RNAi and siRNA in target validation

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Gene silencing by RNA interference (RNAi) technologies has made considerable progress in the last few years [1] and small interfering RNAs (siRNAs) have become a preferred modality for target validation, which was the theme of the *4th International Conference on RNAi and siRNA*, organized by IBC Life Sciences in Zurich, Switzerland from 3–4 February 2004.

Genomic and proteomic technologies have helped to discover a plethora of drug targets, in turn creating a bottleneck in the drug discovery process that is being tackled with target validation using RNAi technologies. However, the problems of delivery and off-target effects, as well as poor tissue distribution, present significant challenges, as pointed out by Clive Jackson of AstraZeneca (http://www.astrazeneca.com) in his opening talk. Success criterion at AstraZeneca is 85% of message knockout and this was achieved for a kinase in the synovial fibroblast. The Global Target Validation Network within AstraZeneca is monitoring the success of siRNA across the company in different biological systems and disease areas. The design of siRNA experiments draws on general observations on gene silencing studies with RNase H antisense as well as experience with siRNA sequence selection and delivery optimization. Jackson pointed out that it is often necessary to confirm function by alternative approaches to siRNA in a full target validation package.

Sumedha Jayasena of Amgen (http://www.amgen.com) reviewed the benefits and drawbacks of RNAi for target validation. Certain siRNAs can silence 'off-targets', induce an interferon response, silence chromatin and lead to false conclusions on end points. To maximize the benefits of using siRNA, Jayasena called for a better understanding of the mechanism of siRNA action, intelligent approaches to siRNA design and chemical modification, and better screening for non-specific effects. To avoid undesirable effects of siRNAs, one should identify highly potent siRNAs that can be effective at low nanomolar concentrations. Internal stability of siRNAs should be achieved during the design stage to reduce participation of the sense strand. Finally, chemical modification of siRNA can reduce or eliminate nonspecific and off-target effects.

RNAi in model systems

Andreas Köpke of Devgen (http://www.devgen.com) described the use of model systems for target validation and identification. *Caenorhabditis elegans* forms the basis of Devgen technology, which involves finding *C.elegans* homologs of potential human gene targets that require validation. Selective RNAi knockdown or knockout technology is applied to these genes to study expression patterns and a phenotypic profile is compiled. This information leads to the identification of pathways influenced by the target and its relevance for the disease. The advantages of this approach include the use of a genome-wide library for every assay, applicability throughout the life cycle, the controllable strength of knockdown, assay speed and low cost, although the disadvantages are that the degree of knockout can vary between genes and it is difficult to tackle neurological targets. Genes involved in obesity have been identified using this approach and mice with a targeted disruption of the stearoyl-CoA desaturase-1 (SCD-1) isoform have reduced body adiposity and increased insulin sensitivity [2]. A genome-wide RNAi screen identified druggable targets that reverse disease and two of these have progressed to lead selection. Validation of these targets has also been conducted in mammalian models using gene knockdown by lentiviral-mediated transduction of short hairpain RNA (shRNA). Christophe Echeverri of Cenix Bioscience (http://www.cenix.com) presented a multispecies platform using *C. elegans* and Drosophila, as well as human cells, for high-throughput RNAi; 94% of siRNAs passed a silencing potency test, which involved high-throughput–high content assays and targets such as kinases, G-protein-coupled receptors and phosphatases, whereas 6% were rejected, and class-focused libraries are available for kinases and G-protein coupled receptors.

An integrated approach

An integrated approach to gene silencing was presented by Bettina Möckel (QIAGEN; http://www.qiagen.com/siRNA). Four siRNA duplexes targeted against the gene of interest were designed using an advanced algorithm and stringent homology analysis. Möckel also discussed the RNAiFect transfection reagent, which is based on a lipid formula developed specially for transfection of siRNA into a wide range of eukaryotic cells. Using a state-of-the-art design algorithm licensed from Novartis Pharma, QIAGEN is increasing the success rate (% active siRNAs) of gene silencing to a high level. Based on this, QIAGEN will be providing two siRNAs against the gene of interest, with the guarantee that at least one of them will reduce gene expression of the target mRNA by at least 70%. This presentation indicated that approaches to gene silencing should take into

