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# Stem Cells in Drug Discovery: Current trends and Emerging Challenges

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## Abstract:

Current development in stem cell research ultimately revolutionizes the way drug discovery and development will be directed in the future and also paves the way for innovative cell based therapies in regenerative medicine. The unique and exquisite feature of both embryonic and adult stem cells can be harnessed to continually derive human somatic cell types *in vitro* which otherwise is difficult to generate from other sources. Recently, enormous attention has been directed towards the identification, generation, characterization and application of hESCs-derived tissue precursor cells. Such potential resources and strategies provide unparalleled opportunities in disease modeling, drug discovery, drug development, toxicology, safety assessment, and cell replacement therapies. This review illustrates underlying mechanisms by which stem cells are being exploited by various chemical compounds to generate potential cell models for both biopharmaceutical research and regenerative medicine. Here we also summarize various strategies and differentiation techniques for dissemination of stem cell population *ex vivo*.

*Key words*: embryonic stem cells, adult stem cells, differentiation, drug discovery and development, biopharmaceutical applications.

## 1. Introduction

Drug discovery in the biopharmaceutical industry is catalyzed by increasing numbers of identified potential drug targets since the advent of human genome sequencing project. Despite a large number of discovered novel drug targets, clinically proven drugs available for the human disorders are abysmally low. In particular, the current trend is such that even for those low numbers of successful drugs do not have unique targets, mostly having common proteins, genes, or pathways as targets. For instance only 43 novel proteins were targeted by more than 100 popular drugs or new molecular entities (NMEs) in 2001. Indeed, a surprisingly small number of, typically not more than 3, novel host targets or therapeutic proteins could be commercialized each year by the entire pharmaceutical industry[1]. For drug discovery and development against human disorders, various animal cell models are exploited at various stages such as target identification, target validation, lead optimization, drug candidate selection, library screening for early hits, leads, pharmacokinetic, and toxicological analysis.

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One of the major obstacles hindering the drug developmental process is lack of screening systems based on normal human functional models. It is not surprising that many clinically approved compounds after trials fail at patient therapy due to either their inefficiency or unanticipated toxicity or side-effects arising during clinical practice. Conventionally, biopharmaceutical research relies on animal cells or immortalized human cell lines representing human system to test drug efficacy or toxicity. From target identification to library screening, lead optimization and drug selection, various functional assays are conducted for drug discovery and development.

In order to establish valid functional cell models, cell cultures derived from tumor cell lines are transformed to simulate human proteins which in turn could be exploited as drug targets. In particular, an accurate report on the metabolic fate of a given drug is a prerequisite during pharmaceutical drug development process. Although, these functional cell models are robust and reproducible, they still fail to represent or mimic the human cellular system, posing an enormous challenge to precise and efficient drug discovery for human disorders[2]. For instance, the complex functionalities of hepatocytes are not reflected by any currently available *in vitro* model which is metabolically competent. Tissue culture system offers an alternative technique to isolate the cells of interest. However, the major limitation is that they dedifferentiate quickly and possess only limited cell divisions *in vitro*. Hence, these problems are largely attributed to imperfect disease models which do not faithfully represent the human diseases. It is therefore not surprising that the clinical outcome of pharmaceutical compound remains low as animal models may not truly representing or lack adequate similarities to the human cell system. To circumvent these problems, embryonic stem cells offer far reaching implications, allowing us to generate a variety of fully differentiated cells, rendering an efficient and diverse tissue population for various pharmaceutical research purposes on the road to drug discovery[3,4]. This hES (human embryonic stem) cell derived differentiated or dedifferentiated system not only rejuvenates cell therapy in regenerative medicine but also paves the way for meaningful insights of underlying signaling or regulatory pathways that regulate major cellular mechanisms. In this review, we highlight current challenges and future opportunities for stem cell research in drug development and discovery process.

### *1.1. Therapeutic impact of embryonic and adult stem cells*

It is apparent there are no simplistic chemotherapeutic approaches available for most debilitating disorders, such as degenerative disorders, cancers, and relevant tissue damage disorders. This roadblock in the treatment drives an enormous attention in the potential application of stem cells. A defining characteristic of stem cells is their impressive self-renewal potential with long-term differentiation capabilities[5]. Embryonic stem cells (ESCs) derived from early embryo possess nearly unlimited self-renewal capacity and developmental potential to differentiate into virtually any cell types in an organism.

