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Stem Cell Therapeutics: Exploring Newer Alternatives to Human Embryonic Stem Cells

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Abstract

Stem cells therapeutics has come a long way since stem cells and their potential was discovered for the first time. Intense research into cellular biology of stem cells has revealed that they possess immense potential for curing many human diseases. Research done in last couple of decades revealed that a particular class of stem cells called, “Human embryonic stem cells (HESCs)” possessed exceptional self-renewal and pluripotency properties. There ability to differentiate into specialized cell lineages of all three embryonic germ layers contributed further towards their popularity. However, in recent times HESCs have come under the cross-hairs of critics, politicians and religious groups due to certain technical and ethical concerns related to them. Such problems with HESCs-research have forced stem cell researchers to start exploring the prospects of using alternatives to HESCs for regenerative medicine and therapeutics. In the present review, various sources of stem cells have been described, which in near future, have the potential to replace HESCs in regenerative medicine.

Introduction

Stem cells have emerged as a revolution in the field of regenerative medicine. In last couple of decades, intense stem cell research has given us important insights into nature of these cells and their potential for organ formation as well as regeneration and repair after injuries (National-Academy-of-Sciences, 2002). Tissue repair systems in mammals are mostly based upon dedifferentiation-independent processes regulated and governed through pre-existing stem cells or progenitor cells, which is the reason why stem cells have been at the heart of regenerative medicine. Regenerative medicine deals with all the tissues in human body, which was the reason why stem cells having the capability of differentiating into any type of human tissue cell with considerable capacity were required. Human embryonic stem cells (HESCs) were found to live up to this requirement, since they exhibited the properties of indefinite self-renewal and pluripotency (National-Academy-of-Sciences, 2002). Over a period of time, it has been proved that HESCs can differentiate into specialized cell lineages of all three embryonic germ layers in relatively simplified cultures, thereby contributing further towards their popularity. However, even though HESCs hold tremendous promise there are certain major ‘technical' obstacles in the successful and safe clinical application of these cells (National-Academy-of-Sciences, 2002). Firstly, the cell differentiation factors responsible for tissue-specific differentiation of HESCs are not fully characterized. Secondly, there is a good possibility of HESCs derived cells facing immune rejection from the recipient's body. Moreover there is also a risk of these cells driving the production of teratoma or teratocarcinomas. Lastly, the use of HESCs has received severe ethical criticism since cultivation of HESCs involves destruction of an embryo, which is religiously considered to be a potential human-being (Reichhardt *et al.*, 2004). While scientific

research has been looking forward to solving various risks and limitations associated with HESCs on behest of its tremendous advantages; yet it has been tough for the researchers to confront the ethical debate over HESCs, as its driven by philosophical and religious ideologies associated with human civilization (Pera and Trounson, 2004). These debates and discussions regarding HESCs have finally led to formulation of stringent laws and crippling of government funds against HESCs-based research (Pera and Trounson, 2004). Such measures against HESCs-research have forced stem-cell researchers to start exploring the prospects of using alternatives to HESCs for regenerative medicine. Scientists have been looking forward to various different alternatives, which can convincingly replace HESCs in regenerative medicine. In the following sections, various types of cells and strategies, which can be used as alternatives to HESCs, have been discussed in details.

Pluripotent Aminotic Epithelium Cells (AECs)

Pluripotent amniotic epithelium cells are kind of stem cells derived from the amniotic membrane. Such stem cell-like cells, have found important application in tissue repair as these cells lack HLA-molecules on their surface, thereby making them non-immunogenic and ideal for regenerative purpose (Strom and Miki, 2003). In fact, amniotic membrane material has found good application in treatment of human corneal injuries (Shimmura and Tsubota, 2002). These amniotic membrane cells have also been reported to exhibit neural characteristics e.g. expression of nestin, BDNF and dopamine (Kakishita *et al.*, 2003). These pluripotent AECs have been regarded as one of the most promising alternatives for HESCs in regenerative medicine (Mimeault *et al.*, 2007).

