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Consideration of the cellular microenvironment: Physiologically relevant co-culture systems in drug discovery☆

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ABSTRACT

There is renewed interest in phenotypic approaches to drug discovery, using cell-based assays to select new drugs, with the goal of improving pharmaceutical success. Assays that are more predictive of human biology can help researchers achieve this goal. Primary cells are more physiologically relevant to human biology and advances are being made in methods to expand the available cell types and improve the potential clinical translation of these assays through the use of co-cultures or three-dimensional (3D) technologies. Of particular interest are assays that may be suitable for industrial scale drug discovery. Here we review the use of primary human cells and co-cultures in drug discovery and describe the characteristics of co-culture models for inflammation biology (BioMAP systems), neo-vascularization and tumor microenvironments. Finally we briefly describe technical trends that may enable and impact the development of physiologically relevant co-culture assays in the near future.

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1. Introduction

Over the past 30 years, robotics and high throughput screening (HTS) technologies have revolutionized the drug discovery process, influencing cell-based as well as biochemical approaches. Cell-based assays enable selection and characterization of compounds based on functional effects in intact cells. These functional effects can be measured in a variety of ways including: changes in cell components (e.g. protein, mRNA, or metabolite levels) or component states (phosphorylation state, methylation, etc.), physical properties of cells (e.g. shape, proliferation, chemotaxis or impedance), or in the subcellular localization of organelles or molecules (e.g. as can be assessed by high content screening). The advantages of cell-based assays over biochemical assays include the ability to (1) assess targets in physiologically relevant settings, (2) evaluate entire pathways and multiple targets in a single assay format, and (3) characterize compounds with unknown targets or targets that are not amenable to biochemical approaches. Cell-based assays can range in their suitability for high throughput compound testing, however, and in the past, the more physiologically relevant but complex assays have been restricted for use in the evaluation of small numbers of test agents. As interest in phenotypic drug discovery increases, so does interest in methods for developing physiologically relevant assays that are also suitable for industrial drug discovery.

In the pharmaceutical industry most cell-based screening is performed in immortalized cell lines, often engineered to overexpress targets or reporter constructs. Cell lines are attractive to use, due to their ease of culture, expansion potential, and suitability for the prosecution of high-throughput screens. However, generation of cell lines involves the identification of cell clones which differ from their in vivo counterparts by proliferating robustly ex vivo, an experimental condition which may select for cell clones exhibiting enhanced growth characteristics and potentially altered regulatory and signal transduction pathways. Since correlation of in vitro and in vivo studies are frequently discordant, efforts to develop more physiologically relevant in vitro assays which better translate to in vivo biology are of fundamental importance.

For assays to be used for phenotypic drug discovery, in programs without prior identification of a molecular target, it is important to establish that the assays to be used are validated to be relevant to the disease process. Although technically challenging, primary human cells are attractive to use for screening as they are phenotypically more similar to normal cells and retain the normal regulation of their growth pathways. Primary human cells are now available from many tissues [1]. And while three dimensional (3D) model systems and engineered tissues are attractive to pursue as they can provide architecture that is considered more physiologically relevant, due to their limited scalability, they are currently more applicable to basic research or transplantation medicine rather than industrial drug discovery.

We have found that the use of primary human cell-based co-cultures provides a significant step towards physiological relevance, but in two-dimensional (2D) formats that are more easily scaled. Here we will focus on the use of primary human cells and co-cultures in industrial drug discovery applications, as their utilization is becoming more widespread.

2. Industrial drug discovery

The pharmaceutical drug discovery process has a number of steps that can be subdivided into preclinical and clinical components (Fig. 1A). In the pre-clinical or discovery phase, pharmacologically active agents are identified and optimized in the lead generation and optimization phases, respectfully. For small molecules, compounds are screened in medium throughput (MTS) or in high throughput (HTS), corresponding to tens of thousands or hundreds of thousands of molecules, respectively. Following initial testing, compound activity is confirmed in dose response experiments and the structures of active molecules of interest are used to identify similar or related untested molecules, a process called hit expansion. Compounds with a combination of promising potency, efficacy, chemical structure, and physical properties and that demonstrate structure activity relationships are tested in pre-clinical disease models and used as the template for subsequent cycles of chemical synthesis and pre-clinical testing in animal models. Identification of a safe and therapeutically efficacious compound in animal models allows selection of a clinical candidate which is scaled up for clinical trial safety testing in healthy human volunteers or patients (Phase 1), dose finding testing in patients (Phase 2) and final efficacy testing in patients (Phase 3). It should be noted that the attrition rates of preclinical, Phase 1, Phase 2, and Phase 3 have been estimated as approximately 80%, 50%, 70%, and 50%, respectively [2] which underlines the many intrinsic hurdles and high risk nature of drug discovery. Indeed, it has been this high rate of failures in clinical testing that drives the interest in pursuing more physiologically relevant screens in drug discovery.

The topic of this review, physiologically relevant co-culture assay systems, is an important component of the discovery, and preclinical phases of the drug discovery process as illustrated in greater detail in Fig. 1B. The goal of preclinical research is to identify a molecule that is safe and efficacious in animal models and that is also likely to be active in humans, where human cellular models of therapeutically relevant conditions can be applied. Although conceptually straightforward, the majority of clinical trials fail due to lack of human efficacy [3]. This illustrates practical difficulties of translating preclinical results to clinical trials and simultaneously underlines the scientific and business drivers for development of preclinical models with enhanced clinical translation such as disease relevant in vitro assay systems.

Contemporary drug discovery research has relied heavily on the identification of a molecular target thought to be physiologically relevant and where in vivo modulation of activity is expected to be therapeutically beneficial. Such hypothesis driven drug discovery approaches have been termed “targeted drug discovery” (TDD) and have been popular since the integration of molecular biology capabilities and the elucidation of novel drug targets from exon and genomic sequencing [4]. Typically in TDD, drug target specific assays are enabled and utilized for MTS or HTS and target selectivity assays frequently follow to establish the specificity of confirmed actives to the molecular target of interest. If biochemical screening/selectivity assays were utilized for screening, cell based assays (frequently using genetically engineered cell lines overexpressing the molecular target and/or substrate) are utilized to determine whether confirmed biochemical actives modulate the molecular target in a cellular context (Fig. 1B). In the TDD strategy the ability of a compound to modulate a therapeutically relevant biomarker or response in a physiologically relevant cellular system is not addressed until several steps beyond MTS/HTS and just preceding in vivo testing (Fig. 1B).

Interestingly, despite the emphasis on hypothesis driven TDD approaches, phenotypic drug discovery (PDD) approaches account for the majority of first-in-class new molecular entities (NMEs) that have attained US FDA approval [5,6]. Unlike TDD, the PDD strategy is empirical and relies on direct chemical interrogation of a physiologically relevant biological system to identify compounds that modulate therapeutically relevant endpoints [4].

Physiologically/therapeutically relevant cellular systems can therefore be positioned at various stages of the preclinical discovery workflow depending on the specific needs of the project and the choice of lead generation strategy (Fig. 1B) which in turn defines the prerequisite operational parameters, throughput, and level of statistical validation required from the assay. For example projects utilizing a PDD strategy may utilize co-culture systems in lead generation and compound screening where tens to hundreds of thousands of compounds are initially tested whereas TDD approaches may utilize the same physiologically relevant cellular model two or three steps following lead generation and may be required to test hundreds of compounds. Co-

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