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PERSPECTIVE

Induced adult stem (iAS) cells and induced transit amplifying progenitor (iTAP) cells – a possible alternative to induced pluripotent stem (iPS) cells?

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Abstract

The successful derivation of iPSC lines effectively demonstrates that it is possible to reset the 'developmental clock' of somatic cells all the way back to the initial embryonic state. Hence, it is plausible that this clock may instead be turned back half-way to a less immature developmental stage that is more directly applicable to clinical therapeutic applications or for *in vitro* pharmacology/toxicology screening assays. Such a suitable developmental state is postulated to be either the putative transit amplifying progenitor stage or adult stem cell stage. It is hypothetically possible to reprogram mature and terminally differentiated somatic cells back to the adult stem cell or transit amplifying progenitor stage, in a manner similar to the derivation of iPSC. It is proposed that the terminology 'Induced Adult Stem Cells' (iASC) or 'Induced Transit Amplifying Progenitor Cells' (iTAPC) be used to described such reprogrammed somatic cells. Of particular interest, is the possibility of resetting the developmental clock of mature differentiated somatic cells of the mesenchymal lineage, explanted from adipose tissue, bone marrow and cartilage. The putative adult stem cell sub-population from which these cells are derived, commonly referred to as 'mesenchymal stem cells', are highly versatile and hold much therapeutic promise in regenerative medicine, as attested to by numerous human clinical trials and animal studies. Perhaps it may be appropriate to term such reprogrammed cells as 'Induced Mesenchymal Stem Cells' (iMSC) or as 'Induced Mesenchumal Progenitor Cells' (iMPC). Given that cells from the same organ/tissue will share some commonalities in gene expression, we hypothesize that the generation of iASC or iTAPC would be more efficient as compared to iPSC generation, since a common epigenetic program must exist between the reprogrammed cells, adult stem cell or progenitor cell types and terminally differentiated cell types from the same organ/tissue. **Copyright © 2009 John Wiley & Sons, Ltd.**

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The successful derivation of induced pluripotent stem cell (iPSC) lines through genetic manipulation of primary explanted somatic cells (Yu *et al*., 2007; Takahashi *et al*., 2007) has aroused much interest because of their potential applications in therapy (Romano, 2008) and in pharmacological and toxicology screening (Nishikawa *et al*., 2008). Nevertheless, significant challenges have to be overcome before the various potential therapeutic and non-therapeutic applications of iPSCs can be realized (Surani *et al*., 2008). Of particular concern is how the epigenetic state of reprogrammed iPSCs might differ from human embryonic stem cells (Surani *et al*., 2008; Han and Sidhu, 2008) and the permanent genetic modification sustained by iPSCs due to viral transduction of recombinant DNA (Heng and Richards, 2008). Besides these two major challenges, it is also imperative to

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question whether reprogramming of somatic cells to the pluripotent 'embryonic stem cell-like' state is absolutely necessary for the various therapeutic and non-therapeutic applications that have been touted for iPSCs. After all, it is expected that tissue engineering, transplantation therapy and pharmacology/toxicology screening would in fact utilize differentiated iPSC progenies of a specific well-defined lineage, rather than undifferentiated iPSCs *per se*.

The successful derivation of iPSC lines effectively demonstrates that it is possible to reset the 'developmental clock' of somatic cells all the way back to the initial embryonic state. Hence, it is plausible that this clock may instead be turned back half-way to a less immature developmental stage that is more directly applicable to clinical therapeutic applications or for *in vitro* pharmacology/toxicology screening assays. Such a suitable developmental state is postulated to be either the putative transit amplifying progenitor stage or adult stem cell stage. Transit amplifying progenitor cells refer to the intermediate stage of cellular differentiation at which stem cells have already committed to a particular lineage and are actively proliferating just before the onset of terminal differentiation. It is most widely used to describe hepatic oval cells (Jensen *et al*., 2004) and keratinocyte progenitors (Turksen and Troy, 1998) but is also likely to be applicable to the differentiation pathways of other somatic lineages.

