacy in cell tracking techniques [71] that is undermining a *bona fide* phenomenon.

Nonetheless, the ever expanding research into somatic cell plasticity has made it hard to draw sharp lines to delineate the difference between pluripotency, lineage commitment and, therefore, what can potentially constitute life sustaining cellular entities. Far more poignant is the notion that somatic cells of bone marrow origin can act as a potential source of germ cells that could sustain oocyte production in adulthood [72].

THE POTENTIAL OF SOMATIC CELL PLASTICITY

Nevertheless, the multitude of diseases by which such technologies can cure or even treat are listless. It was once believed, and not vice versa, that only stem cells give rise to specialised cells that, upon terminal differentiation, will ultimately perish. In the face of dwindling sources of stem cells in an ageing human body, such a bleak ending of differentiated cells is the cause of tissue degeneration, organ failure and eventual demise.

Even more paradoxical is the finding that fully intact differentiated cells can be reprogrammed to become pluripotent stem cells *via* mere cell surface contact. This means that we already have at hand less strenuous means to mend defaced tissue, as well as remedy organ dysfunctions, by unleashing dormant developmental programs through contacting differentiated cells. The process involves harnessing of dormant, more primitive developmental programs whilst silencing those associated with its specialisation, by mere cell surface receptor ligation thus rendering them pluripotent. This involves contacting cells with agents ranging from monoclonal antibody [73], cytokine [74] cellular extract [75] and even small synthetic chemical compounds [76]. From my research, into somatic cell reprogramming I have found that fully intact differentiated cells undergo reprogramming in response to ligation, cross-linking or contacting ubiquitous sites on cell surface receptors. This process, which I have termed retrodifferentiation [73, 77-78] or rather reprogramming by cell surface contact, can be achieved in very short periods of time and is far more pronounced in response to ligation with unconjugated monoclonal antibodies. Interestingly, one such agent which I have noted to induce profound and rapid plasticity in differentiated cells such as white blood cells is a monoclonal antibody that binds monomorphic sites on the MHC class II beta-chain, clone CR3/43. In these experiments, and following exposure to a variety of well established cell culture conditions, the conversion of a more commited heterogeneous cell population such as white blood cells into undifferentiated cells with far more flexible developmental potentials is noted. The exact mechanism behind rewinding the ontogeny of a fully developed cell to a stem cell stage remains unclear. However, the mechanism always involves loss of differentiation-associated antigens such as the pan leukocyte marker, CD45, and haematopoietic lineage-associated antigens including class I and II human lymphocytes antigens [HLA], with the optional acquisition of a multitude of stem cell-associated markers such as OCT4, nestin, Gata 4 and CD34+ obtained from a single source of unmobilised [79] donor blood. In this process, the differentiated cells appear to develop a loose chromatin structure, prominent nucleoli and basophilic cytoplasm prior to acquiring new, more specific differentiated features. This process, which has been termed retrodifferentiation, occurs in exactly the reverse direction to differentiation of a given stem cell and is followed by transdifferentiation into a

variety of specific tissues consisting of a spectrum of differentiated cell types. At various switch points in reverse development or ontogeny, the heterogeneous population of differentiated cells is converted into another heterogeneous population of stem cells with variable specialisation potentials giving rise to undifferentiated, haematopoietic, neuronal or cardiopoietic progenitors capable of engrafting an immunodeficient animal host [80]. As to why the homologous sites on MHC class II antigens [81] should elicit such profound and rapid reprogramming of differentiated cells is intriguing indeed. The variable site of this major histocompatibility complex (MHC) is involved in tissue rejection [82] and antigenic presentation [83] to T lymphocytes. In other words the engraftment or tolerance, respectively, of any graft, be it the product of stem cells [84] or an organ transplant [85] is mediated by the extent of matching between donor and recipient MHC antigens [86]. How can a compound cell surface receptor consisting of an alpha and beta chain such as the MHC class II antigen have different sites implementing completely different and apposing cellular responses; such as stem cell regeneration as is the case with retrodifferentiation or tissue rejection and demise following allogeneic transplant? These compartmentalised signals leading to different cellular functions such as tissue repair or rejection ought to be dissected. An effort may well lead to the understanding of the more complex signal transduction of the cells in response to extant and impending environmental stimuli. Examples of an extant environmental stimuli is the one generated during tissue injury that leads to migration and homing of cells to [87] remote sites while, the cross talk [88] between T-cells and antigen presenting cells is an excellent example of a more intimate microenvironmental stimulus. The deciphering of the relationship between these complex signal transductions involved in cellular reprogramming or extinction may well translate into a more comprehensible cellular language to be harnessed to its full potential in transplantation and regenerative medicine.

