expression from mouse hepatocytes *in vivo*: comparisons between immunocompetent and immunodeficient inbred strains. *Gene Ther.* 2, 151–155

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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.

References

1 Southan, C. (2001) A genomic perspective on human proteases as drug targets. *Drug Discov. Today* 6, 681–688
2 Wolfsberg, T.G. *et al.* (1995) ADAM, a widely distributed and developmentally regulated gene family encoding membrane proteins with a disintegrin and metalloprotease domain. *Dev. Biol.* 169, 378–383
3 Black, R.A. *et al.* (1997) A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells. *Nature* 385, 729–732
4 Moss, M.L. *et al.* (1997) Cloning of a disintegrin metalloproteinase that processes precursor tumor-necrosis factor-α. *Nature* 385, 733
5 Patel, I.R. (1998) TNF-α convertase enzyme from human arthritis-affected cartilage: isolation of cDNA by differential display, expression of the active enzyme, and regulation of TNF-α. *J. Immunol.* 160, 4570–4579
6 Lammich, S. *et al.* (1999) Constitutive and regulated α-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc. Natl. Acad. Sci. U. S. A.* 96, 3922–3927
7 Mathews, D.J. and Wells, J.A. (1993) Substrate phage: selection of protease substrates by monovalent phage display. *Science* 260, 1113–1117
8 Deng, S. *et al.* (2000) Substrate specificity of human collagenase 3 assessed using a phage-displayed peptide library. *J. Biol. Chem.* 275, 31422–31427
9 Billinghurst, R.C. (1997) Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. *J. Clin. Invest.* 99, 1534–1545
10 Peschon, J.J. *et al.* (1998) An essential role for ectodomain shedding in mammalian development. *Science* 282, 1281–1284

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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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