

# Targeted delivery of brain-derived neurotrophic factor for the treatment of blindness and deafness

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**Abstract:** Neurodegenerative causes of blindness and deafness possess a major challenge in their clinical management as proper treatment guidelines have not yet been found. Brain-derived neurotrophic factor (BDNF) has been established as a promising therapy against neurodegenerative disorders including hearing and visual loss. Unfortunately, the blood–retinal barrier and blood–cochlear barrier, which have a comparable structure to the blood–brain barrier prevent molecules of larger sizes (such as BDNF) from exiting the circulation and reaching the targeted cells. Anatomical features of the eye and ear allow use of local administration, bypassing histo-hematic barriers. This paper focuses on highlighting a variety of strategies proposed for the local administration of the BDNF, like direct delivery, viral gene therapy, and cell-based therapy, which have been shown to successfully improve development, survival, and function of spiral and retinal ganglion cells. The similarities and controversies for BDNF treatment of posterior eye diseases and inner ear diseases have been analyzed and compared. In this review, we also focus on the possibility of translation of this knowledge into clinical practice. And finally, we suggest that using nanoparticulate drug-delivery systems may substantially contribute to the development of clinically viable techniques for BDNF delivery into the cochlea or posterior eye segment, which, ultimately, can lead to a long-term or permanent rescue of auditory and optic neurons from degeneration.

**Keywords:** brain-derived neurotrophic factor, neurodegeneration, posterior eye segment, inner ear, targeted drug-delivery, nanoparticles

## Introduction

Secreted protein brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family. This signaling molecule has been well-documented for its ability to regulate neuronal plasticity, cell growth, proliferation, cell survival, and long-term memory.<sup>1</sup> Being involved in pathogenesis of neurodegenerative diseases, BDNF has become a major approach in drug development for the treatment of Alzheimer's disease,<sup>2</sup> Parkinson's disease,<sup>3</sup> Huntington's disease,<sup>4</sup> Rett syndrome,<sup>5</sup> stroke,<sup>6</sup> traumatic brain injury,<sup>7</sup> depression,<sup>8</sup> drug abuse,<sup>9</sup> schizophrenia,<sup>10</sup> amniotic lateral sclerosis,<sup>11</sup> and multiple sclerosis.<sup>12</sup> The crucial limitation pertaining to the use of BDNF in the treatment of central nervous system (CNS) disease is its inability to cross the blood–brain barrier (BBB). In fact, numerous recent reviews have described modern accomplishments in various approaches directed to surmount the BBB, including the modulation of tight junctions or transport systems, or the exploitation of colloid drug-delivery nanosystems.<sup>13–15</sup>

Furthermore, the retina<sup>16</sup> and the inner ear,<sup>17</sup> like the brain, also have their own blood barriers with similar structure and function to the BBB. Those barriers also constrain the usage of BDNF as a treatment for blindness and deafness caused by neurodegeneration of retinal or spiral ganglion cells, respectively. Fortunately, specific anatomical construction of these sensory organs offers the possibility for local drug delivery that can avoid the barriers. Eventually, the main approaches of BDNF delivery to the posterior segment

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of the eye or the inner ear appear to be similar and include direct delivery of the pure protein (intravitreal injection or implantation of the osmotic minipump to the scala tympani), viral gene, and cell-based therapy. Ultimately, this knowledge should be translated to patients, however, regardless of success in preclinical studies, BDNF has yet to prove its potential as a neurorestorative in clinics. In this review, some important points concerning the structure and mechanism of action of the BDNF are discussed. Besides, all published methods of BDNF delivery to the posterior eye segment and to the inner ear are highlighted, and additionally, these methods are analyzed and compared. Finally, future prospects in BDNF delivery that may benefit the potential treatment of blindness and deafness among patients are suggested.

## BDNF

### BDNF structure

BDNF belongs to the neurotrophin family of proteins, and it is the most specific in the biological action family of growth factors. Apart from BDNF, the other group members are: nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). The first neurotrophin, named NGF, was identified by Rita Levi-Montalcini in 1952<sup>18</sup> as “unknown tumoral factor”, which influenced nerve growth in mouse sarcoma and chick embryo. Thirty years later, Barde et al<sup>19</sup> in their study on the survival and the growth of nerve fibers in neuronal cell culture, purified, 2 µg of protein BDNF with a molecular weight (MW) of 12,300 Da from 3 kg of porcine brain. Later, the 252 amino acid (AA) residues in the sequence of BDNF were identified.<sup>20</sup> The three-dimensional molecular structure of BDNF appears to be a homodimer with noncovalent bonds, where each monomer consists of three pairs of anti-parallel β-sheets connected to four loops of β-strands containing 3 disulfide bonds with cysteine knot motif.<sup>21</sup> Besides, the crystallographic BDNF structure is shown in protein databases as heterodimers with NT-3 (code 1B8M) and NT-4 (code 1BND).<sup>22,23</sup> The *BDNF* human gene is mapped on chromosome 11 between 11p13 and 11p14.<sup>24,25</sup> Apparently, BDNF synthesis occurs in the CNS,<sup>20</sup> as well as in the peripheral tissues, including the liver, muscles, pancreas, colon, intestine, lungs, bladder,<sup>26</sup> and placenta.<sup>27</sup> It is well known that BDNF synthesis always follows a precursor molecule (pro-BDNF) synthesis, which is the 35,000 Da protein with its own CNS activity.<sup>28</sup> The polypeptide pro-BDNF that consists of 247 AA residues should be cleaved to form the mature protein with a MW 14,000 Da and 119 AA residues.<sup>29</sup> In fact, there are two existing pathways for BDNF to mature, namely the intracellular and pericellular pathways. The furin, which is located in the Golgi apparatus,

plays a crucial role in the intracellular processing,<sup>30</sup> while the pericellular pathway involves serine protease tissue plasmin, which is synthesized from plasminogen activated by the tissue plasminogen activator.<sup>31</sup> Furthermore, it is important to note that the absence of cleavage leads to the accumulation of pro-BDNF that can act in a manner opposite to BDNF. In addition, studies have shown that pro-BDNF binding with p75 receptor induces neuronal apoptosis,<sup>32</sup> whereas binding with sortilin results in a more stable form of pro-BDNF and the activation of intracellular enzyme furin.<sup>33</sup> Additionally, the Val66Met (valine amino-acid is substituted by the methionine in 66th codon) genetic polymorphism of the pro-BDNF leads to the inability of the pro-BDNF to be bound with sortilin, resulting in a decrease in the production of mature protein that subsequently leads to numerous CNS disorders.<sup>34</sup>

### BDNF receptors

BDNF is a ligand to three different receptors, namely tropomyosin related kinase B (TrkB), p75 neurotrophin receptor (p75NTR), and sortilin. It is well known that TrkB belongs to a large group of tyrosine-kinase receptors, and BDNF as well as NT-4 are the only ligands for this receptor.<sup>21</sup> The human TrkB is a transmembrane glycoprotein type I that consists of 792 AA residues. Its extracellular domain comprises of three tandem leucine-rich motifs, bordered by two cysteine clusters, and trailed by two immunoglobulin (Ig)-like domains, Ig<sub>1</sub> and Ig<sub>2</sub>,<sup>35</sup> where Ig<sub>2</sub>, the closest to cell membrane, is the binding site for BDNF.<sup>36</sup> The receptor is capable of being in a dynamic equilibrium between monomeric and dimeric states and regulates the activity of further intracellular biochemical cascades. Moreover, binding with the ligand results in the conversion of the receptor's monomeric structure into the dimeric form, which is accompanied by autophosphorylation of the intracellular domain.<sup>37</sup>

Basically, the TrkB receptor has three core isoforms in the human brain, specifically a full-length, catalytic form (TrkB.FL), and two isoforms that lack a tyrosine kinase domain called truncated forms, namely, TrkB.T and TrkB.Shc. The truncated forms are synthesized by an alternative splicing of the primary gene and are independently regulated.<sup>38</sup> Furthermore, the TrkB.FL is seen to be expressed in the brain cortex, the hippocampus, the thalamus, the choroid plexus, granule cell layer of the cerebellum, the brainstem, the spinal cord, and the retina.<sup>39</sup> It initiates the survival of neuronal cells and the differentiation and plasticity of synaptic signals, whereas truncated TrkB are capable of inhibiting all these processes when their heterodimerization with the activated TrkB.FL occurs.<sup>40</sup> Additionally, a study has shown that the relationship between levels of the TrkB.FL and the truncated isoforms

influences the cellular response to BDNF.<sup>41</sup> Notably, BDNF binds with TrkB.T, becoming immobilized and unable to bind with TrkB.FL, thus reducing BDNF-signaling.<sup>42</sup> Also, the formation of TrkB.T and TrkB.FL heterodimers affects the signaling by acting as a dominant-negative inhibitor.<sup>43</sup> Therefore, the maximal activity of TrkB is possible only in the case of homodimerization of TrkB.FL.

