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Stem cell-derived islet cells for transplantation

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Abstract

Purpose of review—The promise of islet transplantation for type 1 diabetes has been hampered by the lack of a renewable source of insulin-producing cells. However, steadfast advances in the field have set the stage for stem cell-based approaches to take over in the near future. This review focuses on the most intriguing findings reported in recent years, which include not only progress in adult and embryonic stem cell differentiation, but also the direct reprogramming of non-endocrine tissues into insulin-producing beta cells.

Recent findings—In spite of their potential for tumorigenesis, human embryonic stem (hES) cells are poised to be in clinical trials within the next decade. This is mainly due to the preclinical success of a differentiation method that recapitulates beta cell development. In contrast, adult stem cells still need one such gold standard of differentiation, and progress is somewhat impeded by the lack of consensus on the best source. A concerted effort is necessary to bring their potential to clinical fruition. In the meantime, reported success in reprogramming might offer a “third way” towards the rescue of pancreatic endocrine function.

Summary—Here we discuss the important strategic decisions that need to be made in order to maximize the therapeutic chances of each of the presented approaches.

Keywords

Human embryonic stem (hES) cells; induced pluripotent stem (iPS) cells; mesenchymal stem cells (MSCs); beta cell differentiation; reprogramming; islet transplantation

Introduction

The evolution of islet transplantation has visibly followed each one of the steps of the often cited “Gartner hype cycle” [1]. There is little argument that the invention of the semi-automated method for islet isolation [2] was the “technology trigger” that helped popularize the procedure throughout the world. Indeed, this breakthrough was universally saluted as the onset of a new era of cell therapy for the treatment of autoimmune diabetes. Few disputed that this safe and conceptually simple procedure may lead to the relegation of whole pancreas transplantation for the radical treatment of type 1 diabetes. The “peak of inflated

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expectations” was reached around 2000 with the clinical implementation of a steroid-free immunosuppressive regime that resulted in long-term graft survival (the Edmonton protocol) [3]. Unfortunately, a subsequent follow-up study revealed a much more somber panorama. The realization that only about 20% of the patients remained insulin-independent five years after the procedure [4] dragged the field through a long “trough of disillusionment” that stoked the perception that islet transplantation had taken us as far as it could –which was nowhere close to a cure. Many of the numerous centers that had mushroomed after the first announcements of long-term engraftment closed or drastically reduced operations, and overall funding decreased. A slow “slope of enlightenment” amidst this hostile climate has quietly led to a “plateau of productivity” in which T-cell depleting strategies and other novel immunological interventions now ensure graft survival and function at rates comparable to those observed with whole organ transplantation [5]. While the expectations for this therapy are now considerably lower than just a decade ago, our progressive understanding of its limitations has helped us pave the way for the new generation of cell therapeutics. In fact, it is thanks to islet transplantation that we can now accurately predict not only the nature but also the likely course of the next breakthrough. While stem cell-based advances for other conditions will have to blaze new trails, at the time we manage to effectively and safely convert stem cells into insulin-producing cells, we will already be familiar with the learning curve. The groundwork is laid for the next generation of scientists to dramatically expand the clinical applicability of islet transplantation beyond its current reach (figure 1).

There is a broad consensus on the idea that stem cells will just take the place of islets in the near future, riding along the beaten track set by decades of research on the latter. However, the jury is still out regarding the candidate cell type/s and approach that will ultimately succeed. Since the subject has been recently reviewed in great depth by us [6, 7] and others [8–14], we will focus this article in three of the most rapidly evolving trends, namely the differentiation of embryonic stem (ES)/induced pluripotent stem (iPS) cells, the characterization of some of the most promising additions to the arsenal of adult stem cells (neural crest-derived and adipose cells), and the prospects of direct reprogramming of adult tissues.

ES and iPS cells

By almost any measure, embryonic stem (ES) cells are the gold standard of all stem cells. Both their replicative potential and ability to become any cell type remain unmatched. Detractors of this technology often cite the lack of ongoing clinical trials with ES cells as evidence of the purported superiority of adult stem cells, which undoubtedly are more widely represented in the clinical arena. This assessment is misleading on several counts, chiefly the fact that adult stem cells have been in use for more than half a century, whereas human ES cells were first isolated just twelve years ago [15]. In fact, a careful review of the literature indicates that insulin-producing cells of ES cell origin are closer to true beta cells than any other cell differentiation product from adult stem cells. This is so despite a Gartner hype cycle curve that has fallen precipitously after a peak of overinflated expectations (see figure 2). Following a careful recapitulation of the earlier events of specification towards definitive endoderm [16], D’Amour and colleagues initiated the “slope of enlightenment” by showing that a small percentage of these cells can be converted into beta-like cells with an insulin content similar to that seen in native islets [17]. These studies were followed by others in which transplantation into immunocompromised mice was done halfway through the differentiation process. When the cells were allowed to mature *in vivo*, the efficiency of differentiation was significantly improved, and chemically induced diabetes prevented [18]. California-based ViaCyte (formerly Novocell) has reported preclinical success with Pro-Islet™, which is based on the above strategy in conjunction with a durable, retrievable encapsulation device. Efforts at translating this strategy into clinical therapies have been

