HIGH-THROUGHPUT SCREENING

Screen test

Having more choices is a wonderful luxury but can often end up making the decision process tougher. During the past decade, a growing number of novel assay methods have been developed to aid high-throughput screening of large compound libraries against the wealth of potential targets emerging from genomics. Because of the relatively broad applicability of several of these technologies, the choice of method for a specific assay is generally based on cost, speed, signal quality and ease of use — the assumption being that regardless of choice, similar, if not the same, hits should be generated.

But recently Xiang Wu, Matthew Sills and colleagues at Novartis found that different assay technologies in fact identify different hits from a tyrosine kinase assay. One explanation for this variation is that the differences represent artefacts and the common hits functionally relevant compounds.

To explore this, Wu, Sills and another group of collaborators developed a nuclear receptor antagonist assay for the farnesoid X receptor (FXR) using three commonly used assay formats: AlphaScreen, time-resolved fluorescence (TRF) and time-resolved fluorescence resonance energy transfer (TR-FRET). In the *Journal of Biomolecular Screening*, they again report assay variation in screens against a random selection of 42,240 compounds from the Novartis synthetic compound library. After primary screening of single compounds and a confirmation experiment, 78, 25 and 43 compounds inhibited FXR binding using AlphaScreen, TRF and TR-FRET, respectively. Screening compounds as single entities, rather than mixtures, identified a greater number of hits and produced less false-positives and false-negatives, which shows that any gains from throughput and cost savings in screening mixtures of different compounds could be lost in terms of results output.

All the hits from the three screens were used in dose-response experiments and were evaluated in a cell-based secondary reporter gene assay for functional activity. Of the 128 compounds tested, 35 showed activity in the gene assay, 34, 11 and 15 of which were identified in the dose-response studies by AlphaScreen, TRF and TR-FRET, respectively. Nine of the 35 compounds were positively identified in all three screens; six were identified by AlphaScreen and TR-FRET, two by AlphaScreen and TRF, and 17 with AlphaScreen alone.

So, different and only partially overlapping sets of functional hits can be obtained from screening programmes. The fact that AlphaScreen produced the greatest number of

STROKE

Piecing together a picture of stroke

Unravelling the myriad genetic and environmental factors that contribute to the pathogenesis of complex diseases such as cancer and heart disease remains an outstanding biomedical challenge. Now, Solveig Gretarsdottir *et al.*, reporting in *Nature Genetics*, have provided a new piece of the puzzle of stroke — the third leading cause of death in western countries — through the identification of the gene encoding phosphodiesterase 4D (PDE4D) as a risk factor for ischaemic stroke.

Although there are a few known mutations that cause rare Mendelian forms of stroke, the genes involved are probably not contributing factors to the common forms of stroke, which are usually related to atherosclerosis. In a previous search for the genetic basis of common strokes, the same research team identified the chromosomal region 5q12 as a stroke-risk locus. In the present study, this locus has been finely mapped, leading to the characterization of *PDE4D* as a gene strongly associated with two atherogenic forms of stroke, carotid and cardiogenic stroke.

After establishing *PDE4D* as a risk factor for stroke, Gretarsdottir *et al.* looked for functional variants of *PDE4D* that might play a causal role in stroke formation. No significant genetic variants in the coding exons of *PDE4D* were found, and so attention was turned to the expression of *PDE4D* isoforms. An examination of messenger RNA levels showed that the overall level of *PDE4D* mRNA was decreased in randomly selected affected individuals, a difference that was mainly attributable to reduced levels of the *PDE4D1*, *PDE4D2* and *PDE4D5* isoforms. Association studies using single-nucleotide polymorphisms around the *PDE4D* gene also revealed three haplotypes in the study: wild type, at-risk and protective. Interestingly, affected individuals who possess the at-risk haplotype showed decreased levels of just the *PDE4D7* and *PDE4D9* isoforms.