Another receptor, p75NTR, appears to be the 16th member of tumor necrosis factor (TNF) receptor superfamily.<sup>44</sup> The precursors of all the four neurotrophins, such as pro-NGF, pro-BDNF, pro-NT-3, and pro-NT-4 are the ligands to p75NTR. Mature neurotrophins may also be the ligands to p75NTR, but with substantially lower affinity.<sup>45</sup> The p75NTR structure has an extracellular stalk domain, a single transmembrane domain, and a cytoplasmic domain.<sup>46</sup> The regulation of cell survival, cell cycle, axonal growth, and myelin formation have been considered to be the main functions of the p75NTR.<sup>47,48</sup> It can cooperate with other receptors to form heterodimeric complexes. For instance, the interaction with the TrkB has been shown to increase the affinity of BDNF to its binding site,<sup>49</sup> thus enhancing the cell survival. On the other hand, the interface with sortilin leads to the initiation of the apoptosis by pro-BDNF.<sup>50</sup> Sortilin, as mentioned above, is another receptor that interacts with BDNF. It belongs to type-I transmembrane vacuolar protein-sorting 10 protein (Vps10p) domain containing receptors family.<sup>51</sup> Sortilin operates as a coreceptor that regulates TrkB or p75NTR expression in response to a pro-neurotrophin and initiates the apoptotic cascade.<sup>50</sup> Also, it has been recently shown that the interaction of sortilin with Huntingtin-associated protein-1 causes the activation of intracellular protease furin, which, in turn, cleaves the pro-BDNF to a mature form.<sup>33</sup>

## BDNF-mediated signal transduction

### TrkB receptor

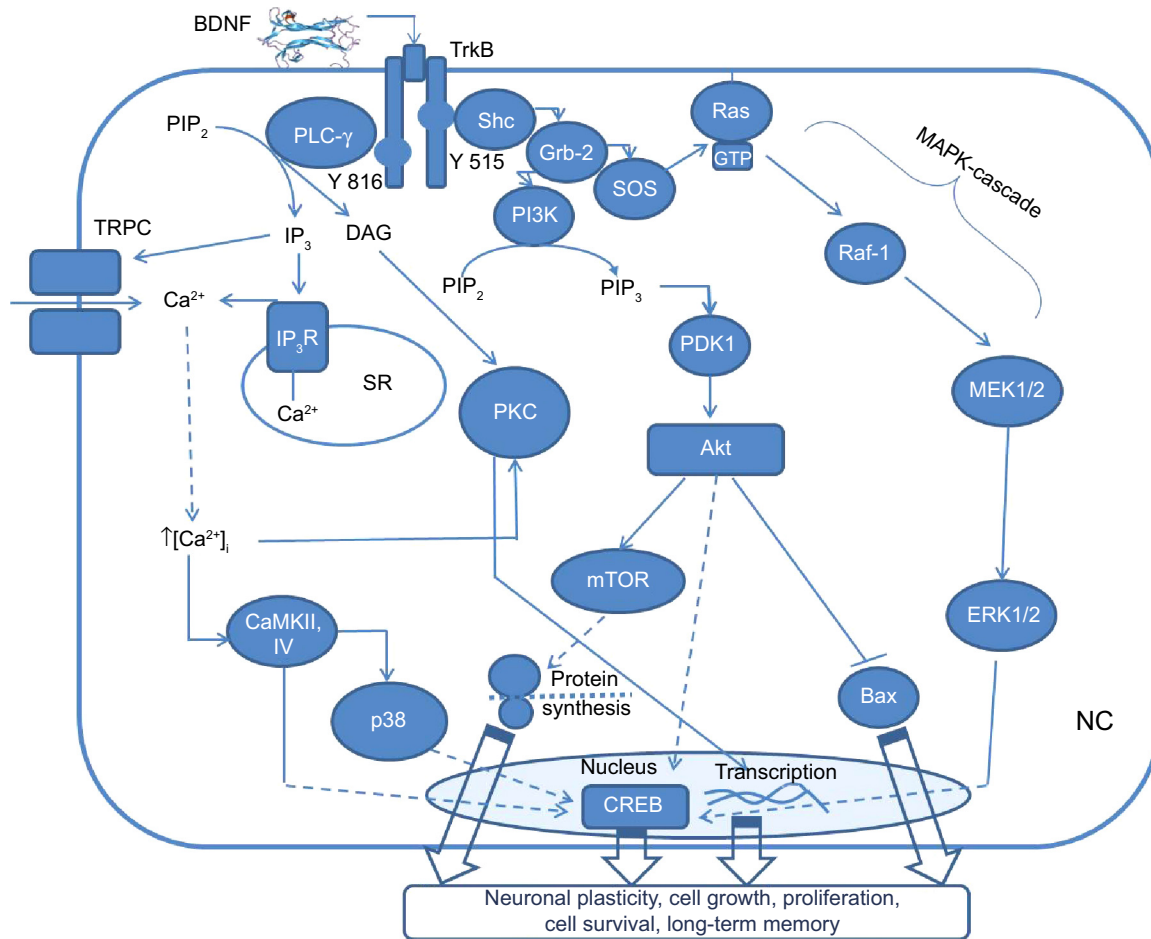
Binding of BDNF to TrkB with picomolar affinity (Figure 1) induces the dimerization of the receptor and the autophosphorylation of the tyrosine residues in the juxtamembrane domain that creates the binding or docking sites for such molecules as Src-homology 2 domain containing transforming protein (Shc), Src-homology phosphatase 2 (Shp2), growth factor receptor-binding protein 2 (Grb2), and phospholipase C $\gamma$  (PLC $\gamma$ ) 1.<sup>52</sup> Further interaction of these molecules initiates the activation of various signaling cascades, which have been described in numerous reviews elsewhere.<sup>53-55</sup> Here, this will be discussed superficially, in order to give a general overview of BDNF's action.

PLC $\gamma$  pathway (Figure 1) is mainly essential for synaptic plasticity.<sup>55</sup> Phosphorylation of the tyrosine residue at position

816 causes PLC $\gamma$  to bind to this site in the juxtamembrane domain. Then, PLC $\gamma$  can hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) to form diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP<sub>3</sub>). IP<sub>3</sub> binds to the calcium (Ca<sup>2+</sup>) ion channel of the endoplasmic reticulum, releasing Ca<sup>2+</sup> into the cytoplasm. High intracellular Ca<sup>2+</sup> initiates protein-kinase C (PKC) activation by DAG as well as activation of membrane transient receptors potential channels (TRPC) that, in turn, induce dendritic remodeling.<sup>56</sup> Furthermore, released Ca<sup>2+</sup> stimulates Ca<sup>2+</sup>/calmodulin-dependent protein kinase 2 (CaMKII) or CAMKIV capable of phosphorylating the activating Ser-133 site of cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) either directly or via activation of downstream kinases p38 of mitogen-activated protein kinases (MAPK) superfamily.<sup>57</sup> The CREB is well-documented as a transcriptional factor involved in the neuronal plasticity, learning and memory, outgrowth of neuronal processes, induction of neurotrophic and neuroprotectant cellular programs, and regulation of circadian rhythms.<sup>58</sup>

Phosphoinositide 3-kinase (PI3K) pathway (Figure 1) is mostly crucial for the survival and proliferation of neuronal cells.<sup>59</sup> Upon phosphorylation of the tyrosine domain at position 515, the adaptor protein Shc binds to this site and is able to interact with Grb2-associated-binding protein 1 (GAB1) that forms a docking site for PI3K (Figure 1). Then active PI3K binds to PIP<sub>2</sub> and converts it to PIP<sub>3</sub> leading to the activation of Akt, also known as protein kinase B (PKB), through phosphoinositide-dependent kinase-1 (PDK1) interaction. The proto-oncoprotein Akt binds with B-cell lymphoma 2 (Bcl-2) associated X protein (Bax)<sup>60</sup> increasing its proportion in the Bcl-2/Bax ratio leading to the antiapoptotic effect. Another signaling cascade of Akt involves the activation of mammalian target of rapamycin (mTOR), a high MW serine-threonine protein kinase that promotes protein synthesis in neuronal dendrites, initiating neurocyte growth, proliferation, and synaptic plasticity.<sup>61</sup> Additionally, a recent study showed that the BDNF/mTOR pathway is also involved in the stabilization of memory traces, which is crucial for the long-term memory (LTM) formation.<sup>62</sup>

MAPK pathway is known to be important in neuronal cell growth, differentiation, protection, and release of neurotransmitters.<sup>63</sup> The activation of this pathway involves recruitment of Shc with further interaction with Grb2. The formation of Grb 2/son of sevenless (SOS) complex results in the initiation of rat sarcoma (Ras) protein by the conversion of guanosine diphosphate to guanosine triphosphate (GTP) (Figure 1). Active Ras turns out to be able to bind the effector protein kinase rapid accelerated fibrosarcoma B (B-Raf), which, in turn, phosphorylates mitogen-activated



**Figure 1** A general scheme, illustrating BDNF-TrkB signaling through three main pathways in the neuronal cell (NC).

**Notes:** PLC $\gamma$  pathway results in activation of neuronal plasticity and long-term memory formation via PKC, TRPC, and transcriptional factor CREB. Also PLC $\gamma$  activates inositol trisphosphate receptor (IP $_3$ R) via IP $_3$  to release intracellular calcium (Ca $^{2+}$ ) from sarcoplasmic reticulum (SR). PI3K-Akt pathway activates CREB and causes further downstream activation of mTOR, which stimulates protein synthesis in neuronal dendrites leading to cell growth, proliferation, and synaptic plasticity. Also, PI3K-Akt pathway blocks Bax protein causing antiapoptotic action. Mitogen-activated protein kinases (MAPK) cascade results in activation of transcriptional factor CREB with further stimulation of cell growth, differentiation, protection, releasing of neurotransmitters, and memory formation.