highlighted by the recent award of more than \$20 million from the California Institute of Regenerative Medicine (CIRM). This makes the goal of having ES cell-based therapies in clinical trials within the next 5–10 years an attainable one. Inasmuch as the formation of teratomas is a common occurrence in pre-clinical experiments [18, 19], safety remains a concern. It is argued, however, that the use of immunoisolation devices will allow for the use of allogeneic material in fully immunocompetent recipients, because any tumorigenic escapees would be promptly destroyed by the host's immune response.

iPS cells have recently stormed in the field almost by surprise [20–27]. Although some claim that the hype associated with these non-controversial ES cell surrogates is now starting to wane [28, 29], there is little not to love about this perfect combination of the best features of adult and embryonic stem cells. Although they are virtually indistinguishable from ES cells, their procurement does not entail the destruction of human embryos, and the fact they could be generated from the prospective patient only adds to their appeal for the design of autologous therapies. Following the main steps of another successful beta cell differentiation protocol [30], Tateishi and colleagues [31] reported recently the generation of insulin-producing islet-like clusters. There is no doubt that ES and iPS cell-based protocols will be largely interchangeable in the near future. Original concerns about the use of integrative viruses for their generation have been addressed by means of the progressive introduction of transducible proteins [32, 33], episomal constructs [26], DNA minicircles [34], modified mRNAs [35] and even small molecules with reprogramming properties [36]. On the negative side, the risks inherent to the use ES cells are also common to iPS cells, and a recent report warned about the potential premature senescence of iPS cell-derived hemangioblasts [37].

Adipose and neural crest-derived stem cells

Mesenchymal stem cells (MSCs) have long been considered the workhorse of adult stem cell research. Easily procurable from virtually every tissue [38], they have been favored by their remarkable (if not unlimited) expandability in plastic surfaces and their readiness to differentiate into a variety of therapeutically valuable tissues, chiefly those of the connective family [39]. Despite the fact that clear-cut criteria should in theory unify MSCs of every possible origin [40, 41], to date there is no consensus on whether MSCs are one and the same. The study of the properties of MSCs from different tissues is additionally complicated by the observation that even discrete clones from the same tissue exhibit intrinsic variability in their differentiation potential [42]. Therefore, the quest for an elusive “best” source for beta cell differentiation may actually entail the screening of hundreds of potential candidates. Considering the current dispersion of differentiation methods for adult stem cells (which span numerous signal-driven approaches [43–52], genetic manipulation [53–58] and even *in vivo* maturation after systemic administration [48, 49, 59–61]), the fact that we still do not have a gold-standard of MSC-to-beta cell differentiation similar to what the ViaCyte protocol [17, 18] represents for hES cells is reason for concern. However, before making a decision to bet on any given MSC source and try to develop a universal protocol that could later be applied to others, we must also consider that the ViaCyte method was indeed optimized for one particular hES cell line, and does not seem to work as efficiently with other lines. As laboratories around the world juggle the centrifugal needs of defining a gold-standard protocol and identifying the ideal MSC source, we anticipate a period of tentativeness before a true breakthrough is reported. Until such time, banking patient-matched MSCs seems like a sensible idea. Those patients for whom the MSC-rich fraction of the umbilical cord blood was not preserved [62–65] may still have the option of harvesting their own adipose stem cells by liposuction later in life. In this context, adipose MSCs obtained from blepharoplasty procedures have recently shown great promise at reversing diabetes in preclinical models [66]. Success in these studies was attributed to the

use of a specific MSC lineage claimed to derive from the neural crest, which is home to highly multipotent cells [67]. This would also be consistent with the reported ability of another neural crest-derived MSC, the periodontal ligament, to differentiate into insulin-producing cells [68]. Whether or not MSCs hold the key to an unlimited supply of beta cells, at the very least they are known to have strong pro-angiogenic [69, 70] and immunomodulatory [71–74] properties, which makes them extremely attractive from a therapeutic perspective. Some investigators even go as far as maintaining that some MSCs will not be rejected in allogeneic and even xenogeneic settings [66]. It is indeed a common misconception that, since MSCs are negative for class II major histocompatibility complex molecules, they should not be rejected. This is not true, as class I mismatches are known to invariably result in rejection in immunocompetent hosts. However, the possibility that these cells are actively hiding from the immune system through other yet-to-be-elucidated mechanisms cannot be ruled out.