Due to their impressive self-renewal and differentiation potentials, embryonic stem cells (ESCs) and adult stem cells hold great promise in cell and gene therapy applications in the treatment of many disorders[6,7]. Embryonic stem cell technology provide a potential platform to develop novel functional models by expanding pluripotent stem cells and also converting the ESC populations to generate large number of differentiated precursor cells of various tissues[4]. Researchers demonstrate that adult or tissue stem cells can survive, migrate, differentiate, integrate and reconstitute within the transplanted organ system. Particularly, stem cells from various developmental organs, including embryonic, neural, hematopoietic, and induced pluripotent cell system were successfully transplanted for variety of clinical purposes.

Interestingly, the differentiation of ESCs could be specifically and systematically controlled in a reproducible manner to perform various drug screens using normal differentiated human cells for appropriate signal transduction systems.

Recent advances in the identification, isolation, characterization and *in vitro* culture techniques highlight the unprecedented potential of stem cells to cure disorders. Translating these potentials into clinical benefits encounters enormous challenges, including efficient engraftment of stem cells into desired tissue system, maintaining the genetic stability for long course of time, and preventing the oncogenic potential during stem cell proliferation. Through their regenerative capability, adult SCs are able to differentiate into residing tissue to partially restore the function. Stem cell therapy principally involves introducing a new cell into the damaged or diseased tissue to replenish or rejuvenate the organ or tissue system. The ability of stem cells to self-renew, proliferate and differentiate to form a functionally competent tissue offers a great potential to replace the diseased or damaged tissues[6]. Mesencymal stem cells from fetal bone marrow, for instance, are capable of differentiating into not only osteogenic, adipogenic and endothelial lineages, but also hepatocyte-like cells, chondrocytes, muscles, neural, and erythroid cells[8]. Interestingly, their regenerative and tissue repair potential are not restricted to their local milieu but also to tissues of distal organs via pro-inflammatory cytokines and growth factors. Here, the added benefit is that both autologous and allogenic stem cells have no immunoreactivity problems in systemic administration and local transplantation, rendering stem cells as an ideal choice to deliver the genes of interest in gene therapy applications in various tissues. Development of cell specific gene therapeutic approaches are now underway to cure various diseases including premature aging diseases, diabetes, atherosclerosis, hematopoietic, cardiovascular, musculoskeletal, gastrointestinal, pulmonary, urogenital, ocular, neurodegenerative and skin disorders[9]. Stem cells offer great promise in the treatment of variety of diseases ranging from heamatological disorders, cancer, neuro, cardiac and nephrological disorders. Current research is directed at exploiting the adult and embryonic stem cell to treat many disorders including cancer, Type 1 diabetes mellitus, Parkinson's disease, Huntington's disease, cardiac failure, muscle damage and neurological disorders. Stem cell treatment remains the only treatment modality for the cure of chronic lymphocytic leukaemia (CLL)[10]. Stem cell transplantation (SCT) remains the only treatment capable of cure, but has traditionally been associated with very high morbidity and mortality. For more than three decades, leukemic and lymphoma patients were successfully treated by using bone marrow and umblical stem cells. Stem cell therapy has an advantage over conventional radiotherapy and chemotherapy which could largely compromise normal hematopoietic cells while killing cancer cells.

### *1.2. Differentiation screens*

Recently, there has been enormous attention to develop methodologies to direct ES cells to derive more specific and differentiated cell population for developmental biology and degenerative medicine purposes. In particular, screen the library to identify a novel compound to sequentially disseminate ESCs in a controlled manner to yield desirable differentiated cells in tissue-culture environment. In a classical experiment, Jessells et al., demonstrates that a precise gradient of extracellular components could dictate the transcriptions factors to tailor the specific neural cell representing defined stage of neural development (reviewed in[11]). Interestingly, ESCs can be differentiated stepwise into each differentiation level such as a neural progenitor fate then early DA neural progenitor, followed by late DA neuron progenitor, and finally DA neuron.