**Abbreviations:** BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B; PLC $\gamma$ , phospholipase C $\gamma$ ; PKC, protein-kinase C; TRPC, transient receptors potential channels; CREB, cyclic adenosine monophosphate response element-binding protein; Shc, Src-homology 2 domain containing transforming protein; Grb-2, growth factor receptor-binding protein 2; IP $_3$ , inositol triphosphate; PIP $_2$ , phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; CaMKII, Ca $^{2+}$ /calmodulin-dependent protein kinase 2; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; PDK1, phosphoinositide-dependent kinase-1; Bax, B-cell lymphoma 2 associated X protein; mTOR, mammalian target of rapamycin; SOS, son of sevenless; Ras, rat sarcoma; GTP, guanosine triphosphate; B-Raf, rapid accelerated fibrosarcoma B; ERK, extracellular signal-regulated kinases.

protein kinase kinase (MEK) 1/2 leading to the activation of extracellular signal-regulated kinases (ERK) 1/2. Finally, the activated ERK 1/2 influences the previously mentioned transcriptional factor CREB.<sup>64</sup>

### p75NTR and sortilin

As described earlier in the “BDNF receptors” section, BDNF has a 1,000-fold lower affinity to pan-neurotrophin receptor p75 that interacts with TrkB enhancing its response to BDNF, thus promoting the cell survival.<sup>65</sup> The opposite action of p75NTR as a cell death receptor occurs when it forms the receptor complex with sortilin.<sup>50</sup> Beside this, p75NTR causes axon degeneration when it forms a triple complex with neurite outgrowth inhibitory protein receptor

(NogoR) and leucine-rich repeat and Ig domain containing NogoR interacting protein 1 (LINGO-1). This complex promotes the suppression of axon growth involving three myelin-associated glycoproteins, specifically Nogo, myelin-associated glycoprotein, and oligodendrocyte-myelin glycoprotein.<sup>66</sup> The signaling cascades, which follow the binding BDNF or pro-BDNF to p75NTR have been shown to promote the neurite outgrowth, proliferation, and apoptosis.<sup>67</sup> All these pathways are set off after the binding of adaptor proteins to activated p75NTR, including neurotrophin receptor-interacting factor (NRIF), p75NTR interacting protein (NRAGE), p75NTR-associated death executor (NADE, NRH2),<sup>67</sup> and TNF receptor-associated factors (TRAF) proteins.<sup>68</sup>

Jun-kinases signaling pathway (Figure 2) results in the activation of phosphoprotein p53 and apoptosis. In response to BDNF, p75NTR promotes Jun-kinases signaling pathway via interactions with NUAGE, TRAF6, and NRIF, stimulating the stress kinase c-Jun N-terminal kinase (JNK). The activation of Jun-kinases cascade involves the initiation of cell division control protein 42 (Cdc42), which, in turn, activates apoptosis signal regulated kinase 1 (ASK1). Additionally, a Jun-kinases kinase named MKK7 is shown to be a link between ASK1 and JNK.<sup>69</sup> JNK activation induces the phosphorylation of transcription factor c-Jun and tumor suppressor p53,<sup>70</sup> resulting in upregulation of proapoptotic genes and caspases<sup>71</sup> that ultimately causes cell death.<sup>72,73</sup>

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) signaling pathway (Figure 2) in contrast to the previous one is proven to be an antiapoptotic.<sup>74</sup> Binding of the neurotrophin promotes the interaction between TRAF6 proteins and p75NTR cytoplasmic domain. Interleukin-1 receptor-associated kinase (IRAK) also binds to p75NTR forming a complex with TRAF6 that results in the activation of atypical PKC, which, in turn, phosphorylates nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (I $\kappa$ B) kinase- $\beta$  (IKK- $\beta$ ).<sup>55</sup> IKK- $\beta$ -mediated phosphorylation of I $\kappa$ B results in the translocation of NF- $\kappa$ B into the nucleus, where genes, induced by NF- $\kappa$ B, promote cell survival or inhibition of the apoptosis.<sup>75</sup>

The signaling molecule ceramide (Figure 2) is a result of the activation of the sphingomyelin cycle.<sup>76</sup> The role of the ceramide in neuronal cell signaling has yet been fully understood.<sup>77</sup> But, it is well-known that ceramide influences Jun-kinases and NF- $\kappa$ B signaling pathways, promoting both apoptotic and prosurvival pathways.<sup>55</sup>

## BDNF as a therapeutic option for blindness

In the year 2010, there were 32.4 million blind people and 191 million vision-impaired people worldwide.<sup>78</sup> Diabetic retinopathy, age-related macular degeneration, and glaucoma are recognized to be the main diseases, which can lead to irreversible blindness.<sup>79</sup> Various stimuli, like high intraocular pressure (IOP), blockage of axonal flow, and retinal ischemia may cause cellular damage in the retina.<sup>80</sup> Glaucoma,<sup>81</sup> ischemia,<sup>82</sup> age-related macular degeneration,<sup>83</sup> diabetic retinopathy,<sup>84</sup> and optic neuritis<sup>85</sup> are the potential contributors to retinal ganglion cells (RGCs) neurodegeneration and subsequent loss. It has been studied that survival of RGCs was enhanced by BDNF *in vitro*.<sup>86</sup> Moreover, at the retina, BDNF receptor, TrkB, has been found to be expressed by a number of cell types, namely, a subset of cone photoreceptors,<sup>87</sup> amacrine cells,<sup>88</sup> Muller

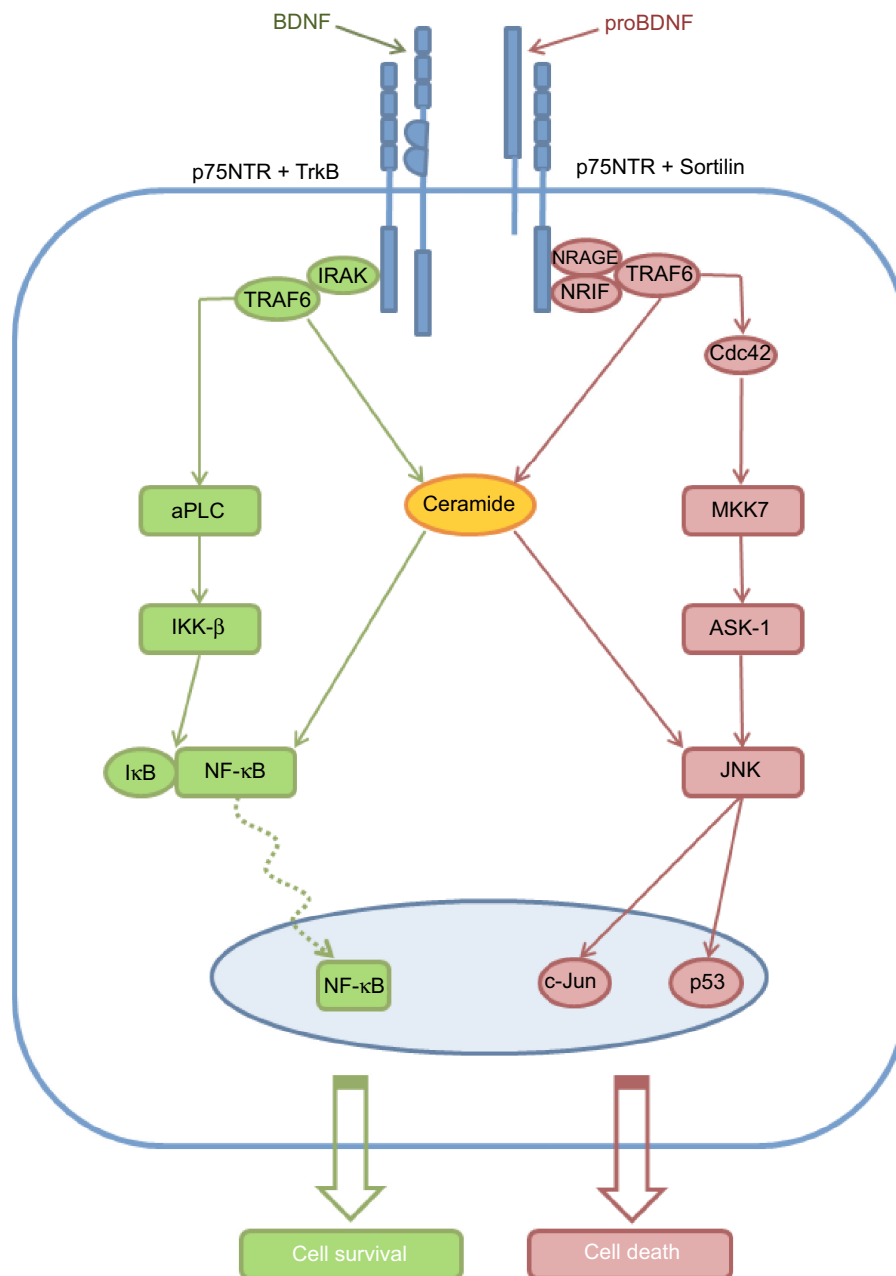
glia,<sup>89</sup> and RGCs.<sup>90</sup> Therefore, in recent years, BDNF appears to be a major therapeutic strategy *in vivo* for ocular or systemic diseases that may lead to cellular damage in the retina. A variety of approaches associated with the delivery of BDNF to the posterior eye segment have been presented in Table 1. BRB, which has similar features with the BBB,<sup>91</sup> limits the movements of BDNF after systemic and periocular administration to the retina. On the other hand, anatomical features of the eye are known to circumvent the need for systemic administration by using relatively straightforward direct access to the retina, like intravitreal injection. This anatomical advantage avoids pharmacological challenges faced by BDNF delivery into the CNS, such as BBB penetration and degradation in the circulation.