Another form of reprogramming mature adult cells into becoming a different type of specialised cell *in vitro* is known as transdifferentiation [89-94]. Though progressive and appearing to exhibit developmental fidelity *in-vitro,* the traversal of the differentiation barrier of such fully developed cells remains unclear, and occurs in response to changes in the biochemical surrounding of the cell. In most instances, the differentiated cell is noted to pass through a

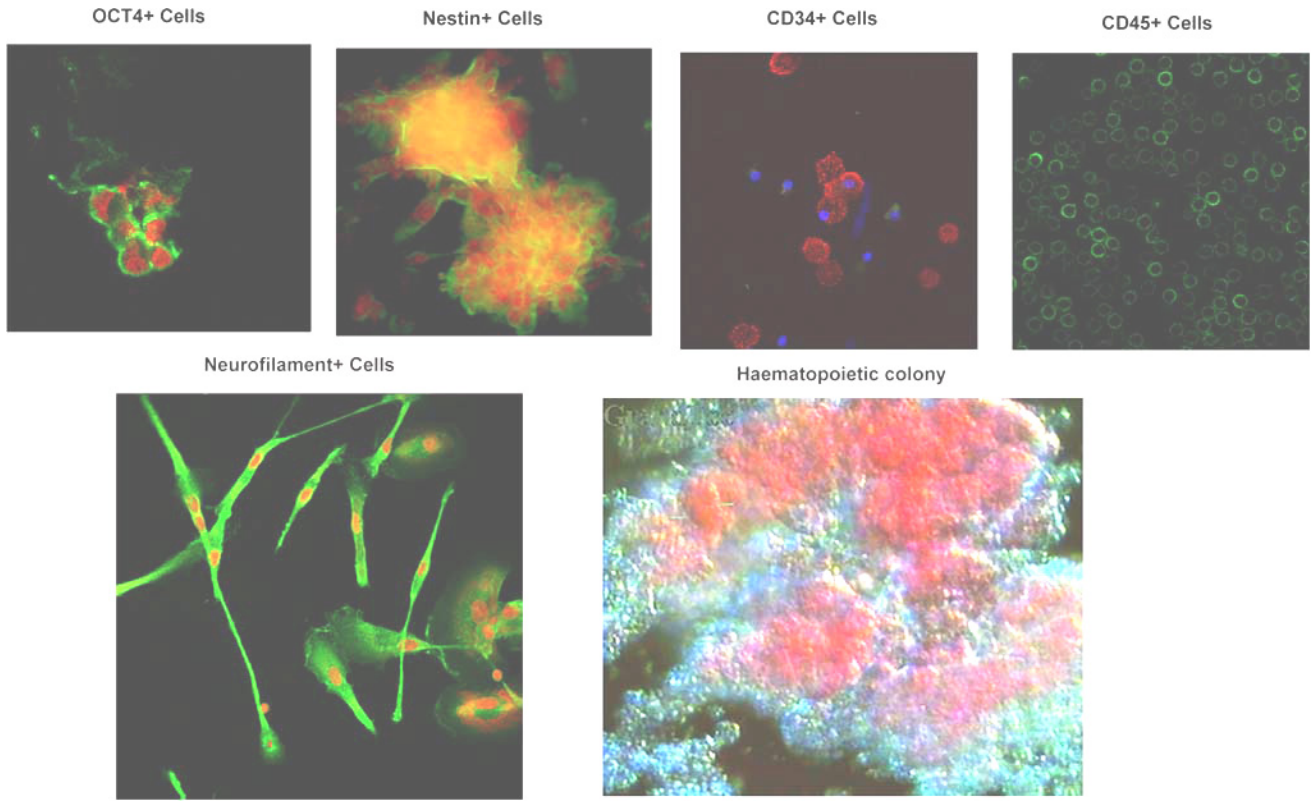

Fig. (1). An illustration of reprogramming commited somatic cells (Retrodifferentiation or Dedifferentiation) such as white blood cells expressing the pan leukocyte marker CD45 (green stain) into a variety of stem cells classes expressing either CD34 antigen (red stain) with the nuclear dye Hoechst, showing Hoechst influx positive or negative cells (blue stain). Further step- wise reversion of leucocytes leads to the production of nestin (green dye) or OCT-4 (green dye) positive cells, respectively, superimposed on a red background staining of nuclei with Propidium Iodide. At these various switch points in reverse development the newly formed CD34 or Nestin positive cells regain the ability to re-differentiate into either pluripotent haematopoietic cells colony or neurofilament positive cell, respectively. The traversal of the differentiation barrier from being CD45 positive into neurofilament positive cells depicts Transdifferentiation.

dedifferentiated state without accounting for whether the differentiated cell has transited a stem cell state to enable the specialised cell to defect into another lineage. The major difference between this phenomenon and retrodifferentiation is that the latter process achieves developmental plasticity *in vitro via* intermediary hierarchical stem cell states, with far more flexible transdifferentiation potentials (Fig. **1**).

Nevertheless, switching of somatic cell developmental allegiance is profoundly beneficial in controlling excessive or diminished tissue regeneration *in-vivo* without involving a controversial or rather cumbersome *in-vitro* step such as therapeutic cloning. For example, in order to eradicate leukaemic cells, one can convert such malignant, and more often genetically mutant, clones [95] into enucleated red blood cells that can be easily removed less invasively from an ailing body *via* simple veni-puncture. In autoimmune disease, the excessive regeneration of an autoaggressive immune response [96] can be curtailed by means of transdifferentiating somatic