Trophoblast-derived Stem Cells (TSC)

Trophoblast's portion that is in contact with inner-cell mass (ICM) of the blastocyst has been found to form extraembryonic ectoderm (ExE) and ectoplacental cone (EPC). Research has found existence of certain stem cells termed as Trophoblast-derived Stem Cell (TSCs) in the ExE (Tanaka *et al.*, 1998). It has been found that these TSCs are maintained under the signal from ICM and epiblast. Such TSCs have been derived mostly from mouse and only recently from Rhesus Monkey (Vandevoort *et al.*, 2007). Though these TSCs haven't been derived from humans, yet there exists a good chance of them being discovered in the near future. Potential of TSCs in regenerative medicine hasn't been demonstrated yet though there is a little bit of scare regarding their role since these are highly invasive and proliferative cells by nature (Hemberger *et al.*, 2004). We need to wait and watch for more research on TSCs to assess their potential for regenerative medicine.

Endometrial Regenerative Cells (ERC)

Endometrial Regenerative Stem Cells (ERCs) are small population of stem-cell-like cells in the menstrual blood, which have been hypothesized to play role in angiogenesis phase of the menstrual cycle in the endometrium (Bulletin-Board, 2008). Research on the differentiation potential of these cells has shown that they are capable of differentiating into endodermal (pancreatic, hepatic, respiratory epithelium), mesodermal (osteocyte, endothelium, adipocyte, myocyte, cardiomyocyte) as well as ectodermal (neuronal) lineages (Meng *et al.*, 2007). Further research has found that ERCs could be promisingly propagated beyond 68 doublings while still maintaining their normal karyotype. ERCs have been demonstrated to have a proliferation rate far better than mesenchymal or umbilical cord stem cells (Bulletin-Board, 2008) and ability to differentiate into cells representing all 3 germ-layers thereby making them potential alternative for HESCs (Meng *et al.*, 2007). ERC's biggest advantage over HESCs is the ease with which these cells may be obtained for creation of patient-specific banking. Potential problems with ERCs however are that, they haven't been confirmed to be complete stem-cells as their telomerase activity and certain other surface markers haven't been assessed (Meng *et al.*, 2007). Moreover, based upon available data the possibility of ERCs giving rise to teratomas may not be ruled out. Thus, since ERCs are relatively newly discovered cells, we need to wait and watch for more research to confirm whether these cells could be effectively used in regenerative medicine.

Placental-derived Stem Cells (PDSC)

Placenta has been reported to contain an important population of multipotent stem cells called Placental-derived Stem Cells (PDSCs), exhibiting characteristics of HESCs including expression of markers like OCT-4, SOX-2, SSEA1 as well as c-Kit (Matikainen and Laine, 2005). These cells have been shown to resemble mesenchymal stem cells and differentiate into various lineages like hepatocyte, vascular-endothelial, pancreatic and neuronal (Strom and Miki, 2003). PDSCs have also been isolated from amniotic membrane. PDSCs seem to be promising for regenerative medicine as they are easy to obtain as well as store yet PDSCs haven't been yet tested in published clinical studies. Moreover, their actual number in a single placenta hasn't been confirmed. To make matters worse, PDSCs have been found to possess unusual property of invasiveness (naturally required during embryo-implantation in placenta), which could increase the threat of teratomas during clinical therapeutic usage. Thus, there is need for extensive research so as to harness the potential of these stem cells.

