

Regulatable gene therapy

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The gene therapist's dream of switching genes on and off at will could be a step closer: an American biotech firm has shown that one of its programmable transcription factors can be activated by an oral drug. Their system can be used to activate and deactivate endogenous genes.

The company behind the new system, Sangamo BioSciences of Richmond, California (<http://www.sangamo.com>), specializes in engineering zinc finger-based transcription factors. Zinc fingers are small DNA-binding peptide motifs that contain one or more molecules of zinc as a structural component; they can be designed to bind to virtually any DNA sequence [1]. Fused to an effector domain, for example the activator protein p65 or the repressor protein KRAB (Kruppel-associated box), they make up artificial transcription factors that can be used to up- or down-regulate a given target gene. 'It is a very general approach for gene regulation that mimics the way most organisms regulate the expression of endogenous genes,' says Sangamo's CEO, Edward Lanphier.

Based on this approach, the firm has several gene activation and repression programmes under way in therapeutic areas such as cardiovascular disease, cancer and infectious diseases. Their most advanced therapeutic programme involves upregulating the endogenous gene for vascular endothelial growth factor (VEGF), which plays a key role in regulating vasculogenesis and angiogenesis and is thus an attractive target for the treatment of ischemic heart and limb diseases. Lanphier says preclinical studies have shown that activating the endogenous VEGF gene generates all the normal isoforms of the VEGF protein in their normal ratios [2]. This leads to the formation of histologically normal vasculature. By contrast,

conventional gene therapy approaches that work with only one isoform of VEGF lead to extremely leaky and histologically abnormal vasculature [3]. Sangamo plans to file an investigational new drug (IND) application in the second half of 2003.

Genetic switch technology

The ultimate goal, Lanphier says, is to be able to control the expression of engineered transcription factors, and thus of endogenous genes, using small molecules. Several genetic switches are

