
Preface

Studies of membrane transporters have had a great impact on our understanding of human diseases and the design of effective drugs. About 30% of current clinically marketed drugs are targeting membrane transporters or channels. This book provides various practical methodologies of the ongoing research on membrane transporters, especially applications of transporter technologies in drug discovery and development. To provide readers the most up-to-date information, new and useful fields and methodologies are embraced in this volume, including pharmacogenomics, nutrigenomics, systems biology, bioinformatics, nuclear magnetic resonance (NMR), imaging, and quantitative real-time PCR. Transporter studies in drug discovery and development for various diseases are discussed, such as neuropsychiatric disorders, cardiovascular diseases, ophthalmic diseases, cancer, and diabetes.

Pharmacogenomics and systems biology studies of membrane transporters are useful in drug discovery and in predicting drug responses in the clinic. In this volume, the current status of these emerging fields in relevance to transporter studies is reviewed and the key issues are discussed.

Bioinformatics is frequently used in transporter studies and has become indispensable for all kinds of research methods. Commonly used bioinformatics methods, such as databases and tools, are collected in this book to facilitate transporter research. Because of heterogeneous sources and tremendous amount of data, data integration has become one of the most important issues in transporter studies. A brief introduction of data integration methodology offered here can help researchers manage their data for further knowledge discovery. The discussion of data modeling approaches can help with the understanding of necessary computational decision support for transporter studies in pharmacogenomics and systems biology.

Bioinformatics analyses may allow definition of phylogenetic relationships in transport protein superfamilies. These novel programs can be used to analyze the major superfamilies of secondary carriers that export hydrophobic and amphipathic compounds including drugs. Applications of these programs are introduced in this volume, including phylogenetic clustering of the families and the analysis of phylogenetic relationships of sequence-divergent drug exporters.

Applications of both *in vitro* and *in silico* techniques are helpful for the understanding of transporter behavior. For example, *in vitro* techniques include mammalian cell-based transporter assays, and *in silico* techniques include ligand-based transporter modeling. The combination of these *in vitro* and *in silico* approaches may assist in faster discovery of ligands for transporter-targeted drug delivery.

Many transporters can be differentially up-regulated in cancer cells compared to normal tissues. Such differential expression of transporters may provide good targets for improving drug delivery, with more focused distribution to the target tissues and enhanced bioavailability. Methodologies to analyze these activities of transporters in cancer are discussed in this volume.

The analysis of gene expression is important for the understanding of interactions between transporters and drugs in the treatment of cancer. Quantitative real-time PCR (qRT-PCR) is a popular technique for assessing gene expression levels with many advantages over microarrays. Gene expression analysis by qRT-PCR has potential as a diagnostic tool for predicting drug responses in cancer patients. In this volume, applications of the qRT-PCR technique are described and compared for analyzing transporter expression levels in various drug-resistant cancer cell lines.

Resistance to multiple drugs is a serious obstacle to chemotherapy treatment of human cancers. Many clinically useful drugs have limited uptake in the intestine and cannot access the brain. For example, it is difficult to directly quantitate drug binding to P-glycoprotein or measure drug transport rates. Fluorescence spectroscopic approaches are very useful in overcoming these problems. Detailed description is provided on using fluorescence tools to quantitate the affinity of binding of various drugs and measure the drug transport activity in real time.

The blood–brain barrier (BBB) is the physiological barrier that regulates the passage of substances into and out of the brain. A useful in vitro BBB model is introduced for the transport studies of the nutrition, physiology, and pharmacology of the brain.

Vesicular monoamine transporters (VMATs) are an important target for biological research in neuropsychiatric disorders. Different structurally related but pharmacologically distinct VMATs have been identified. Various PCR methods are described in this volume for genotyping polymorphisms in these transporters implicated in neuropsychiatric disorders. Human vesicular acetylcholine transporter (hVACHT) in cholinergic nerve terminals stores acetylcholine (ACh) in synaptic vesicles. Methods for characterizing equilibrium binding and transport by VACHT are also discussed.

ATP-binding cassette (ABC) transporters have been linked to Stargardt's disease, fundus flavimaculatus, cone–rod dystrophy, retinitis pigmentosa, and age-related macular degeneration. Genetic pathways involved in the pathogenesis of ABCR-related ophthalmic conditions, as well as diagnostic and therapeutic objectives for these diseases, are discussed in this volume.

Skeletal muscle is crucial in regulating whole body glucose homeostasis. Severe dysfunction in insulin-mediated glucose uptake features insulin-resistant states and type II diabetes. Intravital imaging of protein translocation in skeletal muscle is a very useful technique for the study of insulin signaling. Such analysis may help elucidate the molecular mechanisms of both normal and insulin-resistant conditions.

Glucose transporters have been identified in parasites including *Leishmania*, *Trypanosoma*, and *Plasmodium* that can cause leishmaniasis, African sleeping sickness, and malaria. Hexose transporters are essential for the infectious stage of these parasites and can be important targets for development of novel anti-parasitic drugs.

Because structural and functional studies are essential in understanding the interactions of drugs and the functional transporter proteins, methodologies and protocols are provided from various points of view to tackle structure–function problems. These methods include nuclear magnetic resonance (NMR), site-directed mutagenesis, and the use of *Xenopus* oocytes. These systems can help in the mechanistic and structural characterization of transport proteins and provide insight in signal transduction pathways. Methods to study specific transporters are also provided. For example, the plasma membrane calcium/calmodulin-dependent ATPase (PMCA) plays an important role in signal transduction, especially in the nitric oxide signaling pathway. Different methods in determining PMCA activity are described, such as patch clamp and enzyme assay.

Readers are encouraged to explore integrated views and comprehensive methodologies from different chapters of this book, as these methods are not isolated techniques but rather complementary to each other. One technology may have several other methods and techniques involved. For example, studying the expression system of *Xenopus* oocytes uses fluorescence microscopy. To measure intracellular pH, which is important for understanding the role of membrane transporters in cellular processes, NMR and fluorescent techniques may also be used.

This is an updated and supplemental volume of a previously published book *Membrane Transporters: Methods and Protocols* (Methods in Molecular Biology Series, Volume 227, published by Humana Press, 2003), with emphasis on transporter methodologies in drug discovery and development. This volume strives to deliver to readers not only a collection of practical protocols that can be used immediately in the lab but also critical surveys of key topics by leading researchers in this field. Readers can develop their own workable schemes for their personal studies based on these powerful methodologies. Biomedical researchers in various fields who are interested in membrane transporters and drug development will find the book useful, including chemists, biochemists, molecular biologists, geneticists, physiologists, microbiologists, immunologists, bioinformatics researchers, pharmaceutical scientists, toxicologists, bioengineers, and clinical researchers.

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