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Mini Review

Current State of Regenerative Medicine: Moving Stem Cell Research from Animals into Humans for Clinical Trials

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Abstract

Given the limited capacity of the central nervous system (CNS) and the heart for self-repair or renewal, cell-based therapy represents a promising therapeutic approach closest to provide a cure to restore normal tissue and function for neurological and cardiovascular disorders. Derivation of human embryonic stem cell (hESCs) from the *in vitro* fertilization (IVF) leftover embryos has brought a new era of cellular medicine for the damaged CNS and heart. Recent advances and technology breakthroughs in hESC research have overcome some major obstacles in moving stem cell research from animals towards humans trials, including resolving minimal essential human requirements for *de novo* derivation and long-term maintenance of clinically-suitable stable hESC lines and direct conversion of such pluripotent hESCs into a large supply of clinical-grade functional human neuronal or cardiomyocyte cell therapy products. Such breakthrough stem cell technologies have demonstrated the direct pharmacologic utility and capacity of hESC cell therapy derivatives for human CNS and myocardium regeneration, thus, presented the hESC cell therapy derivatives as a powerful pharmacologic agent of cellular entity for CNS and heart repair. The availability of human stem/progenitor/precursor cells in high purity and large commercial scales with adequate cellular neurogenic or cardiogenic capacity will greatly facilitate developing safe and effective cell-based regeneration and replacement therapies against a wide range of CNS and heart disorders. Transforming non-functional pluripotent hESCs into fate-restricted functional human cell therapy derivatives or products dramatically increases the clinical efficacy of graft-dependent repair and safety of hESC-derived cellular products, marking a turning point in cell-based regenerative medicine from current studies in animals towards human trials.

In the Western World, cardiovascular disease (CVD) and neurological disorders are major health problems and leading causes of death. The estimated annual costs of CVD for the overall United State (US) population are approximately $190 billion. The estimated annual costs of neurological disorders, including Parkinson's disease, Amyotrophic lateral sclerosis (ALS), Spinal muscular atrophy, Alzheimer disease, motor neuron disease, neurodegenerative diseases, stroke, brain and spinal cord injuries, are approximately $1.5 trillion in the US and Europe combined. Currently, there is no treatment option or compound drug of molecular entity that can change the prognosis of those diseases or that will lead to a dramatic functional improvement. Given the limited capacity of the central nervous system (CNS) and the heart for self-repair or renewal, cell-based therapy represents a promising therapeutic approach closest to provide a cure to restore normal tissue and function for neurological and cardiovascular disorders. However, traditional sources of cells for therapy in existing markets have been adult stem cells isolated from tissues or artificially reprogrammed from adult cells, which all have the historical shortcomings of limited capacity for renewal and repair, accelerated aging, and immune-rejection following transplantation. In addition, artificially reprogrammed adult cells have the major drawbacks of extremely low efficiencies and genetic defects associated with high risks of cancers, which have severely limited their utility as viable therapeutic approaches. To date, the existing markets lack a clinically-suitable human neuronal cell source with adequate CNS regenerative potential, which has been the major setback in developing safe and effective cell-based therapies for regenerating the damaged or lost CNS structure and circuitry in a wide range of neurological

disorders. Similarly, the existing markets lack a clinically-suitable human cardiomyocyte (the mature contracting heart muscle cell) source with adequate myocardium (the contractile heart muscle) regenerative potential, which has been the major setback in developing safe and effective cell-based therapies for regenerating the damaged human heart in cardiovascular disease.

Derivation of human embryonic stem cell (hESCs) from the *in vitro* fertilization (IVF) leftover embryos has brought a new era of cellular medicine for the damaged CNS and heart [1,2]. The intrinsic ability of a hESC for both unlimited self-renewal and differentiation into clinically-relevant lineages makes it a practically inexhaustible source of replacement cells for human tissue and function restoration. Therefore, it has been regarded as an ideal source to provide a large supply of functional human cells to heal the damaged or lost tissues that have naturally limited capacity for renewal, such as the human heart and brain. Although a vast sum of government and private funding has been spent on looking for adult alternates, such as reprogramming and trans-differentiation of fibroblasts or mature tissues, so far, only human stem/precursor/progenitor cells derived from embryo-originated pluripotent hESCs have shown such cellular pharmacologic utility and capacity adequate for CNS and myocardium regeneration in pharmaceutical development of stem cell therapy for the damaged CNS and heart.