Similarly, ES cells could either be directed to become pancreatic β cells with initial endodermal induction, then early pancreas, pancreatic endocrine, and then mature β cells secreting insulin (Reviewed in[12]). Another intriguing study demonstrates that human cord blood (UCB)-derived multipotent stem cells regenerated the spinal cord at the injured site accompanied by improved sensory perception and movement upon 5 weeks after cell transplantation[13]. The major advantage of developing gradual differentiating method is they could represent precise target cell population, thus allowing us to develop specific drugs pertaining to the target cells by excluding untoward toxicities or maximum therapeutic benefits.

### *1.3. hES derived cardiac myocytes*

The dissemination of cardiomyocyte precursor from ESCs or adult SCs is invaluable for the development of heart disease models and also can be utilized for repairing damaged heart tissue *in situ*. hES cells could be directed to establish a large number of cardiomyocytes for cardiac drug discovery, development of novel therapeutics for heart diseases, cardiac safety assessment and cardiac modeling.By screening a large number of chemicals, Takahashi and colleagues demonstrated that the putative use of a small molecule, ascorbic acid to enrich the cells with cardiac phenotype which display spontaneous, rhythmic contractile activity, along with presence of cardiac genes such as sarcomeric myosin and alpha-actinin, GATA4, alpha-MHC, and beta-MHC[14]. Similar screening process in a large combinatorial library by Wu and colleagues identified cardiogenol A-D as a potential differentiating agent to derive more specific cardiomyocytes[15]. Some differentiating compounds such as 5-aza-deoxycytidine are unique to hES to differentiate into cardiomyocytes and fail at mouse ES cells[16]. Studies also demonstrate that both iPS and ES cell-derived cardiomyocytes display cardiac functionality and the beta-adrenergic and muscarinic signaling cascade responses exploit them as an autologous cell source for cellular cardiomyoplasty, and myocardial tissue engineering[17].

### *1.4. Hepatocytes derived from ESCs*

At present, liver transplantation is the only effective treatment for severe liver disorder. However, the liver transplantation therapy is severely limited by shortage of donor organs, operative damage, and the risk of immune rejection. This potential problem profoundly catalyzes the demand for alternative approaches such as cell therapies which offer restoration of liver mass and function. Therefore, hESCs are scalable and have the potential to provide an unlimited supply of replacement somatic cells, which possess significant advantages over their adult stem cell counterparts[18]. For last few years, embryonic stem (ES) cells are being widely studied as a promising source of hepatocytes with their proliferative, renewable, and pluripotent capacities.

Direct differentiation approaches use a two-dimensional tissue culture approach employing extracellular matrixes, growth factors, cytokines, and hormones to facilitate the formation of three-dimensional structures termed embryoid bodies (EBs) which consequently differentiate to varying levels of hepatocyte-like cells (HLCs)[19]. In recent years, hESC differentiation to HLCs has been modified with efficient and functional hepatocyte differentiation demonstrated by several groups[20,21]. Recent study by Hay et al. demonstrates that Wnt3a is differentially expressed at critical stages of human liver development to promote the clonal efficiency of hESCs exhibiting functional hepatic differentiation *in vivo*.

Although there has been major progress in the field, there is still the requirement to select HLCs from other contaminating cell types and undifferentiated stem cells in final cell preparations. Recent reports offer significantly improved yields of HLCs to be used in the modeling of human liver development, disease, transplantation, and drug toxicology for cell based therapies. There are other potential methods available to enrich the functional hepatic progenitor cells. For instance, by using the fluorescent activated cell sorting (FACS) for the asialoglycoprotein receptor method, human HLCs through EBs which secreted functional human liver-specific proteins observed in primary human hepatocytes with human hepatocyte cytochrome P450 metabolic activity[22]. Study using purified adult rat primary adult liver stem cells trans-differentiated into pancreatic endocrine hormone-producing cells when cultured in a high-glucose environment[23]. These results indicate that these hepatic stem cells can differentiate in a non-lineage-restricted manner to trans-differentiate into endocrine pancreas which could be directed for future therapies of diabetes. Moreover, these ESCs and derived hepatocytes could be exploited for a variety of potential pharmaceutical applications. For instance, safety and toxicology assessment, using ESC derived hepatocytes for drug metabolism, usage of differentiated cells to identify of surrogate biomarkers, utilizing genotyping the ESCs for varying responses to drugs due to genetic variations and to explore the underlying mechanisms predisposition to disorders, employing transgenic animal models to process target validation and drug discovery.