Meanwhile, axotomy, as an animal model, has been extensively used to discover the survival of RGCs<sup>92</sup> and BDNF signal transduction pathways.<sup>93,94</sup> This model has been reported to be performed in rats,<sup>92,95,96</sup> cats,<sup>97</sup> mice,<sup>98</sup> and hamsters.<sup>99</sup> Apart from this, other animal models, used to establish the BDNF activity in the retina, include light damage,<sup>100</sup> elevated intraocular pressure,<sup>101</sup> potassium cyanide-induced retinal damage,<sup>102</sup> *N*-methyl-D-aspartate (NMDA) excitotoxicity,<sup>103</sup> optic nerve trauma,<sup>97</sup> anterior ischemic optic neuropathy,<sup>104</sup> exploiting the dystrophic royal college of surgeons (RCS) rats strain<sup>105</sup> and investigation of the normal retina development in young rats.<sup>106</sup>

## Direct BDNF delivery to the posterior eye segment

### Pure BDNF

The first intravitreal injection of pure recombinant BDNF was made to the axotomized rats in doses of 0.1–0.15  $\mu$ g/rat/eye.<sup>107</sup> It had shown substantial increase in survival of injured cells 3 weeks after lesion in comparison to control. However, it had been discovered that the single injections could only temporarily rescue RGCs from neuronal death (Table 1). Five weeks after injury, the level of surviving cells did not increase very much, showing the transient effect of BDNF single injection. Later, the multiple injections, as a simple and logical way to surmount this limitation, were demonstrated by Mansour-Robaey et al.<sup>92</sup> Importantly, this study has detected the optimal dose, which is 5  $\mu$ g of BDNF/one rat's eye. As it turned out, the size of the animals is critical for dosing of BDNF, thus for mice, rats, young rats, and hamsters, the dose range was from 0.1 to 5  $\mu$ g/eye/animal, whereas for cats, with the size of eye and vitreal volume comparable to those of primates, 30  $\mu$ g was chosen as an optimal dose (Table 1). Even though the volume of vitreal chamber in cat's eye is around 3 mL, while



**Figure 2** An overall scheme illustrating p75NTR signaling.

**Notes:** Heterodimerization with TrkB in response to BDNF action causes phosphorylation of IκB resulting in liberating of NF-κB, which after translocation into the nucleus promotes gene transcription to support neuron survival. Oppositely, heterodimerization with sortilin in response to pro-BDNF induces activation of Jun-kinase signaling pathway resulting in activation of proapoptotic genes and caspases causing cell death. Signaling molecule ceramide takes part in both NF-κB and Jun-kinases signaling cascades. **Abbreviations:** BDNF, brain-derived neurotrophic factor; p75NTR, p75 neurotrophin receptor; TrkB, tropomyosin related kinase B; NRIF, neurotrophin receptor-interacting factor; NRAGE, p75NTR interacting protein; TRAF, tumor necrosis factor receptor-associated factor; Cdc42, cell division control protein 42; ASK1, apoptosis signal regulated kinase 1; JNK, c-Jun N-terminal kinase; IRAK, interleukin-1 receptor-associated kinase; aPKC, atypical protein kinase C; IκB, kappa light polypeptide gene enhancer in B-cells inhibitor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; MKK7, mitogen-activated protein kinase kinase 7.

rat’s eye is 60-fold smaller (0.05 mL), the concentration of drug at the site of action appeared approximately the same (~0.01 μg BDNF/μL of vitreal volume).<sup>97</sup> Moreover, LaVail et al<sup>108</sup> compared the effectiveness of the intravitreal injection of the BDNF in concentrations of 1, 2, 5, and 10 mg/mL for both mice and rats, but the difference in volume of the injected solution was just 2 fold (0.5 and

1 μL, respectively), while mice have in 6.6-fold smaller vitreal chamber than rats.

Although intravitreal injection of BDNF have been shown as a good strategy for the treatment of various ocular and systemic diseases, some important limitations have been observed. Chen and Weber<sup>97</sup> displayed that at some doses, the increased levels of BDNF had resulted in

**Table 1** Different approaches of application of BDNF in the treatment of posterior eye segment degenerative diseases

Author, year	Animal model	Method administered/ timing	Effect	Dosing
<b>Administration of purified protein</b>				
Mey and Thanos, <sup>107</sup> 1993	Rat: axotomy	Intravitreal/single	Transient survival of injured RGCs	BDNF 50 ng/100 g body weight
Mansour-Robaey et al, <sup>92</sup> 1994	Rat: axotomy	Intravitreal/single vs multiple (on day 0, 3, 7, and 10)	Multiple injections caused better survival of RGCs in comparison to single injection	BDNF 5 µg
Unoki and La Vail, <sup>197</sup> 1994	Rat: pressure-induced ischemia	Intravitreal/single vs multiple (2 days before ischemic insult and 4 or 5 days after)	Transient protection of the retina after single injection, but second injection of BDNF prolonged the protective effect	BDNF 1 µg
Weibel et al, <sup>96</sup> 1995	Young rat: axotomy	Intravitreal/single vs multiple	Positive effect of BDNF on the survival of injured optic nerve axons in comparison to other neurotrophic factors	BDNF 0.1 µg
Sawai et al, <sup>198</sup> 1996	Rat: axotomy	Intravitreal/single	Injection of BDNF or NT-4/5 improved axonal branch growth within the retina, but injection of NT-3 did not	BDNF 5 µg
Peinado-Ramon et al, <sup>199</sup> 1996	Rat: axotomy	Intravitreal/single	Survival, and delaying the onset of RGCs death	BDNF 5 µg
Cellerino et al, <sup>106</sup> 1998	Development of rat retina	Intravitreal/multiple	BDNF regulated the growth of the retinal dopaminergic neurons	BDNF 1 µg ×3
LaVail et al, <sup>108</sup> 1998	Rat/mouse: light damage	Intravitreal/single	Protection of the photoreceptors from degeneration	Rat BDNF: 1, 5, 10 µg; mouse BDNF: 0.5, 2.5, 5 µg
Ikeda et al, <sup>102</sup> 1999	Rat: potassium cyanide (KCN)-induced retinal damage	Intravitreal/single	BDNF attenuated the inner nuclear layer cell death	<sup>125</sup> I-BDNF 1 µg
Cellerino et al, <sup>200</sup> 1999	Development of rat retina	Intravitreal/single	BDNF activated NADPH-diaphorase in dopaminergic neurons and displaced amacrine cells to the ganglion cell layer, but did not protect them	BDNF 1 µg
Kurokawa et al, <sup>201</sup> 1999	Rat: axotomy	Intravitreal/single	Cell death in RGCs and the caspase-2-positive cells were lower	BDNF 5 µg
Klöcker et al, <sup>93</sup> 2000	Rat: axotomy	Intravitreal/multiple 4, 7, and 10 days after axotomy	Inhibition of PI3K did not reduce the BDNF effects on RGCs survival	BDNF 0.5 µg ×3
Kido et al, <sup>103</sup> 2000	Rat: NMDA excitotoxicity	Intravitreal/single	BDNF of 1 and 10 µg has protective effects on retinal damage	BDNF 0.1, 1, or 10 µg
Chen and Weber, <sup>97</sup> 2001	Cat: optic nerve trauma	Intravitreal/single; intravitreal/multiple	30 µg showed highest RGCs survival, whereas high doses showed low survival and inflammation	BDNF 15, 30, 60, 90 µg 30×2, 60×2 µg
Chen and Weber, <sup>202</sup> 2002	Rat: axotomy	Intravitreal/single	No significant effect on glial fibrillary acidic protein or glutamine synthetase expression	BDNF 5 µg
Ota et al, <sup>203</sup> 2002	Rat: axotomy	Intravitreal/single	Smaller change in soma cell-size distribution	BDNF 5 µg
Nakazawa et al, <sup>94</sup> 2002	Rat: axotomy	Intravitreal/single	Both MAPK and PI3K-Akt pathways involved in neuroprotective signaling	BDNF 1 µg
Krueger-Naug et al, <sup>204</sup> 2003	Rat: axotomy	Intravitreal/single	The expression of heat shock protein 27 in RGCs was reduced	BDNF 5 µg

