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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.

Contents

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