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Exploiting pluripotent stem cell technology for drug discovery, screening, safety, and toxicology assessments ☆

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ABSTRACT

In order for the pharmaceutical industry to maintain a constant flow of novel drugs and therapeutics into the clinic, compounds must be thoroughly validated for safety and efficacy in multiple biological and biochemical systems. Pluripotent stem cells, because of their ability to develop into any cell type in the body and recapitulate human disease, may be an important cellular system to add to the drug development repertoire. This review will discuss some of the benefits of using pluripotent stem cells for drug discovery and safety studies as well as some of the recent applications of stem cells in drug screening studies. We will also address some of the hurdles that need to be overcome in order to make stem cell-based approaches an efficient and effective tool in the quest to produce clinically successful drug compounds.

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1. Introduction: rationale for use of stem cells for drug screening

Drug development is a multi-year, multi-million dollar proposition with the vast majority of promising compounds failing to come to fruition. Failure is likely not due to a lack of testable compounds as chemical libraries contain thousands of potentially therapeutic agents just waiting to be explored and scrutinized. Drug screening using in vitro cell culture systems provides the pharmaceutical industry a means to narrow down these large chemical libraries into a list of candidate compounds for further testing. However, in order to generate useful leads, to verify safety, or to verify efficacy against human disease, these cells need to sufficiently recapitulate the characteristics of the intended target tissue. There are a wide variety of sources for cultured cells that can be used in drug screening assays. Each cell type has certain advantages, but they also have characteristics that may contribute to the high compound attrition rate. For example, primary adult tissue would be ideal for in vitro disease modeling and drug screening because compounds could be tested in the specific cells of interest in the patient population of choice; however, these tissues are difficult to acquire, particularly from the numerous organs that are drug targets such as liver, intestine,

Abbreviations: PSCs, pluripotent stem cells; hESCs, human embryonic stem cells; iPSCs, induced pluripotent stem cells; HEK, human embryonic kidney; SMA, spinal muscular atrophy; SMN, survival motor neuron; ALS, amyotrophic lateral sclerosis.

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heart, and brain. Furthermore, their reduced proliferative capacity interferes with obtaining high numbers of cells required for large scale screening. This lack of proliferation also reduces transfection efficiency, which is an important tool often used to modify the cell lines to generate reporter constructs that improve screening productivity. Immortalization of primary cell lines can alleviate the proliferative capacity problem, but the large differences in genetic background that result from transformation call the validity of these as model systems into question [1]. Additional cell sources for drug screening come from readily available animal tissues and have been used to model human relevant physiological events since the beginning of our understanding of human genetics [2,3]. Although mouse models are a popular tool for disease modeling because of the ease of manipulating their genome by targeted genome editing [4], the desire to reduce, reuse, and replace animal models for drug development and species differences at both the genetic and physiological level may reflect their inability to accurately predict clinical failure or success [5–11]. Human cell cultures derived from embryonic sources, such as human embryonic kidney (HEK) lines, have been used to address both species differences and the proliferation problems with primary adult tissue. Furthermore, these cells are readily available and can be easily transfected to express desired targets of interest for drug screening purposes. For example, HEK cells have been used to over-express the human ether-à-Go-Go (hERG) channel to recapitulate electrophysiological function seen in cardiac tissue [12]. Cell lines derived from human fetal tissue are useful, but they have limited ability to fully recapitulate the native tissue environment in which the drug may act. In addition, there are ethical concerns in obtaining and using these cell lines considering the tissue source.

Species differences, alteration of genetic profiles, limited availability of specified cell types, and low proliferative capacity can now be avoided with the use of human embryonic stem cells (hESCs). Since this pioneering work [13], much has been done to examine the ability of hESCs to expand in an undifferentiated state to generate a theoretically infinite source of human cells for drug screening. More recently, information gleaned from ESCs has been used to mimic the pluripotent state in somatic cells through induction. These induced pluripotent stem cells (iPSCs) possess a similar proliferative capacity in an undifferentiated state as ESCs. Collectively, these pluripotent stem cells (PSCs) also have unparalleled capabilities to differentiate into a large range of specified tissue, including heart, liver, and brain (Fig. 1; further reviewed in [7,14]).

