Systematizing Serendipity for Cardiovascular Drug Discovery

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Serendipity has played a significant role throughout the history of drug discovery in general and, in particular, throughout the history of cardiovascular medicine. The anticoagulant properties of dicoumarol were discovered after farmers observed that their cattle were dying of internal hemorrhaging after feeding on rotting sweet clover,1 and digitoxin was identified when the condition of a patient suffering from congestive heart failure dramatically improved after being given an herbal remedy containing extracts of the foxglove plant.2 In fact, many of the most effective pharmacological agents in use today arose through serendipity.

Despite the historical role of serendipity in drug discovery, it is clear that such events occur too rarely to be counted on as consistent sources of future drug discovery. Over the past 3 decades, the standard drug discovery paradigm has shifted more and more toward a target-based approach, in which specific proteins are chosen, on the basis of the current understanding of disease mechanisms, as “targets” for drug discovery efforts. Once a target has been identified, high-throughput screening can often identify small molecules that inhibit the target’s activity, and, in some cases, these molecules can be developed further as drugs.

Well-validated targets can lead to successful drugs, but for many cardiovascular diseases, it remains difficult to predict which targets will offer a therapeutic benefit. Recently developed genomic and proteomic techniques, including gene expression profiling, RNA interference, and in silico molecular modeling, have generated long lists of potential drug targets, but determining which of the potential targets actually have therapeutic value has been much more difficult. Much of the difficulty involves predicting which targets will reverse disease processes, especially when dealing with diseases with complex underlying biology. Indeed, the high rates of cumulative attrition of supposedly validated drug candidates are consistent with a recent study by the Congressional Budget Office, which concluded that despite increased investments, new drug development has been in sharp decline during the last 10 years.3,4 Although there are many contributing factors for this decline, a significant underlying problem involves the process of target selection and validation.5 The supply of new, well-validated targets is dwindling, and many devastating diseases cannot be easily addressed with our current target-based drug discovery paradigm because of the lack of validated targets.4,6

Modern chemical biologists are beginning to develop innovative ways to discover drug leads, even in the absence of well-validated targets. With the use of “phenotype-based” approaches, a disease process of interest can be modeled in cultured cells or animals. Then high-throughput screening can be conducted to identify small molecules that can reverse the disease process (Figure 1). Conducting small-molecule screens that are based on a phenotype (morphological, physiological, or behavioral) holds great potential for discovering novel chemical modulators, regardless of the biological pathway or target affected, because such screens are not limited to a specific, predetermined target and do not require extensive biological validation to be performed. In this way, phenotype-based chemical screens help to discover therapeutic small molecules for diseases without known targets, as well as new targets that can then be fed into more conventional target-based drug discovery programs. Cardiovascular diseases in particular have the potential to benefit greatly from the application of phenotype-based chemical screens because so many different biological pathways are involved in cardiovascular functions and because only a fraction of these pathways have been explored for therapeutic benefit to date. Given the persistent prevalence of cardiovascular disease, its high morbidity and mortality rates, and the total direct and indirect annual economic costs (>$430 billion), it is imperative to develop new methods with which to identify therapeutic small molecules for the treatment and prevention of cardiovascular diseases.7 In this review, we will describe several of the phenotype-based small-molecule discovery approaches that are beginning to complement the more established target-based approaches to cardiovascular drug discovery.

Conducting Phenotype-Based Small-Molecule Screens

Biological Systems

The systematization of serendipity requires a physiologically relevant biological system that is amenable to high-throughput and often high-content small-molecule screens. For disease processes that are largely cell autonomous (that occur at the level of the single cell itself), cell-based biolog-
tical systems can fulfill both criteria. An especially attractive new area of research uses cultured stem or progenitor cells to identify small molecules that induce cardiomyocyte proliferation and differentiation. Given that the adult mammalian heart is composed mainly of postmitotic, terminally differentiated cells, the discovery of small molecules that regulate cardiomyocyte renewal could have important implications for cell-replacement therapies for cardiovascular diseases. In one example, Qyang et al. identified not only 2 unknown compounds but also the glycogen synthase kinase-3 inhibitor 6-bromoindirubin-3-oxime (BIO) as important modulators of cardiomyocyte proliferation and differentiation. Additionally, 2 separate groups used embryonic stem cells as the biological system to discover novel small-molecule regulators of cardiomyocyte differentiation. Takahashi et al. used embryonic stem cells stably transfected with an enhanced green fluorescent protein reporter, under the transcriptional control of the cardiomyocyte-specific α-myosin heavy chain promoter, and screened 880 known drugs for enhanced fluorescence. They found that ascorbic acid (vitamin C), but no other antioxidants, significantly induced embryonic stem cells to differentiate into cardiomyocytes. Sadek et al. performed a similar screen in mouse P19CL6 pluripotent stem cells containing a luciferase reporter gene inserted into the Nkx2.5 locus of a 178-kb mouse bacterial artificial chromosome. After screening roughly 147 000 small molecules, they identified a family of sulfonyl hydrazones that induce cardiac mRNA and protein expression in several biological contexts, including human peripheral blood mononuclear cells, suggesting that such compounds might drive embryonic stem cells toward a cardiac fate and could aid in the generation of functional cardiomyocytes for cell-based therapies.