### *1.5. Neurons derived from ESCs*

For the development of neuronal drug discovery models, it is crucial to enrich derived neural subtype cells from ESCs and optimize the specific culture conditions. For the treatment of Parkinson's disease, human neural precursor cells could be successfully enriched to generate mid-brain dopaminergic phenotype from GABAergic phenotype[24,25]. In this, robustly generated midbrain dopamine neurons from the hES cells were exploited in preclinical models of Parkinson's disease. This experimental system also offers a renewable source of functional human DA neurons for drug screening and development of cell-replacement strategies for disorders affecting the DA system and to explore the molecular mechanisms that control the development and function of human midbrain DA neurons. One potential strategy by which ESCs could be instructed to commit themselves to a particular lineage cell population is to express nurr-related protein 1 (Nurr1) to enrich dopaminergic neurons (sonic hedgehog homologue (SHH), FGF2 and FGF8)[26]. Similar chemical screening of a large number of compounds identified retinoic acid and an activator of sonic Hedgehog signaling as differentiating agents for embryoid bodies generated from ES cells to ultimately generate functional l motor neurons[27]. Relevant differentiation screening approaches were aimed at producing more differentiated specific cell lines by using chemical factors yielded desired cells at target cells for specific disorders[28, 29].

### *1.6. Small molecular compounds in stem cells*

Several research groups have carried out chemical screening for small compounds to modulate self-renewal in ES, neural stem and other adult stem cells. Underlying mechanisms by which certain small molecules regulate the self-renewal in stem cells have been explored to characterize the molecular signatures of self-renewal and to develop potential therapeutics. Retinoic acid, for instance, leads to alterations in HOX gene expression during embryogenesis and is a modifier of the WNT-mediated signaling pathway[30,31].

Prostaglandin E2 (PGE2), a small lipid mediator, has also recently been shown to regulate HSC self-renewal during embryogenesis and can enhance HSC engraftment, as measured by the competitive repopulation studies in mice[32]. Self-renewal can be augmented in stem cells with self-renewal potential. HSCs can execute the self-renewal programme, but the addition of WNT3A, sonic hedgehog (SHH), and angiopoietin-like factors or PGE2 can increase the size of stem-cell pool.

Some low molecular compounds such as ascorbic acid, retinoic acid, 5-azacytidine and glucocorticoids can remodel the adult tissues preferentially through regulating adult stem cell differentiation. For instance, 5-azacytidine is known to induce mouse mesenchymal progenitors to differentiate[33]. Some cancer cells in dedifferentiated state could be differentiated into less potent cells. These drugs potentially target stem cells to differentiate by suppressing their signaling molecules, and cell cycle mediators. This mechanism paves the way for many pharmaceutical agents such as imatinib (marketed as *Gleevec*; Novartis[34]) bortezomib (*Velcade*; Millennium pharmaceuticals[35]) and geldanamycin[36]. Although, the clinical therapeutic applications remain to be validated, these small molecular compounds offer a great platform to mechanistic understanding of underlying stem cell pathways. Growing number of investigations were successfully employed to screen small compounds that largely influences differentiation characteristics of ES cells.

For instance, a large combinatorial chemical library was subjected to phenotypic cell-based screen to identify diaminopyrimidine compounds (cardiogenol A-D) which selectively and efficiently induce mouse embryonic stem cells (ESCs) to differentiate into cardiomyocytes[37]. It is now increasingly evident that screening small molecules to induce ESC differentiation provides more opportunities to derive a variety of progenitor population[38]. By screening a focused active 2,4-disubstituted-pyrrolopyrimidines library, Ding et al. identified GSK-3 which could differentiate neurons in both mouse embryonic carcinoma and ES cells[39].

### *1.7. Dedifferentiation screens*

Both differentiation and dedifferentiation of adult and embryonic stem cells are rapidly transforming concepts, only partly understood at present. Hence, their defining characteristics and the differences between them perhaps should require further study. By screening a chemical library of diverse compounds culminated in the identification of a microtubule-disrupting molecule, reversine was identified that directs the myeblasts to generate mesenchymal stem cells that could ultimately differentiate into a large numbers of bone and adipose cells. Recent study demonstrates that cell-based screen of chemical libraries provides identification of small molecules that control the self-renewal of ES cells. By using this screening method, SC1 an uncharacterized heterocycle, was discovered which propagates murine ES cells in an undifferentiated, pluripotent state under chemically defined conditions in the absence of feeder cells, serum, and leukemia inhibitory factor.