Because these cells are derived from a human source, there is potential for PSCs to provide valuable information about drug safety and efficacy in specified tissues, such as liver or heart. Another application that has more recently been showing promise is their use in high throughput exploratory drug screening. At this point the use of PSCs in drug screening is in its infancy, and progress toward the development of standardized screening methods is still being developed. In this review we will discuss some of the commonly used cell lines for drug screening purposes and discuss how PSCs have or could fit into currently used approaches for drug discovery and development.

2. Drug discovery approaches

2.1. Targeted approach to drug discovery

Targeted and phenotypic approaches are two distinct methods for the identification of drug leads (Fig. 2; Table 1). A targeted approach focuses on identifying drugs that can interact with genes, gene products or molecular mechanisms [15,16]. Therefore, a target based approach relies on what is known about a specific disease, and often requires that a specific mode of action is known, which is generally through the activation or inhibition of a receptor or channel (Fig. 2). The goal of a targeted approach is to develop drugs that affect only one gene or molecular mechanism (i.e. the target) in order to selectively treat the disease without producing side effects. Compounds are then screened to identify a drug with the desired properties. This method has been popular in the pharmaceutical industry because the desired compound properties are identified before screening begins and allows for a systematic search. This also fits well into a workflow for further validation toward clinical application. For example, mutations in leucine-rich repeat kinase 2 (LRRK2) are linked to both familial and sporadic forms of Parkinson's disease, and these mutations have been shown to increase kinase activity. Using LRRK2 as a target, Hermanson et al. generated a cell based, high throughput in vitro assay to monitor a specific phosphorylation event on LRRK2 [17]. Screening 1120 compounds resulted in the identification of 16 inhibitors to this specific phosphorylation event. These compounds can now be further examined for specificity, safety, and efficacy.

Currently, many of the FDA approved molecules have defined targets [18]. However, an understanding of their intended target may not result in effective treatment in clinical trials. For example, succinic semialdehyde dehydrogenase deficiency (SSADHD) is a rare neurological disorder caused by an inability to catabolize the neurotransmitter γ-aminobutyric acid (GABA). In an effort to counteract the excessive GABA in the neural environment, molecules designed to block GABA receptors have been tested, but unfortunately have been ineffective in reducing patient symptoms [19]. In an era where targeted based approaches have been the primary source for drug leads, additional techniques need to be employed to reduce compound failure in Phase II and III clinical trials [20,21]. A report from Swinney and Anthony has highlighted the importance of the development of phenotypic assays for drug discovery [22]. Although targeted drug development approaches have a standardized workflow, Swinney and Anthony report that this approach is currently producing fewer first in class drugs than other methods, which indicates room for improvement.

2.2. Phenotypic approach to drug discovery

Unlike a targeted approach to drug screening, a phenotypic (also described as physiological) screen assesses a compound's effect on specific cellular outcome measures such as cell survival or electrophysiological properties (Fig. 2; Table 1). In this case, molecular mechanisms and protein targets can remain unknown even after the drug's activity and efficacy are determined. The most recognizable phenotypic screens are those using animal models that recapitulate functional and/or behavioral abnormalities due to disease. For example the *Caenorhabditis elegans* model has been used for screening compounds against neuromuscular disorders [23], and small model organisms such as nematodes, fruit flies, and zebrafish allow for medium to high throughput screening options for drug discovery. However, due to the cost of clinical trials and safety and efficacy concerns, lead compounds require further testing in mammalian systems before moving to clinical studies, which adds to the time and expense of drug development only for a candidate to later fail. Therefore, it is advantageous to model disease using cell culture based systems that can be consistently utilized in a high throughput system while avoiding the need for redundant screening because of species differences. Many mammalian based physiological screens involving cell culture have been developed for high throughput drug screening (Table 1). Commonly used physiological assays that have been developed in these different cellular models include cell viability, signaling activity, autophagy, apoptosis, cell cycle analysis, infection rates, cell motility, cellular secretion, cytoskeletal rearrangements, astrocyte activity, nuclear translocation, receptor internalization, neurite outgrowth, mitochondrial health, and electrophysiological function [24]. Immortalized or embryonic derived human primary tissue has been the workhorse of these types of assays in the past, but advances in the use of PSCs may be more relevant for use in physiological assays. For example, Burkhardt et al. discovered that motor neurons derived from three different sporadic (i.e. without a known genetic cause) amyotrophic lateral sclerosis (ALS) patients develop transactive response DNA binding protein 43 (TDP-43) positive aggregates reminiscent of post-mortem ALS pathology [25]. As a proof

