

High throughput screening of small molecule library: procedure, challenges and future

Abstract

High-throughput screening (HTS) of small molecules is used to identify and characterize chemical compounds based on their response on particular assays. The screened compounds can be further used as tools to study complex molecular pathways involved in progression of cancers and potential candidates can be further developed as drugs. Testing of vast number of compounds and fast readout technologies for assays in a timely manner has made this procedure a popular drug-discovery process, which is widely used in the pharmaceutical industry. However, the procedure of screening is a multi-step process and is prone to give false positive and false negative results. Assurance of quality and good analysis of results play critical role in finding a true product. Technological hardware like robotic plate handling is critical for the procedure. Reagents like antibodies and recombinant proteins can also make the whole procedure very expensive. Increased number of available compounds and molecular targets has raised the requirements for even faster procedures, more sensitive assays to run and even smaller sample size assays for screening. Development of new methods like Droplet-based cell encapsulation technology has given hope for a better future of HTS.

Keywords: high-throughput screening (HTS), small molecules, target identification, assay design

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Abhay A Shukla

University of Texas Southwestern Medical Center, USA

Correspondence: Abhay A Shukla, University of Texas Southwestern Medical Center, Dallas, Texas, USA, Tel 2142541993, Email abhay.shukla@utsouthwestern.edu

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Introduction

Cancer kills around 7.6 million people worldwide every year and about a half million people in the US annually. Early detection and more effective chemotherapy have improved cure rate among patients. However, patients diagnosed with advanced stage cancers still have very few options for treatment. It has been shown that there are short and long term adverse effects of chemotherapy. Both adult and pediatric cancer survivors face significant health burdens including but not limited to impaired end-organ function, risk of secondary cancers and shortened life expectancies.^{1,2}

Development of personalized medicine strategy is becoming more popular. It focuses on identifying drugs that can target a molecular pathway or more specifically a protein involved in an uncontrolled or disrupted signaling pathway leading to the progression of particular cancer. Genetics studies are confirming addictions and weaknesses of cancer cells on such pathways and specific molecules. With this approach, normal tissues are spared from getting targeted by toxic chemotherapies.

Drug discovery programs start to find a cure for a disease, which does not have suitable treatment available. Data generated by primary research suggest involvement of specific proteins in the progression of diseases and can be used as target for drug development.³ An assay is then developed that can be used to screen small molecule chemical libraries. The technique of high-throughput screening (HTS) utilizes small molecule chemical compound libraries comprising several hundred thousand compounds. Sorafenib, the first drug identified through HTS, is in the market now. Other drugs for various targets including HSP90 and PI3Kinase are undergoing clinical trials.⁴

The whole process of HTS can be divided into steps of target identification, assay design, primary and secondary screens, data analysis and identification of hits (Figure 1). The role of HTS is

not only limited to identifying new compounds but is also useful in assaying current drugs and their derivatives to improve activity and functioning.⁵ With advancement in the human genome project and synthesis of new chemical compounds, the role of HTS has become even more important in the fight against cancer. Different steps of HTS will be discussed briefly in this review followed by challenges and recent advancement of HTS.

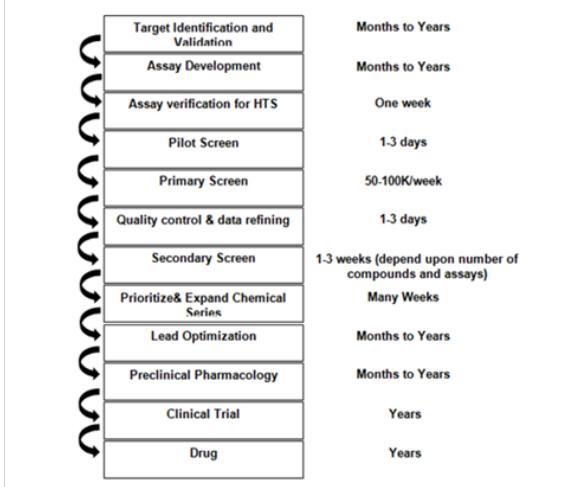


Figure 1 HTS process and drug development. Steps in HTS process and drug development have been given. Approximate time for each step is given.