This methodology potentially expands the number of long-term murine ES cells to derive primary germ layers *in vitro* and in vivo by down-regulating the RasGAP and ERK1[40,41]. These small compounds not only deliver therapeutic advantages of stem cells, but also shed light on novel insights into the underlying molecular mechanisms of stem cells.

### *1.8. Induced pluripotent stem cell (iPS) cells*

Induced pluripotent stem (iPS) cells display pluripotent stem cell characteristics which are artificially derived from adult non-pluripotent cells, by reprogramming their gene expression patterns ex vivo. The generation of human Induced pluoripotent cells (iPS) from human somatic cells revolutionizes the way how regenerative medicine progresses in recent years. Intriguingly, differentiated cells can be reprogrammed to an embryonic-like state by using a defined set of transcription factors to reverse their lineage passage back to a pluripotent state associated with ESC-like phenotype. In a ground breaking study, Shinya Yamanaka's group retrovirally introduced four transcription factors: Oct 3/4, Sox2, c-Myc, and Klf4 in both mouse and human fibroblasts to reprogramme the somatic cells[42]. This technology paved way for unparallel opportunities in regenerative medicine as iPS cells could differentiate into specific progenitor cell types. As it circumvents the use of embryonic stem cells, iPS technology offers a great alternative for the source of differentiated human cells for cell therapeutics in regenerative medicine.

In past decades, gene therapeutic trials against various genetic disorders have not been clinically successful, owing to the paucity and poor quality of adult stem cells in the bone marrow of patients. Now, a combination of gene therapy and induced pluripotent stem (iPS) cell technology could deliver promising therapeutic approaches for the various disorders including fanconi Anemia (FA), cystic fibrosis, and other relevant human genetic diseases. In an elegant experiment, defective genes in cells from patients were rectified using gene therapy[43].

Those repaired cells were then reprogrammed into induced pluoripotent stem cell (iPS) cells using a combination of transcription factors, OCT4, SOX2, KLF4 and cMYC. The resulting FA-iPS cells were indistinguishable from human embryonic stem cells and iPS cells generated from healthy donors, which successfully ameliorate FA phenotype. Importantly, stem cells aspirated from the bone marrow of three CF patients were transfected with Maloney murine leukemia virus carrying CFTR gene[44]. The resulting *ex vivo* co-culture system allows marrow stromal stem cells (MSCs) to differentiate into airway epithelial cells and restores long term functionalities.

This suggests that *ex vivo* gene therapy offers potential advantages such as screen, reprogram, and manipulate the cells before actually delivering them to patients. Similarly, induced pluripotent stem (iPS) cells can be generated from patients with type-1 Diabetes by reprogramming their adult fibroblasts with three transcription factors (OCT4, SOX2, KLF4)[45]. These derived cells, termed DiPS cells have the pluripotent characteristic and therefore can be differentiated into insulin-producing cells.

Interestingly, introduction of microRNAs (miRNAs) – the unique posttranscriptional modulators specific to embryonic stem cells profoundly enhances the production of mouse induced pluripotent stem (iPS) cells. The miRNAs miR-291-3p, miR-294 and miR-295 promote the reprogramming efficiency by Oct4, Sox2 and Klf4 to dedifferentiate the somatic cells into iPS cells[46]. Further analysis of the targets of the miRNAs identified here may offer insights into the reprogramming mechanism. Studies delineate that these ESCs specific miRNAs are highly expressed in ES cells, where they accelerate the cell cycle transition. Various investigations demonstrate that miRNAs regulate the pluripotency of ESCs such that genetic deletion of key miRNA processing enzymes Dicer[47] lose their pluripotency and show defective differentiation perhaps via indirect down-regulation of Oct4, Rex1, Sox2, and Nanog genes.

Besides, murine ESCs with Dicer-deficient mutant ESCs can be partially rescued by the miR-290 cluster miRNAs that downregulate Oct4 indirectly[48]. Current studies are directed towards delineating underlying mechanisms by which reprogramming machineries dictate the somatic cell into pluripotent cell. A growing number of studies are now focusing on iPS cells derived from various patients to offer novel interventions for different diseases, including Type I diabetes, Parkinson's, and Muscular Dystrophy.

