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DNAzymes as molecular agents that manipulate Egr-1 gene expression

Levon M. Khachigian*

Department of Haematology, Centre for Vascular Research, University of New South Wales, Prince of Wales Hospital, Sydney, Australia

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Abstract

In recent years, the arsenal of small-molecule synthetic nucleic acids as gene-specific ''knock-down'' agents has increased in scope and variety. The investigator has the choice of antisense oligonucleotides, ribozymes, siRNA and DNAzymes, each subclass further benefiting from modifications that increase stability and efficiency and decrease toxicity. This review describes our use of DNAzymes in efforts to define the roles of key transcription factor targets, first in cultured vascular cells, then in animal models of neovascularization and arterial thickening.

Keywords: DNAzymes; Catalytic DNA; Egr-1; Angiogenesis; Restenosis; Vascular smooth muscle cells

Early growth response-1 (Egr-1) is a zinc finger transcription factor and the product of an immediate-early gene [1]. Egr-1 transcription is dependent upon several serum-response elements in its promoter [2] that mediate rapid induction under conditions of chemical, biomechanical, immunological or injurious challenge [3]. Egr-1 binds to the promoters of many genes, including those of key growth factors, which in turn stimulate cell proliferation and chemotaxis. Lessons from our own work examining the platelet-derived growth factor A-chain [4] and B-chain [5] indicates that Egr-1's interaction with DNA is associated with the displacement of other transcription factors, such as Sp1, from shared or overlapping binding sites in the promoter.

Regulatory factors, like Egr-1, that control the expression of multiple growth-regulatory genes are by definition ''master-regulators'' and, as such, are attractive targets for gene ''knock-down'' analysis, particularly in the case of Egr-1 where the gene is poorly expressed in the normal vessel wall. The weaponry of synthetic gene silencing agents presently includes antisense oligonucleotides, ribozymes, siRNA and DNAzymes. DNAzymes are single-stranded DNA molecules that bind to their target RNA through Watson–Crick base-pairing and cleave between an unpaired purine and a paired pyrimidine by virtue of a cation-dependent catalytic domain (e.g. the ''10–23'' domain) [6]. Unlike the alternative approaches, DNAzymes do not rely on RNase H for destruction of the mRNA; these agents are stable in serum, have reasonable potency and can be produced at relative low cost. DNAzyme stability can be further increased, without compromising catalytic efficiency, by incorporation of structural modifications (such as base inversions, methylene bridges, etc.) into the molecule.

DNAzymes (with 9 + 9 nt arms, with a 3′-linked inverted T at the 3′-end) targeting Egr-1 inhibit the growth of primary arterial smooth muscle cells [7] and endothelial cells [8] in culture and impair their capacity to regenerate after wounding in vitro. We used these molecules in a number of animal models involving smooth muscle and endothelial cell growth (Table 1). When delivered adventitially (from the outside of the vessel) to the carotid arteries of rats, Egr-1 DNAzymes inhibited intimal thickening, which involves aggressive smooth muscle cell growth in the arterial intima, 2 weeks following balloon catheter angioplasty [8] and 3 weeks after permanent ligation injury [9]. Moreover, when delivered endoluminally in pig arteries by catheter (from the inside of the vessel), Egr-1 DNAzymes blocked in-stent restenosis 1 month after coronary stenting [10].

Besides inhibiting endothelial cell growth and repair in vitro, Egr-1 DNAzymes perturbed spontaneous tubule formation within hours of plating more effectively than

Abbreviations: Egr-1, early growth response-1

* Tel.: +61 2 9385 2537; fax: +61 2 9385 1389.
E-mail address: l.khachigian@unsw.edu.au (L.M. Khachigian).

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Table 1
Efficacy of Egr-1 DNAzymes in animal models

Species	Model	Reference
Restenosis and in-stent restenosis		
Rat	Balloon injury (carotid)	[7]
Rat	Permanent ligation (carotid)	[9]
Pig	Stenting (coronary)	[10]
Angiogenesis		
Mouse	Solid tumor growth	[8]
Mouse	Plug neovascularization	[8]
Rat	Neovascularization (cornea)	[8]

Table 2
Examples of other DNAzyme targets

Target	Reference
bcr-abl	[11]
c-myc	[12]
Integrins beta$_1$ and beta$_3$	[13,14]
PKC-alpha	[15]
PKC-epsilon	[16]
c-Jun	[17]
Twist	[18]
VEGF receptor-2	[19]
Laminin gamma$_1$ chain	[20]
HIV-1	[21,22]
Platelet-type 12-lipoxygenase	[23]

DNAzymes targeting integrins beta$_1$ and beta$_3$ [8]. Egr-1 DNAzymes also caused regression of preformed tubules, whereas their scrambled arm counterparts had no activity compared with the vehicle controls. The Egr-1 DNAzymes inhibited vascularization of subcutaneous matrigel plugs in mice and reduced angiogenesis in rat cornea after VEGF stimulation. The DNAzymes impaired solid tumor growth in athymic nude mice, decreasing vessel density in the tumors, without adversely affecting body weight, wound healing, blood coagulation or reproduction [8]. Therefore, using DNAzymes we have determined that microvascular endothelial cell growth, neovascularization, tumor angiogenesis and tumor growth are processes critically dependent on Egr-1. In the plugs, the tumors and in cultured microvascular endothelial cells, these DNAzymes inhibited the expression of FGF-2, which is Egr-1-dependent, but not that of VEGF. While DNAzymes have great promise as gene-specific and versatile therapeutic agents (Table 2), the issue of delivery, like other synthetic nucleic acid-based drugs, remains to be resolved. Our studies involved local, rather than systemic delivery of DNAzyme, which presently limits the wider therapeutic application of these molecules in clinical settings.

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