Maintaining undifferentiated hESCs in a defined biologics-free culture system that allows faithful expansion and controllable direct differentiation is one of the keys to their therapeutic utility and potential, which requires a better understanding of the minimal essential components necessary for sustaining the pluripotent state and well-being of undifferentiated hESCs [1-4]. The hESC lines initially were derived and maintained in co-culture with growth-arrested mouse embryonic fibroblasts (MEFs). The need for foreign biologics for derivation, maintenance, and differentiation of hESCs makes direct use of such cells and their derivatives in patients problematic. Without an understanding of the essential developmental components for sustaining hESC pluripotence and self-renewal, hESC lines are at risk for becoming unhealthy and unstable after prolonged culturing under animal feeders, feeder-conditioned media, or artificially-formulated chemically-defined conditions [1,3,5]. To avoid those shortcomings, we have resolved the elements of a defined culture system necessary and sufficient for sustaining the epiblast pluripotence of hESCs, including bFGF, insulin, ascorbic acid, laminin, and activin-A, serving as a platform for *de novo* derivation and long-term maintenance of animal-free therapeutically-suitable hESCs and well-controlled efficient specification of such pluripotent cells exclusively and uniformly towards a particular lineage by small molecule induction [1,3-5]. Establishing defined platform for *de novo* derivation and long-term stable maintenance of clinical-grade pluripotent hESCs has overcome some of the major obstacles in moving stem cell research from animals into human trials. Good manufacturing practice (GMP) quality, defined by both the European Medicine Agency (EMA) and the Food and Drug Administration (FDA), is a requirement for clinical-grade cells, offering optimal defined quality and safety in cell transplantation. Resolving minimal essential requirements for the maintenance of pluripotent hESCs allows all poorly-characterized and unspecified biological additives, components, and substrates in the culture system, including those derived from animals, to be removed, substituted, or optimized with defined human alternatives for *de novo* derivation, long-term maintenance, and optimal production GMP-quality xeno-free hESC lines and their therapeutic derivatives [1,3-5].

Pluripotent hESCs themselves are unspecialized non-functional cells that cannot be used directly for therapeutic applications in humans. It has been recognized that pluripotent hESCs must be turned into fate-restricted specialized stem/progenitor/precursor cells or functional mature cells, a process known as differentiation, before use for cell therapy in patients. However, how to channel the wide differentiation potential of pluripotent hESCs exclusively and predictably to a desired phenotype has been a major challenge to clinical translation. Conventional hESC differentiation methods require uncontrollable simultaneous multi-lineage differentiation of pluripotent cells, which yield embryoid bodies (EB) or aggregates consisting of a mixed population of cell types of three embryonic germ layers, among which only a very small fraction of cells display targeted differentiation [1-4]. Those conventional hESC differentiation methods require laborious, costly, and time-consuming purification or isolation procedures to generate only a small quantity of desired cells, impractical for commercial and clinical applications. Growing scientific evidences indicate that those conventional methods result in inefficient, instable, and incomplete hESC differentiation, and poor performance and high tumor risk of such cell derivatives and tissue-engineering constructs following transplantation [1-4]. Under conventional protocols presently employed in the field, hESC-derived cellular products consist of a heterogeneous population of mixed cell types, including fully differentiated cells, high levels of various degrees of partially differentiated or uncommitted cells, and low levels of undifferentiated hESCs, posing a constant safety concern when administered to humans.

Clinical applications of hESC cell therapy derivatives provide the right alternative for many major health problems that have not been resolved by any conventional compound drugs of molecular entity. Recent advances and technology breakthroughs in hESC research have overcome some major obstacles in bringing hESC therapy derivatives towards clinical applications [1,3-16]. Such breakthrough stem cell technologies have demonstrated the direct pharmacologic utility and capacity of hESC cell therapy derivatives for human CNS and myocardium regeneration, thus, presented the hESC cell therapy derivatives as a powerful pharmacologic agent of cellular entity for CNS and heart diseases [1,3-16].

One of the major milestones towards human trials of hESC therapy derivatives is the discovery that formulation of minimal essential defined conditions renders pluripotent hESCs be directly and uniformly converted into a specific neural or cardiac lineage by small signal molecule induction [1,3,4]. We found that pluripotent hESCs maintained under the defined culture conditions can be uniformly converted into human neuronal progenitors and neurons by small molecule induction [1,3,4,12-16]. Retinoic acid (RA) was identified as sufficient to induce the specification of neuroectoderm direct from the pluripotent state