cells into more naïve cells [97] capable of taming any auto-aggressive assault on cells, tissues and organs. Even in the absence of engraftment, stem cells have been shown to impart a protective effect by nursing degenerate neuronal tissue through secreting biochemicals, such as glial cell derived neurotrophic factor (GDNF) which may ameliorate or halt disorders such as motor neuron and Parkinson's diseases [98]. In type I diabetes, which is caused by the demise of insulin-producing cells, one can drive the developmental process of another differentiated cell within the vicinity of the pancreas to transdifferentiate into islet cells capable of resuming insulin synthesis [99]. Theoretically speaking, examples of reprogramming fully intact commited cells into another lineage in order to correct, or impact cell deficit or proliferation, respectively are endless. The multitude of developmental trajectories by which one can send signals towards driving a specific developmental program of a given tissue or somatic cell into another more useful destiny will transfer regenerative medicine into the healing buttons of a receptor ligand interaction and promote mending of tissues with utmost precession and fidelity. On the other hand, rewinding somatic cell ontogeny to a more immature stage within a specific lineage should silence adult genes and allow re-expression of an alternative fetal form of a given gene. For example, the upregulation of functional foetal haemoglobin [100] or utrophin instead [101] of the dysfunct adult haemoglobin or dystrophin in genetic disorders such as beta thalassemia and muscular dystrophy, respectively, (due to reversion of nucleated erythroid or skeletal muscle cells to a more immature stage in their respective ontogeny), may induce amelioration of the clinical sequel associated with such devastating congenital disorders.

However, the implementation of such wonderful technologies will be heavily dependent on the understanding of the forward journeys of embryonic, as well as tissue-specific, stem cells - most of which are not readily accessible or well characterised as cells of the haematopoietic system. Finally, the combination of reprogramming technologies and easy access of this fluid tissue using standard cell separation [102], harvesting [103] and washing [104] devices will undoubtedly render regenerative and transplantation medicine alike amenable to automation and the construction of novel robotic designs replacing the human donor.

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