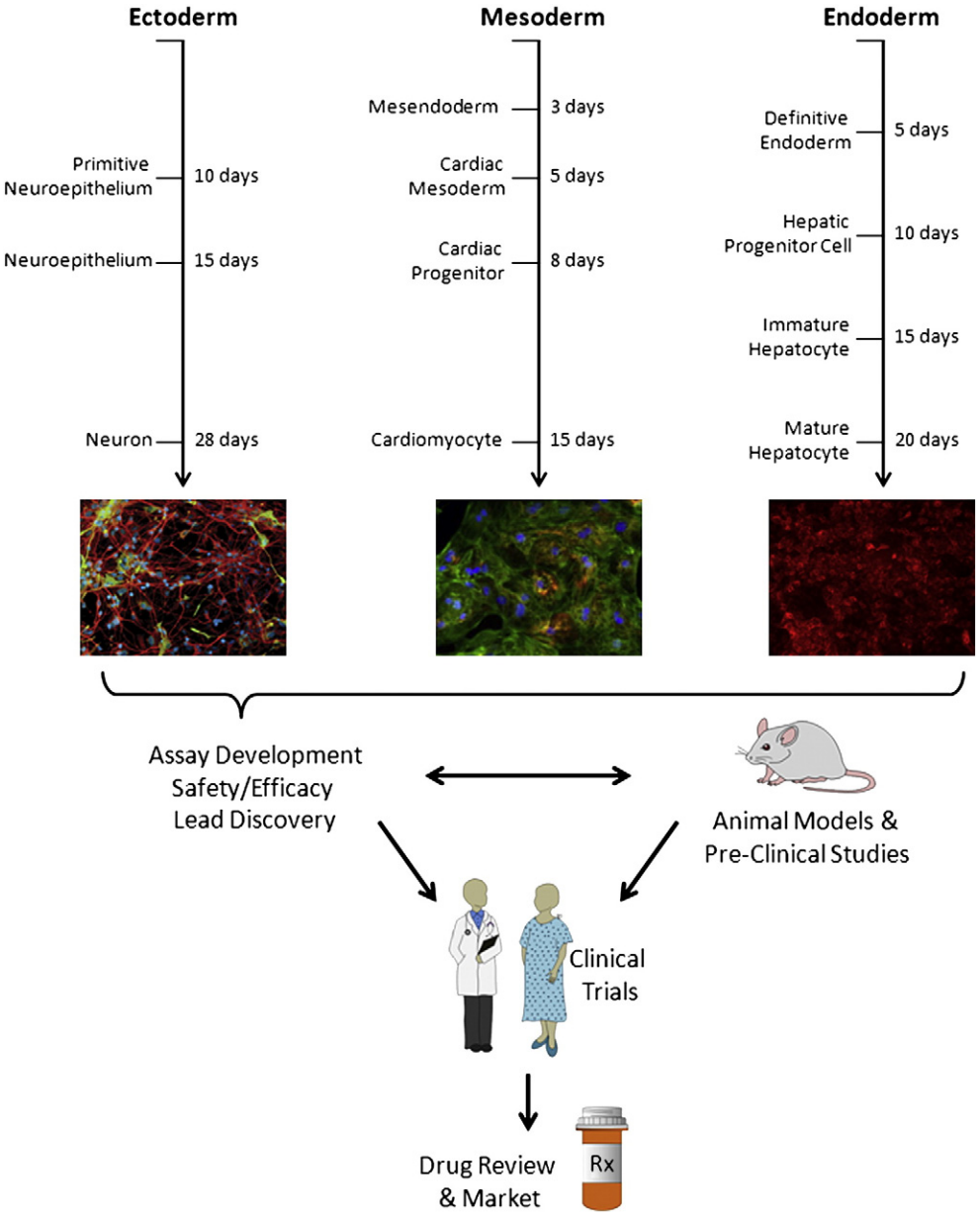

Fig. 1. Stem cells and their use in drug development. Pluripotent stem cells can be restricted to generate the three primary germ layers using developmentally directed pathways leading to virtually all cell types in the human body. Developmental timelines vary for the production of mature cells including neurons [102], cardiomyocytes [103], and hepatocytes [104]. These terminally differentiated tissues are currently being used for safety and efficacy screens as disease models using these cells are demonstrating their usefulness for lead identification. Leads can then be further tested using animal models and clinical trials to generate a marketable drug.
Images reproduced with permission from [102–104].

