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Embryonic Stem Cells for Therapies – Challenges and Possibilities

Ronne Wee Yeh Yeo and Sai Kiang Lim
*Institute of Medical Biology, Agency for Science, Technology and Research (A*STAR); Yong Loo Lin School of Medicine, National University of Singapore*
Singapore

1. Introduction

The successful establishment of human embryonic stem cells (hESCs) in culture (Thomson et al., 1998) has raised unprecedented public interest and expectation of treating intractable diseases such as diabetes, spinal cord injuries, neurodegenerative and cardiovascular diseases. Much of this enthusiasm was predicated on the unlimited self-renewal capacity of hESCs and their remarkable plasticity in differentiating into every cell type in our body. These features presented the tantalizing possibility of an unlimited cell source in regenerative medicine to generate any tissues to replace injured or diseased tissues. However, translating the potential of hESC into therapies has been challenging. Although translation of hESC has been severely impeded by social and political constraints placed on hESC research through ethical and religious concerns over the destruction of viable blastocysts during hESC isolation, the main challenges have been safety and technical issues.

2. Challenges in ESC therapy

2.1 Overcoming tumor formation

The two defining characteristics of ESCs are: 1) their pluripotency, or the potential to differentiate into all cell types in the adult body; and 2) their unlimited self-renewal capacity, or the ability to remain in an undifferentiated state and divide indefinitely. For mESCs, pluripotency is often demonstrated by the production of mESC-derived animals through germline transmission by chimeras resulting from injection of the cells into blastocysts or through tetraploid complementation. In hESCs, proof of pluripotency has been limited to formation of teratomas or teratocarcinomas, which are tumors composed of randomly distributed tissues from the three primordial germ layers in immunologically incompetent mice (Lensch et al., 2007). Karyotypically normal, low passage hESCs form benign teratomas that do not contain undifferentiated tissues and are less invasive (Blum et al., 2009; Reubinoff et al., 2000; Thomson et al., 1998) while high passage hESCs which have become karyotypically abnormal give rise to highly invasive, malignant teratocarcinomas (Herszfeld et al., 2006; Plaia et al., 2006; Werbowetski-Ogilvie et al., 2009; Yang et al., 2008).
Pluripotency coupled with unlimited self-renewal not only define ESCs, they are also the main appeal of ESC as the cell source for regenerative medicine but at the same time, pose

significant challenges to the transplantation of differentiated ESCs to replace injured or diseased tissues. The propensity of ESC to differentiate into teratomas necessitates the need to eliminate any residual ESCs in the differentiated cell preparation. There have been many strategies to eliminate residual ESCs or enhance the purity of differentiated ESC preparations. The use of heterologous selectable gene markers such as antibiotic resistance gene or fluorescent protein markers (Klug et al., 1996; M. Li et al., 1998; Muller et al., 2000; Soria et al., 2000) is generally not a strategy of choice as this could introduce potentially deleterious gene mutations. Most of the strategies centered around the use of endogenous markers that are unique or highly expressed on ESCs and not on their differentiated progeny. For example, SSEA-4 and TRA-1-60 which are highly expressed on hESCs have shown to be highly efficient in physically removing contaminating ESCs by magnetic or fluorescence-activated cell sorters (MACS or FACS) (Fong et al., 2009b). Another strategy exploit the flotation density of cell on discontinuous density gradients such as Puresperm- or Percoll-based gradients (Fong et al., 2009a). Using a relatively novel strategy, Choo et al. has raised antibodies against undifferentiated hESCs (Choo et al., 2008) and identified an antibody that was cytotoxic against hESCs by oncosis. This antibody was an IgM that recognizes podocalyxin-like protein-1(PODXL). hESCs that were treated with mAB 84 did not form teratoma when transplanted into SCID mice even after 18-24 weeks. Therefore, there are viable technologies to remove or reduce residual hESCs in differentiated hESC preparation and mitigate the risk of teratoma formation in patients receiving hESC-based cell therapy.