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consideration design, synthesis and delivery of siRNA as well as downstream analysis, and the last two factors should be automated processes.

siRNA delivery issues

Hans Winkler of Johnson & Johnson (http://www.jnj.com) emphasized that the delivery into cells was still the biggest hurdle in using siRNA. Cationic liposomes are promising for this purpose but are toxic. Although polyamines and peptides are less toxic, they are difficult to attach to siRNA. MPG is derived from the fusion peptide domain of HIV-1 gp41 protein and MPG peptide–siRNA complexes have been used for *in vivo* acute silencing studies. Lentiviral vectors and herpes simplex viruses (HSV) are used as vectors for central nervous system targets. There is insufficient experience with transgenic expression of shRNA and it is limited mainly to Pol II promoters, which have no regulatory or tissue specificity. Winkler cited previous work showing that it is possible to express long double-strand RNA (dsRNA) from an RNA polymerase II (Pol II) promoter using a vector, named pDECAP [3] Because the transcripts from pDECAP lack both the 5′-cap structure and the 3′-poly(A) tail that facilitate dsRNA export to the cytoplasm, long dsRNA does not leave the nucleus and hence does not induce the interferon response. It was concluded that the *in vitro* potential of RNAi is being met but more phenotypic *in vivo* data is needed to make a final judgment on the utility of RNAi.

Antisense and RNAi

Tatjana Achenback (Aventis; http://www.aventis.com) discussed the *in vitro* applications of RNAi and antisense oligonucleotides (ASOs) as a means of target validation. Transfection protocols using FITC-labeled control siRNAs and ASOs show that even differentiated cells are susceptible to knockdown technologies. Mouse 3T3-L1 fibroblasts were used as an *in vitro* model of diabetes, which were changed into adipocytes by treatment with dexamethasone and insulin. However, adipocytes are fully differentiated cells with no proliferation and are thus difficult to transfect by either RNAi or ASO approaches. Moreover, RNAi in model systems for metabolism interferes with functional assays. A proposed solution for this situation is retroviral vectors for antisense delivery as an alternative to the use of lipid vectors. In most of the validation assays, siRNAs were found to be superior to ASOs owing to greater efficacy or lower toxicity but general problems of delivery, stability of proteins and cellular system were applicable to both approaches.

Adenoviral-mediated functional screening

The use of adenoviral vectors for the delivery of small RNAs into cells was discussed by Frank Weise of the Natural and Medical Science Institute, University of Tübingen (http://www.nmi.de). The endogenous adenoviral expression of short hairpin RNA (shRNA) acts as a precursor to the formation of siRNA. The infection protocol is standardized by multiple assays. Parallelization and miniaturization is achieved by immobilization of adenoviral vectors. Adenoviral-mediated knockout thus meets the requirement of functional genomics.

Functional screening of the genome can also be accomplished using arrayed adenoviral libraries, as discussed by Helmuth van Es of Galapagos Genomics (http://www.galapagosgenomics.com). SilenceSelect™ and FlexSelect™ platforms, which are being applied to core disease programs that include Alzheimer's disease, rheumatoid arthritis, osteoarthritis and osteoporosis.

Non-viral RNAi-mediated gene knockdown

James Hagstrom of Mirus Corporation (http://www.RNAinterference.com) described RNAi delivery to target tissues *in vivo* using intravascular delivery under hydrodynamic pressure. Following a single tail vein injection, endogenous gene knockdown of 15–40% was achieved in 30–70% of hepatocytes. The key issue with this approach is the identification of effective knockdown sequences of siRNA and shRNA. Non-viral particles (plasmid DNA) and synthetic vectors can be used for *in vivo* target validation using RNAi and for therapeutic delivery of RNAi, and siRNA-containing particles exhibited increased serum stability and decreased toxicity. As well as intravascular delivery, an injection can be made directly into tissues or, alternatively, aerosol instillation can be used. Mirus has developed a transfection reagent (TransIT-TKO), an amphipathic polyamine and lipid mixture, specifically designed for siRNA delivery *in vivo*. Formulations have also been designed and optimized for individual cell lines.

siRNA-based therapeutics

Although RNAi is a dominant target validation technology, little effort has yet been made in the development of siRNA-based therapeutics. Nassim Usman of Sirna Therapeutics (http://www.sirna.com) reviewed preclinical studies of chemically modified siRNAs in animal models. Sirna is developing RNAi-based therapeutics that selectively target disease-causing genes and viruses. The focus is on developing siRNAs that target vascular endothelial growth factor for the treatment of macular degeneration.

