The *Discussion Forum* provides a medium for airing your views on any issues related to the pharmaceutical industry and obtaining feedback and discussion on these views from others in the field. You can discuss issues that get you hot under the collar, practical problems at the bench, recently published literature, or just something bizarre or humorous that you wish to share. Publication of letters in this section is subject to editorial discretion and company-promotional letters will be rejected immediately. Furthermore, the views provided are those of the authors and are not intended to represent the views of the companies they work for. Moreover, these views do not reflect those of Elsevier, *Drug Discovery Today* or its editorial team. Please submit all letters to Rebecca Lawrence, News & Features Editor, *Drug Discovery Today*, e-mail: Rebecca.Lawrence@drugdiscoverytoday.com

Gene therapy versus protein-based therapy: a matter of pharmacokinetics ▲

Gene therapy for pro-angiogenic and anti-angiogenic therapy is attractive for several reasons and was covered recently by a review in *Drug Discovery Today* by Liau and colleagues[1]. The recent negative publicity around viral gene therapy has cast a shadow on its development which is unfortunate, not only because of the personal impact on specific patients, but also because it has and will for some time, impede a rational and scientific approach to this modality.

Conceptually, there is no essential difference between gene therapy and protein therapy, as it is the goal of gene transfer to stimulate transduced cells to locally produce and secrete the desired proteins. However, the two major claims of gene therapy – local delivery and favorable pharmacokinetics – need further consideration.

The success of local delivery, i.e. obtaining a high ratio of local versus systemic drug levels and presumably a higher therapeutic index, depends on the targeted organ. Heart and blood vessels can be particularly challenging because of their role in the systemic circulation. Despite ten years of research, the maximum efficiency of local delivery achieved by an intramyocardial injection of fibroblast growth factor 2 (FGF-2) protein, a very 'sticky' angiogenic growth factor, is ~20% of the total dose[2]. While comparable efficacy data is unavailable for virus-based delivery, for these viruses to transduce cells, local binding via ligand–receptor interaction (such as the RGD–integrin system) or to cell surface heparan sulfates, has to take place[3]. This process, to the best of our knowledge, is not any more efficient than protein binding to the same class of cell surface proteins. It is, therefore, not easy to understand why viral therapy should be more localized or efficient than protein therapy.

Recent experiments with intra-arterial and peri-adventitial delivery of adenovirus showed low local myocardial transfection efficiency that was almost equal to that of the liver[4]. The development of FGF receptor 1 (FGF-R1)-targeted adenoviruses has further emphasized the need for a higher local/systemic transfection ratio. The assumption that this will improve the therapeutic index depends on relative expression of FGF-R1 in the target and other areas likely to generate side effects (tumors, wounds, retina, atherosclerotic plaque), as well as on local and systemic factors that could determine the efficiency of this binding.

The long-term expression of transduced genes, although heavily dependent on the viral construct, is the major potential advantage over protein therapy. The length of exposure to an angiogenic protein required for its effectiveness has not been established but is widely assumed to be at least a week, a time frame ideally achieved with adenoviral constructs. Slow-release biocompatible polymers have been developed to achieve the same goal for protein therapy. Recent advances in drug-coated stents and injectable biodegradable matrices can make this option even more appealing. In contrast to predictable pharmacokinetics achieved using these protein-based approaches, viral gene therapies carry a high inter-individual variability in gene expression[5]. This variation is explained, in part, by variable quantities of circulating anti-adenoviral antibodies and by other, poorly understood parameters regulating cellular and humoral host immune responses to adenoviruses. The same factors also lead to the uncertain duration of gene expression.

Thus, the differences between gene and protein approaches for therapeutic angiogenesis are primarily of a practical nature and mundane, nonscientific arguments are likely to become decisive in the near-term.

References

1 Liau, G. *et al.* (2001) Clinical efforts to modulate angiogenesis in the adult: gene therapy versus conventional approaches. *Drug Discov. Today* 6, 689–696

2 Laham, R.J. *et al.* (2000) Tissue and myocardial distribution of intracoronary, intravenous, intrapericardial and intramyocardial ^{125}I-labeled basic fibroblast growth factor (bFGF) favor intramyocardial delivery. *J. Am. Coll. Cardiol.* 35, 10A

3 Di Giovine, M. *et al.* (2001) Binding properties, cell delivery, and gene transfer of adenoviral penton base displaying bacteriophage. *Virology* 282, 102–112

4 Hiltunen, M.O. *et al.* (2000) Biodistribution of adenoviral vector to nontarget tissues after local *in vivo* gene transfer to arterial wall using intravascular and periadventitial gene delivery methods. *FASEB J.* 14, 2230–2236

5 Barr, D. *et al.* (1995) Strain related variations in adenovirally mediated transgene

1359-6446/01/$ – see front matter PII: S1359-6446(01)01904-3

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

Mark J. Post and Michael Simons
Angiogenesis Research Center
Dartmouth Medical School
Hanover
NH, USA

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

Marcia Moss
Cognosci
2 Davis Drive
PO Box 12076
Research Triangle Park
NC 27709, USA

Transgenic gene knockouts: a functional platform for the industry ▲

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

1359-6446/01/$ – see front matter PII: S1359-6446(01)01902-X