Numerous studies have demonstrated the importance of altered gene expression in disease pathophysiology. Thus, modification of gene expression has emerged as an important therapeutic strategy. Agents that inhibit the transcription of disease-mediating genes or transactivate the expression of genes whose products disrupt pathophysiological processes are being developed. Regulation of gene expression is a complex biological process involving transcription factor–DNA interaction that initiates gene transcription. Transcription factors are proteins residing in the cell nucleus or cytoplasm that upon activation bind with specific DNA motifs in the promoter region of the target gene. The activation process may involve phosphorylation, dimerization, proteolytic cleavage, ubiquitination, and/or translocation. Accordingly, molecules that block one or more steps of the transcription machinery can potentially modify the expression of a specific gene or group of genes, yielding a functional response(s).

It has been shown that short sequences of DNA containing the consensus binding site, even in the absence of surrounding DNA, can bind transcription protein in a highly specific manner. Indeed, such oligonucleotides have been used as radiolabeled probes for the detection and characterization of transcription factors (TFs) in electrophoretic mobility shift assays. Specific DNA sequences have been used successfully as “decoys” to bind specific TFs in cultured cells or in vivo, rendering the TFs incapable of subsequent binding to the promoter region of target genes. This approach has been shown to be effective in modulating gene expression in vitro and in vivo. Indeed, the use of transcription factor decoy (TFD) as a tool to study gene expression in vivo was first demonstrated for tissue-specific regulation of renin gene expression. The data showed that decoy mimicking a specific negative regulatory element (CNRE) in the promoter region of the renin gene resulted in the derepression of renin expression in extra renal tissues such as liver and submandibular gland, thereby documenting the functional role of CNRE in suppressing renin expression in these tissues. Similar approaches have been used by others in studying the regulation of gene expression.

The in vivo effectiveness of TFD in modulating gene expression prompted the consideration of its use in therapy. We reported the development of a decoy that targeted the E2F family of transcription factors that regulate cell cycle at the G1/S checkpoint with the aims of blocking vascular smooth muscle cell (VSMC) proliferation in vitro and inhibiting neointimal formation in vivo. Since this initial report, numerous TFDs have been successfully used to modulate the expression of a variety of target genes yielding therapeutic actions. These include TFDs to E2F, NF-κB, CREB, AP-1, AGE, etc., that have been shown experimentally to be effective in treating vascular proliferation, myocardial infarction, tumor growth and invasiveness, and hypertension, etc.

In this issue of Circulation Research, Ahn et al. reported the inhibitory effects of AP-1 decoy oligonucleotide on VSMC proliferation in vitro and neointimal formation in vivo. The rationale for targeting AP-1 is based on the data that vascular injury activates JNK and ERK that translocate to the nucleus to activate c-Jun and c-Fos, which subsequently dimerize to form the AP-1 complex. AP-1 binds to specific DNA motif in a number of genes involved in VSMC proliferation. The AP-1 decoy strategy used by these authors is an extension of our earlier work as well as those of others demonstrating the effectiveness of AP-1 decoy in inhibiting target gene expression and VSMC growth in vitro and neointimal formation in vivo. Thus, AP-1 decoy joins E2F and NF-κB as TFDs that show much promise as molecular therapy for vascular proliferative disorders including restenosis, atherosclerosis, bypass graft failure, and transplant vasculopathy.

What is the evidence that TFD can exert biological effect and produce therapeutic benefit? Will TFD be effective as human therapy? Among the TFDs reported to date, E2F decoy has undergone the most extensive evaluation with successful completion of preclinical and toxicology studies, as well as phase I/II human trials. E2F was targeted because it is hypothesized that blocking cell cycle, the common denominator of the pathophysiological process of vascular proliferation, is the most effective strategy to prevent neointimal hyperplasia. Indeed the first clinical trial (PREVENT-I) on human bypass vein grafts demonstrated that E2F decoy inhibited PCNA and c-myc expression and blocked vascular cell proliferation of the graft. PREVENT-I studied a cohort of patients at high risk for lower-extremity graft failure in a prospective, randomized, and controlled fashion. The study results met the primary end points, which were safety and feasibility. A second larger phase IIB clinical trial was performed to study the effect of E2F decoy on coronary artery bypass grafts (CABGs). The result demonstrated a 30% to 40% decrease in CABG failure as documented by angiography and intravascular ultrasound. Two

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phase III trials evaluating efficiency in CABG and peripheral arterial bypass are now underway.