Umbilical Cord Blood Stem Cells (UCB-SC)

Umbilical Cord Blood (UCB) has been recognized as a prime source of haematopoietic stem cells for a long time (Matikainen and Laine, 2005). Biggest advantage of UCB is that, it can be easily collected from the umbilical vein of the placenta which would be otherwise discarded after the birth. UCB has been routinely used in treatment of haematopoietic malignancies as an alternative for bone-marrow transplantation (Grewal *et al.*, 2003). Public cord blood banks have also been set-up in many countries to collect & store UCB after full-term pregnancies for future patient care. UCB-SCs have been found to be almost non-immunogenic thereby making them an important asset in regenerative medicine (Matikainen and Laine, 2005). UCB-SCs have been shown to differentiate into cells representing all 3 germ layers e.g. osteoblast-, neural-, chondrocyte-, adipocyte- and hepatocyte-like cells (Lee *et al.*, 2004). In certain diseased mouse-models, UCB-SCs have been shown to assist in recovery from myocardial and hind-limb ischemia (Botta *et al.*, 2004) as well as in recovery of motor function (Chen *et al.*, 2001). The above discussion shows that, UCB-SCs may be regarded as potential alternatives to the HESCs in regenerative medicine. Only drawback however is that when compared to adult stem cells, UCB-SCs have been involved in a very small number of clinical regeneration investigations. However, in coming years it's expected that studies on UCB-SCs would place it on fore-front of regenerative-medicine research.

Amniotic Fluid Stem Cells (AFSCs)

Amniotic Fluid Stem Cells (AFSC), as the name suggests are novel cells derived from the amniotic fluid. Research has revealed that AFSCs could differentiate into at least 6 cellular lineages representing all three germ layers (De Coppi *et al.*, 2007). AFSCs have been shown to differentiate along, Neural, Hepatic, Osteogenic, Myogenic, Adipogenic as well as endothelial lineage. AFSCc were also found to grow stably in culture such that they exhibited property of self-renewal but without presence of senescence. AFSCs were found to maintain long telomeres beyond 250 population doublings (p.d.), which exceeds the typical “Hayflick limit” of about 50p.d. (De Coppi *et al.*, 2007). This is quiet a significant property when compared to ESCs or Adult Stem Cells (ASCs). Potential of AFSCs in regenerative medicine has also been tested. AFSCs differentiated into neural-lineages and osteogenic-lineages were transplanted in the brain of a mouse suffering from Twitcher disease and intra-peritoneal cavity of another mouse respectively. Later it was shown that the respective differentiated AFSCs started healing the brain as well as started forming bones (De Coppi *et al.*, 2007). Further it has been elucidated that, AFSCs could be cultured without feeder layers, they have a short doubling time (36hrs), they don't form tumours and moreover they are obtained from a source (amnion or amniotic fluid) which otherwise would be discarded (De Coppi *et al.*, 2007). Thus they represent an ‘ethical' and renewable source of stem cells that could be potentially used as an alternative for HESCs in regenerative medicine (Domestic-Policy-Council, 2007, Apr 2). The only thing here is that, we need to wait for more research papers and find out whether the results obtained above are reproducible elsewhere or not.

Adult Stem Cells (ASCs)

Adult Stem Cells (ASCs) are tissue-resident cells found in all mammalian organisms. ASCs carry out a critical function of maintaining homeostasis in many human-tissues by assisting in wear and tear of the

body and constantly acting as source of newer mature cells, which take place of old cells. ASCs have been at the top of the list as alternatives for HESCs in the field of regenerative medicine (Mimeault *et al.*, 2007). Most well characterized ASCs are Bone-marrow derived stem cells (BMSCs), hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs), Mesenchymal Stem Cells (MSCs), Cardiac Stem Cells (CSCs) and Neural Stem Cells (NSCs) (Mimeault *et al.*, 2007). HSCs are the most classic of all the ASCs since they have been used in clinical medicine since 1970s (Matikainen and Laine, 2005). MSCs are other highly promising ASCs, which have ability to differentiate into neuron-like cells, multiple mesodermal-tissue types like muscle, marrow stroma, tendon, cartilage, bone, fat, ligament and a number of other connective tissues (Bongso and Richards, 2004). MSCs are currently regarded as the most preferred choice in patient-specific regenerative medicine since they are pluripotent, easy to culture and have a favourable doubling time (Bongso and Richards, 2004, Jiang et al., 2002). Various differences between ASCs and HESCs have been summarized in Table 1.