Direct reprogramming

The basic idea behind reprogramming (also termed transdifferentiation) is that even a terminally differentiated tissue might be converted into another under the appropriate conditions. Following Waddington's imagery [75, 76], if the determinants of normal differentiation were like boulders rolling downhill until they found their final accommodation, reprogramming would require a rearrangement of the cell's epigenetic landscape akin to pushing the boulders over the top of the mountain and down another valley. Such relocation is possible, and indeed has been described under specific circumstances between the pancreas and the liver [77–82], perhaps because of the shared ancestry of both organs [83–91]. However, it is clear by now that accomplishing this in a consistent fashion entails much more aggressive interventions (namely, the introduction of “master genes” from the desired tissue) than those used for standard differentiation. Earlier this decade, Ferber and colleagues pioneered this approach by delivering the Pdx1 gene (a vital regulator of pancreatic development [92] and beta cell homeostasis [93]) into recipient mice by means of adenoviral vehicles. Ectopic expression in the liver led to the activation of beta cell genes and dramatic reductions in blood glucose levels, which outlived the period during which the adenovirus was expected to remain in the system [94, 95]. Subsequent studies confirmed that an initial trigger was sufficient to unfold a self-sustainable beta cell differentiation program [96]. Following their lead, other groups reported similar results either with Pdx1 alone or together with other reprogramming genes [97–104]. However, with the possible exception of the transgenic frog setting described by Horb *et al* [105] (which, strictly speaking, was a “redirecting” of embryonic development rather than proper transdifferentiation), the majority of these approaches did not yield true, glucose-responsive beta cells. This changed recently with a report showing that the transfer of three factors (Pdx1, Ngn3 and MafA) led to the reprogramming of pancreatic acinar tissue towards beta cells [106]. Although similar combinations of genes had already been tested with only moderate success in liver [84, 99, 107–110], the developmental proximity between pancreatic acinar and endocrine cells may have made the difference. These results are very exciting from a therapeutic perspective, because the harnessing of direct reprogramming may offer a less cumbersome alternative to differentiation, especially if methods that do not rely on the use of viruses (such as protein transduction [111]) are developed. Although *in vivo* reprogramming strategies would be a stretch in a therapeutic context, the plentiful leftover tissue that is currently discarded after islet isolation procedures could be an excellent source of newly created beta cells. Alternatively, candidate tissues could be biopsied from the patient, reprogrammed *ex vivo* and then implanted back. While autoimmunity would still have to be addressed separately, allorejection would no longer be of concern. This is further discussed in the next section.

Conclusion

To the long-standing controversy between the use of adult or embryonic stem cells, a key decision to be made is whether to bank on autologous cells customized from the patient we intend to treat, or to focus on a universal donor cell line that could be taken “over the counter” in the context of a robust tolerance induction or immunoisolation method. This question is most timely. Millions are invested each year in strategies that will potentially involve the use of patient-derived autologous adult stem or iPS cells. But are these approaches scientifically justified for diabetes? Are they really worth the trouble? On the one hand, it could be argued that taking allorejection out of the picture would single out autoimmunity as the only remaining challenge, one that perhaps could be amenable to more targeted interventions. On the other, wouldn’t meeting such challenge be akin to stopping the onset of the disease on its tracks –in other words, *curing the disease*? If we follow this reasoning, years and millions of dollars spent on the development of autologous beta cell transplantation strategies may just lead us to the realization that we would *still* need to find a cure for diabetes. Despite the uniqueness of this condition when compared to other possible targets of stem cell therapies, the momentum of novel techniques such as nuclear reprogramming may have contributed to the perception that some approaches must be pursued just because they *can* be pursued. Gathering the necessary data to decide between allogeneic and autologous is a strategic decision that should be made now, to prevent precious resources from being spread too thin in too many directions. The prospect of personalizing regenerative medicine strategies for type 1 diabetes is appealing, but complicated and, at the end of the day, perhaps useless from the perspective of getting us closer to a cure. The alternative of a well-defined allogeneic stem cell substrate may enormously simplify the problem –not because the patient would not need immunosuppression, but because immunosuppression would be also needed for autologous transplantation anyway, or because advances in immunoisolation [112, 113] will render the entire autologous approach obsolete.

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Figure 1.
Gartner innovation curve representing the defining steps of the evolution of clinical islet transplantation.

Figure 2.
Gartner innovation curve representing the key milestones of embryonic stem cell differentiation along the insulin-producing cell lineage.