of concept, they utilized TDP-43 aggregation as a phenotypic endpoint for high-content screening using iPSC-derived motor neurons [25]. A critical aspect of this work is that they found this phenotypic marker in a model of sporadic ALS, a feat not attainable with standard animal model systems. In the neurodegenerative disease Spinal Muscular õ-Atrophy (SMA), motor neuron survival has been directly correlated with a reduction of the survival motor neuron (SMN) protein [26]. We have demonstrated reduced SMN protein "gems" as well as decreased survival of motor neurons derived from SMA patient iPSCs compared to control lines [27,28]. Furthermore, we have recently demonstrated that both SMA mice and SMA iPSCs display abnormal morphological changes in astrocytes (Table 2; [29]). Specifically in the SMA iPSC derived astrocytes, there are also physiological changes leading to functional deficits. Compared to a targeted screening for gem counts, which correlates directly with SMN protein levels [30–32], physiological assays could be used for the screening of drugs that alleviate either motor neuron degeneration or astrocyte activation. Given that motor neurons rely on astrocyte function for survival, an approach that measures multiple physiological aspects of this disease would likely be most successful for the identification of an effective drug. Assays that look at the physiological outcome may also provide valuable information about the disease that can suggest the use of combinatorial therapy that may improve treatment outcome. For example, SMN gene replacement strategies in SMA mouse models demonstrate that there is a therapeutic window in which gene replacement is effective. Phenotypic assays may allow for the screening of treatments that expand or eliminate this "window" to allow better clinical outcomes. A targeted based approach identifies SMN as the needed gene, but does not easily address complicated treatment paradigm issues.

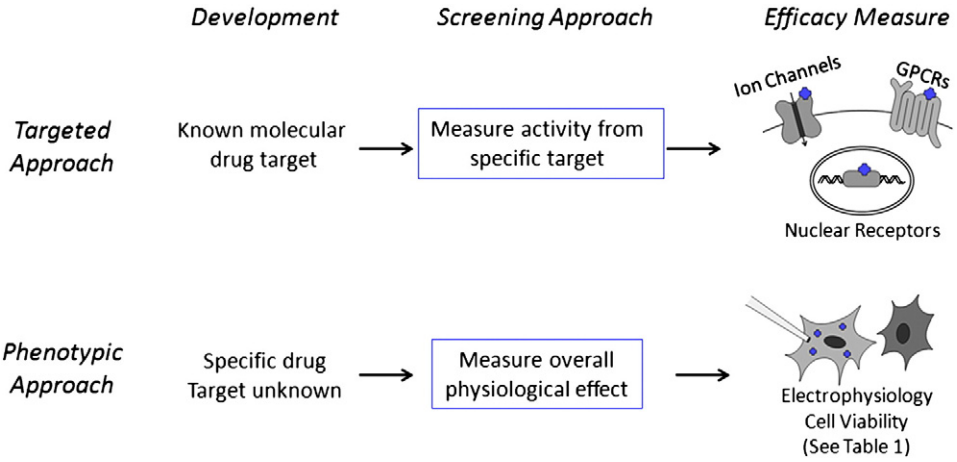

Fig. 2. Targeted versus phenotypic drug screening approaches. Drug lead identification using a targeted approach relies on the identification of drugs that interact with a pre-determined target [105]. The most common targets of FDA approved drugs include ion channels, G protein coupled receptors (GPCRs), and nuclear receptors [18]. A phenotypic approach relies on identification of drugs that generate a desired physiological change without knowing the target [105]. Additional examples of phenotypic screens can be found in Table 1.

2.3. High throughput and high content screening in PSCs

One of the advantages of PSCs is their ability to proliferate indefinitely, which allows for the generation of an adequate supply of cells for high throughput screening (HTS) techniques. HTS typically involves automated screening of chemical libraries and data capture from a single well. Target based approaches often use reporter assays for live cell and rapid lead identification. However, advances in automated liquid handling, detection, and computer programming now allow for the use of high content screening (HCS). High content screening is different than HTC in that it can report multiple readouts on a cell-by-cell basis in a microscopy based system [33,34]. This provides quantitative data from multiple endpoints and allows for more thorough examination of each drug hit and its mode of action.