So what are the therapeutic advantages of utilizing transit amplifying progenitor cells? As discussed previously (Heng and Cao, 2005a, 2005b), there probably exists a subtle balance somewhere between the undifferentiated and fully differentiated state that would be optimal for achieving maximal efficacy of cell transplantation therapy. Ideally, the transplanted cells should be mature enough to be committed to a particular well-defined lineage, expressing lineage-specific markers that could facilitate their engraftment and integration within the recipient tissue/organ (e.g. gap–junction connexin proteins), whilst at the same time be immature enough to be actively mitotic and less fastidious in their nutritional and oxygen requirements. Such an intermediate state of differentiation is thus speculated to be the putative transit amplifying progenitor stage. Regenerative medicine strategies utilizing transit amplifying progenitors would be particularly useful for cell and tissue lineages that have proved to be difficult to obtain via adult or embryonic stem cell differentiation, e.g. pancreatic β cells. Additionally, such an approach would also be promising in the design of treatment strategies for tissues and organs that may be devoid of an adult stem cell subpopulation, e.g. kidney and pancreas.

The initial challenge is therefore to identify the various intracellular factors (e.g. transcription factors) responsible for maintaining the adult stem cell state or differentiating stem cells in the transit amplifying progenitor stage of various specific lineages. Once the genes encoding these various factors have been identified, these may possibly be used to reprogramme mature and terminally differentiated somatic cells back to the adult stem cell or transit amplifying progenitor stage, in a manner similar to the derivation of iPSCs. It is proposed that the terminology 'induced adult stem (iAS) cells' or 'induced transit amplifying progenitor (iTAP) cells' be used to described such reprogrammed somatic cells.

Of particular interest is the possibility of resetting the developmental clock of mature differentiated somatic cells of the mesenchymal lineage, explanted from adipose tissue, bone and cartilage (Peng *et al*., 2008; Choi *et al*., 2008; Kobayashi *et al*., 2008). The putative adult stem cell subpopulation from which these cells are derived, commonly referred to as 'mesenchymal stem cells', are highly versatile and hold much therapeutic promise in regenerative medicine, as attested by numerous human clinical trials and animal studies (Pelagiadis *et al*., 2008; Bobis *et al*., 2006; Chen *et al*., 2008). Perhaps it may be appropriate to call such reprogrammed cells 'induced mesenchymal stem cells (iMSCs)' or 'induced mesenchymal progenitor cells (iMPCs)'.

Identifying the appropriate combination of transcription factors and choosing the right somatic cell types to reprogramme will be key to the success of generating iAS or iTAP cells. Recent advancements in the generation of iPSCs have suggested that endogenous levels of critical genes required for reprogramming by some somatic cells may be sufficient to replace the corresponding exogenous gene expression (Shi *et al*., 2008a). Given that cells from the same organ/tissue will share some commonalities in gene expression, we hypothesize that the generation of iAS or iTAP cells would be more efficient as compared to iPSCs generation, since a common epigenetic programme must exist between the reprogrammed cells, adult stem cell or progenitor cell types and terminally differentiated cell types from the same organ/tissue.

Subsequently, the next challenge would be to identify and develop appropriate *in vitro* culture conditions for maintaining the reprogrammed cells in the adult stem cell or transit amplifying state for prolonged durations, so as to enable extensive expansion of cell numbers. No doubt the adult stem cell and transit amplifying progenitor stages of cellular differentiation are highly transient phases of development. However, it must be remembered that the embryonic stem cell state is equally brief and transient during normal development. Hence, if appropriate culture conditions for maintaining and propagating human embryonic stem cells in the pluripotent state can be identified and developed (Skottman *et al*., 2007; Chase and Firpo, 2007), there is no reason why the same cannot be achieved for adult stem cells or transit amplifying progenitor cells of various lineages. Indeed, much progress has already been made in the development of appropriate culture conditions for the *ex vivo* expansion of the putative bone marrow-derived mesenchymal stem cells (Beyer *et al*., 2006; Brinchmann, 2008; Pountos *et al*., 2007).

Undoubtedly, the theoretical possibility of iAS or iTAP cells will also be dogged by the same concerns faced by iPSCs, in particular their uncertain epigenetic state

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and permanent genetic modification with recombinant DNA. Currently, the major method of gene transfer is virus-based, and the transgenes are more often than not randomly inserted within the host genome. Hence, there is a risk of viral infection, as well as of the transgenic cells becoming malignant. There is no completely fool-proof method to prevent reprogrammed somatic from becoming cancerous upon infusion *in situ* within the recipient, particularly if they are proliferating rapidly. Nevertheless, we can mitigate the risk of tumourigenesis by avoiding permanent genetic modification to reprogrammed somatic cells. Moreover, the tumourigenic potential of iTAP and iAS cells should also be theoretically lower than that of pluripotent iPSCs. Like their naturally-occurring adult stem cell counterparts, such reprogrammed somatic cells should not possess the ability to form tumours or teratomas, thus making them a safer alternative to iPSCs.

