Molecular Biology in Medicinal Chemistry

Edited by Th. Dingermann, D. Steinhilber and G. Folkers

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Methods and Principles in Medicinal Chemistry

Recently Published Volumes:

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Contents

Preface

Why address molecular biology and related technologies in a series named "Methods and Priciples in Medicinal Chemistry"? It was the advent of the silicon chip and the detection of DNA processing enzymes that jointly started an evolutionary track in the 1970s which boosted the whole variety of methodologies in what is today known as the life sciences. Also, the classical field of medicinal chemistry has been augmented and today comprises a huge range of techniques and methodologies from QSAR and structure-based design to the recently developed "high-throughput" synthesis and screening. A paradigmatic change in the 1990s gave rise to a focus on the molecular level of drug action and hence demanded the development of appropriate biological assay technology. This is the point where the present book starts.

In the first part, molecular targets are dealt with, going deep into cellular assay technologies. Cell-based assays imply not only the "simple" detection of one cellular product, but the tracking of a variety of metabolic processes, finally resulting in a multidimensional phenotypic characterization of cellular behavior. In a hierarchical step, the second chapter introduces the "gene knock-out" models, a technique that allows to "design" a disease model within a complex organism to generate a more relevant analytical tool for medicinal chemistry. The subsequent chapter deals with a fascinating readout technology for molecular assays, the so-called reporter genes.

The recent elucidation of the human genome has provided another boost to the whole field. Suddenly, a huge amount of targets was available for study. The question, however, which still remained was: What does the target do within the cellular biochemistry and how is it controlled? Those are the questions tackled in chapter 4, which deals with orphan receptors of the GPCR type and shows the challenges and the opportunities for finding new ligands with hitherto unknown biological activity.

The second part of the book is devoted to synthesis. Two important fields can benefit tremendously from molecular biology and its techniques: Stereoselective synthesis of natural compounds and of their mimics, and synthesis of DNA-derived drugs or protein drugs. The first chapter within this section gives a comprehensive overview about the use of enzymes in stereoselective synthesis, emphasizing recombinant technologies, which greatly enhance the selection of

working tools. The fascinating field of nucleic acid drugs, the design and synthesis, their mimics and their mechanisms of action are the topics of chapter 6.

The third part deals with questions of analysis. Invaluable contributions have come from use of proteins for enantioseparation and affinity chromatography. The use of NMR and associated techniques for structure elucidation is another analytical topic to read about in this section.

Kinetics, metabolism, toxicology, and the very rapidly growing fields of pharmacogenomics and toxicogenomics form the contents of the final part of this book which is unique in its presentation of today's entanglement of the biosciences with medicinal chemistry.

The editors are indebted to the volume editors and the authors, whose work and motivation adds an highly important and fascinating facet to the series and gratefully acknowledge the ongoing support from Frank Weinreich, Wiley-VCH, during the whole project.

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Foreword

Where to start and where to end was the initial question when we selected the subjects which should be included into a volume dealing with the application of molecular biology in medicinal chemistry. The enormous progress in molecular biology during the last decades has led to the development of many methods with impact on drug discovery and drug development as well.

Modern target identification and validation on the one hand, and drug development and characterization on the other hand require an overlapping spectrum of methods and assays which goes far beyond the classical methodological repertoire of medicinal chemistry. The detailed molecular characterization of interactions of drugs with their targets is as important and demanding as detailed knowledge on drug interactions with the entire physiological system. Concepts and assays are available that provide us with information on drug transport, metabolism and stability. But we also can determine and predict how a system will react upon the application of a drug, even if this drug is considered to act very specifically at a certain target. Methods and concepts of modern pharmacogenomics and toxicogenomics have clearly demonstrated this.

Considering all this we finally decided to organize this volume in four parts: (I) Molecular Targets, (II) Synthesis, (III) Analysis and finally (IV) Kinetics, Metabolism and Toxicology.

The first chapter in part I sets the biological stage by providing an up-to-date description of available ''Cellular Assays'' and their use and impact on ''Drug Discovery''. Assays for membrane proteins and fast cellular responses, assays for gene and protein expression profiling in high-throughput formats, spatio-temporal assays and subpopulation analysis as well as phenotypic assays are introduced.

More complex systems are addressed in the second chapter of part I, which deals with gene knockout mice and techniques available for the generation of such animals.