2.2 Overcoming immunorejection

Like all tissue transplants, hESC-based cell therapy will have to circumvent host immune rejection to engraft in the recipients. One proposed strategy was to establish ESC repositories with lines expressing the combinations of HLA molecules that are compatible with HLA haplotypes present in the population (Nakajima et al., 2007; Taylor et al., 2005). Alternatively, the host's immune system could be manipulated to induce tolerance to foreign tissues by ablation of donor-reactive T cell in the thymus, generation of tolerogenic dendritic cells and induction of T_{reg} cells [reviewed in (Chidgey et al., 2008)]. However, with the development of induced pluripotent stem cell technology that makes the creation of "patient-specific" pluripotent cells containing the same genetic material as the recipient a highly viable and practical option, the issue of host rejection has become a non issue.

The quest to create "patient-specific" pluripotent cells began with therapeutic cloning or somatic cell nuclear transfer (SCNT) where the diploid nucleus of a somatic cell was injected into a haploid enucleated egg to be reprogrammed by soluble factors in the host cell. Upon stimulation, the re-programmed cell divides to form a blastocyst with an inner cell mass that has identical nuclear genetic composition as the nucleus donor. Although this approach has worked to generate ESCs from different animals such as mice, rabbits, cats, sheep, cattle, pigs, goats [reviewed in (Wilmut et al., 2002)] and even primates (Byrne et al., 2007), no hESC has been generated through this approach as it remains a highly inefficient process and the use of human oocytes is ethically controversial (French et al., 2008; J. Li et al., 2009b). ESCs generated through SCNT are in principle, heterogeneous in their genetic composition as they contain nuclear DNA of the nucleus donor and mitochondrial DNA of the egg donor (Evans et al., 1999). This raises the possibility that SCNT-derived ESCs could be rejected by the innate immune system of the host with which the ESCs share the same nuclear but not mitochondrial genetic material (Ishikawa et al., 2010).