Table 1: Differences between Human Adult Stem Cells and Human Embryonic Stem Cells (Bongso and Richards, 2004)

Human Adult Stem Cells	Human Embryonic Stem Cells
Stem Cells are hard to access & purify	Once isolated, the cells show high degree of proliferation
Mostly multipotent with MSCs acting as pluripotent	Pluripotent
Telomerase levels low	Telomerase levels high
Chromosomes tend to shorten with ageing	Chromosome length is maintained across serial passage
Apoptosis may be early	Apoptosis is late
No Teratoma risk	Significant Teratoma risk
No ethical issues	Serious ethical issues
Patient-specific hence less chances of immune rejection	High chance of immune rejection

ASCs are definitely less controversial than HESCs. Moreover, there have been 1,373 publicly available human clinical trials related to ASCs (As of April 2, 2007) while there have been no such trials for HESCs (Domestic-Policy-Council, 2007, Apr 2), which further adds upto the importance of ASCs. However only disadvantage of ASCs is that they are scarce and hence hard to isolate and culture. This actually increases the overall cost and time for the treatment. In next couple of decades, it's expected that the regenerative-therapy based on ASCs would improve several folds.

Induced Pluripotent Stem Cells (iPS)

Induced Pluripotent Stem Cells (iPS) are by far the most exciting alternatives proposed for HESCs in the field of regenerative medicine (Cibelli, 2007). The initiative for the production of these cells was taken solely with the purpose of circumventing the ethical issues associated with the HESCs (Takahashi *et al.*, 2007). These iPSs are results of *in vitro* dedifferentiation wherein certain transcription factors like OCT3/4, SOX2, KLF4 etc. are used to reprogram the chromatin of a fully differentiated cell so as to induce it or dedifferentiate it back to the embryonic stem cell (ESC) form. Such cells which are induced to reprogram from differentiated form to ESC-form are termed as iPS (Cibelli, 2007). Researchers have managed to produce iPSs from differentiated human fibroblasts by transfecting them (viral-mediated) with relevant trans-acting factors. Two groups have recently made use of Oct3/4, Sox2, c-Myc, Klf4 (Takahashi *et al.*, 2007) as well as Oct4, Sox2, Nanog, Lin28 (Yu *et al.*, 2007) respectively to reprogram human somatic cells into iPS. These reprogramming breakthroughs are exciting since they promise an ethical source of HESCs which in future may further develop to give patient-specific iPS (Cibelli, 2007). However, this initiative of replacing HESCs with iPSs has still a long way to go. There are many problems such as teratoma formation, immune-rejection etc. which still need to be addressed (Cibelli, 2007). Further, the researchers need to prove the complete ‘stemness' of these iPS cells. However, in light of current standing iPS do offer new vistas for advancement of regenerative medicine.

Conclusion

Human Embryonic Stem Cells have enormous potential yet the research relating to them has been crippled largely due to the ethical debate against them. These ethical concerns have been the reason behind recent initiative to find alternatives for HESCs in regenerative medicine. Of all the alternatives proposed for HESCs most impressive ones have been, induced Pluripotent Stem-Cells, Amniotic Fluid Stem-Cells, Umbilical Cord Blood Stem-Cells and Adult Stem Cells. For a particular alternative to replace HESCs in regenerative medicine, it ought to have almost all properties of HESCs along with other properties like non-immunogenic and non-teratomic nature. These cells have tremendous potential as well as power and they need to be handled carefully. Present trends have proved to be encouraging and it's expected that in coming years there would be more such breakthroughs, which would allow researchers to replace HESCs with other alternative stem cells. Such breakthroughs may revolutionarize the field of regenerative medicine and provide relief to millions of patients who are currently in need of stem cell based treatments.

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