Decoy oligonucleotides: silencing the negativity in gene therapy?

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Throughout the last 15 years, the concept of replacing deficient genes or knocking down the expression of genes that cause disease has been consistently developed and is under scrutiny by the public. This concept is known as gene therapy. Several strategies of gene therapy have been developed, ranging from the use of retroviral vectors carrying a deficient gene of interest whose aim is to re-insert the deficient gene into the patient genome, to the use of DNA technology to inhibit target gene expression. In particular, the application of DNA technology, such as RNA interference and antisense strategies, to silence or regulate transcription of disease-related genes *in vivo* has important therapeutic potential. This perspective highlights both the positive and negative aspects of gene therapy and raises questions as to how effective the use of retroviral vectors and decoy oligonucleotides can be as gene-therapy strategies.

During the last 5 years, the transfer of *cis*-element double-stranded oligonucleotides, termed 'decoys' or 'aptamers', has proven to be a powerful tool in a new class of anti-gene strategies [1]. As illustrated in **Figure 1**, decoy oligonucleotides (dODN) usually mimic the consensus binding site of a specific transcription factor. Gene expression controlled by this transcription factor is effectively prevented, thereby effectively silencing gene expression and preventing the protein from being produced. Therefore, being less specific in comparison with the small interfering (si)RNA or antisense ODN method, the dODN technique can be considered as a gene silencing approach. Nevertheless, decoys have been shown to be highly specific for their target transcription factor.

In comparison with the use of decoys, earlier gene-therapy trials demonstrated the harmless and practical methodology of using retroviral vectors to transduce cells, such as peripheral blood lymphocytes and hematopoetic stem cells *ex vivo*, in order to express a gene of interest. The engineered cells would then be returned to the patients and monitored for integration and expression of the viral vector, as well as recovery effects of the genetic abnormality.

The use of viral vectors has been shown to be useful in clinical trials of children affected by rare inherited disorders that cause severe combined immunodeficiency (SCID). The use of a retroviral vector in these clinical trials was aimed at replacing a functional copy of the defective gene in some hematopoetic and progenitor cells, and appeared to cure the immunodeficiency [2,3]. Despite these positive results, a fraction of children treated for the X-linked form of SCID were later diagnosed with leukemia due to consequent oncogene activation at a vector-insertion site [4]. It is also difficult to forget the sudden death of Jesse Gelsinger in Pittsburgh (PA, USA) in 1999 during an adenoviral gene-therapy trial to correct a single gene deficiency known as ornithine transcarbamylase enzyme deficiency. Jesse started showing signs of severe immune system reactivity 24 h after the adenoviral vector was administered, which resulted in multiple-organ system failure; he then became the first human victim of this technology 4 days after gene-therapy treatment. Thus, despite the positive results obtained to date, it is no wonder that the use of viral vectors for gene therapy spur new debate and public scrutiny.

The use of decoys provide researchers and clinicians with a new opportunity to target the genetic roots of pathogenesis and disease progression. Since decoys have been used to stimulate gene expression by targeting transcriptional repressors, several other novel applications of transcription-factor decoys include their use as adjuvants to modify either the immune reaction, metabolism of other active drugs and delivery vehicles, or to influence the biological response to cell-based therapeutics; lyophilized decoys are also very stable and readily water soluble, thus making them more clinically favorable. Taking these points into consideration,

Keywords: antisense oligonucleotide, decoy oligonucleotide, gene expression, retroviral, RNA interference, transcription factor, viral vectors

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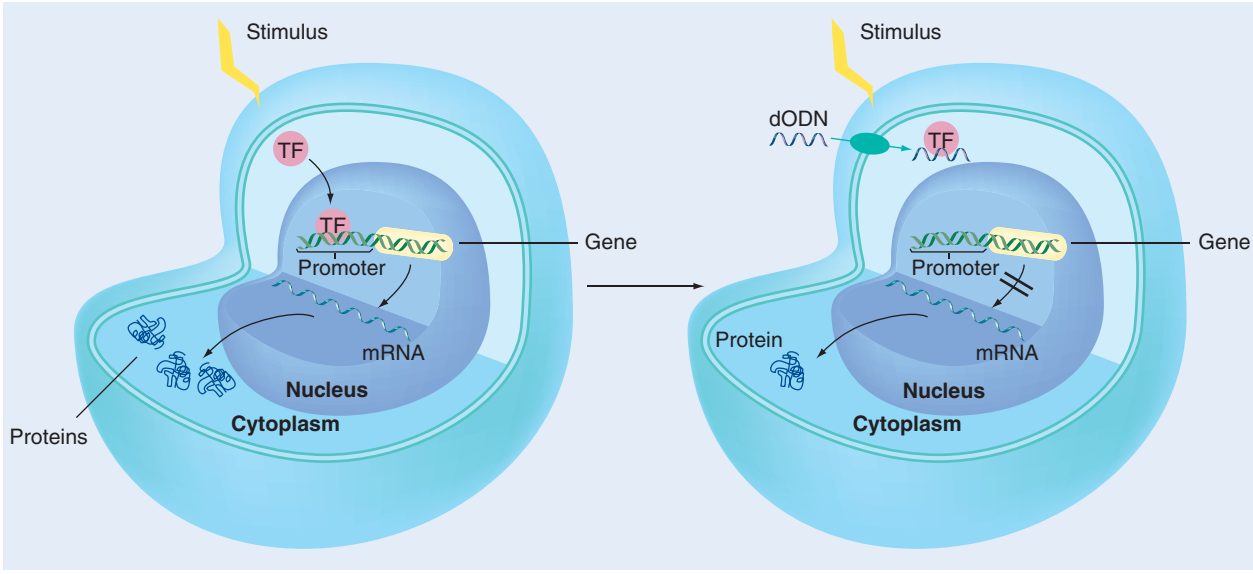
Figure 1. The gene-silencing action of dODN.