Another company that is developing therapeutic applications for RNAi is Intradigm Corporation (http://www.intradigm.com). Patrick Lu described Intradigm's approach for siRNA delivery to disease models, particularly tumors via systemic delivery. siRNA can be used for the validation of tumorigenic targets as well as for therapeutic development and because siRNA is highly

sequence-specific, selective knockdown of tumor-causing mutants has tremendous therapeutic potential.

Concluding remarks

This was an excellent conference that included important contributions from several experts over two days. The advantages and limitations of RNAi approaches to the important topic of target validation were discussed. More importantly, measures were suggested to remedy some of the drawbacks of the new technologies.

References

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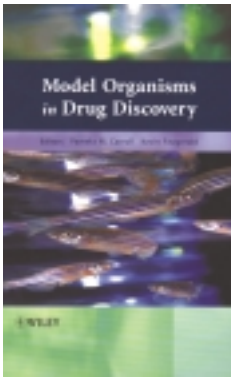

Model organisms in drug discovery

Edited by Pamela M. Carroll and Kevin Fitzgerald, Wiley Europe, 2003, 302 pages in hardback, ISBN: 0-470-84893-6

Over the past decade, huge advances in science have been made in areas such as genomics and proteomics, including, most significantly, the completion of the draft human genome sequence. Additionally, there has been an increase in spending in the pharmaceutical industry. However, these monumental steps forward have to date had little impact on our ability to combat and cure many human diseases and over the last ten years the number of marketable drugs has not significantly increased.

The process of getting a drug to market is long and arduous. Many promising targets and drugs fail along the way, costing time and millions of dollars. The process involves identifying and then validating a target, ultimately in human clinical trials, which still take the same amount of time as they did in years past. However, many inroads have been made in trying to shorten the time taken to identify targets and the use of model organisms has helped in this regard. These systems provide several advantages, including available genetic and molecular tools, cost, and short reproductive and generation times. Additionally, experiments in model systems are conducted in an intact organism.

Model systems have been used for decades for scientific study. Often, they provide an advantage for processes that seem too complex for study in more complex eukaryotes. Additionally, many of these studies have been groundbreaking (for example, the discovery of cell death genes in *Caenorhabditis elegans*), opening up new areas of study in mammals, including humans. Recently, many drug companies have begun to use model organisms as a faster, cheaper method to identify new drug targets. In the new book edited by Carroll and Fitzgerald, the use of model organisms in drug discovery is reviewed.

The book begins with a brief overview and comparison of each model system discussed in the book. The chapters are then organized in such a way as to start from the simplest to the most complex organism when compared with humans, including budding yeast (*Saccharomyces cerevisiae*), nematodes (*C. elegans*), flies (*Drosophila melanogaster*), zebrafish (*Danio rerio*) and mice (*Mus musculus*). Chapters are written by a researcher from the pharmaceutical field who has worked or works with that organism and provides details on almost all of the methods that have been or can be used for that organism in the pursuit of drug discovery. Moreover, both the advantages and disadvantages of the organism in this effort are discussed. Chapter 3 is particularly enlightening because the authors discuss in great detail how one goes from model system to target identification and validation using *C. elegans* as a model for unipolar depression.

This book is an invaluable resource for any researcher in the academic or private sector looking to expand into a model organism work because it reviews all available techniques for each model system. This is both an advantage as well as a limitation because, at times, chapters provide too little detail and are more like a good survey of available experimental techniques. However, this book should also be essential for any graduate level course on drug discovery or any researcher wanting to understand how model systems can be used in the laboratory.

The availability and understanding of model organisms might provide new tools for both academic researchers and drug companies. One watches the next few years with interest to see if this impacts on our ability to combat the complex diseases that ail our society.

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