G-protein coupled receptors are addressed in the third and fourth chapter. In the first of these two chapters, the focus lies on the characterization of G-protein coupled receptors and the application of reporter genes as read out systems, while the subsequent chapter, as a reference to the postgenomic era, discusses strategies for the identification of ligands for orphan G-protein coupled receptors.

Part II of the volume covers several aspects of drug synthesis. A classical overlap

between organic synthesis and biotechnology is the stereoselective synthesis of drugs with the help of recombinant enzymes. The first chapter in this part gives an overview on this topic and provides many examples, underscoring the impact of such strategies.

Nucleic acid drugs eventually will come of age. Their attractiveness as potentially very specific ligands was always in conflict with numerous pharmacokinetic problems. However, various concepts for stabilizing these molecules, the fascinating potential of RNAi and the first approved drugs were strong reminders of these molecules, e.g. as manipulators of cellular signaling. The chapter by Engels and Parsch touches many aspects, including synthesis and application of this type of compounds, including the RNAi technology.

Part III of the book focuses on analytical aspects. Enantioseparation of chiral drugs and affinity chromatography are extremely important tools in drug development and comprehensive reviews on these topics are presented in chapters 7 and 8.

Analytical methods related to structural biology are included in a series of three articles. Two of these papers deal with NMR technologies that have strongly developed during the last decades. This led to the establishment of NMR as an relevant tool for the determination also of macromolecular structures and for the detection and characterization of ligand-target interactions. Chapters 9 and 10 summarize the application of NMR in drug discovery and describe techniques for ^{13}C- and ^{15}N-isotopic labeling of proteins.

Rational drug design depends on exact structure knowledge, which is still best provided by X-ray crystallography. Despite of extreme methodological improvements in this field, structures of membrane receptors, which represent the most important drug targets, are not available. A strong move towards solving this problem might be the use of antibody fragments as crystallization enhancers. Details of this exciting new technique are described in the final chapter of part III.

Pharmacogenomics and toxicogenomics are new fields with considerable impact on future drug development. The last section of the book covers these hot topics, which will eventually initiate the change from the "one size fits all" concept to the "right drug, right size, right person" concept.

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Part I
Molecular Targets

1
Cellular Assays in Drug Discovery

Hugo Albrecht, Daniela Brodbeck-Hummel, Michael Hoever, Beatrice Nickel and Urs Regenass

1.1
Introduction

1.1.1
Positioning Cellular Assays

Cell-based assays allow to study the function of pharmaceutical or disease targets within complex environments and in the overall biological context. The application of techniques in molecular biology together with the introduction of new tools and analytical devices has moved readouts of cellular assays from phenotypic endpoints such as cell proliferation, cell death, respiration and functional differentiation to the cellular analysis of functional states of specific signaling molecules and metabolic components.

This transition of cellular assays from ''black box'' systems to specific target-associated, mechanistic measurements allows the identification of chemical compounds with target-specific or closely associated modulatory function. It can therefore be assumed that cellular assays will play an increasingly important role in early drug discovery, in particular in discovering compounds for target validation (''chemical genetics''; [1] for review) and in hit-to-lead selection as well as in lead optimization. In contrast to biochemical testing of chemicals for modulatory activity on molecular targets, cellular systems can deliver additional information with respect to drug transport, metabolism and stability. Most importantly, pharmacological targets remain in their natural environment when probed for modulation and data should therefore have a higher predictive value (Fig. 1.1).

However, it remains to be said that, in order to use cellular assays in high throughput, cells need to be taken out of their natural habitat and put into culture. This will inevitably lead to alterations in the repertoire of gene and protein expression, and therefore in their phenotype. Cells used in assays should therefore be characterized with respect to the functionality of their intracellular networks, responses to external stimuli, at least for the signaling pathway of interest, and compared with responses and signaling events at the tissue or organ level. In many cases this is unfortunately not yet fully possible.