(Continued)

**Table 1** (Continued)

Author	Animal model	Method administered/ timing	Effect	Dosing
Chen and Weber, <sup>109</sup> 2004	Rat: nerve crush	Intravitreal/single	BDNF decreased in TrkB.FL (both mRNA and protein)	BDNF 5 µg
Lee et al, <sup>205</sup> 2005	Rat: axotomy	Intravitreal/single	BDNF affected the expression of anti-tyrosine hydroxylase immunoreactivity	BDNF 5 µg
Chen et al, <sup>206</sup> 2012	Mouse: light damage	Intravitreal/single	Dose and time-dependent c-Jun expression in the retina	BDNF 0.1 µg; 0.2 µg
Galindo-Romero et al, <sup>98</sup> 2013	Mouse: axotomy	Intravitreal/single	Neuroprotective effect on the axotomized RGCs	BDNF 2.5 µg
<b>Administration of purified protein in combinations</b>				
Klöcker et al, <sup>116</sup> 1998	Rat: axotomy	Intravitreal/single	Single BDNF treatment rescued 27% of the RGCs transiently. Combined treatment of BDNF with free radical scavenger <i>N</i> -tert-butyl-(2-sulphophenyl)- nitron (S-PBN) improved survival up to 68%, and with NOS-inhibitor <i>N</i> -ω-nitro-L- argininemethylester (L-NAME) – up to 55%	BDNF 0.2, 0.5, 2 µg + L-NAME, S-PBN
Yan et al, <sup>207</sup> 1999	Rat: axotomy	Intravitreal/multiple 1, 7 days	Combination GDNF and BDNF showed better protection than each factor individually	0.5 µg GDNF +0.5 µg BDNF ×2; 1.0 µg GDNF +1.0 µg BDNF ×2
Ko et al, <sup>101</sup> 2000	Rat: high IOP	Intravitreal/multiple 5, 13, 21, and 29 days after IOP elevation	Combination of BDNF and S-PBN substantially improved survival of RGCs up to 90.1%	BDNF 0.5 µg ×4 BDNF 1.0 µg ×4 BDNF 0.5 µg + S-PBN ×4 BDNF 1.0 µg + S-PBN ×4
Koeberle and Ball, <sup>113</sup> 2002	Rat: axotomy	Intravitreal/single	Neurturin, GDNF, and BDNF had different cellular targets in the retina to enhance RGCs survival	BDNF 0.05, 0.25, 0.5, 1.0, 2.5 µg 1.0 µg neurturin +1.0 µg BDNF 1.0 µg GDNF +1.0 µg BDNF
Watanabe et al, <sup>114</sup> 2003	Cat: axotomy	Intravitreal/single	Combined injection of BDNF, CNTF, and forskolin (raises intracellular concentration of cAMP) resulted in a 4.7-fold increase of surviving increase of β-cells of RGCs	BDNF 0.5, 1 µg BDNF 1 µg + CNTF 1 µg +0.1 mg forskolin
Tropea et al, <sup>115</sup> 2003	Long–Evans rats: retina lesion	Intravitreal/single; osmotic minipump	BDNF in a combination with chondroitinase ABC (C-ABC), which promotes CNS regeneration via degradation of chondroitin sulfate proteoglycans side chains, results in reinnervation of the collicular scotoma by unlesioned RGCs axons	BDNF 10 µg + C-ABC
Zhang et al, <sup>99</sup> 2005	Hamster: axotomy	Intravitreal/single	Protection RGCs and increasing NOS activity by BDNF	BDNF 1.25, 5 µg or BDNF + CNTF
Fu et al, <sup>112</sup> 2009	Rat: high IOP	Intravitreal/single	Long-term protection for RGCs	BDNF 1 µg + LINGO-1-Fc
Weber et al, <sup>117</sup> 2010; Weber and Harman, <sup>208</sup> 2013	Cat: optic nerve trauma	Intravitreal/single; osmotic minipump into brain vision cortex 14, 28 day	Treatment eye + cortex animals showed better RGCs survival	BDNF 90 µg; 12.6, 25.2 µg

(Continued)



Table 1 (Continued)

Author	Animal model	Method administered/ timing	Effect	Dosing
Goldenberg-Cohen et al, <sup>133</sup> 2014	Mouse: anterior ischemic optic neuropathy	Intravitreal/single	Enhancing of neuroglial differentiation of the RGCs	BDNF 5 µg + BMSCs
<b>Viral mediated BDNF delivery systems</b>				
Di Polo et al, <sup>121</sup> 1998	Rat: axotomy	Intravitreal/single	Ad.BDNF increased survival of RGCs in 4–5 fold in comparison with pure BDNF	BDNF 5 µg Ad.BDNF
Mo et al, <sup>125</sup> 2002	Rat: axotomy	Electroporation of BDNF gene to RGCs	Protection axotomized RGCs against apoptosis	BDNF gene
Martin et al, <sup>124</sup> 1996	Rat: high IOP	Intravitreal/single	Protection of RGC	AAV-BDNF-WPRE
Schuettauf et al, <sup>123</sup> 2004	Rat: NMDA excitotoxicity	Intravitreal/multiple	Retinal cell survival	AAV2 3 µL ×3
Gauthier et al, <sup>122</sup> 2005	Rat: light damage	Intravitreal/single	BDNF gene delivery enhanced the survival and structural integrity of damaged photoreceptors	BDNF 5 µg or 5 µL AAV
Wang et al, <sup>209</sup> 2009	Rabbit: high IOP	Intravitreal/single	Protecting the retinal structure	10 µL AAV
Wan, <sup>210</sup> 2010	Rat: streptozotocin induced diabetes	Intravitreal/single	Increase in tyrosine hydroxylase and density of retinal dopaminergic amacrine cells	5 µL AAV
Ren et al, <sup>127</sup> 2012	Rat: high IOP	Intravitreal/single	Sustained rescue of RGCs and visual function	BDNF 0.5 µg + AAV-BDNF
<b>Cell-based BDNF delivery systems</b>				
Lawrence, <sup>105</sup> 2004	Dystrophic Royal College of Surgeons rats (animal with genetically transmitted progressive retinal dystrophy)	Transplantation into the subretinal space	Preserving photoreceptors from the cell death	SCTM41-BDNF Schwann Cell Line; SCTM41-GDNF Schwann Cell Line
Abe et al, <sup>132</sup> 2008	Rat: retinitis pigmentosa	Transplantation into the subretinal space/topical doxycycline	BDNF expression possible to be controlled by doxycycline (DOX) for photoreceptors' protection	Retinal pigment epithelial cell line that can express BDNF by exposure to DOX (Tet-BDNF-RPE)
Zhou et al, <sup>130</sup> 2009	Rat: normal	Intravitreal subretinal/ single	BDNF can promote neural stem cells (NSCs) migration and differentiation into neural cells	10 µL NSC-BDNF suspension (10 <sup>5</sup> cells)
Zhang and Wang, <sup>128</sup> 2010	Rat: retinitis pigmentosa	Intraocular transplantation/ 2 weeks	Inhibition of photoreceptor apoptosis and slowing down retinal damage	5 µL BMSC-BDNF suspension (5×10 <sup>4</sup> cells)
Park et al, <sup>129</sup> 2012	Rat: axotomy	Subretinal or intravitreal/ 4 weeks	Increased BDNF mRNA and protein expressions	20 µL rat bone marrow mesenchymal stem cells (rMSC)-BDNF (3×10 <sup>5</sup> cells)

