Exercise as Gene Therapy: BDNF and DNA Damage Repair

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Abstract

DNA damage is a common feature of neurodegenerative illnesses, and the ability to repair DNA strand breaks and lesions is crucial for neuronal survival (see Jeppesen et al1 and Shiwaku et al2 for reviews). Interventions aimed at repairing these lesions, therefore, could be useful for preventing or delaying the progression of disease. One potential strategy for promoting DNA damage repair (DDR) is exercise. Though the role of exercise in DDR is not understood, there is increasing evidence that simple physical activity may impact clinical outcomes for neurodegeneration. Here we discuss what is currently known about the molecular mechanisms of BDNF and how these mechanisms might influence the DDR process.

Neuronal DNA Damage and BDNF

The ability to efficiently repair strand breaks and other lesions in DNA is essential to the survival of eukaryotic cells.3 Accordingly, the accumulation of such breaks and lesions is associated with the development or progression of neurodegenerative diseases.4–8 Neuronal cells are especially vulnerable to DNA damage. These cells are highly metabolically active and thus produce DNA-damaging reactive oxygen species at a greater rate than other cell types.9 At the same time, they are also terminally differentiated, so the inability to repair DNA damage may lead to an irreversible loss of vitally important neurons.10

Emerging evidence suggests that it may be possible to enhance the inherent DNA repair capabilities of neurons through regulation of BDNF. In its mature form, BDNF is a 14 kDa polypeptide that acts primarily by binding highly specifically and selectively to the TrkB receptor.11,12 Binding to TrkB sets off a series of intracellular signals that promote cell survival, and several studies have concluded that BDNF protects neurons from death and degeneration, both in vitro and in vivo.13–16 Proposed explanations for BDNF-mediated neuroprotection include maintenance of the cytoskeleton through Rho family GTPases17 and inhibition of apoptosis through suppression of p5318 or increased expression of the apoptosis-mediating protein Bcl-2.19 In addition to its antiapoptotic effects, there is increasing evidence that BDNF may protect neurons through regulation of DNA damage repair (DDR).20 In fact, BDNF may be so effective against DNA damage that it is...
detrimental to cancer chemotherapy: cisplatin, for example, kills tumor cells by inducing cross-linkage of DNA, and exogenous BDNF has been shown to induce resistance to cisplatin treatment.\textsuperscript{21,22} Nonetheless, the role of BDNF in normal, noninvasive cells is largely beneficial.\textsuperscript{23}

A major obstacle to the clinical applications of BDNF, however, is that it is difficult to administer directly.\textsuperscript{24} Even in experimental settings where BDNF is able to be delivered to the desired site of action, as by local injection, the half-life of BDNF is short, limiting its clinical potential.\textsuperscript{25} The following paragraphs will discuss alternative strategies for harnessing the therapeutic utility of BDNF for DDR.

**Exercise and BDNF in Neuronal DNA Damage Repair**

Exercise is consistently shown to upregulate BDNF,\textsuperscript{26} and certain forms of physical activity have demonstrated the ability to evoke BDNF release.\textsuperscript{27,28} It is hypothesized that the effect of exercise on BDNF is an adaptive response to low-level stressors; exposure to mildly harmful stimuli (eg, reactive oxygen species generated by aerobic metabolism) may be triggering a protective response.\textsuperscript{29} Instead of administering exogenous BDNF, then, it may be possible to increase BDNF levels—and thereby offset DNA damage—through exercise.

The evidence for an exercise-based approach to the treatment of neurodegeneration is only recently emerging. Though the upregulation of DDR pathways was previously observed in skeletal muscle\textsuperscript{30,31} and liver,\textsuperscript{32} there are only a few studies that have specifically looked at the connection between exercise and DNA damage in neurons. In 2011, Koltai et al\textsuperscript{33} examined DDR in exercised rats and found increased levels of the protein Ku70, which aids in the repair of DNA double-stranded breaks. Later, Yang et al reported that voluntary running elevated levels of both BDNF and Ape-1, a major component of the base excision repair (BER) pathway.\textsuperscript{20} A similar effect of exercise has also been noted in APP/PS1 mice, a transgenic model of Alzheimer disease.\textsuperscript{34} After 20 weeks of treadmill running, Bo et al isolated hippocampal mitochondria from the APP/PS1 mice to study another BER enzyme, Ogg-1. Treadmill running increased both total protein levels of Ogg-1 and Ogg-1 activity and also led to reduced levels of the 8-oxo-dG DNA lesion.\textsuperscript{34}

**DNA Damage Repair and Gene Editing**

The recent rise in popularity and availability of CRISPR/Cas9-based gene editing systems has renewed interest in gene therapy. The goal of gene therapy is to excise or replace genes that create a disease phenotype. In the past, these goals have been achieved with the use of zinc-finger nucleases (ZFNs) or transcription activator–like effector nucleases (TALENs).\textsuperscript{35} Gene editing with ZFNs or TALENs requires synthesis of unique proteins that are capable of recognizing and cleaving specific DNA sequences. CRISPR/Cas9 technology, in contrast, exploits a bacterial defense mechanism against viral DNA wherein RNA sequences guide a single protein, the Cas9 endonuclease, to cut specific sites in DNA.\textsuperscript{36} Once DNA cutting takes place, it is possible to insert new DNA sequences into the genome.\textsuperscript{36} For inherited neurodegenerative diseases, CRISPR/Cas9 and other oligonucleotide delivery technologies hold great promise.\textsuperscript{37} Still, there are major challenges to using any gene editing systems in

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neurons and other nondividing cells. First, the efficiency of DNA repair in nondividing cells is already low in comparison with actively dividing cell populations.\textsuperscript{38} This means that there are few opportunities for access to the intracellular enzymes governing DNA repair, and the ability to make changes to the sequence of a gene of interest will be correspondingly limited. Beyond this, effective introduction of new genes with CRISPR/Cas9 technology relies on homology-directed repair (HDR) of DNA strand breaks.\textsuperscript{39} Homology-directed repair is guided by a template to rebuild broken DNA, so pairing Cas9 with a short oligonucleotide can “retrofit” DNA with a new coding sequence. The predominant endogenous repair mechanism in neurons, however, is nonhomologous end joining (NHEJ).\textsuperscript{40} In contrast to HDR, NHEJ is error-prone and may not allow for functional introduction of new genes. Hypothetically, then, exercise could be used to enhance alternate DNA repair pathways, thereby improving the overall efficiency of gene editing in neuronal cells. However, it remains to be seen whether exercise or any other BDNF-modulating techniques will advance the practical use of CRISPR/Cas9 and related systems.

**Future Strategies for Enhancement of DNA Repair**

Recent research increasingly indicates that exercise could be a simple and cost-effective means of enhancing DDR rates via increased circulating or local BDNF. As yet, however, there is no standardized exercise regimen to enhance DDR capability. In the animal studies that have explored this relationship, the intensity and duration of exercise has varied widely. Neither has the relationship between DDR and exercise been adequately studied in humans. Furthermore, as mentioned previously, exercise can induce mild oxidative stress. Intensive exercise may not only be ineffective for DDR, but could itself induce DNA damage.\textsuperscript{41} Therefore, determining an appropriate level of activity will be essential if exercise is ever to be prescribed clinically to enhance DNA repair. However, next-generation gene therapy approaches, though potentially exquisite in their precision, will need augmentation to increase the rate of DNA repair.

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