Beyond using cell identity as a phenotypic readout, it is now feasible to assess the effects of small molecules on the electrophysiological properties of cells, including cardiomyocytes, in a high-throughput manner. Despite the validation of ion channels as promising targets for the treatment of cardiovascular diseases, a major challenge for the discovery of new ion channel modifiers has been the difficulty in establishing a high-throughput screening platform that uses electrophysiology to characterize the effects of small molecules on ion channel function. Several companies have now developed instruments for performing high-throughput, automated patch clamping to evaluate the effects of small molecules on both voltage-gated and ligand-gated ion channels. An additional motivation for the development of high-throughput cellular electrophysiology is to have the ability to screen potential drugs for cardiotoxic side effects. Many drug failures, including notable cases like terfenadine (Seldane), are caused by off-target interaction with the potassium channel human Ether a-go-go Related Gene (hERG), causing QT prolongation and cardiac arrhythmias. The ability to screen compounds for off-target effects on ion channels is critical for evaluating potential drugs. However, the development of high-throughput screening platforms utilizing electrophysiology also holds the promise to discover novel therapeutic modifiers of ion channel physiology.

The cardiovascular system offers a highly diverse palette of phenotypes that can be used as the basis for phenotype-based small-molecule discovery. An important challenge for future cardiovascular drug discovery is the generation of cellular systems can fulfill both criteria. An especially attractive new area of research uses cultured stem or progenitor cells to identify small molecules that induce cardiomyocyte proliferation and differentiation. Given that the adult mammalian heart is composed mainly of postmitotic, terminally differentiated cells, the discovery of small molecules that regulate cardiomyocyte renewal could have important implications for cell-replacement therapies for cardiovascular diseases. In one example, Qyang et al. identified not only 2 unknown compounds but also the glycogen synthase kinase-3 inhibitor 6-bromoindirubin-3-oxime (BIO) as important modulators of cardiomyocyte proliferation and differentiation. Additionally, 2 separate groups used embryonic stem cells as the biological system to discover novel small-molecule regulators of cardiomyocyte differentiation. Takahashi et al. used embryonic stem cells stably transfected with an enhanced green fluorescent protein reporter, under the transcriptional control of the cardiomyocyte-specific α-myosin heavy chain promoter, and screened 880 known drugs for enhanced fluorescence. They found that ascorbic acid (vitamin C), but no other antioxidants, significantly induced embryonic stem cells to differentiate into cardiomyocytes. Sadek et al. performed a similar screen in mouse P19CL6 pluripotent stem cells containing a luciferase reporter gene inserted into the Nkx2.5 locus of a 178-kb mouse bacterial artificial chromosome. After screening roughly 147 000 small molecules, they identified a family of sulfonyl hydrazones that induce cardiac mRNA and protein expression in several biological contexts, including human peripheral blood mononuclear cells, suggesting that such compounds might drive embryonic stem cells toward a cardiac fate and could aid in the generation of functional cardiomyocytes for cell-based therapies.

