

but treatable disease. To do this, strategies that include the prolonged administration of multiple angiogenesis inhibitors with other biological agents during and after conventional modalities will be required. The translation of anti-angiogenic and anti-vascular therapies into the clinic is now inevitable. However, only by continued study and improved understanding will this occur rapidly so that anti-angiogenic agents can achieve their full potential.

1. Folkman, J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nature Med.* **1**, 27–31 (1995).
2. Boehm, T., Folkman, J., Browder, T. & O'Reilly, M.S. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. *Nature* **390**, 404–407 (1997).
3. Niethammer, A.G. *et al.* A DNA vaccine against VEGF

- receptor 2 prevents effective angiogenesis and inhibits tumor growth. *Nature Med.* **8**, 1369–1375 (2002).
4. Alon, T. *et al.* VEGF acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. *Nature Med.* **1**, 1024–1028 (1995).
  5. O'Reilly, M.S. & Fidler, I.J. The development of anti-angiogenic agents for the clinic. in *Progress in Oncology 2002* (eds. DeVita, V.T., Hellman, S. & Rosenberg, S.A.) 129–157 (Jones and Bartlett Publishers, Sudbury, 2002).
  6. Kuonen, B.C. *et al.* Dose-finding and pharmacokinetic study of cisplatin, gemcitabine, and SU5416 in patients with solid tumors. *J. Clin. Oncol.* **20**, 1657–1667 (2002).
  7. Yu, J.L., Rak, J.W., Coomber, B.L., Hicklin, D.J. & Kerbel, R.S. Effect of p53 status on tumor response to antiangiogenic therapy. *Science* **295**, 1526–1528 (2002).
  8. Relf, M. *et al.* Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor  $\beta$ -1, platelet-derived endothelial cell growth factor, pla-

- centa growth factor and pleiotrophin in human primary breast cancer and its relation to angiogenesis. *Cancer Res.* **57**, 963–969 (1997).
9. Kaban, L.B. *et al.* Antiangiogenic therapy of a recurrent giant cell tumor of the mandible with interferon  $\alpha$ -2a. *Pediatrics* **103**, 1145–1149 (1999).
  10. O'Reilly, M.S., Holmgren, L., Chen, C. & Folkman, J. Angiostatin induces and sustains dormancy of human primary tumors in mice. *Nature Med.* **2**, 689–692 (1996).
  11. Kerbel, R. & Folkman, J. Clinical translation of angiogenesis inhibitors. *Nature Rev. Cancer* **2**, 727–739 (2002).

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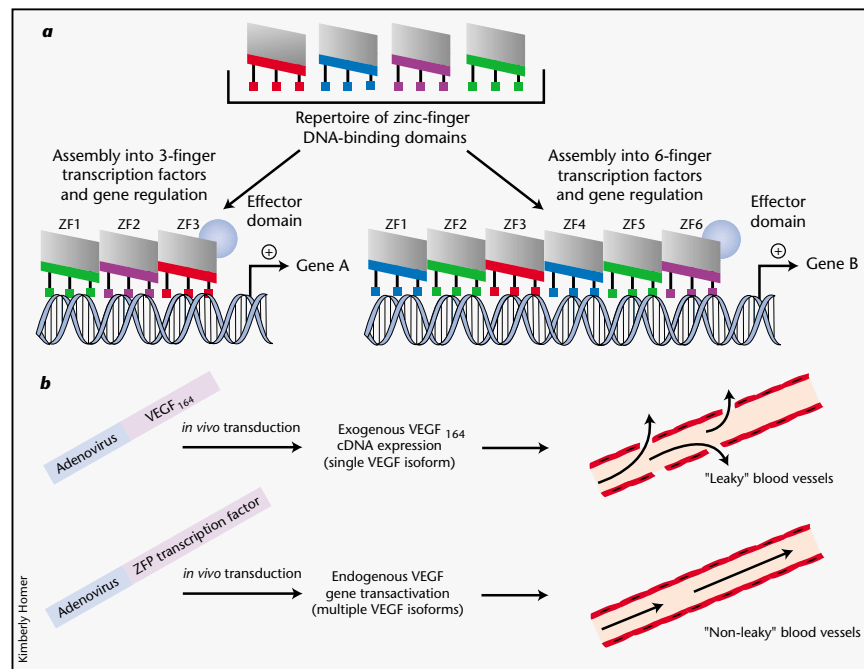
## Vessel maneuvers: Zinc fingers promote angiogenesis