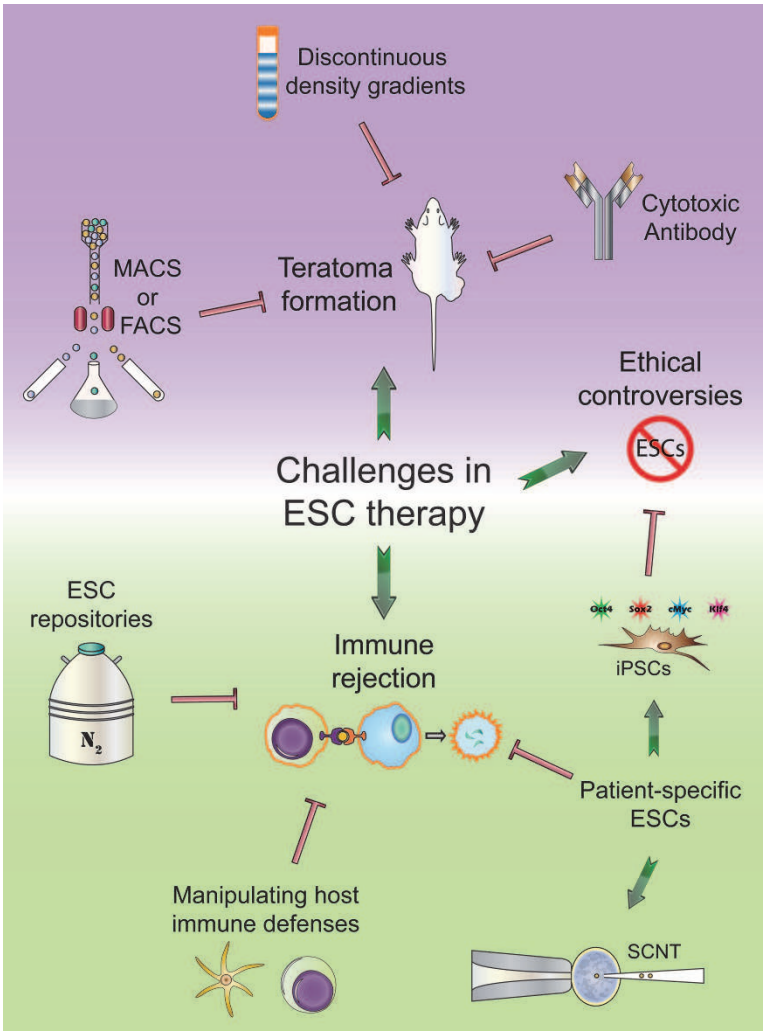

Fig. 1. Mitigating tumor formation and immune rejection. Two of the major challenges to the translation of ESCs into clinical applications are teratoma formation by residual undifferentiated ESCs in the cell preparation and immune rejection of ESC-derived cells or tissues due to incompatible HLA profiles of ESC and recipient. To mitigate the risk of teratoma formation, several methods to remove residual hESCs have been developed using either physical or biological methods. Some of the physical separation methods are based on magnetic- or fluorescence-activated cell sorters (MACS or FACS) that sort against cells with ESC-associated surface markers, SSEA-4 and TRA-1-60 or on cellular density using discontinuous gradients of Percoll or PureSperm. Alternatively, residual ESCs can be destroyed using a cytotoxic antibody (mAb 84) specific for undifferentiated hESCs. To prevent immune rejection, one strategy proposed the establishment of ESC repositories to carry lines expressing HLA combinations compatible with all possible haplotypes in the

population. Alternatively, donor cell tolerance can be induced by manipulating host immune defenses, such as eliminating donor-reactive T cells in the thymus, generating tolerogenic dendritic cells and inducing T_{reg} cells. An ideal approach would be to generate patient-specific ESCs. Some of early efforts include the use of somatic cell nuclear transfer (SCNT). More recently, induced pluripotent stem cell (iPSC) technology has enabled with great ease the generation of self pluripotent stem cells without the destruction of oocytes or embryos, hence bypassing ethical controversies.

The breakthrough in creating "patient-specific" pluripotent cells was achieved when Yamanaka demonstrated that the introduction of transcription factors which regulate ESC self-renewal, including Oct3/4 and Sox2 was sufficient to reprogram somatic cells into ES-like cells (Takahashi & Yamanaka, 2006). These induced pluripotent stem cells (iPSCs) are karyotypically normal with gene expression profiles highly similar to ESCs and can differentiate into cells of all three germ layers (Takahashi et al., 2007; Yu et al., 2007). Apart from being patient-specific, the major attraction of iPSCs lies in their derivation from somatic tissues and not from ethically contentious tissues such as human oocytes or embryos. However, retroviral and lentiviral vectors were required to express the transcription factors for reprogramming of the somatic cells and this carries a risk of insertional mutagenesis. To circumvent the need for viral vectors, non-viral genetic modification approaches were developed (Okita et al., 2008; Soldner et al., 2009; Woltjen et al., 2009). Recently iPSCs were obtained via a direct delivery of reprogramming factors into cells using poly-arginine protein transduction domains (Zhou et al., 2009) or mRNA (Plews et al., 2010), thereby circumventing any form of genetic manipulation. These improvements have essentially abrogated the issue of host/donor cell immune compatibility and considerably enhanced the prospects of generating patient-specific iPSCs for regenerative medicine. However, a recent study demonstrated that some hiPSC derivatives exhibit limited expansion capability, increased apoptosis and early cellular senescence as compared to their hESC-derived counterparts, raising doubts about the clinical value of this reprogramming technology (Feng et al., 2010). Also, it remains to be determined if the progeny of these cells, which are genetically identical to the reprogrammed cell, will trigger any immune response when reintegrated into the donor.

2.3 ESC differentiation

ESC owes its allure as the source of stem cells for regenerative medicine to two important potentials: 1) unlimited self-renewal potential and 2) the potential to differentiate into all the cell types in an adult. Unfortunately, the recent technological advances to circumvent the risks associated with transplantation of ESC-derived cells, namely teratoma formation and host immune rejection, were not matched by similar progress in differentiating hESCs into cells suitable for regenerative medicine. In contrast to adult stem cells where hundreds of clinical trials have been conducted to evaluate their clinical efficacy, the first testing of a hESC-based therapeutic candidate has only just been initiated. In Oct 2010, Geron Corp announced the enrollment of the first patient to test the safety of human embryonic stem cell (hESC)-derived oligodendrocyte progenitor cells, GRNOPC1, in treating spinal cord injury. With the progress made in reducing the risk of teratoma formation by residual ESC in differentiated ESC preparations and the generation of patient-specific iPSC, the major impediment to the development of hESC-based cell therapies remains the general lack of progress in developing protocols for efficient and reproducible differentiation of hESCs into