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phenotypes that not only faithfully recapitulate specific aspects of cardiovascular disease but also are amenable to high-throughput screening. Cell-based approaches have distinct advantages over cell-free systems, including built-in selection for cell permeability and lack of cellular toxicity. Cell-based systems have already been used to discover novel regulators of cardiac hypertrophy involving the nuclear factor of activated T cells signaling pathway, to decipher the role that Wnt signaling plays in cardiomyocyte specification and proliferation, and to discover novel enhancers of myocardial repair and regeneration.8,10,11,14,16

In the future, it will be important to develop multidimensional bioassays that enhance the overall content coming from screens and hence increase the amount of useful information gleaned from small molecule–induced phenotypes. Bioassays will be designed to detect multiple phenotypic changes simultaneously (eg, myocardial disarray, gene expression, cardiomyocyte proliferation, nuclear localization of fluorescently tagged proteins). Fortunately, the potential for multidimensional bioassays has progressed greatly in the last decade, in part as a result of the development of automated microscopes and cell image analysis software. Although it is still quite time consuming to perform image-based small-molecule screens (ranging from 10 000 to 100 000 compounds), the ability to obtain large amounts of information will undoubtedly improve the selection of high-quality small-molecule hits. Indeed, multidimensional, image-based chemical screens are no longer stuck in the theoretical realm of chemical biology, and promising successes are beginning to be reported.17–22

Crossing the In Vitro/In Vivo Divide

The advantages of a cell-based approach to cardiovascular drug discovery include the ability to maximize screening throughput and the versatility to design and automate new assays. For disease processes that are cell autonomous, cell-based assay systems are often the best choice. However, cell-based approaches are limited in their ability to model complex diseases involving cell-cell interactions or other aspects of systemic physiology. For these diseases, the appropriate physiological context only exists in vivo. Fortunately, many of these diseases can be modeled in animals, and in vivo small-molecule screens with a whole-organism approach are becoming more commonplace. It is apparent that identifying novel chemical modulators of a complex cardiovascular disease state (in contrast to a simple biochemical interaction) may best be achieved with the use of these in vivo, phenotype-based screens. Because every new drug must eventually be tested in vivo, the added information on compound specificity, toxicity, and target validation more immediately gleaned from organism-based small-molecule screens may make them the most efficient option for future drug discovery efforts.

In Vivo Chemical Biology

Rodents

Rodents have been among the favorite choices for modeling cardiovascular diseases. For decades, the idea has existed that one could find treatments for these diseases by systematically testing thousands of small molecules for their ability to suppress the disease phenotype in the appropriate rodent disease model. In fact, the drug isoniazid, which has been used since 1952 as a first-line antimicrobial for tuberculosis, was discovered by screening 5000 compounds in 50 000 mice.23 The approach was successful in that it enabled discovery of isoniazid, but the success came at a high cost in time, resources, and animals.

More recently, discovery of the cholesterol-lowering drug Zetia (ezetimibe) was achieved almost exclusively through in vivo assays. Dozens of compounds were tested for their ability to reduce serum cholesterol in cholesterol-fed hamsters. Once an active compound was discovered, all of the medicinal chemistry optimization was guided by the results from in vivo studies in these hamsters.24 Despite the fact that the scientists had no a priori knowledge of what biological pathway they were targeting, they were able to develop a novel class of cholesterol-lowering drug because the compounds they discovered modulated a phenotype in vivo.

Invertebrates

Despite the positive outcome with ezetimibe, the process was laborious and expensive. Generally speaking, it is extremely difficult to test thousands of small molecules in rodent assays (Figure 2). Therefore, alternative biological systems that retain physiological relevance but are suitable for high-throughput, phenotype-based small-molecule screens are needed. New methods are continually being developed that are enabling the major invertebrate animal model systems, including Caenorhabditis elegans and Drosophila, to contribute to the drug discovery process (Figure 2). The simple body plan (959 total cells in the adult hermaphrodite), high fecundity, short generation time, and genetic tractability make the roundworm C elegans an especially useful model for rapidly dissecting the in vivo action of a drug. C elegans has been used to identify the targets of farnesyl transferase inhibitors,
An example of this approach was the discovery of compounds that can reverse a congenital vascular defect in zebrafish. The zebrafish gridlock mutant exhibits a dysmorphogenesis of the dorsal aorta that inhibits blood circulation to the trunk and tail of the embryo. The gridlock mutant animals have been proposed as a model of aortic coarctation. The gene responsible for the phenotype is hey2, a hairy enhancer of split-related transcriptional repressor implicated in a signaling pathway controlling vascular cell fate decisions. Screening of several thousand small molecules was performed to identify compounds that could suppress the phenotypic effects of the gridlock mutation. Two classes of compounds were discovered that completely restore normal circulation to mutant animals. Further experimentation revealed that the compounds function by activating the vascular endothelial growth factor pathway (in 2 distinct ways) and identified opposing actions of ERK (p42/44 mitogen-activated protein kinase) and PI3K (phosphatidylinositol-3-kinase) signaling in the venous/arterial cell fate decision. In this instance, phenotype-based screening in vivo led to discovery of novel molecules that can activate the vascular endothelial growth factor pathway. It also led to discovery of novel connections between PI3K and arteriogenesis. The ability of these compounds to correct congenital vascular diseases and/or promote formation of new arteries in the context of ischemia is currently under investigation with the use of mammalian model systems. Beyond cardiovascular disease, in vivo chemical screens with zebrafish have been used to discover several other compounds with therapeutic potential. These include compounds that expand the hematopoietic progenitor pool (of potential utility for improving chord or marrow transplants) and compounds that mobilize iron from intracellular stores (of potential use for treating anemia).