**Abbreviations:** BDNF, brain-derived neurotrophic factor; RGCs, retinal ganglion cells; NT, neurotrophin; NMDA, N-methyl-D-aspartate; MAPK, mitogen-activated protein kinases; PI3K-Akt, phosphoinositide 3-kinase; GDNF, glial cell-derived neurotrophic factor; IOP, intraocular pressure; CNTF, ciliary neurotrophic factor; cAMP, cyclic adenosine monophosphate; C-ABC, chondroitinase ABC; CNS, central nervous system; NOS, nitric oxide synthase; LINGO-1, leucine rich repeat and immunoglobulin domain containing NogoR interacting protein 1; NogoR, neurite outgrowth inhibitory protein receptor; BMSC, bone marrow stem cell; AAV, adeno-associated virus; DOX, doxycycline; NSCs, neural stem cells; rMSC, rat bone marrow mesenchymal stem cells; WPRE, woodchuck hepatitis posttranscriptional regulatory element; Ad.BDNF, adenovirus vector containing BDNF.

less neuronal survival. They also revealed that continuous application of BDNF intravitreally led to a decrease in the TrkB.FL receptors in rat retina, but not the “truncated” forms.<sup>109</sup> They concluded that BDNF limits its own long-term

neuroprotective efficacy by initiating downregulation of its TrkB receptor. Consequently, it is possible to assume that BDNF acts in a dose-dependent manner, however, multiple applications do not inevitably have an additive effect on

RGCs survival. Furthermore, the safety profile of intravitreal injections, which depends on the surgical technique, the risk of cataract and retinal ischemia, as well as endophthalmitis, appears to be the major limitation of multiple injections.<sup>110</sup> Therefore, in order to overcome difficulties with BDNF short half-life, dosing, and undesirable effects of multiple injections, other methods of BDNF delivery have been explored for long-term maintenance. One of them was a proposal to use hyaluronic acid aqueous solution of BDNF instead of conventional phosphate buffered saline or Hank's balanced salt solutions. BDNF was dissolved in 0.5% hyaluronic acid aqueous solution, resulting in a long-acting 1 mg/mL BDNF solution with sustained release of BDNF for ~1 week.<sup>111</sup> In this study, long-acting BDNF improved the differentiation of the hippocampus-derived neural stem cells embedded into the developing rat retina in comparison to BDNF dissolved in Dulbecco's phosphate buffered saline.

### Combinations with BDNF

The usage of combinations of BDNF with other growth factors or biological molecules (Table 1) has been proven to prolong half-life,<sup>112</sup> increase effectiveness,<sup>113,114</sup> enhance sprouting,<sup>115</sup> and reduce side effects.<sup>116</sup> Of interest, Weber et al<sup>117</sup> have demonstrated that BDNF improves the survival of RGCs after optic nerve injury, as well as appearing to be a key factor in the protection of structure and visual function of ganglion cells. In this study, cat's eye with a crushed nerve was treated by BDNF (90 µg) intravitreally, while an infusion cannula was connected to an osmotic minipump to deliver the BDNF (100 µL of 0.3 µg/µL solution) into the visual thalamus. Such a combination substantially enriched the survival of RGCs and preserved central visual function.

### Viral mediated BDNF delivery

To achieve stable and long-lasting transduction of retinal cells, researchers have utilized gene therapy by using viral vectors (Table 1). Among them, adeno-associated virus (AAV) was considered to be the most appropriate viral vector for gene therapy due to its sustained expression<sup>118</sup> and safety.<sup>119</sup> Furthermore, a research had shown that intravitreal injection of adenovirus (Ad) vector containing BDNF had resulted in the transduction of the retinal Muller cells, which then began to secrete Ad-mediated BDNF.<sup>120</sup> Muller cells are essential for the maintenance and function of RGCs, moreover, they span across the entire sickness of the retina. Meanwhile, Di Polo et al<sup>121</sup> reported a persistent expression of Ad-mediated BDNF by transduced Muller cells which had promoted survival of axotomized RGCs, but not for long-term. Next, Gauthier et al<sup>122</sup>

showed that BDNF gene transfer by AAV to Muller cells had served as a promising strategy to protect photoreceptors from light-induced retinal degeneration. Another study that explored the possibility of using recombinant AAV serotype 2 (AAV2) to deliver BDNF, revealed that this virus was able to provide efficient transduction of ganglion cells rather than Muller cells.<sup>123</sup> Furthermore, Martin et al<sup>124</sup> suggested the incorporation of woodchuck hepatitis posttranscriptional regulatory element (WPRE) in AAV-BDNF to facilitate transfection of neurons in rats' RGCs. It has also been reported that electroporation was utilized to deliver *BDNF* gene into the RGCs.<sup>125</sup> Such transport may decrease unwanted effects of the intravitreal injection technique, and *BDNF* cDNA can be delivered into RGCs without detectable damage to the RGCs. Although cells transduced by Ad show a more sustained release of BDNF within the posterior eye segment in comparison with a single intravitreal administration of pure BDNF protein, the onset of gene expression appears to be the major limitation for the usage of viral vectors.<sup>126</sup> On the other hand, the combination of gene therapy with intravitreal injection of BDNF can compensate for the slow onset of viral-mediated expression. One study found that the combination of BDNF with AAV-BDNF had increased amount of survived RGCs by up to 97.4%, which was greater than using AAV-BDNF alone.<sup>127</sup>

### Cell-based BDNF delivery

The employment of stem cells (SCs) to transfer specific genes has been proposed as an alternative to the viral gene delivery approach (Table 1).<sup>105,128-130</sup> The essential benefits of SCs are their extensive survival and the possibility to create cell and stem cell-based delivery systems for diverse therapeutics.<sup>131</sup> It has been shown that bone marrow stem cells (BMSCs) transplanted into the subretinal space can express BDNF, thus executing trophic and protective effects on light-damaged rats' retinas.<sup>128</sup> In addition, one study established a positive effect of usage of retinal-pigment epithelial cells carrying vectors transduced with the gene of a BDNF that were transplanted into the subretinal space of rats. This vector, which included a tetracycline-responsive element (TRE) and the cDNA of BDNF enabled upregulation of the expression of BDNF through the exposure of Tet-BDNF-RPE cells by topically applied doxycycline.<sup>132</sup> Another study suggested that the usage of genetically modified Schwann cell lines would enhance the release of several growth factors.<sup>105</sup> The combination of BMSCs with BDNF has been shown to promote differentiation of primitive cells derived from the bone marrow to the glial cells and neurons.<sup>133</sup> Park et al<sup>129</sup>

applied a retroviral vector carrying rats' *BDNF* cDNA to transduce rat bone marrow mesenchymal stem cells (rMSCs). After intravitreal or subretinal injection of modified rMSCs, the incorporation of the cells into the retina with further production of BDNF was detected. It is important to note that these methods present complications of subretinal injection technique, such as retinal folding and splitting, as well as rejection, accidental tumor growth, proliferative vitreoretinopathy, and choroidal neovascularization.<sup>134</sup>

## Nanoparticulate delivery

Ophthalmic drug delivery via nanoparticulate systems has the potential of being a great discovery as such systems accomplish the four important criteria of ophthalmic drug delivery, namely, improved drug permeation, low toxicity, controlled sustained drug release, and selective drug targeting.<sup>135</sup> Despite numerous promising results of using liposomes, solid lipid nanoparticles, polymeric nanoparticles, polymeric nanomicelles, and nanoemulsions for the treatment of posterior eye segment, the application of BDNF has not been published yet.<sup>136</sup> On the other hand, a similar approach has been reported where intravitreal injection of the gelatin nanoparticles (NPs) with basic fibroblast growth factor (bFGF) was used for the treatment of photoreceptor degeneration in dystrophic RCS rats.<sup>137</sup>

## BDNF as a therapeutic option for deafness

Hearing loss has affected over 360 million people worldwide,<sup>138</sup> causing substantial burden for the economy, and has a high impact on quality of life and causes emotional distress.<sup>139</sup> The existing conventional therapy for patients with injured hair cells (HCs) leading to hearing loss is a cochlear implant (CI) auditory prosthesis. CI electrodes directly stimulate spiral ganglion cells (SGCs) soma and probably their central axons, delivering partial restoration of sensory organ function in patients.<sup>140</sup> In such cases, the maximal preservation of the population of SGCs becomes essential because severe SGC degeneration constrains the effectiveness of hearing rehabilitation by a CI. BDNF has been shown to take part in both the development and the maintenance of SGCs.<sup>141</sup> Besides, BDNF has also been well-documented to be expressed by HCs, as well as by supporting cells of the organ of Corti.<sup>142</sup> Obviously, the damage of HCs or supporting cells in the auditory epithelium leads to a decrease in BDNF expression, causing degenerative changes in SGCs.<sup>143</sup> Therefore, BDNF has a major place within different therapeutic approaches of deafness treatment (Table 2). However, systemic application

of BDNF as a treatment for hearing loss was not very effective as it has short half-life, poor pharmacokinetics, and high risk of unwanted effects of high dose.

