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Novel proteases for drug discovery ▲

I read with great interest the articles published in *Drug Discovery Today* on the impact of the Human Genome Project on drug discovery. As I enjoy working with proteases, I would like to add some additional comments to the recent article in *Drug Discovery Today* by Christopher Southan entitled *A genomic perspective on human proteases as drug targets*[1].

The mapping of the human genome has uncovered many novel proteases for which the physiological role is unclear. One way forward to determine if the proteases are valid targets for drug discovery would be to take partial sequences of the genes that encode such proteases and carry out tissue distribution experiments to determine where they are expressed. This can be done either at the mRNA level using antisense transcripts, or at the protein level by generating antibodies to orphan proteases. Tissues from diseased individuals should also be screened to enable a comparison of the regulation of orphan proteases in normal and diseased states. Once this is done, a target candidate list could be generated and prioritized.

Many orphan proteases exist in the ADAM (a disintegrin and metalloprotease) family[2]. For example, if TNF-α-converting enzyme (TACE)[3,4] were an orphan protease it would be on a candidate list because levels of the enzyme are upregulated in disease states such as osteoarthritis and rheumatoid arthritis[5]. In addition, ADAM 10 is an orphan protease that has been postulated to have a role in Alzheimer's disease as it might be an α-secretase for the processing of amyloid precursor protein (APP)[6], and further evidence for ADAM 10 in Alzheimer's disease is accumulating. For example, mRNA levels of ADAM 10 are found in the brains of Alzheimer's patients and the enzyme processes a peptide substrate for APP at the α cleavage-site[6].

To uncover the physiological substrates for orphan proteases, substrate mapping using phage display can be performed[7]. This has been done with collagenase 3 (Ref. 8), a protease that has some known physiological substrates such as type II collagen[9]. With this technique, clones are generated that encode for sequences that are processed by the enzyme of interest. Several peptide substrates are generated and specificity constants (k_{cat}/K_m) are determined. A structure–activity relationship (SAR) can be generated and, subsequently, BLAST searches can be performed using predicted substrate sequences for the enzyme. When BLAST searches were performed on the clones from collagenase 3, putative substrates that are reasonable candidates were revealed, such as biglycan and the latency-associated peptide of transforming growth factor-β (TGF-β).

Finally, in addition to substrate mapping to determine the physiological role of an enzyme, knockout experiments either by generating transgenics or using antisense mRNA can be used. A transgenic knockout of TACE has been used to discover a role for this enzyme in the processing of other substrates such as transforming growth factor-α (TGF-α)[10]. The knockouts, coupled with direct biochemical experiments such as specific-inhibitor studies and processing of putative substrates, can ultimately be used to validate an orphan protease in a physiological role, as well as demonstrating its function in a disease state.

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Transgenic gene knockouts: a functional platform for the industry ▲

Steve Harris[1] recently provided a good overview in this journal of the use of

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transgenic knockouts as a functional tool in the drug discovery process and a source of information for pharmaceutical companies. The first part of the paper described the challenges faced by the industry in selecting and validating the key human therapeutic targets from ~30,000 genes identified by the two human genome sequencing projects[2,3]. The goal of this process it to select targets with both a strong therapeutic rationale and a good chemical tractability in the drug discovery pipeline. Unfortunately, a large number of potential targets are lacking the information necessary to make informed decisions. Therefore, the use of functional genomics platforms has become fashionable to add value to the targets being pursued by the industry. As described by the author, transgenic knockouts had a major impact in the exploratory phases of drug discovery. More recently, the technology has also had an impact on preclinical candidate gene selection.

However, more challenges are faced by transgenic gene knockouts. After the publication of the estimation of human genes, which is a smaller estimate than initially expected, it is understood that a more subtle level of complexity exists. For example, it is likely that splice variants are relatively common and will produce more than one protein for a given gene. Therefore, it is important to ask whether the correct splice variant is used in the targeted region of the gene of interest. In the future, one could also argue the use of the correct allelic variant.

Towards the end of his discussion, Steve Harris highlighted the options available to access high-throughput knockout phenotyping capabilities. A few companies are currently offering the possibility of outsourcing transgenic knockout activities by using two typical business models: (1) by subscription to a database or (2) on a fee-for -service basis. These are attractive options and, for example, the access to a database could alleviate the problem of the optimal timing of a gene knockout in the target selection and validation processes. However, the costs should not be underestimated, and a thorough analysis of internal capabilities and return on investment must be undertaken. Integrating external data with internal information and long-term support is not without challenges. Another aspect to clarify up-front is the respective intellectual property position of the provider and the customer.

As with any functional genomics platforms, transgenic gene knockouts obviously comprise an important piece of the jigsaw needed to validate and select targets, but there are still many challenges remaining.

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