Transcriptional regulation: a risk worth taking?

It's just too risky. That is the attitude of many large pharmaceutical companies when appraising the regulatory elements of transcription as targets for drug discovery. However, two biotech companies, Signal Pharmaceuticals (La Jolla, CA, USA) and Tularik (South San Francisco, CA, USA) are walking where many of the giants of their industry have feared to tread. Both are confident that they will discover highly useful new drugs, and many of the previously skeptical pharmaceutical companies are now lining up to support their efforts.

Reticence toward targeting transcriptional regulation for drug discovery is often justified by several well-worn arguments. First, transcription is too complex and too fundamental to tinker with. Second, specificity will be difficult to achieve because a particular transcription factor may regulate multiple genes. And third, there is the redundancy argument, which is based on the fact that genes are often activated by multiple factors, any one of which is sufficient to initiate transcription.

New opportunities

But Signal Pharmaceuticals and Tularik have seen opportunities where many large companies see danger. They argue that while it is true that transcription is complex and a given transcription factor may regulate multiple genes, understanding how to control such factors is an opportunity to control the overproduction of a host of coordinately regulated proteins, all of which may be involved in a particular disease process.

The transcription factor NFκB is a good example. This controls the production of cell adhesion molecules, interleukin-1, tumor necrosis factor and interleukin-8, all of which play important roles in the inflammatory response. A compound that inhibits NFκB would be expected to find utility in the treatment of a wide variety of inflammatory diseases including rheumatoid arthritis, osteoarthritis, psoriasis and inflammatory bowel disease. In fact, an inhibitor of NFκB should mimic the effects of the glucocorticosteroids, one of the most highly prescribed group of drugs for the treatment of inflammatory reactions, such as pink eye, lupus, reaction to poison ivy and transplant rejection. As it turns out, recent research indicates that the mechanism of action of glucocorticosteroids is through inhibition of NFκB [*Science* (1995) 270, 283–290]. This discovery provides legitimacy to those who advocate looking towards the regulation of transcription for new drug discovery, and has most likely played an important role in enticing large companies to reconsider transcription factors as targets for drug discovery.

Signal Pharmaceuticals was founded in 1993 based upon the extensive expertise in both signal transduction and transcription factor biology of a group of investigators at the Salk Institute (San Diego, CA, USA) and The University of California (San Diego, CA, USA). The founding scientists were Drs Michael Karin, Inder Verma, Tony Hunter, Stephen Heimemann and Fred Gage. Today, the company has 44 employees including 20 PhD level scientists, who work at the research facility in San Diego. Signal has raised $18 million in venture capital financing and, in April of this year, entered into a research collaboration in the areas of osteoporosis and inflammation with Tanabe Seiyaku Co. – an agreement that is reported to be worth $46 million.

Key transcription factors

Drug discovery at Signal Pharmaceuticals focuses on the signal transduction pathways that regulate specific transcription factors. The major transcription factors of interest and their indications are: NFκB for transplantation and inflammation, AP-1 for autoimmunity, transplantation and cancer, C/EBPB for osteoporosis, AIDS and dementia, HPV E2 for genital warts, CMV IE86 for retinitis, HSV ICP4 for herpes infections, and HCV for hepatitis and hepatocellular carcinoma.

Whole cell screening

Signal's screening program revolves around a battery of specially engineered cell lines that have been transfected with DNA constructs containing a binding region for a specific transcription factor coupled to a gene that encodes a reporter molecule. When the cells are activated through hormone or antigen binding, the intracellular signaling pathways mobilize the appropriate transcription factor, which then initiates the synthesis of the reporter gene. Using the same reporter gene in each assay allows the use of a single highly automated assay protocol that can

Dr David W. Anderson, Vice President, Drug Discovery & Preclinical Development, Signal Pharmaceuticals.

measure the activation of many different transcription factors just by changing the cell line.

The company's highly engineered cell lines are used to screen a 60,000 compound small molecule library, a 100,000 compound nonpeptide combinatorial library, and a collection of 50,000 natural product isolates. Of course, a hit in such a screening assay may act anywhere along the signal transduction pathway or directly perturb the interaction of the transcription factor with its DNA binding site. Signal has the expertise, through its in-house scientific staff and the academic laboratories of its founding members and collaborators, to determine the mechanism of action of interesting active compounds. But, 'There is a lot more to know about signal transduction than we currently know,' according to Dr David Anderson, Vice President of Drug Discovery and Preclinical Development at Signal. Thus, a major advantage of the whole-cell screening strategy is that it may identify a compound that acts through a signal transduction enzyme that has not yet been discovered.

