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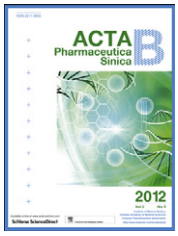

REVIEW

Quality control of cell-based high-throughput drug screening

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Abstract The pharmaceutical industry is presently suffering difficult times due to low productivity of new molecular entities. As a major source of drug leads, high-throughput screening (HTS) has been often criticized for its 'dead end' lead compounds. However, the fruitful achievements resulting from HTS technology indicate that it remains a feasible way for drug innovation. Because of increasing considerations of earlier stage ADMET (absorption, distribution, metabolism, excretion and toxicity) in drug development, cell-based HTS is highly recommended in modern drug discovery for its ability to detect more biologically relevant characteristics of compounds in living systems. This review provides a systematic and practical description of vital points for conducting high quality cell-based HTS, from assay development to optimization, compound management, data analyses, hit validation as well as lead identification. Potential problems and solutions are also covered.

Abbreviations: R&D, research and development; ADMET, absorption, distribution, metabolism, excretion and toxicity; cAMP, cyclic adenosine monophosphate; GPCR, G protein-coupled receptor; NR, nuclear receptor.

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1. Introduction

High-throughput screening (HTS), driven by the great progress in automation technology and combinatorial chemistry, has been widely implemented in drug discovery since the early 1990s and rapidly became one of the major sources of drug leads. Pharmaceutical companies, such as Pfizer and Glaxo SmithKline, were among the early leaders. In the past twenty years or so, many academic institutions joined the 'screening mania' and simultaneously, hundreds of screening centers appeared, as molecules available for screening continued to increase. However, in spite of constant increases in research and development (R&D) expenditures, the number of new chemical entities (NCEs) that reached to the market has actually decreased[1,2]. Analyses show that leads originating from HTS are responsible for over 60% of clinical trial failures[3,4]. Nonetheless, the data also indicate that among 58 drugs that were approved between 1991 and 2008, 19 were attributed to HTS[5]. Without question, HTS is still a feasible approach to drug innovation. The problem becomes one of how to improve the quality of leads arising from drug screening that may result in increased productivity of new molecular entity (NME) entering to the market place.

Since HTS has not substantially improved the drug discovery process and increased R&D spending has not led to a proportionate increase in new drug output, the pharmaceutical industry is looking back to the golden age of phenotype-oriented drug discovery[6] and considering ADMET (absorption, distribution, metabolism, excretion and toxicity) of leads at earlier stages in drug development. A potential way to do this is through the use of cell-based assays. Cell-based assays not only obtain potencies of compounds but also detect cytotoxicity, permeability and effects on growth at the same time, which can be viewed as predictors for late development. Cell-based assays accounted for 52.6% of all HTS efforts in 2006[7] and became more favorable in recent years. However, cell-based assays are generally more complicated than biochemical ones and their performance could be undesirable under certain circumstances. Thus, quality control is of paramount importance and will be discussed in detail below. Although several comprehensive reviews are accessible in the public domain, this article attempts to give key points relevant to carrying out high quality cell-based HTS in a systematic and practical manner with potential problems and solutions highlighted.

Basically, HTS program consists of five parts: target identification, reagent preparation, assay development, compound management and high-throughput screening[8]. Among them, target identification and reagent preparation are beyond the interest of this review, although both of them are vital for successful HTS. Instead, we will cover topics such as assay development and optimization, compound management and data analysis, as well as hit identification and lead validation. High content screening (HCS), as an important part of cell-based HTS, has attracted significant attention recently because of its multiplexing and functional cell based characteristics[9]. However, considering the complexity of its data analysis, HCS is not included in this discussion.

2. Assay development

Cell-based assays or screening models, as the fundamental ingredient of HTS, are approaches used for sensing functional changes of targets under the stimulatory or inhibitory effects of compounds. In biochemical assays, targets are generally specified, while for cell-based assays, the exact target is not required. It could be a specific molecule or a particular signaling pathway, even the whole cell. For example, in cell death assays[10], organisms such as bacteria, fungi, parasites and mammalian cells are directly used as screening models. These whole cell based screenings are highly physiologically relevant, thus providing opportunities to discover entirely novel drugs and drug targets. However, subsequent pharmacological characterization and target identification could be exhaustive. Most of the time, specific targets are decided as soon as screening assays are proposed.

Cell lines used for HTS can be roughly divided into two classes, primary and engineered cells. With technology advancements, such as HCS, ion channel patch-clamp and atomic force microscopy, screenings with primary cells become increasingly feasible and trendy[11]. Several selected primary cell types, originating from human or other species, are commercially available (e.g., Clonetics, Walkersville, MD, USA) and amenable to HTS. As far as mammalian cell based assays are concerned, large-scale primary cell culture still poses some difficulties. Therefore, engineered cells remain the major type of cell lines used in HTS. In the following discussion, we offer some general ideas and tips for generation of engineered cell lines and related detection methods.

2.1. Cell line generation

To provide sufficient signal output for detection, cell-based assays require high expression of targeted proteins, which in naive cells is often low and needs to be up-regulated through either transient or stable gene transfection[12]. The transient transformed cell lines briefly express high level of targeted proteins, but display relatively larger variances in expression quality due to transfection inefficiencies. Stable transformed cell lines consistently express targeted proteins over a long period of time, while their expression levels are usually not as high as transient ones. Both strategies can be employed in HTS[13], but stable expression is much more preferred because of reduced cost and less assay variation[14].

Generally, gene transfection requires primary knowledge about the sequence of a targeted protein for vector construction. It is noted that some genes of interest are protected by patents that prohibit commercial use. Alternative strategies must be sought under such a situation: one can either increase the expression level of a particular gene through activation of internal gene scripts[15,16] or introduce specific transcriptional factors[17]. Sometimes, simply increasing the expression of a targeted gene is not sufficient to yield a high signal output and genes involved in the same signaling pathway may also need to be enhanced. For instance, G protein enriched cell lines, such as $G_{\alpha}16$ or $G_{qi}5$ transfected CHO cells, are preferred to screening G protein coupled receptor (GPCR) modulators.

2.2. Detection methods

A variety of methods, such as reporter gene, fluorescence/bioluminescence resonance energy transfer (F/BRET), calcium mobilization and label-free detection, have been applied to