The long-term success of TFD oligonucleotide as a broadly utilized therapeutic modality will depend on several critical factors. These include the specificity of the TFD, the stability of the oligonucleotide, and the efficiency of tissue/cellular delivery. Theoretically, TFD can modulate the expression of all genes that contain the target consensus DNA sequence and in all tissues. However, this broad effect may not be desirable in all circumstances. Thus, careful selection of the target TF and the development of tissue-directed delivery are important ways to address this concern. The duration of TFD action is dependent, to a large extent, on the intracellular stability of the TFD oligonucleotide. Most of the studies have used double-stranded linear DNA (PS ODN) with chemical modification that involves phosphothioation and methylphosphonation. These modifications increase resistance to nuclease digestion but may render insensitivity to RNaseH and induce immune response.25,26 To address these concerns, Ahn et al27 synthesized circular dumbbell decoy oligonucleotide (CD ODN) that is covalently modified with the enzymatic ligation of the two identical DNA stands. It has been reported that CD ODNs are more resistant to exonuclease and can be transported into cells with more efficiency. Indeed, Ahn et al demonstrated that CD ODN was more stable than PS ODN in vitro and was more effective in inhibiting VSMC growth and migration in vitro. However, the differential efficacy in vivo between CD ODN versus PS ODN (using a single concentration) in inhibiting neointimal formation was quite modest. A dose-ranging study with full dose-response curves both in vitro and in vivo would be more informative and convincing. Nevertheless, this study highlights the importance of addressing the appropriate chemical modification of TFD ODNs to optimize their use in human therapy in vivo.

Another major determinant of TFD effect is the efficiency of cellular uptake and delivery. ODNs are internalized into cells by endocytosis that is associated with ODN degradation by nuclease. Attempts at enhancing ODN uptake using cationic, anionic, or other lipid formulations as delivery vehicle have met with variable success in vitro and in vivo. In this report, the authors used HVJ-liposome for increased vascular delivery. Indeed, the efficiency of this approach for arterial gene or ODN transfer has been demonstrated previously.27,28 Sendai virus (HVJ) contains two fusigenic proteins on its viral coat that enable fusion at neutral pH with plasma membrane. Thus, liposomes containing UV-inactivated HVJ or recombinant fusigenic proteins can facilitate intracellular uptake of ODN or gene that is encapsulated in the HVJ-liposome. More recently, we have also developed a novel approach of pressure-mediated transfection.29,30 ODN can be delivered into tissues in a pressure-dependent manner achieving up to 80% efficiency. This approach has been important in our E2F decoy strategy for preventing bypass graft failure.

Finally, the timing of treatment with TFD may also be important in influencing the therapeutic effect. The report of Ahn et al.27 pretreatment with AP-1 decoy before vascular injury resulted in more effective inhibition of neointimal formation than treatment after injury. The explanation for increased efficacy of the pretreatment approach is the early inhibition of genes and cellular processes that are immediately activated in response to injury leading to vascular proliferation. This observation has potential implications in the clinical sequence of TFD administration before interventional/surgical procedure. Indeed, in our study of bypass graft, we transplanted the vein graft with TFD ex vivo, before implantation in vivo. The magnitude of inhibition of vascular proliferation in both experimental and human grafts is in excess of 75% using this pretreatment approach.10,11

There has been an explosion in the use of TFDs as tools for studying gene regulation and as experimental therapy to treat a variety of pathological conditions. The results to date are very encouraging. Careful and systemic planning and improvement of this technology by addressing the method and timing of delivery, ODN stability, and specificity of action will facilitate the development of TFD as an important therapeutic modality for human diseases.

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