Whole cell screening is not the only type of assay utilized by Signal for drug discovery. The company is now using more and more biochemical screening assays as the specific molecular players of the various signal transduction pathways are being identified. The MAP kinases are of particular interest to Signal, according to Anderson.

Dual NFκB and AP-1 inhibitor

A lead compound has been identified at Signal that acts as a dual inhibitor of both NFκB and AP-1. Interestingly, it is specific for T cells, even though NFκB and AP-1 are found in all tissues. Anderson believes the compound will be useful for treating autoimmune diseases or transplant rejection. Currently, scientists are working to establish its mechanism of action. A binding protein has been identified, and the compound is known to work by inducing the biosynthesis of IκB, the cytoplasmic protein inhibitor of NFκB. 'It acts by a mechanism similar to glucocorticosteroids, except it does not bind to the steroid receptor,' said Anderson.

The Tularik approach

Tularik is a slightly older company than Signal, having been founded in November 1991 by Drs David Goeddel, Steven McKnight and Robert Tjian. Dr Goeddel came to Tularik from Genentech (South San Francisco, CA, USA), where he held the distinction of being the first scientist hired. During his 15 year tenure, Goeddel did much of the gene cloning that led to five products that are now marketed by Genentech. Goeddel came to Tularik upon its founding in 1991 as Vice President of Research; in March of this year he took over responsibilities as Tularik's President.

Dr McKnight was formerly a Howard Hughes Investigator at the Carnegie Institution (Washington, DC, USA), where he headed an extremely productive academic research program on transcription factor biology. He left the academic world in 1992 to join Tularik, where he is now Director of Biology. Dr Tjian, the third founder, remains in academic research at the University of California (Berkeley, CA, USA), where he continues to head an active research program on transcription factors. He currently serves as Chairman of Tularik's Scientific Advisory Board.

Tularik has gone through three rounds of private equity funding, raising a total of $38 million. In addition, it has established collaborations with several major pharmaceutical companies for specific therapeutic indications including: Yamanouchi Pharmaceutical Co. for development of small-molecule drugs to modulate the transcription of cellular-adhesion molecules; Merck and Co. for the development of compounds to control virus targets including Herpes Simplex Virus, Human Papilloma Virus and Human Immunodeficiency Virus; Sumitomo Pharmaceuticals Co. to discover compounds that upregulate the LDL receptor gene and thereby lower serum LDL cholesterol; and Taisho Pharmaceutical Co. to discover novel methods for modulating immune system disorders.

Focus on biochemical assays

Like Signal Pharmaceuticals, Tularik relies on a combination of whole cell and biochemical assays for high-throughput screening of its library of approximately 115,000 defined compounds and natural product extracts. But, unlike Signal, Tularik places its primary focus on biochemical assays. According to Dr Andrew Perlman, Tularik's Vice President of Medical Research, this philosophy has been adopted because the identification of active compounds during the initial screening period is not affected by technical problems associated with cell permeability and metabolism. Also, the biochemical assay defines a likely mechanism of action at the same time as the hit is obtained, and a biochemical assay is much more amenable to the rapid generation of a structure–activity relationship. Of course, the trick to using this approach successfully is a thorough understanding of the signal transduction mechanisms and transcriptional regulatory pathways so that an intelligent choice can be made in selecting biochemical targets. This is a particular strength at Tularik, according to Perlman. Currently, Tularik has three lead compounds that came out of the three original antiviral, adhesion protein and low-density lipoprotein drug discovery programs.

According to Anderson of Signal Pharmaceuticals, the biggest question in targeting signal transduction and transcription pathways remains safety and specificity, which were the aspects that originally dissuaded many of the large pharmaceutical companies from performing research in this area. But today, there is a great deal of interest from the major pharmaceutical companies, because clear-cut examples exist of highly specific compounds that work by blocking transcription of disease-causing proteins. Undoubtedly, both signal transduction and transcription will be fiercely competitive areas for drug discovery research in the immediate future.

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