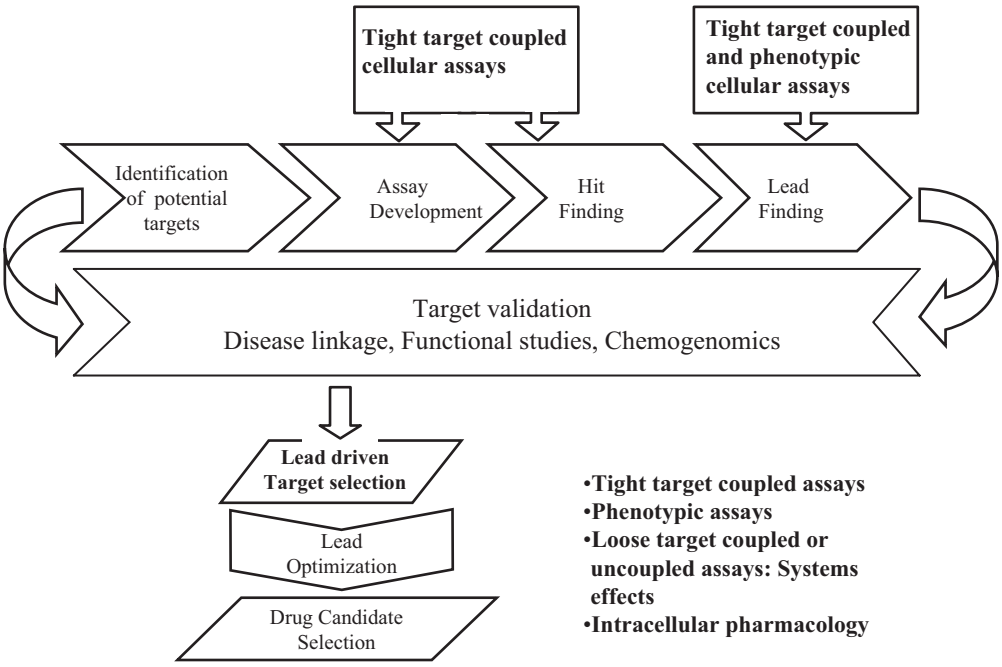

Fig. 1.1 Chemistry-driven target selection. The increased number of potential pharmaceutical targets emerging from the functional annotation of the human genome requires an efficient process to select those that are valid for modulating disease-relevant pathways and phenotypes. Cellular assays which are tightly coupled in the readout with the target of interest allow us to qualify the hits identified in cellular or biochemical high-throughput assays with respect to functionality in a physiologically relevant environment. Cellular assays which are not tightly coupled to the target will indicate system effects of compounds and can be interpreted as a cellular "safety" classification. Cellular assays can be used for target and compound prioritization.

1.1.2 Impact on Drug Discovery

Many compounds are lost during the discovery and development process due to undesirable effects or toxicity and lack of required efficacy. A large part of these effects can be related to interactions of the potential drug with targets other than the one initially selected to modulate disease. Cellular test systems have the potential to elucidate the multiplicity of interactions of drugs, and therefore contribute to a better understanding of drug action and drug selection.

The cell is organized in metabolic and signal transduction cascades. Most of these cascades are complex and nonlinear [2]. In addition, different cascades communicate with each other, and form intracellular circuits and domains. Most diseases cannot be explained by one altered step in a pathway, but are due to several alterations that affect networks in a complex way [3, 4]. Similarly, drugs, whether they interact specifically with one single target or with several molecules, will disturb signaling and metabolic cascades in a complex fashion. In addition, since

many cell types make use of the same types of signaling molecules, in a different context, many cell types might be affected to different extents by the same drug. The recent introduction of high-content screening, high-throughput gene and protein expression, as well as metabolite analysis [5, 6], offers the possibility to study system interactions in a multiparallel fashion, and allows a better understanding of the degree and location of interference. Cell-based assays therefore provide a solution to study the modulation of a pharmaceutical target within its complex natural network as well as to assess potential effects on other networks.

Human diseases are characterized by one or several alterations in metabolic and signaling pathways important to maintain cellular homeostasis and the differentiated functions. Cellular assays can offer a representation of such alterations and therefore become relevant functional models to assess drug action.

Nowadays, cellular assays offer the opportunity to identify the intervention points that lead to phenotypic or endpoint alterations. Often, multiple intervention sites are necessary to alter the outcome of biological networks. Similar rules might apply for the number of drug targets that need to be modulated; in particular, for complex diseases. The right targets and drug combinations can be selected only in the case where each single component of a particular pathway can be individually studied, and this is only rendered possible by the use of cellular systems.

The combination of compound that lead to the desired endpoint can be identified by analyzing multiple parameters. The analysis can, moreover, facilitate the assessment of the effect of individual compounds on multiple pathways. This can be taken as a measure for ''unwanted effects'' (safety profile), but can also lead to the identification of applications in new indications. The analysis of targeted and focused chemical libraries in cellular systems may allow a rapid selection of compounds with various and desired profiles.