Zinc-finger transcription factors can be engineered to target specific genes. Now this approach is applied successfully to a clinically relevant setting in mice—promotion of angiogenesis. (pages 1427–1432)

Many factors impinge on the formation of blood vessels, but the ultimate control of angiogenesis resides in the nucleus. It is there that the activity of multiple signaling pathways is evaluated, resulting in changes in transcription-factor activity and gene expression. In a study in this issue, Rebar *et al.* take control of the transcriptional program of blood-vessel growth<sup>1</sup>. Using engineered zinc-finger transcription factors, the authors drove blood vessel formation in mice by targeted expression of a pro-angiogenic molecule, vascular endothelial growth factor (VEGF). This study demonstrates for the first time in animals that a designed transcription factor can modulate expression of its intended target and also induce a potentially useful clinical effect.

Of the known DNA-binding motifs, zinc-finger domains have the greatest potential for incorporation into a universal

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**Fig. 1** Designer fingers. **a**, Zinc-finger transcription factors can be designed to target specific sequences by combining finger modules that each bind a three-base-pair sequence. **b**, Transcription factors designed to drive VEGF expression result in multiple VEGF isoforms, unlike VEGF-containing cDNAs. Engineered transcription factors also produce apparently normal vasculature.

system for gene regulation<sup>2,3</sup>. Polydaetyl zinc-finger proteins can now be readily assembled through the combination of zinc-finger domains of predefined specificity (Fig. 1). The combination of such domains

provides for the rapid assembly of a protein that can bind an 18-base-pair (bp) DNA sequence—a DNA address with sufficient complexity to be unique within the genome. Although recent years have seen explosive progress in the design of engineered transcription factors with exquisite specificity, only recently has this approach been applied *in vivo* in animals and transgenic plants<sup>4,5</sup>. Because the chromatin configuration of a particular gene locus can rely heavily on context, successful design of transcription factors that bind a specific DNA locus *in vitro* may not necessarily ensure access to the same gene *in vivo*.

Engineered transcription factors present an advantage over commonly used gene transfer techniques relying on expression of an exogenous cDNA. Engineered factors can activate or repress endogenous genes in the appropriate dose and splicing-variant stoi-

chiometry. This is particularly important if altering the expression levels of different transcripts results in highly heterogeneous phenotypes. Indeed, ectopic expression of VEGF can result in blood vessels with unpredictable properties, including hyperpermeability<sup>6</sup>.

Rebar *et al.* seem to have largely circumvented such problems. They found that their adenoviral-delivered zinc-finger transcription factor induces expression of native VEGF isoforms, apparently leading to the production of a physiologically normal vasculature (Fig. 1). Moreover, this vasculature was functional; compared with a control adenoviral reporter gene construct, the engineered transcription factor accelerated wound healing, which relies on angiogenesis.

The new study verifies the value of transcription factors as targets for drug development and highlights their potential to control angiogenesis in a therapeutic context. But two immediate questions remain. First, is this strategy applicable to other diseases that might benefit from angiogenesis induction, such as ischemia? Second, would it be possible to inhibit angiogenesis by turning off the transcription of pro-angiogenic molecules? Potent and selective gene suppression has already been achieved for the endogenous proto-oncogenes *ERBB-2* and *ERBB-3* in cell culture<sup>7</sup>. Moreover, present engineered transcription factors may do better than

RNA interference—another attention-grabbing technique—when it comes to silencing gene expression (although such approaches must still be compared side-by-side).

The potential to either activate or repress gene transcription is a major advantage of a transcription factor-based approach. Simply changing the effector domain fused to a zinc-finger protein can alter the protein's properties. For example, gene activation could be achieved by fusion of a targeted zinc-finger protein to an activation domain (such as VP-16), whereas repression could be achieved by fusing the same DNA-binding motif to a repression domain (such as the Kruppel-associated box (KRAB)). Additionally, transcription factors could be chemically modified allowing fine-tuning of gene activation or repression<sup>2,7</sup>.

*De novo* design of transcription factors with biological function is still in its early stages. However, preclinical and clinical applications are likely to appear in the future. The low intrinsic toxicity of designed transcription factors in transgenic organisms further supports their clinical potential<sup>5</sup>. Indeed, with proper design it should be possible to regulate multiple genes in a biosynthetic or developmental pathway with a single designed transcription factor. Artificial transcriptional factors might eventually be used to direct the formation of particular, desirable endothelial-cell

phenotypes in blood vessels of tissues—or even whole organs<sup>8</sup>—in order to artificially program protein-expression profiles within selective vascular beds. The work of Rebar *et al.* effectively sets the stage for these developments.