clinically relevant cell types in sufficient quantity and purity suitable for transplantation studies in clinically relevant large animal models.
The pluripotent differentiation potential of hESCs has always been predicated on their ability to form teratomas in immune-compromised animals and embryoid bodies consisting of tissues from the three germ layers. This ability suggest that differentiation of ESC into the various cell types in the adult animal was not contingent on the presence of an embryonic microenvironment. Instead, it relies on a rather minimal environment that did not support the pluripotent and self-renewing state of the ESCs and bore little resemblance to the dynamically evolving microenvironment of a developing embryo. Nevertheless, much effort to direct differentiation of hESCs into potentially therapeutic cell types have focused on the recapitulation of the embryonic microenvironment based on a yet to be tested rationale that the embryonic microenvironment represents the optimal micro-environment for directed *in vitro* differentiation of ESC.

2.3.1 Recapitulating embryonic development to induce lineage commitment

Embryogenesis is a highly dynamic complex process that is still being unraveled despite years of intensive research and much progress in elucidating the molecular and cellular processes involved in formation of an embryo. From a developmental perspective, the ESC represents cells that were frozen in the developmental state of a late-stage embryo just prior to differentiation and lineage commitment. The ability of ESC to re-enter the developmental process and differentiate when returned to the micro-environment of a blastocyst has provided compelling impetus to use the developing embryo to guide and direct *in vitro* differentiation of ESC to a specific cell type. Much effort has therefore been devoted to identifying the molecular cues that were involved in the differentiation of pluripotent cells in the blastocyst into specific terminally differentiated cells. The underlying rationale has always been that a temporal and spatial recapitulation of these cues *in vitro* will direct differentiation of ESC towards a specific cell type.
An early and critical phase of embryogenesis is gastrulation. During this process, the mono-layered blastula undergoes a series of transformation to form the tri-layered gastrula. The formation of these three germ layers (endoderm, mesoderm and ectoderm) marks the first stage of cell fate determination. This is followed by organogenesis when tissues and organs are formed from further differentiation of the germ layers. The endoderm gives rise to the epithelia of the gut and respiratory system, and organs such as liver and pancreas; the mesoderm gives rise to muscles, the circulatory system, bone and connective tissues; and the ectoderm gives rise to the nervous system and the epidermis. Similarly, the initial step towards deriving functional cells and tissues from ESCs may involve germ layer induction *in vitro*.
The first visible sign of gastrulation is the formation of the symmetry-breaking structure called the primitive streak (PS). Epiblast cells, which are derived from the inner cell mass, ingress through the PS to form the mesoderm and definitive endoderm. The remaining epiblast cells that do not ingress form the ectoderm. Many molecular factors have been implicated in this process and they include members of the large transforming growth factor β (TGFβ) and Wnt signaling families (Conlon et al., 1994; Hogan, 1996; Schier, 2003; Yamaguchi, 2001). Painstaking research has revealed some of the temporal and spatial effects of these factors during embryogenesis and many of these factors exerted similar effects on the differentiation of ESC cells. As reviewed by Murry and Keller (Murry &

Keller, 2008)], differentiation of ESCs into each of the three germ layers could be induced by the same factors known to induce them during gastrulation. For example, Wnt, Nodal or BMP4 which have been shown to be important in the formation of epiblast cells in the PS of a developing embryo (Kispert & Herrmann, 1994) could similarly induce the formation of PS-like cells from ESC (Kubo et al., 2004; Lindsley et al., 2006; Ng et al., 2005; Nostro et al., 2008). As in gastrulation, exposure of the PS-like cells to high levels of Nodal further differentiate these cells to a Foxa2^{hi} cells that are comparable to cells in the anterior PS that forms the definitive endoderm (D'Amour et al., 2005; Kubo et al., 2004). In contrast, exposure to Wnt, low level of activin (which activates Nodal) and BMP4 causes the PS-like cells to differentiate into a Flk-1^{+} posterior PS-equivalent population that forms the mesoderm (Nostro et al., 2008). Therefore, the three germ layers can be induced in ESCs by exposing the cells to factors known to be important in the formation of these three germ layers during embryogenesis. Further, by modulating these factors in a concentration and temporal manner that recapitulates early embryonic development, commitment of ESCs to one of the germ layers could be enhanced.