A challenge for the future will be to expand the number of screenable cardiovascular phenotypes that can be modeled in zebrafish, with the ultimate goal of identifying different classes of small molecules that perturb all aspects of cardiovascular biology. These efforts will likely benefit from emerging technologies that enable a more directed approach to modeling cardiovascular disease by creating transgenic lines in which human disease genes are overexpressed (Figure 3). For example, the Cre/Lox system of conditional expression developed in rodents has been used successfully to model acute lymphoblastic leukemia in zebrafish. Moreover, this system has been modified further to model various types of hyperplastic tumors by adding the inducible heat shock Hsp70 promoter.

Spatial and temporal control of gene expression can also be accomplished in the zebrafish with the use of the Gal4/UAS system. This allows for the generation of separate lines of transgenics in which Gal4 is placed downstream of a tissuespecific promoter and crossed with a separate transgenic line harboring a gene of interest downstream of a UAS activating sequence. Thus, 100% of the embryos produced from this cross will express the gene of interest only in cells where that promoter is active. By keeping the Gal4 and UAS lines separate and modular (and hence the gene of interest inactive), researchers can bypasses the problems of premature lethality and variation in phenotypic penetrance, which are significant confounders for conducting high-throughput
screens with mutant zebrafish lines (Figure 3). To achieve temporal control of gene expression using the Gal4/UAS system, Esengil et al\textsuperscript{51} fused the insect-derived ecdysone receptor to Gal4. Only the addition of a chemical derivative of ecdysone (tebufenozide) can facilitate the dimerization of the Gal4/ecdysone chimera and activate gene expression.

Finally, several independent groups have now demonstrated the ability to introduce targeted mutations into the germline of zebrafish to create genetic loss-of-function mutants, similar to gene knockout technology used in mice, but without the need for establishing embryonic stem cell lines.\textsuperscript{52–54} This technology makes use of the properties of zinc-finger nucleases and represents an exciting step forward in the capacity to perform reverse genetics in the zebrafish, an often-cited weakness of the model system (Figure 3).

The zebrafish has emerged as a powerful model organism in which to conduct phenotype-based chemical screens. The establishment of molecular and genetic techniques that allow precise temporal and spatial control of gene expression greatly enhances its versatility for modeling human diseases, including, but not limited to, cardiovascular diseases. These qualities provide a unique context in which to design unbiased small-molecule screens and to identify chemical modifiers of novel disease pathways.

**Assay Design**

Historical examples of serendipitous drug discovery begin with an initial observation that subsequently guides the process of discovery. For example, Sergio Ferreira and Sir John Vane\textsuperscript{55} observed that the application of venom from the Brazilian pit viper lowered blood pressure. Despite knowing little about the underlying biology, they continued to use this phenotype as the basis for future experiments, ultimately leading to the dissection of the renin-angiotensin system. Systematizing serendipity requires the capability to convert this type of phenotypic observation into a high-throughput readout that is amenable to the application of small molecules.

For phenotype-based small-molecule screens taking place in cells, it is common to use fluorescent reporters activated by tissue-specific promoters, as mentioned previously in the examples of small-molecule activators of cardiomyocyte proliferation and differentiation. This allows for rapid detection either visually or with the use of automated microscopy. Additionally, image-based screening assays can also be used to detect the effects of small molecules on the localization of subcellular structures. For example, Li et al\textsuperscript{56} developed an assay to detect inhibitors of gap-junction communication based on the relocalization of a membrane-impermeant fluorescent dye from donor cells to acceptor cells.