of hESCs maintained under the defined culture, without going through a multi-lineage embryoid body (EB) stage, and trigger a cascade of neuronal lineage-specific progression to human neuronal progenitors (Xcel-hNuP) and neurons (Xcel-hNu) of the developing CNS in high efficiency, purity, and neuronal lineage specificity by promoting nuclear translocation of the neuronal specific transcription factor Nurr-1 [1,3,4,12-16]. Neuroectoderm specification transforms pluripotent hESCs uniformly into a more neuronal lineage-specific nuclear Nurr1-positive embryonic neuronal progenitor than the prototypical neuroepithelial-like nestin-positive human neural stem cells (hNSCs) derived either from CNS or hESCs [3,15]. Genome-scale profiling of microRNA differential expression showed that the expression of pluripotence-associated hsa-miR-302 family was silenced and the expression of Hox miRNA hsa-miR-10 family that regulates gene expression predominantly in neuroectoderm was induced to high levels in those hESC-derived neuronal progenitors [4,13,14]. Following transplantation, those hESC neuronal derivatives engrafted widely and yielded well-dispersed and well-integrated human neurons at a high prevalence within neurogenic regions of the brain [3,14,15]. This technology breakthrough enables well-controlled generation of a large supply of neuronal lineage-specific progenies across the spectrum of developmental stages direct from the pluripotent state of hESCs with small molecule induction, providing an adequate neurogenic human cell source for developing safe and effective stem cell therapy for CNS repair [3,4].

To date, the lack of a suitable human cardiomyocyte source with adequate myocardium regenerative potential has been the major setback in regenerating the damaged human heart, either by endogenous cells or by cell-based transplantation or cardiac tissue engineering [1,3-9]. There is no evidence that adult stem/precursor/progenitor cells derived from mature tissues, such as bone marrow, cord blood, umbilical cord, mesenchymal stem cells, patients' heart tissue, placenta, or fat tissue, are able to give rise to the contractile heart muscle cells following transplantation into the heart [1,3-9]. Despite numerous reports about cell populations expressing stem/precursor/progenitor cell markers identified in the adult hearts, the minuscule quantities and growing evidences indicating that they are not genuine heart cells and that they give rise predominantly to non-functional smooth muscle cells rather than functional contractile cardiomyocytes have caused skepticism if they can potentially be harnessed for cardiac repair [1,3-9]. Due to the prevalence of heart disease worldwide and acute shortage of donor organs or adequate human myocardial grafts, there is intense interest in developing hESC-based therapy for heart disease and failure. We found that pluripotent hESCs maintained under the defined culture conditions can be uniformly converted into a specific cardiac lineage by small molecule induction [1,3-11,13]. Nicotinamide (NAM) was identified sufficient to induce the specification of cardiomesoderm direct from the pluripotent state of hESCs maintained under the defined culture, without going through a multi-lineage EB stage, by promoting the expression of the earliest cardiac-specific transcription factor Csx/Nkx2.5 and triggering progression to cardiac precursors and beating cardiomyocytes with high efficiency [1,3-5]. Cells within the beating cardiospheres expressed markers characteristic of cardiomyocytes, including Nkx2.5, GATA-4, α-actinin, cardiac troponin I (cTnI), and cardiac troponin T (cTnT) [1,3-5]. Electrical profiles of the cardiomyocytes confirmed their contractions to be strong rhythmic impulses reminiscent of the p-QRS-T-complexes seen from body surface electrodes in clinical electrocardiograms [3,5]. Our breakthroughs have overcome some major obstacles in bringing hESC therapy to clinics, enabling *de novo* derivation of clinical-grade cGMP compatible stable hESC lines from human blastocysts that have never been contaminated by animal cells and proteins, and direct conversion of such pluripotent hESCs into a large supply of clinical-grade functional human heart precursors and cardiomyocytes to be translated to humans trials for cardiac repair [1,3,4].

Our novel approach of hESC lineage-specific differentiation direct from the pluripotent stage using small molecule induction is a major milestone towards clinical application of hESC cell therapy derivatives, offering the benefits in efficiency, purity, stability, safety, and scale-up production of clinical-grade hESC cell therapy products in cGMP facility over all other existing conventional approaches. Such milestone advances and medical innovations in hESC research enable high efficient generation of a large supply of high purity clinical-grade hESC neuronal and heart muscle cell therapy products in commercial scales as powerful cellular medicines to offer adequate pharmacologic utility and capacity for CNS and heart regeneration in the clinical setting that no conventional drug of molecular entity can.

Development and utilization of multi-cellular 3D human embryonic models using hESCs will provide an authentic and reliable *in vitro* tool targeted for rapid and high fidelity safety and efficacy evaluation of human therapeutic candidates and products, and thus reduce the reliance on animal models to test potential therapeutic strategies and lead to advances in technologies used in the regulatory review [1,3]. It will dramatically increase the overall turnover of investments in biomedical sciences and facilitate rapid progress in identification of therapeutic targets and approaches for the prevention and treatment of human diseases. On July 9, 2012, the Food and Drug Administration Safety and Innovation Act (FDASIA) was signed into law, which gave FDA a new and powerful expedited drug development tool, known as the "breakthrough therapy" designation. This new designation helps FDA assist drug developers to expedite the development and review of new drugs with preliminary clinical evidence that indicates the drug may offer a substantial improvement over available therapies for patients with serious or life-threatening diseases. In addition, the FDA has established a Fast Track program that is intended to facilitate the development and expedite the review of new drugs and biological products that are intended to treat a serious or life-threatening condition or disease and demonstrate the potential to address unmet medical needs for the condition. Many of those serious or life-threatening diseases, such as heart disease, stroke, Parkinson's disease, ALS, Spinal muscular atrophy, Alzheimer disease, have relied on stem cell research to drive the advance of medicine to provide future regeneration and reconstruction treatment options for the damage or lost functional tissues and organs. Because of interspecies differences, conventional preclinical studies using animal models are often poor predictors of human efficacy and safety. Animal models are xeno-hosts for transplantation