dODN: Decoy oligonucleotides; TF: Transcription factor.
Adapted with permission from Markus Hecker.

there are no limiting factors for their formulation and development as aerosols, droplets, ointments or injectables.

Rapid expansion of our understanding of the diversity of transcription factors and the roles that these regulatory proteins play in normal and abnormal cellular function has demonstrated the design, investigation and development of transcription factor decoy technology as appealing. Papers published on the ability to use decoys as research tools or as potential therapies continue to grow each year and a few examples of models in which decoys have been studied include inflammation, ranging from arthritis and sepsis (nuclear factor [NF]κB), to allergic airway reactivity and asthma (signal transducer and activator of transcription [STAT] 1 and NFkB), to glomerulonephritis (E2F and AP1) [5]. A large number of decoy applications in cancer have also been investigated, including colorectal and ovarian cancer (CRE), melanoma (SP1) and breast cancer (CRE, estrogen receptor and NFkB).

One of the best-studied applications of decoys to date is targeted to inhibit NFκB [5]. In particular, this target is attractive since it has been reported to act as a 'master switch', not only in response to proinflammatory stimulation to drive a cellular response, but also towards intra- and extra-cellular processes. Numerous stimuli are known to transactivate NFκB, including oxidative stress, ultraviolet radiation, proinflammatory cytokines, such as interleukin-1 and tumor necrosis factor-α, and lipopolysaccharide. Thus, strategies and agents to inhibit NFκB may present attractive therapeutic targets that have greater specificity compared with other anti-inflammatory drugs, such as the corticosteroid family of drugs.

The most advanced clinical program involving the use of transcription factor decoys reported to date involves the modification of the biology of bypass vein-graft failure via intraoperative delivery of decoys that inhibit postoperative neointimal hyperplasia. In this case, decoys specifically targeting NFκB, C/EBP and AP1 have all been studied [5,6]. Other clinical trials are also underway and initiated to examine specifically the STAT1 decoy on reactive airway disease and NFκB on atopic dermatitis.

Future perspective

During the next 5 years, we should start to observe an increase in the number of clinical trials involving treatment with decoys around the globe, in its movement from bench to bedside. Despite the fact that decoys are seemingly proving to have positive effects in ongoing clinical trials, an important point to consider is that hundreds of transcription factors have been described and grouped, primarily by the consensus DNA sequences to which they bind

within the promoter/enhancer regions of the genes they regulate; a degree of overlap and promiscuity exists regarding the classes and roles of transcription factors in cell division, growth and differentiation. The degree of overlap and promiscuity existing within families of transcription factors raises the following questions: how effective is the use of transcription factor decoys when the target gene of interest is positively and negatively affected by two transcription factors, where both transcription factors recognize similar binding sites? How efficient are decoys when targeting a transcription factor that acts as both a repressor and inducer of gene expression? Gaining a deeper understanding of the complex network involved in gene activation and repression to predict the use of decoys is a promising and exciting development in gene therapy.

Disclosure

Nicole Nogoy is a Commissioning Editor with Future Drugs Ltd. She has received no financial or other incentives for this work.

Executive summary

- The regulation of transcription of disease-related genes *in vivo* has important therapeutic potential.
- Current disease models where the use of decoys have been employed include arthritis, sepsis, asthma, glomerulonephritis and a variety of cancers, such as colorectal, ovarian, melanoma and breast cancer.
- One of the major transcription factors targeted is nuclear factor κB, as it has a tendency to act as a 'master switch' in response to proinflammatory stimulation and has the ability to drive specific intra- and extra-cellular responses.

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