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 antivascular 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.


Vessel maneuvers: Zinc fingers promote angiogenesis

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.

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 system for gene regulation. Polydactyl 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. 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 at least two substantial advantages 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 stoichiometry. 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.

Rebar et al. seem to have largely circumvented such problems with ectopic VEGF expression. They found that their adenoviral-delivered zinc-finger transcription factor induces expression of native VEGF isoforms leading to the production of an apparently 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. Moreover, present engineered transcription factors 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.

De novo design of transcription factors with biological function is in its early stages. However, preclinical and clinical applications are certain to appear in the future. The low intrinsic toxicity of designed transcription factors in transgenic organisms further supports their clinical potential. 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—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.


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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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