Furthermore, BCB is well-known to be critical in restricting the passage of large molecular weight substances into perilymph. The protein concentration in perilymph is ~1/20 that of blood.<sup>17</sup> Of interest, protein penetration is about ten times higher than that of cerebrospinal fluid and four times higher than that of aqueous humor.<sup>144</sup> Based on this, the delivery of pure BDNF from the systemic circulation into the inner ear should be easier than into the retina or brain, however, it still remains a challenge. In addition, the anatomical features do not allow large proteins to easily bypass this barrier using local direct injection, like intravitreal injection, as the organ of Corti is located inside the dense otic capsule of the temporal bone thus, limiting straight access. Direct drug delivery to patients using intratympanic delivery or diffusion through the round window membrane (RWM) or via cochleostomy, across the stapes footplate, or the endolymphatic sac seems to be a challenging task.<sup>145</sup> Beside this, there are two advantages of such anatomical structures: first, the peri- and endolymphatic fluids provide a means for the distribution of BDNF throughout the whole cochlea and, second, BDNF delivered to the inner ear will not reach the systemic circulation, therefore, reducing adverse reactions to the organism.<sup>146</sup>

Many experimental models have been presented to cause hearing loss. Traditionally, the combination of aminoglycosides and diuretics is considered to be a classical one.<sup>147</sup> Systemic administration of gentamicin, kanamycin or neomycin, and furosemide or etacrynic acid has been widely used by different researchers for producing loss of SGCs (Table 2). Besides this, other experimental models of deafness include, intraperitoneal injection of cisplatin,<sup>148</sup> noise exposure,<sup>149</sup> or exploiting of deaf *Pou4f3* mutant mice.<sup>143</sup> Although guinea pigs were established to be the most suitable animals for the experimental deafness, cats, rats, chinchillas, mice, and pigeons were also used (Table 2).

## Direct BDNF delivery into the inner ear Pure BDNF

One of the most popular approaches to deliver BDNF into the inner ear is the implantation of osmotic minipump into scala tympani (Table 2). The main benefit of the mini-osmotic pump is that it allows regulated duration of the infusion of therapeutics from days to weeks, whereas injections offer discrete infusions and are followed by latent periods. Additionally, the opportunity to put in new full drug reservoir instead of consumed one by a

**Table 2** Different approaches of application of BDNF in the treatment of inner ear degenerative diseases

Author, year	Animal model	Method of administration/ timing/dosing	Effect	Total dose
<b>Administration of purified protein</b>				
<i>Implanted osmotic minipump (single)</i>				
Miller et al, <sup>153</sup> 1997	Guinea pigs: kanamycin (400 mg/kg) and ethacrynic acid (40 mg/kg)	14 days/BDNF (50 ng/mL)	Statistically significant enhanced SGCs survival	BDNF 8.4 ng
Ruan et al, <sup>152</sup> 1999	Guinea pigs: kanamycin (400 mg/kg)	15, 30, 60 days/BDNF (100 µg/mL)	Protective effect on HCs was observed at 30 days, but not at 15 and 60 days	BDNF 9 µg BDNF 18 µg BDNF 36 µg
Shoji et al, <sup>149</sup> 2000	Guinea pigs: 5-hour noise exposure (4 kHz octave band noise, 115 dB sound pressure level)	12 days/BDNF (1 µg/mL; 10 µg/mL)	BDNF did protect the sensory epithelium	BDNF 0.144 µg; 1.44 µg
Gillespie et al, <sup>155</sup> 2003	Guinea pigs: kanamycin sulfate, 400 mg/kg + furosemide 100 mg/kg	28 days/BDNF (62.5 µg/mL)	SGCs survival is increased; decrease in SGCs survival after cessation of treatment	BDNF 10 µg
McGuinness and Shepherd, <sup>211</sup> 2005	Rats: gentamicin, 350 mg/kg + furosemide, 175 mg/kg	28 days/BDNF (5.4 µg/mL)	Significant rescue of SGCs	BDNF 1.35 µg
Radeloff and Smolders, <sup>151</sup> 2006	Adult pigeons ( <i>Columba livia</i> ): collagen sponges loaded with 0.5 mg gentamicin placed in front of the round window membrane	56 days/BDNF (625 ng/mL)	BDNF did not enhance the time course or the extent of functional recovery and did not decrease the evoked ABR threshold	BDNF 0.42 µg
Agterberg et al, <sup>212</sup> 2008	Guinea pigs: kanamycin sulfate, 400 mg/kg + furosemide 100 mg/kg	28 days/BDNF (100 µg/mL)	Increased SGCs shape and survival rate and density; no change in ABR	BDNF 16.8 µg
Song et al, <sup>213</sup> 2008	Rats: gentamicin, 250 mg/kg + furosemide 85 mg/kg	Delayed intervention (30 days after deafening)/ 28 days/BDNF (5.4 µg/mL)	BDNF enhances SGCs bodies and peripheral processes survival	BDNF 1.35 µg
Agterberg et al, <sup>214</sup> 2009	Guinea pigs: kanamycin sulfate, 400 mg/kg + furosemide 100 mg/kg	28 days/BDNF (100 µg/mL)	Increased SGCs survival rate; preserved SGCs shape, response comparable	BDNF 16.8 µg
Sly et al, <sup>215</sup> 2012	Guinea pigs: kanamycin sulfate, 400 mg/kg + furosemide 100 mg/kg	28 days/BDNF (62.5 µg/mL)	Improved survival of SGCs, but not an increase in SGCs somal area; decreased tone and click eABR thresholds	BDNF 10.5 µg
Leake et al, <sup>216</sup> 2011	Young cats: neomycin sulfate, 60 mg/kg	70 days/BDNF (94 µg/mL)	Improved survival of the cochlear SGC neurons; reduction in thresholds for electrical ABR	BDNF 37.5 µg
Waaier et al, <sup>217</sup> 2013	Guinea pigs: kanamycin sulfate, 400 mg/kg + furosemide 100 mg/kg	28 days/BDNF (94 µg/mL)	Quantitative and qualitative improvement of peripheral processes	BDNF 16.8 µg
van Loon et al, <sup>218</sup> 2013	Guinea pigs: kanamycin sulfate, 400 mg/kg + furosemide 100 mg/kg	28 days/BDNF (100 µg/mL)	SGCs significantly bigger in comparison with normal throughout the cochlea	BDNF 16.8 µg
<i>Implanted osmotic minipump (combinations)</i>				
Shinohara et al, <sup>192</sup> 2002	Intracochlear infusion of 24 µL of 10% neomycin	26 days/BDNF (100 µg/mL) + CNTF (100 ng/mL)	SGCs survival rate is increased; ABR threshold is decreased	BDNF 31.2 µg + CNTF <sub>AX1</sub> 31.2 µg
Yamagata et al, <sup>219</sup> 2004	Guinea pigs: intracochlear infusion of 10% neomycin sulfate for 2 days	14 days/BDNF (100 µg/mL) + CNTF <sub>AX1</sub> (100 ng/mL). Delay 2 or 6 weeks	Density of SGCs is increased; ABR threshold is decreased; 6-week delay increases in ABR threshold in comparison with 2-week delay	BDNF 8.4 µg + CNTF <sub>AX1</sub> 8.4 µg
Wise et al, <sup>220</sup> 2005	Guinea pigs: kanamycin sulfate, 400 mg/kg + furosemide 100 mg/kg	Delay treatment 5 or 33 days/28 days/BDNF (50 µg/mL) + NTF (50 µg/mL)	Increased SGCs survival rate	BDNF 8.4 µg + NTF

(Continued)