### *1.9. Stem cell mediated prodrug drug delivery*

One of the potential problems associated with stem cell transplantation is adverse inflammatory responses in host animal to contradict the therapeutic benefits. To circumvent this problem, recent study designs microencapsulated stem cells in which genetically engineered neural stem cells (NSCs) are delivered in a time-controlled manner. More interestingly, the encapsulated system could also efficiently be programmed to regulate the rate and extent of proliferation and migration of the NSCs. Adult stem cells could be exploited as potential targeted drug delivery system for anticancer drug as they have the tendency to migrate to distal, diseased, and metastatic cancerous tissues. In principle, well-designed NSCs display both the ability to differentiate *in vivo* in a controlled fashion and to sustain their self-renewal, propagation and expansion capabilities at the target sites. NSCs should be immortalized to avoid the transformation into cancer stem cells. In this, stem cells tender great advantage over other therapies as traditional cancer therapeutics are facing increasing difficulties to access the remote and inaccessible cancerous sites in various tissues. For instance, human fetal primary stem cells generate the tumor-targeting neural cell line, HB1.F3.C1, which were then programmed to secrete a form of rabbit carboxylesterase (rCE)[49], which in turn activates an anti-cancer prodrug *Campto/Camptosar* (irinotecan; Pfizer). This study also demonstrates that administration of modified NSCs followed by *Camptosar* profoundly enhances survival rate to almost 100% in mice bearing cancer.

In particular, gene-directed enzyme prodrug therapy (GDEPT) is based on the delivery of a gene that encodes an enzyme which is non-toxic per se, but is able to convert a prodrug into a potent cytotoxin. MSCs can be employed as a vehicle for Prodrug gene therapy to deliver the candidate genes encoding enzymes that convert nontoxic prodrugs into toxic anti-metabolites. Human adipose tissue-derived mesenchymal stem cells (AT-MSC) with enhanced tumor tracking properties provide an attractive opportunity for targeted transgene delivery into the sites of tumor formation and also serve as a potential source of autologous stem cells[50,51]. In this system, Cytosine Deaminase (CD), HSV-1 Thymidine kinase and carboxyesterase genes render sensitivity to anticancer drugs 5-fluorocytosine 5-FC, ganciclovir (GCV) and camptothecin-11 (CPT-11), respectively. The potentiating effect of prodrug 5-FC observed in a recent investigation by Kucerova et al. suggests that human adipose tissue-derived mesenchymal stem cells (AT-MSCs) could be used as a cellular vehicle for CD:UPRT gene (CDy-AT-MSC) to suppress the HT-29 tumor cells *in vitro*[52]. Interestingly, engineered CD-AT-MSCs combined with 5-FC were efficiently controlled human colon cancer xenograft growth *in vivo.*

Besides, this CDy-AT-MSC/5FC augmented the bystander effect and selective cytotoxicity on A375 human melanoma, glioblastoma, HT29 colon, MDA-MB-361 breast cancer cells and bladder carcinoma targets *in vitro*. Similarly, AT-MSC (TK-MSC) expressing Herpes simplex virus - thymidine kinase (HSV-tk) could exert cytotoxic effect on tumor cells upon treatment with prodrug ganciclovir (GCV)[53]. AT-MSC (TK-MSC) displayed both bystander cytotoxic effect on tumor cells and prodrug ganciclovir conversion-mediated suicide effect on TK-MSC.

This supports the idea that mesenchymal stem cells could be utilized for tumor-targeted cancer gene therapy. Due to extensive tropism of neural stem cells (NSC) toward malignant gliomas, NSCs could target medulloblastoma and be used as a cellular therapeutic delivery system which disseminates therapeutic agents to medulloblastoma.

The HB1.F3 cells (an immortalized, clonal human NSC line) were engineered to secrete the prodrug activating enzyme Cytosine Deaminase (CD) and allowed to target medulloblastoma. In this, CD enzyme converts non toxic substrate antifungal agent 5-FC to antitumor agent 5-fluorouracil (5-FU), allowing newly generated 5-FU diffuse into target the surrounding medulloblastoma cells and melanoma brain metastases models[54]. The results confirm the potential clinical utility of these cells and the CD gene as a cell-directed approach for enzyme-mediated prodrug conversion in the field of molecular cancer chemotherapy.