Data from four major pharmaceutical companies suggest that HTS is one of the most productive techniques pharmaceutical companies have for the identification of novel lead compounds [35]. It has been reported that the average time from target identification to FDA approval is 13.5 years [35,36]. Given that human ESCs were first isolated in 1998 and iPSCs described in 2007, it makes sense that advances through HTS/HCS with regard to stem cell research would have not yet made it through this pipeline. However, valuable work in HTS involving stem cells has first come in the form of identification of molecules involved in either stem cell maintenance [37–42] or cellular differentiation [43–46], and now demonstrating the feasibility of iPSCs in HTS and HCS applications for phenotypic screens, such as for cardiac hypertrophy through expression of B-type natriuretic peptide [47]. Despite the recent advent of hESC and hiPSCs and therefore few HTS/HCS successes to date, there have still been a number advances in drug safety and efficacy studies, disease modeling, and drug lead identification using stem cells.

Table 1
Examples of cell lines used for phenotypic screening. This table contains a list of cell lines that have been used in phenotypic screens for specific diseases. These physiological outcome measures could then be used as a read-out of drug efficacy.
Modified with permission from [24].

Examples of cell types used in phenotypic screens			
Disease	Cell type	Assay type	References
Primary cells			
Thyroid cancer	Thyrocytes	TSH responsive proteins	[106]
Cystic fibrosis	Bronchial epithelial cells	Electrophysiology	[107]
SMA	Fibroblasts	Gem counts	[108]
Immortalized primary cells			
Respiratory papillomatosis	Tumor cells	Cell viability (ATP content)	[109]
Cystic fibrosis	Bronchial epithelial cells	Electrophysiology	[110]
Engineered cell lines			
Huntington disease	PC12	Protein aggregates (GFP)	[111]
SMA	U2OS	RNA splicing (luciferase)	[112]
Pluripotent stem cells			
ALS	iPSCs	TDP-43 aggregation	[25]

3. Stem cells in safety pharmacology

The pharmaceutical industry generates large libraries of new chemical entities each year with the intent on identifying drugs that either interact with a chemical target or induce a desired physiological effect. When effective drugs are identified, further preclinical safety screening is necessary, both for the safety of the patients and to avoid large costs associated with failed clinical trials. Yet, even after exhaustive safety screening for proper adsorption, distribution, metabolism and excretion, drugs have failed in the clinic due to the safety screens not effectively recapitulating human pharmacokinetics and pharmacodynamics [48]. This is true of 36 drugs that were withdrawn from the market between 1994 and 2006 at a great cost in terms of time, money, and confidence in drug development. Of these withdrawn drugs, 45% were cardiotoxic and 37% were hepatotoxic [49]. In the heart, at least some of these withdrawn drugs acted through inhibition of the hERG ion channel. Drug-induced prolongation of the electrocardiographic QT interval through binding to hERG can lead to delays in ventricular repolarization and arrhythmia causing sudden cardiac death [50–54]. Primary canine or rabbit cells have been predominantly used in preclinical in vitro tests [55–57]; however, the inability of these cells to predict human sensitivity suggests that there is a need for the development of additional model systems.

Due to their ability to readily differentiate into cardiomyocytes, human PSCs are being recognized for their value in predicting cardiotoxic drugs (reviewed in [7]). A number of researchers have established in vitro screening assays using human ESC derived cardiomyocytes that examine hERG channel blockers, beta-adrenergic modulators, and cardiac action potential modulators for the identification and prediction of toxic agents [50,58–61]. Similarly, others have recently assessed alterations in calcium flux and beating properties in iPSC-

Table 2
Disease modeling using iPSCs. This table lists a sampling of studies using disease specific iPSCs for the purpose of disease modeling and any associated phenotypic characterization. Modified with permission from [7,85].