2.3.2 Enhancing lineage commitment

The intensive research efforts to induce a bias in differentiating pluripotent ESCs towards one of the germ layers would, in principle, enhance the subsequent production of specific tissue cell types of this germ layer e.g. muscles from mesoderm. However, enhancing commitment of differentiating ESC to one of the three germ layers may not be the limiting factor in generating clinically useful cell types in sufficient number and purity for therapeutic or screening applications. For example, the most efficient derivation of clinically useful cell types from ESC is neural cell types and not surprisingly, the first ESC-derived cell type to be clinically tested is oligodendrocytes. The relative efficiency of generating neurons, astrocytes and oligodendrocytes from ESC probably lies not in the ease of generating neural progenitor cells but in the relatively high expansion capacity of ESC-derived neural progenitor cells (Dottori & Pera, 2008; Studer, 2009). The high expansion capacity of neural progenitor cells would easily circumvent a limiting supply of rare neural progenitor cells formed during ESC differentiation and obviates the need to first bias differentiation of pluripotent ESCs towards an ectodermal germ lineage. Therefore, the rationale underlying the intensive research efforts to bias differentiating pluripotent ESCs towards one of the germ layers may be redundant at least for the derivation of neural cell types. Unlike ectodermal differentiation which is generally considered the default differentiation pathway for ESC, the derivation of mesodermal or endodermal cell types from ESC could still be enhanced by the recapitulation of early embryonic development processes to enhance mesodermal or endodermal commitment.

2.3.3 Terminal differentiation of ESC

In 2005, D'Amour et al reported the use of a multi-stage protocol that attempts to temporally recapitulate embryonic development for the differentiation of hESC into insulin-producing pancreatic cells for diabetes treatment. During this differentiation regime, they observed the formation of sequential transient cell populations with markers that mapped onto the developmental pathway of pancreatic endoderm. The final cell population representing pancreatic endoderm was transplanted in mice for further differentiation and maturation. When these transplanted animals were treated with streptozotocin, the induction of

hyperglycemia was attenuated. It was observed that some of the transplanted mice developed teratomas, suggesting the cell preparation was heterogenous and contaminated with ESCs that could differentiate into all cell types. In contrast, differentiation of mouse ESCs to insulin-producing cells is often a three-step protocol consisting of the formation of embryoid bodies, spontaneous differentiation into ecto-, endo- and mesoderm lineages and finally induction of pancreatic differentiation (Schroeder et al., 2006). However, the cell populations generated using this protocol have low insulin content. Using an approach that combines elements from this protocol and that used in neural differentiation, we first derived highly expansible E-RoSH cell lines with meso-endoderm potential from spontaneously differentiating EBs (Lian et al., 2006; Yin et al., 2004). Like neural stem cells, these E-RoSH cell lines are highly proliferative and provide unlimited supply of cells for differentiation. Serum starvation and nicotinamide supplementation induce differentiation of E-RoSH cells to form a heterogenous, insulin-producing culture. Limiting dilution of such cultures yielded independently derived clonal insulin-producing ERoSHK cell lines. These cells contain equimolar of insulin and C-peptide that was stably maintained over 30 passages at a high concentration of 300-500 pmol/10^6 cells. The insulin-producing ERoSHK cells resemble pancreatic cells and display the defining functional properties of bona fide pancreatic beta cells (G. Li et al., 2009a). They synthesize and store insulin in typical intracellular vesicles. Under stimulation by secretagogues such as glucose, tolbutamide and glibenclamide, these cells close their ATP-sensitive K+ channels, leading to membrane depolarization, opening of Ca2+ channels and the subsequent release of insulin and C-peptide in equimolar ratio, a mechanism resembling that of primary beta cells. Most importantly, these cells can reverse hyperglycemia when grafted into streptozotocin-treated mice. Relative to their progenitor E-RoSH cells, ERoSHK cells also exhibit enhanced activity in biochemical pathways that are also highly characteristic of beta cells such as the pentose phosphate pathway, clathrin-mediated endocytosis and PPAR signaling (T. S. Chen et al., 2010). Importantly, transplantation of ERoSHK cells in hyperglycemic streptozotocin-treated mice reverses the hyperglycemia and removal of the transplanted cells restores the hyperglycemia. The transplanted cells do not form teratomas. Together, these studies illustrated the diversity of approaches that have been taken to differentiate ESCs to insulin-producing cells and the relative potential of each approach in generating the desired end product on a scale to support potential therapeutic application. They also prompted doubts on the need to recapitulate the precise developmental pathway when differentiating ESC.