## 2. Other relevant Pharmaceutical applications

The use of embryonic stem cells for cell-replacement therapy in diseases like diabetes mellitus requires methods to control the development of multipotent cells. Cell therapeutic strategies for long have been exploiting variety of stem cell technologies to gain major benefits. Though, there are several strategies employed to generate pancreatic islet cells, only the strategy using forced expression of PAX4 was successful in promoting the development of insulin-producing cells *in vitro*[55]. Here, the constitutive expression of Pax4 influences ES cells to differentiate into pancreatic lineage, which leads to the formation of islet-like spheroid structures that produce increased levels of insulin. By inhibiting the intracellular signaling regulator PI3-K, pancreatic β-like cells were developed from mouse embryonic stem cells. Although not identical to pancreatic islets of Langerhans, these cells produced significantly higher level of insulin, and displayed glucose-dependent insulin release *in vitro*. They enhanced the circulating insulin levels, controlled weight loss, improved glycemic control, and dramatically rescued survival in mice with diabetes mellitus. These observations demonstrate that embryonic stem cells can serve as a repository of insulin-generating tissue for cell replacement therapy in diabetes mellitus. In co-culture with endothelial cells, embryonic neural progenitor cells (NPCs) show reduced neurogenesis and elevated self-renewal. The adult neural stem cells could even produce progeny that exhibited an endothelial phenotype with enhanced barrier properties. The co-culture of endothelial cells, pericytes and astrocytes adopt the anatomical condition of the blood-brain barrier (BBB) *in vivo*. This set-up can be used as a model of the BBB to study the pharmacokinetics of several neurological drugs which typically transport across the barrier[56,57]. Similar studies were directed to generate ESCs-derived membrane model with ABC efflux pumps to assess the membrane permeability of certain pharmacological agents.

### 3. Toxicity studies

Due to the limited availability of precise human cell or tissue models *in vitro*, drug toxicity investigations are preferentially carried out in other animal models which typically lead to inaccurate results or misinterpreted toxicology outcomes. To test carcinogenicity of various genotoxic as well as nongenotoxic carcinogens, the Syrian hamster embryo (SHE) cell transformation assay is the only available option which often yields imprecise toxicology outcomes. Although hindered by ethical roadblocks in the past decades, many investigations are now preferentially using either human or mouse embryo cells for embryo cell test (EST)[58]. Since the advent of high-throughput screens during the drug discovery phase, a large number of lead candidates are being selected for drug development.

This catalyzes an enormous need for *in vitro* alternative test models to determine the pharmacokinetics and toxicology profile of compounds in the late- and/or early-development phase. In particular, embryotoxic or teratogenic new chemical entities (NCEs) could be implemented for reproductive toxicology studies. An ECVAM (European Centre for the Validation of Alternative Methods) validated system[59], embryonic stem cell test (EST) utilizes the differentiating potential of murine embryonic stem (ES) cells to test embryotoxicity *in vitro*[60].

## 4. Conclusions and future directions

Despite the fact there has been tremendous progress in our understanding of stem cells in the past few years, stem cell therapeutics is still a young field such that there are many intriguing aspects of stem cells are still remain to be elucidated to fully understand the therapeutic potential of stem cells. Importantly, mechanisms by which low molecular compounds, signaling pathways, and *ex vivo* culture conditions regulate stem cell behavior is still poorly understood. In this context, unraveling the molecular mechanisms of stem cells is a prerequisite to optimize their therapeutic potentials in drug discovery and development. Recent insights into the differentiation of embryonic, adult, and induced pluripotent stem cells offer great benefits but also raise several fundamental questions in regard to their clinical applications. It is evident that major challenges still remain in deriving potential hESCs by exploiting the iPS technology; how somatic cells fate is reversed into lineage non-specific iPS stem cells and how stem cells reciprocate to certain signals are yet to be answered. It is also fascinating to know how an organism drives stem cell mobilization and their reestablishment at distal tissue organs in response to variety of stress signals. Given that complex degenerative disorders persist despite the conventional therapies further propagates our immense interest in the development of novel strategies based on stem cell therapeutics. Taken together, it is increasingly apparent that combinatorial, multifaceted, and sophisticated approaches should be directed to gain more insights of the stem cells to develop most-promising targeted therapies for various chronic degenerative disorders.

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