1. Rebar, E.J. *et al.* Induction of angiogenesis in a mouse model using engineered transcription factors. *Nature Med.* **8**, 1427–1432 (2002).
2. Beerli, R.R. & Barbas, C.F. III. Engineering polydactyl zinc-finger transcription factors. *Nature Biotechnol.* **20**, 135–141 (2002).
3. Pabo, C.O., Peisach, E. & Grant, R.A. Design and selection of novel Cys2His2 zinc finger proteins. *Annu. Rev. Biochem.* **70**, 313–340 (2001).
4. Xu, L. *et al.* A versatile framework for the design of ligand-dependent, transgene-specific transcription factors. *Mol. Ther.* **3**, 262–273 (2001).
5. Guan, X. *et al.* Heritable endogenous gene regulation in plants with designed polydactyl zinc finger transcription factors. *Proc. Natl. Acad. Sci. USA* **99**, 13296–13301 (2002).
6. Thurston, G. *et al.* Angiopoietin-1 protects the adult vasculature against plasma leakage. *Nature Med.* **6**, 460–463 (2000).
7. Beerli, R.R., Dreier, B. & Barbas III, C.F. Positive and negative regulation of endogenous genes by designed transcription factors. *Proc. Natl. Acad. Sci. USA* **97**, 1495–1500 (2000).
8. Pasqualini, R. & Arap, W. Vascular targeting. in *Encyclopedia of Cancer*, Vol. 4, 2nd edn. (ed. Bertino, J.R.), 501–507 (Academic Press, San Diego-Oxford, 2002).

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## Breaking up the biofilm

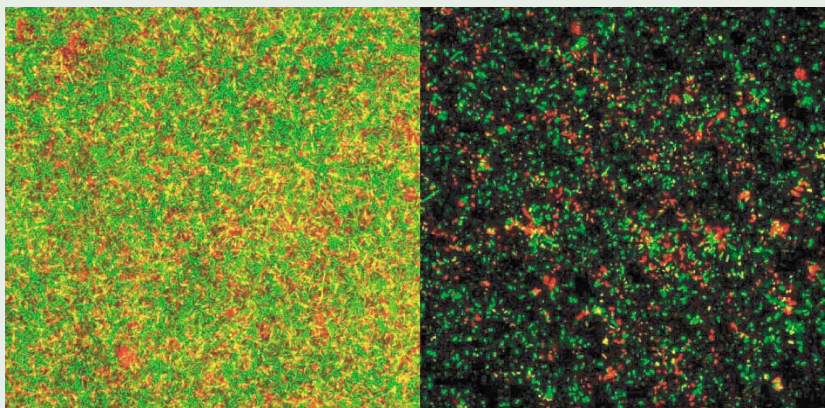
The lungs of patients with cystic fibrosis can contain slimy biofilms of the bacterium *Pseudomonas aeruginosa*, enmeshed in thick airway mucus. These biofilms present a front against antibiotics and other treatments, and patients succumb to complications from such bacterial infections, often before their mid-30s.

Recent data have suggested that in the lung, biofilms persist under anaerobic conditions. In the October *Developmental Cell*, Sang Sun Yoon *et al.* describe experiments replicating these anaerobic biofilms in culture. They find that *P.*

*aeruginosa* form denser, more robust biofilms under anaerobic (left) than aerobic conditions (right). In both images, live bacteria are stained green and dead bacteria are red. The authors went on to identify genes that assist in biofilm formation in anaerobic conditions. Among these were the outer membrane protein F (*OprF*) gene, which was upregulated 5-fold during aerobic biofilm growth but 39-fold during anaerobic growth. Bacteria without *OprF* produced very poor anaerobic biofilms. Yoon *et al.* provide hints that bacteria lacking *OprF*, a channel-forming protein, are defective in a respiratory pathway critical for anaerobic growth.

Anaerobic conditions impair the effectiveness of many 'front-line' antibiotics such as tobramycin. If anaerobic biofilm formation could be effectively inhibited, say the authors, this might give these antibiotics a second chance to work in patients with particularly resilient *P. aeruginosa* populations. Indeed, vaccination with *OprF* has been shown to protect mice against *P. aeruginosa* infection.

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