This question was previously raised by Burns et al (Burns et al., 2004). From their perspective, developmental events directing duodenal endoderm towards an insulin-expressing β-cell phenotype are the result of millions of years of evolutionary selection, driven by environmental pressures rather than by conscious design. Therefore, instead of mapping experimental protocols on to the known developmental pathways of pancreatic endocrine cells, they proposed that conscious design may be a less circuitous route to arrive at the same end-point. However, in lieu of known developmental pathways, there is no obvious source to guide and rationalize such a design. In essence, a conscious design would inevitably have to be an empirical approach of careful observation, trial and error, and high throughput screens.

2.3.4 Empirical differentiation of ESC

Despite a pervasive belief that a high fidelity recapitulation of developmental process represents the best strategy for efficient differentiation of pluripotent stem cells to

therapeutically useful cell types , the two human ESC-derived cell types ready for testing in man were derived by empirically formulated protocols. Fortuitously, some elements in these protocols were subsequently found to map onto similar pathways in embryonic development.

In the basic protocol for deriving Geron Corporation's GRNOPC1 which is already in Phase I clinical trial, one of the key elements in inducing neural commitment in ESCs to form neurospheres is retinoic acid (RA) (Nistor et al., 2005). RA was first observed to be an inducer of neural differentiation in embryonal carcinoma cells (ECs) (Jones-Villeneuve et al., 1982) before the first retinoic acid receptor (now known as RARa1) was cloned in 1987 (Giguere et al., 1987; Petkovich et al., 1987). Based on the empirical observation that RA induced neural differentiation in P19 tetracarcinoma cells, RA was used to enhance neural lineage commitment in ESCs (Bain et al., 1995). Today, RA is often used to enhance neural lineage commitment in ESC to generate neurospheres containing neural stem cells and for the subsequent terminal differentiation of neurospheres to produce neurons, oligodendrocytes and astrocytes. Therefore, the use of RA to induce neural differentiation in ESCs was rationalized on empirical observation of their effects on EC cells and this preceded the cloning of RA receptors and our understanding of its role in embryonic development.

To date, there is little evidence that RA plays a significant role in neural differentiation during gastrulation. The first RA signaling in the gastrulating vertebrate embryo occurs in the posterior mesodermal cells when RA is first synthesized by retinaldehyde dehydrogenase 2 (RALDH2) (Niederreither et al., 1997). There is however no RA signaling in the anterior regions of the embryo due to the presence of RA metabolizing enzymes such as CYP26A1 and CYP26C1 (Hernandez et al., 2007; Ribes et al., 2007; Uehara et al., 2007). In fact, RA receptors in the prospective head region of the Xenopus gastrula function as transcriptional repressors to prevent inappropriate activation of genes acting as posterior determinants. Also, the absence of endogenous RA synthesis in mice affect primarily forebrain development but did not compromise the early neural lineage commitment or differentiation (Natalia Molotkova et al., 2007; N. Molotkova et al., 2005; Niederreither et al., 2000; Sirbu et al., 2005). In fact, the pathway for neural differentiation during embryonic development could not have informed on the usefulness of insulin, triiodothyroidin, EGF and FGF in enhancing the *in vitro* proliferation and differentiation of ESC-derived oligodendrocyte precursors and increase oligodendrocyte survival.

The second ESC-derived cell type most likely to be tested in man is Advanced Cell Technology's retinal pigment epithelial (RPE) cells which has been given FDA clearance to initiate a Phase I/II multicenter clinical trial to treat patients with Dry AMD. The derivation of these RPE cells relies primarily on spontaneous differentiation of hESCs (Klimanskaya et al., 2004). RPE cells are formed as colonies of pigmented cells when hESCs undergo spontaneous differentiation by FGF2 withdrawal or embryoid body formation. These colonies of pigmented cells were then picked and expanded using very unremarkable culture medium.