of human cells, not ideal for testing the safety and efficacy of therapeutic outcomes of human stem cells. Large primate models are very costly and often taken years to obtain results. In addition, the results of animal studies can be highly variable and difficult to reproduce, making them unreliable as benchmarks for decisions on human trials. Preclinical data using animal models, even results of large animal models, do not necessarily provide the benchmarks or indictors for safety and efficacy in human trials. Those FDA expedited programs do not specifically require evidences from animal models or animal proof-of-concept data to support FDA accelerated approval and priority review, which may help fast-track the development and review of human stem cell therapy products that have the potential to provide safe and effective therapy where no satisfactory alternative therapy exists or a significant improvement in the treatment, diagnosis or prevention of a disease compared to the marketed human stem cell therapy product.

The ability of a human stem cell, by definition, to both self-renew and differentiate makes it a practically inexhaustible source of replacement cells for many devastating or fatal diseases that, so far, have been considered as incurable, such as neurodegenerative diseases and heart diseases. The driving force behind stem cell research is the therapeutic utility of stem cells intrinsic only to human cells, not just the science. Only human stem cells have the potential to become the next blockbuster drugs for many incurable diseases. For successful pharmaceutical development of stem cell therapy, the human stem cell therapy product must meet certain commercial criteria in plasticity, specificity, and stability before entry into clinical trials. Moving stem cell research from current studies in animals into human trials must address such practical issues for commercial and therapeutic uses: 1) such human stem cells or their progenies or derivatives must be able to be manufactured in a commercial scale; 2) such human stem cells and their progenies or derivatives must be able to retain their normality or stability for a long term; and 3) such human stem cells must be able to differentiate or generate a sufficient number of the specific cell type or types in need of repair. Those practical issues are essential for designating any human stem cells as human stem cell therapy products for investigational new drug (IND)-filing and entry into human trials. Compared to conventional compound drugs of molecular entity, cell therapy products have very different benchmarks or indicators regarding to safety and efficacy in clinical trials. It is hard to imagine that cell therapy products or human cells would have any serious toxicity or lethal side effect of chemicals or compound drugs. Rather, the safety of a human stem cell therapy product is evaluated by whether it can retain a stable phenotype and karyotype for a long period of time and whether there is no tumor or inappropriate cell type formation following transplantation. The efficacy of a human stem cell therapy product is measured by its pharmacologic activity or cellular ability to regenerate the tissue or organ that has been damaged or lost. Therefore, the pharmacologic utility of human stem cells cannot be satisfied only by their chaperone activity, if any, to produce trophic or protective molecules to rescue existing endogenous host cells that can simply be achieved by a drug of molecular entity. Recent advances and technology breakthroughs in hESC research have overcome some major obstacles in bringing hESC therapy derivatives towards human trials [1, 3-16]. We have established novel human stem cell technology platforms, including defined culture systems for derivation and maintenance of clinical-grade pluripotent hESCs and lineage-specific differentiation of pluripotent hESCs by small molecule induction [1,3-16]. Our milestone advances and medical innovations in hESC research enable high efficient direct conversion of non-functional pluripotent hESCs into a large supply of clinical-grade high purity functional human neuronal cells or heart muscle cells for developing safe and effective stem cell therapies as treatments or cures for a wide range of neurological and cardiovascular diseases [1,3,4]. Currently, these hESC neuronal and cardiomyocyte cell therapy derivatives are the only available human cell sources in commercial scales with adequate cellular pharmacologic utility and capacity to regenerate CNS neurons and contractile heart muscles, vital for CNS and heart repair in the clinical setting. The availability of human stem/progenitor/precursor cells in high purity and large commercial scales with adequate cellular neurogenic or cardiogenic capacity will greatly facilitate developing safe and effective cell-based regeneration and replacement therapies against a wide range of CNS and heart disorders. Transforming non-functional pluripotent hESCs into fate-restricted functional human cell therapy derivatives or products dramatically increases the clinical efficacy of graft-dependent repair and safety of hESC-derived cellular products, marking a turning point in cell-based regenerative medicine from current studies in animals towards human trials.

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