Cell type	Disorder	Gene(s)	Phenotype	
Cardiac	Long QT-syndrome type 1, Long QT-syndrome type 2	KCNQ1, KCNH2	Prolonged action potential duration (APD) in atrial/ventricular cardiomyocytes and trafficking defect with reduction in I_{ks} and I_{kr} currents.	[77, 113-115]
	Catecholaminergic Polymorphic Ventricular Tachyhardia (CPVT)	RYR2	Elevated diastolic Ca^{2+} concentrations, reduced SR Ca^{2+} content, increased frequency and duration of elementary Ca^{2+} release events leading to increased susceptibility to delayed after depolarizations (DADs) arrhythmia.	[78]
	LEOPARD syndrome (includes Noonan syndrome)	PTPN11, RAF1, SHOC2	Increased sarcomeric organization and preferential localization of NFATC4 in the nucleus, which correlate with potential hypertrophic state. Study of molecular insights into disease mechanism	[83]
Skeletal muscle	Duchene muscular dystrophy (DMD)	Dystrophin	Reduced dystrophin expression	[116-119]
	Becker muscular dystrophy (BMD)	Dystrophin	Undefined	[116]
Immune	Multiple-sclerosis (MS)	MHC	Functional neurons with some differences in electrophysiological characteristics.	[120]
Neural	Spinal muscular atrophy (SMA)	SMN1	Lack of nuclear gems, motor neuron degeneration, abnormal neurite outgrowth and astrocyte activation.	[27-29,121]
	Schizophrenia (SCZD)	DISC1	Reduced neuronal connectivity, decreased neural number, and altered gene expression profiles.	[122,123]
	Alzheimer's disease (AD)	PS1, PS2	Increased amyloid Ab42 secretion in neurons	[124,125]
	Parkinson's disease (PD)	PINK1, LRRK2	Dopaminergic neurons with morphological alterations, reduced neurite numbers, neurite arborization, impaired Parkin recruitment to mitochondria, increased mitochondrial copy number, upregulation of PGC-1a.	[116,126-128]
	Huntington's disease (HD)	Huntingtin	Neurons with elevated caspase activity	[116,129,130]
	Autism spectrum disorders (ASDs)		No identified phenotype in GABAergic neurons to date.	[131]
	Amyotrophic lateral sclerosis (ALS)	SOD1, VAPB	Reduction in VAPB protein in motor neurons.	[86-88,132,133]
Metabolic	Juvenile onset type 1 diabetes-mellitus (T1D), Type 2 diabetes (T2D)		Differences in insulin-producing cells not defined	[116,134,135]
Blood	Sickle cell anemia	b-Globin alleles, b(s)/b(s)	Erythroid cells expressed low levels of normal HBB transcripts after gene correction	[136,137]
Eye	Retinitis pigmentosa (RP)	RP1, RP9, PRPH2, RHO	Rod photoreceptor cells recapitulated disease through in vitro degeneration and revealed ER stress	[138]

derived cardiomyocytes exposed to over 130 different compounds [62]. However, using PSC derived models is not without challenges. The current patent landscape around PSCs can make it difficult for corporate entities to freely operate. Furthermore, developing the expertise in the maintenance and differentiation of stem cells toward specified cells such as cardiomyocytes can make large-scale transition difficult. Therefore, commercial sources of stem cell derived cardiomyocytes are available from GE Healthcare (Cytiva Cardiomyocytes; ESC) and Cellular Dynamics International (iCell Cardiomyocytes; iPSC) and will likely ease the technical burden for pharmaceutical companies. Importantly, these commercially available stem cell derived cardiomyocytes exhibit similar functional properties as one would expect from heart tissue. For example, iCell Cardiomyocytes, generated from human iPSCs, were found to recapitulate relevant pharmacological functions in regards to hERG, Na^{2+} and Ca^{2+} channel blocking properties [63]. Similarly, Cytiva cardiomyocytes responded appropriately with changes in plasma membrane integrity, calcium homeostasis, nuclear phenotype, and mitochondrial status when treated with the known cardiotoxic compounds doxorubicin, amiodarone, and antimycin [64,65]. Considering the high cost of failed clinical trials, screening for multiple physiological outputs using physiologically relevant cells may justify the use of human stem cells in pre-clinical cardiotoxic studies to replace less reliable methods.

The liver, because of its exposure to high concentrations of drug, is also very susceptible to toxicity and was the second most common cause for drug withdrawn from 1994 to 2006 [49]. Stem cell derived hepatocytes would also be an ideal system for pre-clinical screening for potential hepatotoxic molecules. Although there are a number of protocols for the differentiation of hepatocytes from stem cells (Table 1; [66–71]) a major issue has been to generate hepatocytes that reflect adult like liver functions. There is currently a large research focus into generating mature hepatocytes for cell based therapies and for drug safety screening [64,72]. Medine et al. have just recently developed a method for culturing metabolically active stem cell derived hepatocytes that are functionally comparable in toxicology assays to the gold standard primary tissue [73]. In addition Shan et al. recently utilized HTS to identify small molecules that help mature PSC derived hepatocytes [46]. These breakthroughs in the maturation of hepatocytes were hurdles that needed to be overcome for their use as a model for adult liver and these advances pave the way for their use in hepatotoxic assays in the future.