With organism-based screens, it is possible to perform high-content phenotypic evaluations in both wild-type and disease contexts and to use virtually any type of biological phenotype as the assay readout. These phenotypes could include the presence or absence of a particular anatomic structure (eg, screening for compounds that stimulate new blood vessel growth), organ size or morphology (eg, screening for suppressors of cardiac hypertrophy), or a physiological end point (eg, heart rate or contractility). Published examples include a screen for inhibitors of bone morphogenetic protein signaling in wild-type zebrafish embryos to look for small molecules that induce dorsalization of developing embryos.\textsuperscript{47} In another example, Min et al\textsuperscript{57} developed an assay to find activators of insulin signaling by screening for small molecules that suppress dauer formation in \textit{daf}-2 mutants (a gene encoding an insulin receptor–like protein). Their strongest suppressor, GAPDS, was subsequently found...
to target GAPDH and effectively alter insulin signaling in human cells. Finally, Milan et al. developed an automated screening assay to measure the effect of small molecules on zebrafish heart rates. This assay allowed the authors to establish a correlation between drugs that induce bradycardia in zebrafish and induce QT prolongation in humans.

Small Molecules

Historically, the main source of biologically active small molecules has been nature. In an effort to systematize the discovery of naturally occurring small molecules with therapeutic utility, drug discovery scientists have for decades been collecting plant and microbial extracts and testing them for therapeutic effect. Indeed, many currently prescribed drugs used to treat cardiovascular disease, including the statins, enalapril, Coumadin, and cyclosporine, were originally derived from natural products. Because natural products are limited in number and can be difficult to obtain and purify, systematized drug discovery now depends on synthetic organic chemists, who are helping to create vast collections of synthetic small molecules that can be used in screening. Often, the objective is to build a small-molecule “library” that contains compounds representing as many diverse structural classes as possible, on the assumption that a large, diverse library is likely to contain compounds capable of modulating a variety of therapeutic targets (Figure 4A).

Most current chemical libraries are collected libraries, in which compounds synthesized individually or in small groups by teams of chemists are added into a library over time. However, diversity-oriented synthesis (including combinatorial chemistry/split-and-pool synthesis) is an alternative that in principle could ultimately replace the library collection approach by making it possible to synthesize entire libraries at once. Several such libraries have been synthesized, and as the remaining technical hurdles are overcome, this approach may contribute to or even supersede collected libraries as the source of compounds for systematic small-molecule screening.

Much analysis and debate continue around questions of what small-molecule attributes are ideal for a small-molecule library, and existing libraries may have significant differences in size, diversity, and composition.

Frequently, the compounds in chemical libraries are predominantly based on structures of previously successful drugs, including those targeting G-protein–coupled receptors.
and enzymes, which share the property of having natural substrates that are themselves small organic molecules (metabolites and neurotransmitters). It is therefore not surprising that many of the compounds identified by screening these libraries function by targeting a relatively small number of target types. Much effort is now being directed at improving the diversity of libraries by expanding the amount of "chemical space" that is represented by molecules in a library.51,56,67

Another recent movement involves constructing libraries of small molecules with more potential for targeting protein-protein interactions68,69 (Figure 4B). Although protein-protein interactions do not often have well-defined small-molecule binding pockets, inhibitors have been identified for several different protein-protein interactions, including those involving interleukin-2, HDM2, Bcl-Xl, HPV E2, ZipA, and tumor necrosis factor.68 As chemical libraries and screening methodologies evolve to facilitate discovery of protein-protein interaction disruptors, this class of molecule is likely to become more prevalent.

Once the exclusive purview of large pharmaceutical companies, libraries have fortunately become more accessible to academic researchers. Chemical libraries are available commercially, through many academic institutions, and through the National Institutes of Health, which has invested heavily in the assembly, maintenance, and provision of small-molecule libraries through the Molecular Libraries Roadmap Initiative. This proliferation of available small-molecule libraries is enticing many academic scientists to join the systematic search for novel compounds with therapeutic activities.

Tools for Determining Mechanisms of Action

A frequently cited limitation of phenotype-based small-molecule discovery is the lack of a systematic method for identifying the mechanism of action of a small molecule. An inherent consequence of in vivo, phenotype-based drug discovery is that the small-molecule hits generated by a screen often carry little information about their particular mechanisms of action; however, they are also, by definition, already biologically relevant and validated for their efficacy in vivo. Nevertheless, better methods for target pathway identification are needed, and owing to the growing momentum of phenotype-based small-molecule screens, the techniques available for determining mechanisms of action are continuing to improve. Affinity chromatography remains a standard option for isolating potential protein binding partners from a cellular extract via their ability to bind to a particular small molecule that has been immobilized onto a solid support matrix.70 The major drawback of this technique is the necessity for a "chemical handle" on the small molecule, which facilitates its attachment to the matrix but does not significantly alter its binding properties. To address this issue, some libraries have been designed with an affinity handle built into each molecule. In other libraries, the inclusion of numerous structurally related compounds enables rapid assessment of which functional groups are required and which are dispensable for activity. This information can frequently suggest the most likely positions for nondisruptive attachment to solid support.