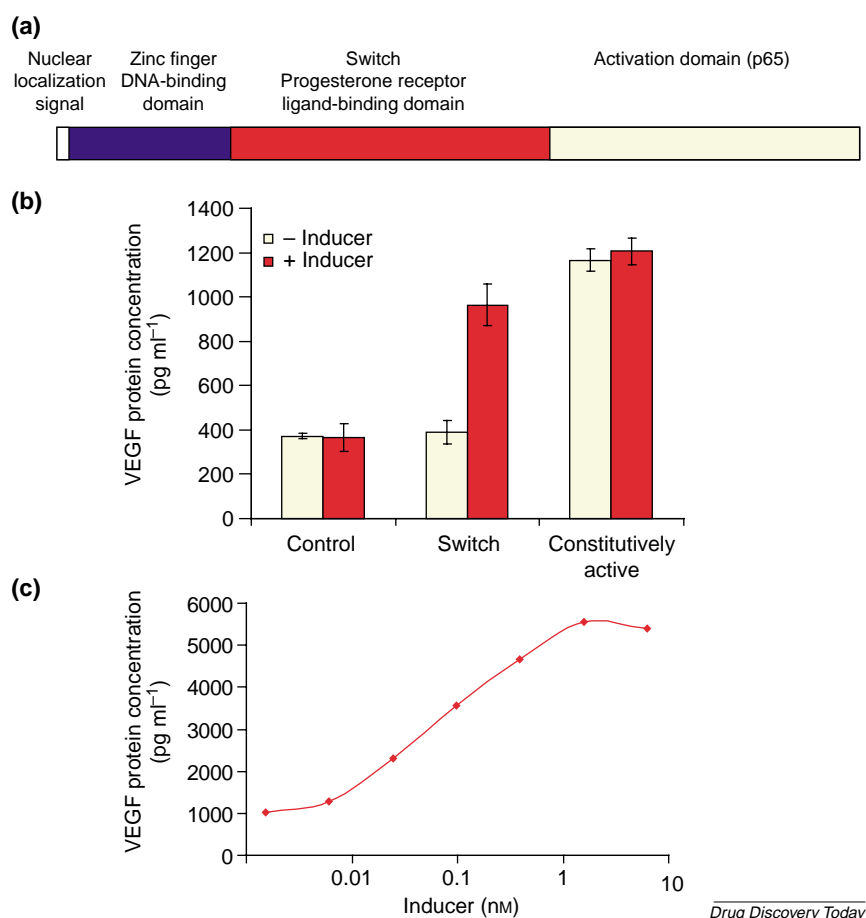


Figure 1. Small-molecule regulation of endogenous gene expression. Engineered transcription factor (a) comprises a nuclear localization signal, a DNA-binding domain that recognizes a sequence within the vascular endothelial growth factor (VEGF) promoter, a switch domain (progesterone receptor ligand-binding domain) and a transcriptional activation domain. (b) Transient transfection into human cells. The control protein contains the same activation and switch domains, but a different DNA-binding domain that does not target the VEGF gene. The constitutively active protein contains the same DNA-binding and activation domains without the switch domain. In the presence of inducer (red bar), the switch-containing protein induces the expression of VEGF, but there is no change in VEGF expression with the control or constitutively active proteins. (c) The magnitude of response depends on the dose of the inducer [5]. Figure kindly provided by Carolyn Dent, Sangamo BioSciences (<http://www.sangamo.com>).

already available to control the transcription of inserted transgenes. One of the lead technologies is the GeneSwitch® system developed by Bert O'Malley [4] and commercialized by Valentis (<http://www.valentis.com>). This plasmid-based system has the following components:

- a mutant form of the human progesterone receptor's ligand-binding domain, which enables the transcription factor to be activated allosterically by low concentrations of the anti-progestin drug mifepristone;
- the transcriptional activation domain from the p65 subunit of human NF- κ B; and
- the DNA-binding domain from the yeast GAL4 protein, which enables the transcription factor to bind to a transgene with a promoter containing sequences specific to the GAL4 DNA-binding site.

Carolyn Dent at Sangamo replaced the GAL4 DNA-binding domain with the zinc finger motifs specific for the endogenous *VEGF* gene. She then tested the system in both transiently transfected and stable human and mouse cell lines. The new system works in all of these experimental systems, according to Dent. 'In the absence of the inducer, there is no increase in the amount of VEGF; so there is no background expression,' she reported at the fifth annual meeting of the *American Society of Gene Therapy* in Boston, Massachusetts. 'In the presence of the inducer, there is an increase in the amount of VEGF, and that increase is titrable [Fig. 1],' [5].

Implications

Gene Liao, Programme Director at Genetic Therapy (http://www.us.novartis.com/inno_discovery/) believes this is an exciting result: 'If you can turn on VEGF and define exactly how long you want it activated and then shut it off, you are in pretty good shape.' He points to studies that show that long-term induction of VEGF can have deleterious side effects, and that VEGF expression must therefore be carefully modulated [6]. Liao adds that it has always been one of the big issues with gene therapy in general – to be able to regulate the timing and level of gene expression.

However, he cautions that there are still many hurdles to be overcome before this approach can be applied to humans. 'One of the key issues is going to be how you get these transcription factors to the right place to express them at sufficiently high levels *in vivo*.' (Dent says they are considering two delivery methods: intramuscular injection of naked DNA and delivery via virus vector systems, for example based on adenovirus or lentivirus.)

However, if this technology can be shown to be a safe and effective means of fine-tuning the expression of endogenous genes, there will be many therapeutic applications. For instance, Liao suggests, 'This technology could be used to turn on the endogenous expression of erythropoietin, which would be great for cancer patients. One could also inhibit pro-inflammatory genes such as COX-2

or NF- κ B. Regulated nitric oxide synthase (eNOS) expression would be potentially useful in a variety of cardiovascular settings.'

He adds that the technology might also be of benefit for patients with type 1 diabetes, whose pancreatic islet cells have been destroyed; one could induce a second organ such as the liver to express and secrete insulin. 'You do not want to express steady-state levels of insulin all the time,' he explains. 'Ideally, you could take a pill after you eat and thereby induce your insulin. A whole field opens up,' he says.

References

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Corrigendum

In the article *Text-based knowledge discovery: search and mining of life-sciences documents* by Robert Mack and Michael Hehenberger [*Drug Discov. Today* 7 (Suppl.), S89–S98], the authors refer in Ref. 26 to work by Soumya, R. *et al.* The correct citation is as follows:

- 26 Raychaudhuri, S. *et al.* (2002) Associating genes with gene ontology codes using a maximum entropy analysis of biomedical literature. *Genome Res.* 12, 203–214

The authors would like to apologize for any confusion that this might have caused.

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