Target identification and validation

Target identification is one of the most important steps in developing a new drug.³ Recent advances in published studies discussing whole genome sequencing, exome sequencing, and copy number analysis and RNA sequencing using multiple tumors and normal tissues are

generating data that is very useful in target identification.³ This leads to a specific target based approach for HTS. Another commonly used approach is to perform phenotypic screening. This screen can potentially lead to the identification of compounds that change or identify a disease phenotype.³

Target validation is achieved by either using cell or animal based models. Identified targets can be regulated using gene over expression and knockdown methods. Cell culture based cancer progression assays can quickly confirm the role of particular targets. Likewise, xeno grafts in mice using established tumor cells or patient derived tumors having either over or under expressed genes can suggest the role of particular targets in progression of cancer. Use of transgenic animals with advancement in inducible and tissue specific knockout of genes makes it a very popular model for validation.³

Assay development and verification

Cell based assays are most commonly used for HTS. A cell based assay could use a reporter gene assay, in which the readout is a luminescent protein (Luciferase proteins) under control of a specific gene promoter. Small molecules can be identified which specifically increase or decrease expression of the luciferase gene. Phenotypic assays are another option where a change in phenotype caused by abnormal signaling pathways can be measured.⁶ Zebra fish embryos have been successfully used for this kind of assay.⁷ Other commonly used assays are second messenger assays that monitor changes in signal transduction and cell proliferation assays that result in changes in growth rate of cells.⁵ Biochemical assays are another kind of assay used in HTS. Commonly used ones are fluorescence resonance energy transfer (FRET), fluorescence polarization (FP), homogeneous time resolved fluorescence (HTRF), etc.⁵ A comparison of cell based and biochemical assays has been reviewed by Moore & Rees.⁸

Verification of assays is a critical step in HTS. Careful selection of cells for the assay is important. Reproducibility of the assay is another requirement for a successful screen. Use of proper positive and negative controls and/or drug vehicle only (e.g. DMSO) controls are essential. A statistical factor Z' determines the performance and quality of the assay. Signal to background ratio and assay signal variation can change the Z' factor. The acceptable value of Z' factor varies from 0.5-1. A Z' factor value close to 1 confirms a robust screen.^{6,9} Micro well plates are used to run assays. 384-well plates are the most preferred format to run screens. Decreased number of cells also results in decreased signal intensity, which can result in loss of potential good compounds. A machine to dispense compounds is a must in a robotic-centric HTS system.

Software used in HTS

Spot fire by TIBCO and Vortex by DOTMATICS are two commercial available software packages available to conduct analysis of HTS data. HTS Navigator is software which is freely available and has been shown to efficiently process and analyze the diverse data.⁹ Besides commercially available comprehensive analysis tools, there are also numerous open-access software packages designed for HT screening data management (eg. Screen saver, K-Screen, HTS-Corrector). More information is available at <http://dx.doi.org/10.5772/52508>.

Challenges

One of the challenges that researchers face is toxicity induced by the compound. Small molecules can be very toxic in nature and produce

cell death even at low concentrations. Toxicity of a compound is also a model specific. Finding specific targets of screened small molecules is another challenge. Basic biochemical methods are used to identify specific targets but may lead to false identification. Nonspecific targets of a compound are another problem. Expanding chemical series and lead optimization work (structure activity relationship analysis (SAR) may take years. Cost of reagents like antibodies and recombinant proteins can make the whole screening process very expensive and lengthy.

Future

Miniatrization of the assay format could resolve the cost issue and can also make the whole process of library screening very fast. Several companies have managed to adopt 1536 well plate for assays. There are also some examples of screening using 3456 well plates.¹⁰ More use of primary cells for HTS is in the future. Strategies to design the assay to target multiple genes at the same time are required to attain a phenotype change by small molecules.¹¹ Whole animal testing via cassette dosing is a procedure adapted by HTS to rapidly access pharmacokinetics of many drugs. Drug affinity responsive target stability (DARTS) and Target Identification by Chromatographic Co-Elution (TICC) methods can make target identification easier.^{12,13} Droplet-based micro fluidic approaches involve working with reduced sample volumes and single-cell analysis capabilities. Droplet micro fluidics uses a 2-phase system. Cells are captured in a micro droplet (1 pL to 10 nL) covered by immiscible oil. Small number of cells can be analyzed in separate droplets; making this technology suitable for working with stem cells and primary cells.¹⁴

In summary, multiple target approaches, better quality data, miniaturization of the assay and quicker SAR analysis will make HTS a commonly used method in not only identifying new drugs and drug validation but also in academic drug discovery.

Acknowledgments

None.

Conflicts of interest

Author declares that there is no conflict of interest.

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