Though heart and liver may be the primary targets for toxicity screening, there have been efforts in developintg systems to examine nervous system toxicity. For example, there is a great interest in examining metabolic markers that predict developmental toxins in PSC derived neural lineages [74–76]. These valuable studies demonstrate the versatility of PSCs as tools for toxicity screening and provide a system for vastly reducing downstream costs of failed clinical trials. The next step in the development of these screens would be to generate greater genetic diversity of stem cell lines to better represent the world population. Since genetic variability can influence pharmacodynamics and pharmacokinetics, ideally, drug screening would be completed on large cohorts of diseased and healthy PSCs in an effort to segregate responders from non-responders and ensure that any compound effects are affecting the intended disease target. PSCs provide a robust system in which to do this, but as we discuss later, technical and financial challenges need to be addressed before biomanufacturing facilities could take on such a large endeavor.

4. Disease modeling and drug screening

Much work has gone into identifying disease relevant defects in iPSC derived models that can be targeted for drug screening efforts. To date, most advances in cardiomyocyte drug screening have come in the form of toxicity screening rather than lead identification for specific cardiac related disease. However, iPSC derived cardiomyocytes have been used to evaluate the therapeutic efficacy potential of new and existing pharmacological agents. For example, Itzhake et al. generated iPSCs from patients with long-QT syndrome (LQT) and demonstrated that LQT-iPSC-derived cardiomyocytes increased arrhythmogenicity in the presence of potassium channel blockers, whereas calcium channel

blockers ameliorated this phenotype [77]. Similarly, the disease catecholaminergic polymorphic ventricular tachycardia (CPVT) was modeled with patient derived iPSCs [78]. The CPVT-iPSC derived cardiomyocytes recapitulated the expected arrhythmias, and were rescued with the drug Dantrolene. It may be that screening drug libraries on iPSCs and monitoring electrophysiological properties are hampered by the relatively low throughput capabilities of this type of analysis. However, development of automated systems and high throughput physiological assays may allow this to be a more prominent screening system in the future [79–81]. Modeling cardiac disease caused by mutations in genes other than ion channels has relied on phenotypic assays that may be more amenable to HTS including cardiomyocyte size, nuclear redistribution of proteins, force of contraction, or mitochondrial health [82–84].

Certainly, targeted approaches for drug development from PSC derived disease models are possible. However, the novelty of human PSCs is in their ability to recapitulate a disease relevant phenotype in a dish. Neurodegenerative diseases have provided a vast source for future drug exploration opportunities because of the great complexity involved in the central nervous system. Progress has been made in phenotype evaluation of human iPSCs in regard to neurodegeneration (Table 2; further reviewed in [7,85]). For example, Amyotrophic Lateral Sclerosis (ALS) is a condition in which the loss of upper and lower motor neurons becomes fatal, generally within 5 years of diagnosis. iPSCs have been generated from a number of different familial forms of ALS (Table 1), and it has been found that stressors or single-cell longitudinal studies are necessary to see reductions in motor neuron survival [86,87]. These groups acknowledge the importance of using complementary assays to detect subtle phenotypic changes in ALS derived iPSCs compared to control lines. In a different study examining ALS, Yang et al. used mouse iPSCs carrying a motor neuron reporter gene to screen motor neuron survival after trophic factor withdrawal in the presence of approximately 5000 small molecule compounds at varying concentrations [88]. Two compounds, tyrphostin A9 and kenpaullone, not previously identified as motor neuron protective were identified. They chose to further characterize kenpaullone because of its protective capacity for not only the ALS motor neurons, but for the wild type motor neurons as well. Interestingly, a follow-up study exploring neuroprotection in human derived ALS-iPSCs also demonstrated the effectiveness of kenpaullone compared to two other compounds that had each failed to demonstrate efficacy in clinical trials. Importantly, human iPSC derived motor neuron cultures appear to have predicted this lack of efficacy.

5. Challenges to PSC implementation in drug development

As demonstrated above many academic and industry laboratories have made substantial efforts toward developing systems for modeling human disease, exploring novel treatment paradigms, and testing the safety of current drugs using human PSCs. Despite these advances and the great promise PSC model systems provide, there is still much work to do. Some of the challenges moving forward include: 1) reducing cost, 2) standardizing HTS/HCS by reducing differentiation variability and enriching specified cell types, 3) improving maturation of cells toward more adult phenotypes, and 4) amassing more characterization and validation data of toxicity, safety, and disease phenotypes against known compound standards.