Hierarchical, spatio-temporal readouts will generate system-level insight into mechanisms of drug action (intracellular pharmacology) and allow us to predict systemic effects (Fig. 1.2). The development and application of computational tools as well as models of intracellular pathways are a prerequisite to secure data evaluation and interpretation [7, 8]. System-level understanding is only possible by seeking experimental results at the cellular level or beyond.

The recent developments of cellular high-throughput systems, which allow specific measurements of molecular events, render applications in primary screening, and in hit-to-lead selection and lead optimization, in particular, possible. This will enable the development of structure–activity relationships at the system level as a new paradigm in compound selection and decision-making.

1.1.3 Classification of Cellular Assays

Cellular assays have the advantage that the pharmacological targets do not need to be purified. However, in many instances, cellular assays require cell manipulation to allow for a specific signal readout. Overexpression of cell-surface receptors or intracellular proteins can have a substantial effect on the cell physiology and

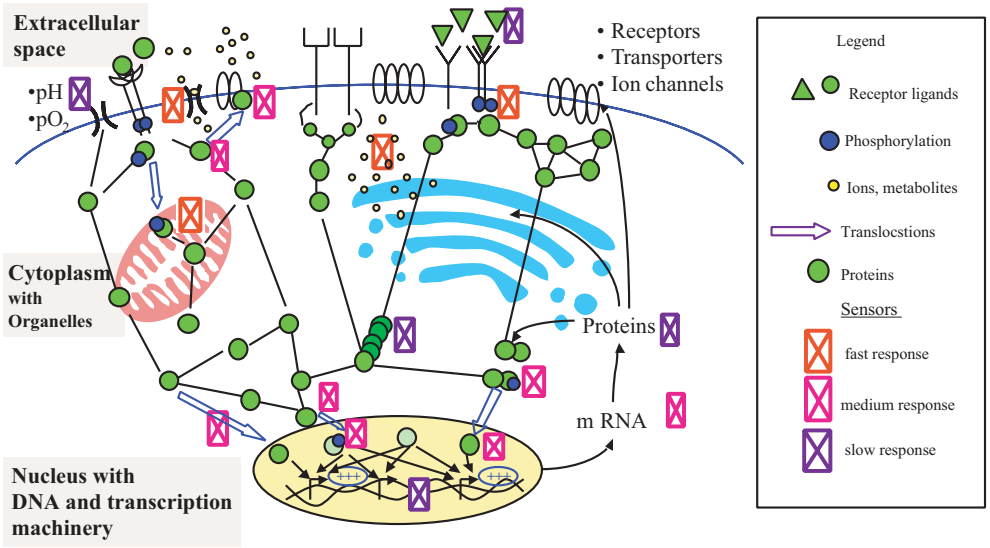

Fig. 1.2 Schematic cellular circuit and cell systems analysis concept. Cells connect to their environment with a wealth of receptors, channels and transporters. Intracellular signaling and metabolic pathways and networks react to intra- and extracellular stimuli in a spatio-temporal manner. Different cell types can react differently to the same stimuli. It can be envisaged that different cell types, cells from normal or pathological tissue, or genetically manipulated cells, can be exposed to different stimuli with and without drugs and the cellular reaction can be assessed by sensor systems in a spatio-temporal fashion, from fast responses to phenotypic changes. Multiparametric analysis will identify compounds with target-specific, target-unrelated, reversible or irreversible effects on cellular homeostasis.

have to be taken into account when using the systems for the discovery of novel drugs.

Early reactions to hormones, growth factors and neurotransmitters are often mediated via second-messenger systems or rapid influx and efflux of ions. Post-translational protein modifications and translocation of proteins are the next steps that alter the physiological state of cells. Such changes are able to lead to flow changes in metabolic pathways, and to alterations in gene and protein expression, ultimately leading to new cellular phenotypes, including cell proliferation and cell death.

Cellular assays can be classified by the temporal events occurring when a cell is exposed to alterations in the extra- and intracellular environment (Tab. 1.1). Fast responses indicated by changes in ion or second-messenger concentrations ultimately lead to functional and phenotypic changes.

Some of the cellular events can be recapitulated in so-called model systems, which might be easier to handle and to manipulate genetically than mammalian cells. Yeast has turned out to be an organism of choice, where metabolic signals and protein–protein interactions relevant for signal transduction can be translated