2.3.5 Strategizing differentiation of ESC for therapeutic applications

The progress of ESC-derived oligodendrocytes and RPE cells to clinical testing attests to the robustness and efficiency of the empirically-driven differentiation protocols. In contrast, differentiation of ESCs by meticulous mapping on embryonic development pathway has not yielded cells that are ready for clinical testing. Despite this dichotomy in outcomes, there is

still a prevalent belief that high fidelity recapitulation of embryonic development process is the route to generate the most physiologically relevant cells. Embryonic development is a time tested success with defined milestones. In contrast, an empirically driven differentiation strategy is an inherently inefficient chance event.

The success of a differentiation strategy based on embryonic development is predicated not only on the elucidation but also the recapitulation of the highly dynamic temporal and spatial changes in the embryonic microenvironment that is influenced by both intra- and

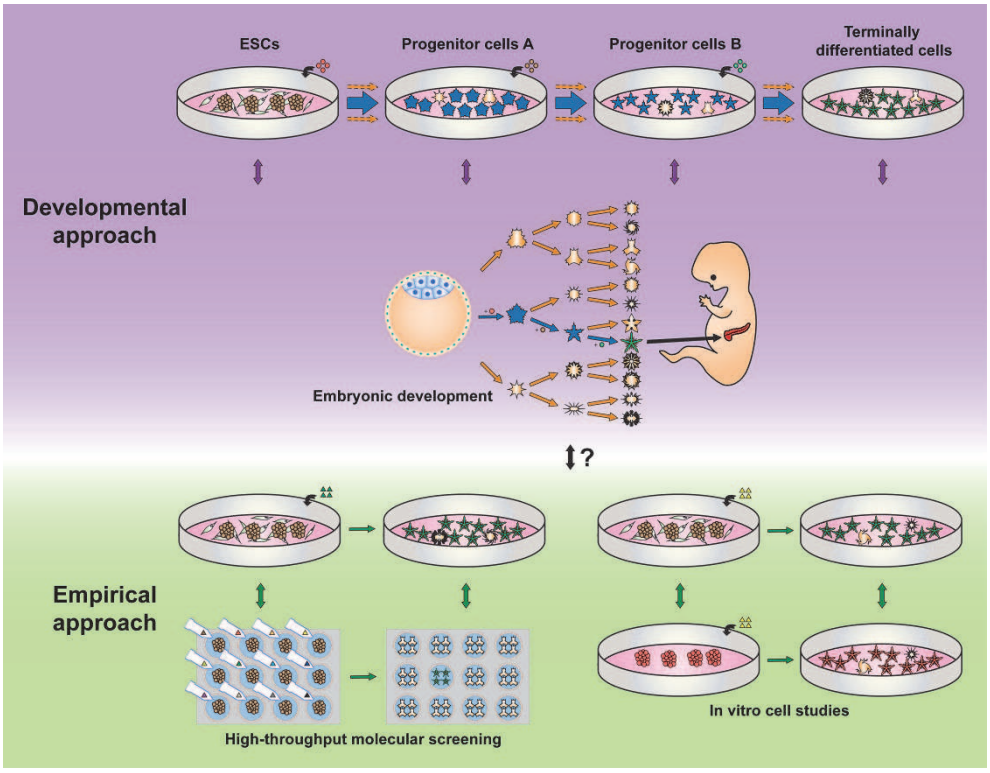

Fig. 2. Strategies for differentiation of ESCs into therapeutically useful cell types. The strategies currently being used could be broadly classified into a developmental or an empirical approach. The developmental approach (upper panel) to produce a desired cell type (green stars) relies on the recapitulation of the developmental pathway (blue arrows) during embryogenesis that produces that desired cell type. The general expectation is that identifying the cues that direct the developmental process during embryogenesis and recapitulating these cues spatially and temporally *in vitro* will be most optimal in yielding physiologically functional cell type (e.g. pancreatic insulin-producing cells). The empirical approach involves the differentiation of ESCs either spontaneously, or using novel factors identified empirically, such as through high-throughput molecular screening or in vitro cell studies (e.g. neural induction of EC cells by RA). These factors may or may not play a role in development.

extra-embryonic factors. Notwithstanding this, translating such a complex differentiation strategy to a scalable commercially viable manufacturing process will be an equally confounding unknown. On the other hand, developing a differentiation strategy using an empirical approach is a chance process of trial and error and fortuitous observation. This inherent inefficiency can be circumvented by high throughput screens to identify inducing molecules or combinations of molecules. There is also a likelihood that such a strategy would provide for a potentially scalable manufacturing process that will support clinical applications.