There is a huge financial burden of growing, maintaining, and differentiating PSCs compared to other rodent or human cell lines. First, PSCs require specific reagents for both undifferentiated colonies as well as differentiated cell types that can be 2–10 times the cost of reagents used to grow other cell lines. Second, culturing times can last upwards of 2–6 months to generate fully differentiated cell types, which use large amounts of the costly medium and specialized reagents. Third, PSCs require daily maintenance to ensure that the cells are performing as expected, which either requires several experienced technicians or expensive automated systems. High content screening requires a large number of differentiated cells, and the workflow from undifferentiated to specified cell type requires a great deal of maintenance. The creation of scalable culture systems that require less maintenance and increase yield would be beneficial for drug screening. To this end, multiple groups have developed systems for maintaining PSCs as aggregate cultures [89–92]. These systems allow scaling up of differentiation while reducing maintenance time and are worth further examination. Alternatively, industrial sources of specified cells are also available. For example, Cellular Dynamics International currently offers differentiated and qualified cardiomyocytes, endothelial cells, hepatocytes, and neurons. The commercial availability of these cells removes concerns for the end user over consistent maintenance and differentiation protocols. Furthermore, for commercial use, acquiring these cells could simplify licensing agreements allowing for greater freedom to operate. However, expense will likely still be an issue because the vendor must recoup the cost of generating the PSCs, which will be passed onto the consumer. Nevertheless, as new culture protocols are developed, there is hope that costs will begin to decline.

The use of PSCs is still relatively new, and differences in differentiation efficiencies are a concern [93]. However, improvements in differentiation techniques have come from a number of different sources. Rather than converting somatic cells to the fully undifferentiated iPSC state and then advancing forward to a specific cell type, multiple groups have demonstrated the ability to directly convert somatic cells into neurons and cardiomyocytes, effectively bypassing undifferentiated iPSCs [94–98]. This process generates more purified populations of the desired cell type, but each differentiation requires genetic manipulation that can introduce batch-to-batch variability. As such, this method may not be ideally suited for large scale drug screening and discovery, but it does demonstrate our increased ability to understand and manipulate developmental biology. As our knowledge of these developmental systems increase, so will our ability to improve differentiation of PSCs.

There is also a need to identify and isolate specified cell types from PSCs, especially in the neural field. Unfortunately, many antibodies that have historically been used to identify subsets of neurons are not entirely specific. In vivo applications take advantage of discrete anatomical locations to aid neuron identification, such as in the case of motor neurons in the ventral horn of the developing neural tube. To differentiate PSCs in vitro, cells are exposed to specific chemicals in a time and concentration-dependent fashion designed to simulate in vivo patterning processes. However, dissociated cells in vitro lack the anatomical context, which can complicate cell identification. The development of additional markers for mature subsets of cells may be possible with mass spectrometry techniques to identify unique extracellular surface proteins [99]. These targets would provide the field with unique tags upon which to generate antibodies for both identification and subsequent isolation through flow activated cell sorting.

Once the differentiated cell types are identified and isolated, there is a need to ensure that the cells are behaving in a physiologically relevant manner. Numerous studies have shown that PSC-derived cardiomyocytes beat and PSC-derived neurons generate action potentials indicative of a basic physiological function, but the majority of the data suggest that cells behave more similar to immature fetal cells rather than adult cells [100]. For example, cardiomyocytes derived from multiple hESC and iPSC lines all showed action potential properties of immature cells due to the lack of the inward rectifying potassium channel Kir2.1. Importantly, when Kir2.1 was over-expressed in PSCs, the electrophysiological phenotype was indistinguishable from an adult cardiomyocyte [100]. In the neural field, studies have shown that astrocyte contact, not just astrocyte-derived factors, is necessary for hESC-derived neurons to form mature action potentials [101]. This raises a concern that PSC-derived neuronal cultures will not actually recapitulate physiological functions in isolation. Taken together, more research is needed to identify biological pathways that control cell

maturation in order to ensure that PSC-derived cells are fully exhibiting the particular properties of interest.

6. Conclusions

The development of iPSC technology spurred the use of PSCs in disease modeling and drug exploration. As scientists begin to better understand the use of these cells and develop more reliable methods for differentiation, modeling, and screening, PSCs will likely aid the drug discovery process and reduce attrition rates. Though one must be aware of the challenges and caveats of using PSCs, recent advances and proof of concept retrospective studies provide the framework for improvements in drug discovery.

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