Platform technologies that avoid the integration of recombinant DNA within the host cell genome now exist. These include the use of adenoviral vectors (Stadtfeld *et al*., 2008), plasmids (Okita *et al*., 2008), transposons, e.g. *piggybac* (Yusa *et al*., 2009) and small molecules that can facilitate reprogramming (Marson *et al*., 2008; Shi *et al*., 2008b). An even more novel alternative would be the direct delivery of recombinant transcription factors to the cytosol (Heng and Richards, 2008; Heng *et al*., 2005), through the use of either protein transduction domains (Heng and Cao, 2005c) or immunoliposomes (Sullivan *et al*., 1986). Indeed, Bosnali and Edenhofer (2008) successfully generated transducible versions of two transcription factors, *Oct-4* and *Sox-2*, which have previously been used to reprogramme somatic cells to iPSCs (Yu *et al*., 2007; Takahashi *et al*., 2007). This was achieved through recombinant fusion of the corresponding genes with the HIV TAT protein transduction domain. More recently, Zhou *et al*. (2009) successfully reprogrammed murine fibroblasts to the pluripotent stem cell state with recombinant transcription factors fused to a poly-arginine protein transduction domain. Yet another possible means of cellular reprogramming without the use of recombinant DNA is through RNA interference (Heng and Cao, 2004). Perhaps transducible transcription factors may be combined together with siRNA, as a novel integrated strategy for reprogramming somatic cells to either iAS or iTAP cells (Cao *et al*., 2005). A more intriguing possibility is the screening and identification of small molecules that can induce somatic cells to revert to a less differentiated state. Preliminary work by Shi *et al*. (2008b) and Lyssiotis *et al*. (2009) have identified a variety of small molecules that can mimic the action of the reprogramming factor Klf4 in the generation of new iPSC lines.

With regard to the uncertainty in the epigenetic reprogramming of somatic cells, it is speculated that this would be less of a concern with iAS or iTAP cells as compared to iPSCs. After all, the purpose of having 'half-way' reprogramming of iAS or iTAP cells instead of a full reprogramming to the embryonal state of iPSCs is that we want these cells to be epigenetically predisposed and committed to a particular lineage or subset of lineages. Hence, e.g. in liver tissue engineering, we would require transit amplifying progenitors of the hepatic lineage that are highly proliferative, e.g. hepatic oval cells (Jensen *et al*., 2004). It will not be such a great concern if mature differentiated hepatocytes retain much of their original epigenetic signature upon reprogramming to the transit amplifying state of hepatic oval cells, provided that this does not compromise the proliferative capacity expected of such reprogrammed cells (Jensen *et al*., 2004). The same cannot be said of iPSCs, which should ideally return to the epigenetic signature of the embryonic state upon reprogramming, so as to be considered truly pluripotent.

The use of iAS or iTAP cells may also circumvent theoretical but potentially devastating problems associated with incomplete epigenetic reprogramming in iPSCs. It has been proved experimentally that *Hox* gene expression, which is subjected to epigenetic control, confers positional memory on adult cells. Skin fibroblasts, for instance, retain a *Hox* code similar to that of their site of origin, even after prolonged passage in culture (Chang *et al*., 2002; Rinn *et al*., 2008). More recently, it has been demonstrated that *Hox* status and embryonic origin can influence the behaviour of skeletal stem cells during tissue-grafting procedures (Leucht *et al*., 2008). The broad implication of these findings is that incomplete epigenetic reprogramming in iPSCs derived from skin fibroblasts, for example, could potentially adversely affect the success of a tissue graft because of molecular differences between the graft and recipient site. Furthermore, the possibility that committed iPSC progenitors could revert to their original somatic phenotype at the graft site does exist, and this reversion in identity could potentially be catastrophic to the host tissue.

In conclusion, more intensive research effort is needed to characterize the much less studied transit amplifying stage of cellular differentiation. The theoretical possibility of iAS or iTAP cells of various lineages is intriguing indeed, and would certainly be a big boon to the field of regenerative medicine if these were to materialize.

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