3. ESC therapeutics: cell versus biologic

A much overlooked form of ESC-derived therapeutics is biological products or biologics from ESC. To date, the predominant or only forms of ESC-derived therapeutics that are being evaluated are primarily cell-based. The capacity of ESC to undergo spontaneous differentiation in a minimal culture medium to form tissues from all three germ layers suggest that differentiating ESCs can produce an inductive and sustaining microenvironment for the various cell types that are being formed. It is conceivable that some of this microenvironment may also induce or sustain some tissue regeneration and repair in adult. However capturing this microenvironment and translating it to a scalable manufacturing process would be a challenge.

Biologic-based therapeutics have several advantages over cell-based therapies. Biologics eliminates the need to preserve viability during manufacture, storage and transport, and administration to the patient. This substantially reduces the cost and complexity of production and delivery. Maintaining cell viability before and after transplantation has always been an important consideration in cell-based therapy. Although preserving the activity of biologics is not a minor consideration, it is, nevertheless more tractable than preserving cell viability. Cell therapy is generally a permanent or long term therapeutic sustained by the replicative capacity of the transplanted cells with little recourse for termination of therapy except when removal of the graft is possible. In contrast to biologics, cell therapy presents increased risks of tumor formation and acute immunological rejections. All things considered, ESC-based biologics is an attractive alternative to develop ESC-based therapeutics.

As an illustration of a potential ESC-derived biologic, we have demonstrated that mesenchymal stem cells derived from hESCs (Lian et al., 2007) secrete factors (Sze et al., 2007) that are cardioprotective in pig and mouse models of myocardial ischemia/reperfusion injury (Timmers et al., 2008). The active component in this secretion was small lipid vesicles of 50-100nm known as exosomes (Lai et al., 2010). Immortalization of these mesenchymal stem cells did not compromise the production or activity of the exosomes (T.S. Chen et al., 2011). These studies provided for the development of a sustainable scalable manufacturing process to produce potentially therapeutic exosomes for testing in the clinic.

4. Conclusion

ESC is a versatile cell that has exerted significant impact on our understanding and investigation of cell biology, differentiation and development. It has provided exciting possibilities for the treatment of highly intractable diseases. As the first ESC-derived cell

type makes its way into clinical testing, there is an apprehensive hope that ESC will justify its hype not only as a therapeutic agent but one that will treat a multitude of intractable diseases as wide ranging as its differentiation potential. The establishment of iPSC technology by Shinya Yamanaka (Takahashi & Yamanaka, 2006) represents a paradigm shift in not only our understanding of stem cell biology but also in overcoming the ethical and immune challenges that had stymied the translation of ESC into clinical applications. His approach of re-programming terminally differentiated cells into pluripotent stem cells contradicted fundamental principles in developmental biology. This approach of exploring beyond the obvious and logical using empirical and experimental strategies may be necessary to transcend this current bottleneck in generating the quantity and quality of ESC-derived cells for therapy.

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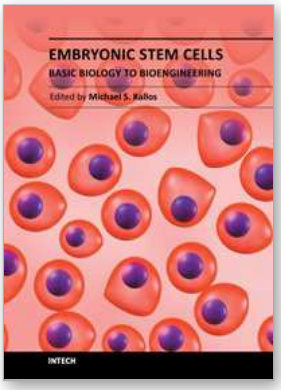

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Embryonic stem cells are one of the key building blocks of the emerging multidisciplinary field of regenerative medicine, and discoveries and new technology related to embryonic stem cells are being made at an ever increasing rate. This book provides a snapshot of some of the research occurring across a wide range of areas related to embryonic stem cells, including new methods, tools and technologies; new understandings about the molecular biology and pluripotency of these cells; as well as new uses for and sources of embryonic stem cells. The book will serve as a valuable resource for engineers, scientists, and clinicians as well as students in a wide range of disciplines.

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Phone: +86-21-62489820
Fax: +86-21-62489821