COMMENTARY

Natural Antisense Makes Sense for Gene-specific Activation in Brain

Miguel A Varela¹, Thomas C Roberts¹, Samir EL Andaloussi¹ and Matthew JA Wood¹

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The upregulation of specific genes in vivo has been an elusive goal for gene therapy when compared with the wide repertoire of methods available to silence genes or modify mRNA splicing patterns. In the latest issue of Nature Biotechnology, Modarresi and colleagues¹ accomplished in vivo upregulation of brain-derived neurotrophic factor (BDNF), a relevant therapeutic target for a number of neurodegenerative diseases. Rather than using small molecules or microRNA inhibitors, which could lead to activation of off-target genes, Modarresi et al.¹ upregulated BDNF by inhibiting a natural antisense transcript (NAT) in response to the local delivery of oligonucleotides to the central nervous systems of mice. It is now clear that mammalian genomes are much more extensively transcribed than was once thought, and that the vast majority of cellular transcriptional output is noncoding RNA. Katayama et al.² reported widespread antisense transcription in the human and mouse genomes and showed, for the first time, that targeting antisense transcripts with small interfering RNAs influenced the expression of overlapping sense mRNA transcripts. As such, NATs function to regulate expression of neighbouring genes in cis. Further studies from the laboratories of David Corey, Kevin Morris, and Long-Cheng Li demonstrated that antisense transcripts are epigenetic regulators of their corresponding sense strand protein-coding genes, and in the cases of the progesterone receptor and the tumor suppressor p21, targeting these transcripts with small interfering RNAs resulted in a loss of epigenetic repression and consequently gene activation.³,⁴ More recently, a landmark in vivo study by Turunen et al.⁵ reported transcriptional activation of vascular endothelial growth factor by lentiviral expressed promoter-targeting short hairpin RNAs in a mouse hindlimb ischemia model, thus demonstrating the therapeutic relevance of this gene activation approach. However, the present study by Modarresi et al.¹ is the first demonstration of oligonucleotide-mediated transcriptional gene activation in vivo and is an elegant and important step towards translating this approach into novel molecular therapies, especially for neurological disorders.

Targeting the NAT of BDNF to increase BDNF levels in the central nervous system has therapeutic potential. BDNF plays a central role in neurogenesis, neuronal development, and synaptic plasticity. Neurogenesis occurs mainly during development, but in adulthood it is involved in the consolidation of memory for which BDNF is also a critical factor.⁶ Higher levels of BDNF have also been found to be predictors of a slower cognitive decline in Alzheimer’s disease patients.⁷ Additionally, activation of the BDNF signaling pathway is responsible, at least in part, for the neuroprotective effects of physical exercise in Parkinson disease, promoting neuronal survival, and facilitating the recovery of brain functions after injury.⁸ However, little is known about the function of this NAT in humans and Modarresi et al.¹ now identify this transcript in mouse for the first time.

This study showed that inhibiting the NAT of BDNF stimulated neuronal outgrowth in vitro and improved neuronal survival and proliferation in vivo as a direct result of BDNF gene activation and increased BDNF protein levels. Additionally, the authors showed in vitro activation for two other genes using the same approach: glial-derived neurotrophic factor and ephrin receptor B2. Modarresi et al.¹ tested small interfering RNAs and an array of oligonucleotides composed of locked nucleic acids⁹ either mixed with 2’OMe RNA chemistry for steric block or designed as gapmers to induce RNase H cleavage of complementary NATs. The locked nucleic acid gapmers proved to be most potent oligonucleotides to date, with high affinity for the targeted transcript.

In contrast to the positive data in these brain regions, Modarresi et al.¹ observed that the levels of BDNF NAT and BDNF mRNA were unaltered in the hypothalamus, and explained this by suggesting that oligonucleotide delivery was more effective to regions immediately adjacent to the third ventricle. However, the hypothalamus is in very close proximity to the third ventricle; thus alternative explanations for this lack of response could include differential expression of BDNF or its corresponding NAT, which the authors have shown to have highly tissue-specific patterns of expression.

Since regulation of gene expression by NATs can be either transcriptional or post-transcriptional, Modarresi et al.¹

¹Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK
Correspondence: Matthew JA Wood, Department of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford, OX1 3QX, UK.
E-mail matthew.wood@dpag.ox.ac.uk
potential long-term. However, in this study the effect was relatively transient (96 hours), suggesting that the presence of NAT is required to maintain silencing.

Although representing an extremely encouraging strategy to interfere with gene expression, the use of oligonucleotides as therapeutic agents is hampered by their limited bioavailability. This is evident also in this study where relatively high total doses (1.5 mg/kg/day for 28 days) were administered, despite being delivered locally using an invasive procedure. From a therapeutic perspective, it would be more feasible to exploit delivery vehicles for systemic delivery of these compounds. However, bypassing the blood-brain barrier remains a major hurdle. An attractive approach is to use biological membrane vesicles termed exosomes for delivery of therapeutics to the brain. This can be accomplished by using modified exosomes that display brain-homing peptides on their surface for systemic delivery or using unmodified exosomes for intranasal administration.

As to whether the transcriptional activation of BDNF represents an effective therapeutic approach, it will be important to extend these observations in further studies comparing this approach with other methods such as administering synthetic BDNF molecules as the authors acknowledge. Indeed, Pardridge and colleagues\textsuperscript{12,13} have previously exploited the “Trojan Horse” approach to convey BDNF protein to the brain by systemic delivery. By using a monoclonal antibody targeting the transferrin receptor conjugated to BDNF, significant increases in BDNF levels were observed in brain and phenotypic improvements were detected in a rat model of transient forebrain ischemia.\textsuperscript{12,13} Regarding the safety of increasing BDNF levels, it would seem to be a relatively safe target to explore the benefits of transcriptional modulation since fluctuations in the levels of this molecule can also occur naturally without adverse effects. For example, upregulation can also be triggered by exercise.\textsuperscript{6} It will be extremely interesting to see whether the increases in BDNF protein levels (50–100% over background) reported by Moderessi \textit{et al.}\textsuperscript{1} are sufficient to mediate phenotypic improvements in a relevant disease model. If this is the case, this study will have opened a new avenue for therapeutic intervention of various neuronal disorders.

In summary, the study by Modarresi and colleagues\textsuperscript{1} is a welcome and elegant addition to the field and advances the concept of transcriptional gene modulation by antisense transcripts to \textit{in vivo} studies on a relevant therapeutic target. However, the generality of this approach remains uncertain since NATs have been annotated in only a minority of genes to date, and the percentage of these that is relevant for gene regulation is unknown.\textsuperscript{14} Nevertheless, the results of this study encourage a search to discover more therapeutically relevant NATs. These are exciting times for gene therapy as it now appears that the same natural mechanisms that regulate gene expression in the nervous system could be manipulated in order to protect it from the effects of neurodegenerative disease.

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Supplementary Information accompanies this paper on the Molecular Therapy–Nucleic Acids website (http://www.nature.com/mtna)