PDE4D is expressed in the principal cell types involved with atherosclerosis, and is known to selectively degrade the second messenger cyclic AMP, which is ubiquitous in signal transduction and reduced levels of which are implicated in atherosclerosis. Reciprocally, the PDE4D isoforms identified as under-expressed in affected individuals — *PDE4D1*, *PDE4D2* and *PDE4D5* — are upregulated by cAMP. Taken together, these results indicate that dysregulation at the level of cAMP could be a key causal factor. The authors suggest that decreased cAMP concentrations, caused by greater activity of one or a few different isoforms of PDE4D, could lead to the lower levels of expression of *PDE4D* isoforms observed in stroke patients. As such, a small molecule that reduced the overall activity of PDE4D, or which specifically targeted the overactive isoforms, could result in elevated levels of cAMP, and a decreased risk of atherosclerosis and stroke.

Daniel Jones

ORIGINAL RESEARCH PAPER Gretarsdottir, S. *et al.* The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. *Nature Genet.* 2003 Sep 21 (doi:10.1038/ng1245).

functional antagonists in this assay does not necessarily mean that it will be the optimal method for other targets as there could be technology-specific interference with each screen. To find the best method, the authors suggest two approaches: either to use replicate determinations in the primary screening process, which should hopefully reduce the variability during this part of the process, or to use more than one assay for a specific target, an approach that should become more feasible owing to reduced screening times and cost reductions through miniaturization.

Simon Frantz

References and links

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CHEMICAL GENETICS

Knowledge = power

Chemical genetics — the use of small molecules to mimic the cellular effects of genetic mutations — has emerged as an important approach for unravelling biological pathways and also for providing chemical starting points for the development of potential drugs that modulate these pathways. However, this strategy typically requires tens of thousands of molecules to be screened in order to identify a few active molecules, and then considerable further effort to establish their underlying mode of action.

Turning this problem on its head, Brent Stockwell and colleagues have now assembled a library of ~2,000 compounds with known and well-characterized biological activities, and developed an annotation system that captures all of the available published information on these activities. As described in their paper in *Chemistry and Biology*, having this knowledge associated with each compound can greatly aid the identification of the mechanisms underlying interesting effects — for example, antiproliferative activity — in cellular screens.

The 2,036 biologically active compounds, which include 514 US FDA-approved drugs, represent 169 broad, primary biological mechanisms, such as antihypertensive, anti-inflammatory and antifungal. Each compound was annotated with a score for each of 12,755 biological mechanisms — comprising the 169 primary mechanism descriptors, 200 Medline terms related to pharmacology and more than 12,000 human gene names — by counting the number of abstracts in Medline that contain both the compound name and a given biological mechanism using automated algorithms.

A comparison of the annotated compound library (ACL) with a commercial library typical of those used in high-throughput chemical-genetic screens revealed that it is significantly more structurally diverse. But would it yield more hits in a biological screen? To test their hypothesis that compounds with known biological activity would have a greater probability of being active in new cellular assays than random compounds because their molecular mechanism might be operative in a new context, the authors evaluated the ability of the two libraries to selectively inhibit the proliferation of engineered human tumour cells — an assay that none of the compounds in the ACL had previously been tested in. And indeed, 1% of the ACL compounds were at least fourfold

selective for killing tumour cells over normal cells, compared with only 0.01% of the compounds from the commercial library.

Next, the authors tested the ability of the ACL to uncover mechanisms associated with cellular processes. Lung tumour cells were treated with each compound in the library, and 85 compounds had an antiproliferative effect. In a conventional chemical-genetic screen, these compounds would have been the starting point for 85 separate time-consuming target identification projects. However, using the information associated with each compound in the ACL, the authors were able to rapidly identify 28 biological mechanisms that were statistically over-represented in the 85 active compounds. These included both known anticancer mechanisms, confirming the utility of the approach, and also several mechanisms with no previously recognized relationship with cell death, highlighting its potential to identify novel associations. Follow-up experiments with one such novel mechanism showed that several compounds with this mechanism, which would not have been selected *a priori* as antitumour agents, could selectively kill tumour cells.

So, this method can considerably accelerate the evaluation of numerous mechanisms that might underlie the cellular effects of the compounds in this ACL (information for which is publicly available on the Stockwell lab website). Expanding such ACLs, and introducing further information for each compound, such as effects on gene expression from microarray experiments, has the potential to allow the mechanisms regulating cellular processes to be defined with ever-increasing precision.

Peter Kirkpatrick

References and links

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WEBSITE

Stockwell lab: http://staffa.wi.mit.edu/stockwell/