An exciting new extension of this biochemical approach for identifying mechanisms of action is called SILAC (stable isotope labeling of amino acids in cell culture). SILAC is a mass spectrometry–based approach in which amino acids (eg, lysine) are labeled with a stable isotope (eg, 13C) that is then metabolically incorporated into the proteome of a group of cultured cells.71 After a protein digest and mass spectrometry analysis, one can make quantitative assessments of protein levels by comparing the isotopically labeled samples with unlabeled controls. Recently, Ong et al72 described a SILAC-based method designed to identify the specific protein targets of a small molecule, not just its effects on protein expression. By taking SILAC-labeled lysates and performing pull-down experiments with affinity matrices loaded with a specific small molecule, Ong et al were able to compare the relative enrichment of target proteins between a small molecule and its nonbinding controls. Although SILAC has been used extensively with cultured cells, there are an increasing number of reports describing its application to yeast,73 zebrafish,24 and mouse.75

Another approach to identifying mechanisms of action uses yeast and relies less on the modification of the small molecule of interest. This approach uses yeast “synthetic lethal” screens, in which small molecules compromise the growth of yeast only in the presence of genetic mutations that reduce the activity of the targets of the small molecules.76 Lum et al77 used this strategy to analyze the activities of 78 medicinal compounds in which they not only correctly identified the targets of many well-characterized compounds but also identified lanosterol synthase (sterol biosynthetic pathway) as the target of the antianginal drug molsidomine, thus providing a potential mechanism for its cholesterol-lowering effects. In a more recent example, Hoon et al78 demonstrated the feasibility of integrating 3 separate genome-wide dosage assays that measure the effects of increasing and decreasing gene dosage on chemical sensitivity. Applying this platform to 188 novel synthetic small molecules identified novel potential protein targets and structure-activity relationships, underscoring the utility of this in vivo, systems-level approach for defining small-molecule bioactivity.

Bioinformatics and computer modeling are also becoming powerful tools for predicting the potential binding partners and mechanisms of action for small molecules. For example, Lamb et al79 have developed the “connectivity map,” a bioinformatic resource that assembles gene expression profiles reflecting diverse cell states, including those caused by small-molecule treatments, genetic mutations, and diseases. Among other things, the resource enables searching for known perturbations that generate expression profiles similar to the profile generated by a small molecule of interest. In some cases, these similarities reveal important clues about the mechanism of action of the small molecule.80 In a separate effort, Keiser et al81 used a database of 65 000 annotated ligands to cluster receptors on the basis of the chemical similarity among their ligands. This allowed unexpected links to be made among targets, including the finding that methadone, emetine, and loperamide (Imodium) antagonize muscarinic M3, α2-adrenergic, and neurokinin NK2 receptors, respectively. These results were confirmed experimentally and highlight the potential utility of using ligand structures as
a means to organize putative protein targets in ways that lead to novel small molecule–protein interactions. The exact method to use for target pathway identification is rarely a clear-cut choice. More likely, researchers will draw from several methods not only to narrow down the list of potential mechanisms of action but also to obtain a genetic and biochemical profile of the effects of the chemical. Ultimately, understanding the full suite of phenotypic changes induced by a specific small molecule may be more important than identifying a specific molecular target when it comes to placing the effects of that small molecule into the appropriate biological context. 

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Conclusion

A goal for future cardiovascular drug discovery is to build on past successes in a manner that systematizes serendipity. The potential for modeling cardiovascular diseases in vivo is increasing rapidly with the continued development and application of new genetic technology, as are the techniques for chemical synthesis, high-throughput assay development, and target identification. Now more than ever, researchers who wish to develop high-throughput, phenotype-based small-molecule screens for cardiovascular drug discovery are not limited by the techniques available but by their own creative ability to discover new ways to use these techniques to probe the underlying mechanisms of cardiovascular disease.

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