**Table 2** (Continued)

Author, year	Animal model	Method of administration/ timing/dosing	Effect	Total dose
Shepherd et al, <sup>193</sup> 2005	Guinea pigs: kanamycin sulfate, 400 mg/kg + furosemide 100 mg/kg	28 days/BDNF (62.5 µg/mL)	Increased SGCs survival rate; ES did not provide trophic support of SGCs	BDNF 10 µg + ES
Miller et al, <sup>221</sup> 2007	Guinea pigs: kanamycin (420 mg/kg) and ethacrynic acid (60 mg/kg)	Delay treatment: 4 days, 3 weeks or 6 weeks/26 days/ BDNF (100 µg/mL)	Increased SGCs survival rate at different treatment delays, but longer delay led to decreased overall SGCs density	BDNF 31.2 µg + FGF <sub>1</sub>
Shepherd et al, <sup>163</sup> 2008	Guinea pigs: kanamycin sulfate, 400 mg/kg + furosemide 100 mg/kg	28 days/BDNF (62.5 µg/mL) +14 or 28 days of ES	Chronic ES averted the rapid loss of SGCs that occurred after the withdrawal of exogenous BDNF	BDNF 10 µg + ES
Glueckert et al, <sup>165</sup> 2008	Guinea pigs: kanamycin (450 mg/kg) followed (2 hours later) with ethacrynic acid (60 mg/kg)	Immediate and delay (21 days) treatment/26 days/ BDNF (100 µg/mL) + aFGF (50 ng/mL)	SGCs survival is enhanced, number of afferent peripheral processes is increased	BDNF 15.6 µg + aFGF
Song et al, <sup>161</sup> 2009	Rats: gentamicin, 250 mg/kg + furosemide 85 mg/kg	Delayed intervention (30 days after deafening)/ 28 days BDNF (5.4 µg/mL)	Combination of ES and BDNF had a synergistic effect: SGCs soma and peripheral process survival was increased	BDNF 1.35 µg + ES
Landry et al, <sup>162</sup> 2011	Guinea pigs: kanamycin sulfate, 420 mg/kg + furosemide 130 mg/kg	28 days/BDNF (30 µg/mL)	Greater SGCs survival and lower response thresholds for eABR. ES in combination with BDNF did not enhance SGCs	BDNF 5 µg + ES
Leake et al, <sup>160</sup> 2013	Young cats: neomycin sulfate, 60 mg/kg	70 days/BDNF (100 µg/mL)	SGCs numerical density had been increased	BDNF 37.5 µg + ES
<b>Viral mediated BDNF delivery systems</b>				
Staecker et al, <sup>170</sup> 1998	CBA/6J mice: 10 µL of neomycin 10 <sup>-1</sup> mol/L solution	Intracochlear injection through RWM into scala tympani of 10 µL HSV containing BDNF cDNA for transfection of the SGCs	Up to 95% of the original population of SGCs survived at 28 days	HSV.BDNF
Lalwani et al, <sup>222</sup> 2002	Guinea pigs: kanamycin (400 mg/kg) and ethacrynic acid (40 mg/kg)	Implanted osmotic minipump/8.3 days/AAV-BDNF/GFP (3×10 <sup>6</sup> viral particles/mL)	SGCs survival was increased in basal turn	AAV-BDNF/GFP
Nakaizumi et al, <sup>172</sup> 2004	Guinea pigs: kanamycin (400 mg/kg) and ethacrynic acid (50 mg/kg)	5 µL of an adenoviral suspension injected into the scala tympani through the RWM/28 days	SGCs survival was increased; no protective action of CNTF was observed	Ad.BDNF; Ad.BDNF + Ad.CNTF
Rejali et al, <sup>171</sup> 2007	Guinea pigs: with kanamycin (420 mg/kg) and ethacrynic acid (52.5 mg/kg)	CI electrode coated by fibroblast cells transduced by a viral vector with a BDNF gene insert/48 days	Spiral ganglion survival was enhanced in basal turn. Cells infected by Ad.BDNF placed on the CI in an agarose matrix could survive for weeks	Ad.BDNF, fibroblast cells
Chikar et al, <sup>173</sup> 2008	Guinea pigs: direct infusion of 60 µL of 10% neomycin into the perilymph	Single injection + intracochlear implant/80 days expression	Increased SGC survival rate; decrease in eABR threshold	5 µL Ad.BDNF (20×10 <sup>9</sup> adenoviral particles) BDNF Ad vectors
Wise et al, <sup>169</sup> 2010	Guinea pigs: kanamycin sulfate, 400 mg/kg + furosemide 100 mg/kg	Single injection of viral vectors into the scala media	Detection of localized gene expression; protection SGCs from degeneration	
Shibata et al, <sup>177</sup> 2010	Guinea pigs: 10 µL of 10% neomycin sulfate solution into the scala tympani perilymph	Inoculation with viral vectors into the scala media (adenoviral or adeno-associate viral particle)/14 or 30 days	Increase in the density of SGCs and inducing of nerve fibers growth	5 µL of Ad.BDNF, 20×10 <sup>9</sup> Ad particles; 5 µL of AAV2.GFP-BDNF, 8.4×10 <sup>9</sup> AAV2 particles

(Continued)

**Table 2** (Continued)

Author, year	Animal model	Method of administration/ timing/dosing	Effect	Total dose
Fukui et al <sup>143</sup> 2012	Deaf <i>Pou4f3</i> mutant mice	Adenoviral vector with a mouse BDNF gene insert, driven by the cytomegalovirus promoter	Increased number of nerve fibers in the auditory epithelium and survival rate of the auditory neurons in Rosenthal's canal	1 mL of Ad.BDNF (4×10 <sup>9</sup> adenoviral particles)
<b>Other delivery systems</b>				
Li et al <sup>123</sup> 2005	Rats: the induction of bacterial meningitis by <i>Streptococcus pneumoniae</i>	Intracerebroventricular administration 6 µg BDNF once a day, 7 days + antibiotic therapy	Decrease in the eABR threshold	BDNF 42 µg
Ito et al <sup>178</sup> 2005	Guinea pigs: kanamycin (420 mg/kg) and ethacrynic acid (25 mg/kg)	Biodegradable hydrogel immersed in 84 µg BDNF was placed on the RWM	Successful development of a novel strategy for drug application to the inner ear; number of surviving SGCs was increased; significant reduce in the eABR threshold was detected	BDNF 84 µg
Meen et al <sup>148</sup> 2009	Guinea pigs: 15 mg/kg intraperitoneal cisplatin	Cochleostomy, single placement of pure BDNF into the cochlea	Prevention of hearing loss	BDNF 0.05 µg
Meen et al <sup>154</sup> 2010	Guinea pigs: 15 mg/kg intraperitoneal cisplatin	Cochleostomy, single placement of pure BDNF into the cochlea	No changes in eABR click thresholds	BDNF 0.05 µg
Havenith et al <sup>179</sup> 2011	Guinea pigs: kanamycin sulfate, 400 mg/kg + furosemide 100 mg/kg	Gelfoam® (Pharmacia and Upjohn) containing BDNF, placed on the round window membrane/28 days	Increased SGC survival rate in basal turn, but no effect on SGC shape and size; no effect on eABR thresholds	BDNF 6 µg
Pettingill et al <sup>182</sup> 2011	Guinea pigs: kanamycin sulfate, 520 mg/kg + furosemide 130 mg/kg	Shwann cell-based gene transfer combined with alginate encapsulation technology/28 days	Greater auditory SGCs survival	BDNF-Schwann cells encapsulated in a biocompatible matrix

**Abbreviations:** BDNF, brain-derived neurotrophic factor; SGCs, spiral ganglion cells; HCs, hair cells; ABRs, auditory brainstem responses; CNTF, ciliary neurotrophic factor; NTF, neurotrophic factor; ES, electrical stimulation; FGF, fibroblast growth factor 1; aFGF, acidic fibroblast growth factor; eABR, electronic ABR; HSV, herpes simplex virus; RWM, round window membrane; AAV, adeno-associated virus; GFP, green fluorescent protein; CI, cochlear implant; Ad.BDNF, adenovirus vector containing BDNF.

minor surgery may provide long-lasting delivery of more than 2 weeks.<sup>150</sup> Technically, direct delivery of pure BDNF protein is carried out by exploiting implanted osmotic minipump with drug primed cannulas that release of 0.5 or 0.25 µL/h of human recombinant BDNF. The overall dose of BDNF appeared to be important when using experimental animals with different sizes. The total effective dose range for guinea pigs was 10–16.8 µg/ear, for rats was 1.35 µg, and for cats was 37.5 µg (Table 2). Of interest, Radeloff and Smolders<sup>151</sup> investigated the effectiveness of the treatment in deaf pigeons using a very small dose of 0.42 µg BDNF/ear. They revealed no reduction in the auditory brain stem responses (ABR) threshold, thus concluding that BDNF was not a limiting factor during HC regeneration and reinnervation. Nonetheless, the analysis of the corresponding doses, used for other animals showed that the application of such small amount of drug was the main reason for the unsuccessful result rather than other factors. Shoji et al<sup>149</sup> also

showed that 0.144 and 1.44 µg BDNF were not efficacious in the protection of the sensory epithelium in guinea pigs with acoustic trauma. The authors admitted that they had used five times less dose than other researchers. However, they had compared BDNF with NT-3 action and had assumed that BDNF did not have any specific action of sensory epithelium. Meanwhile, another study claimed that BDNF had no protective effect on HCs at 15 days (total 9 µg of BDNF) and 60 days (36 µg), but some effects at 30 days (18 µg).<sup>152</sup> One more study demonstrated that direct placement of 0.05 µg of BDNF into the inner ear after cochleostomy did not improve or treat hearing loss after cisplatin-induced deafening.<sup>148</sup> At the same time, the positive effect of 8.4 ng BDNF on SGCs survival in deaf guinea pigs has remained controversial in the context of dose-dependent manner of BDNF action.<sup>153</sup> As an alternative approach cochleostomy was performed. The posterior part of the tympanic membrane was separated, then

the underlying cochlea was opened into the scala tympani just anterior to the round window using a small diameter cutting drill and, finally, pure BDNF was placed there.<sup>148</sup> Due to lack of therapeutic effectiveness,<sup>154</sup> this method needs further investigation to establish an effective scheme of BDNF administration.

### Combinations with BDNF

One of the main limitations of using osmotic minipumps is the transient protection of SGCs.<sup>152</sup> Indeed, the survival effects of BDNF on neuronal cells were not observed after the treatment phase.<sup>155</sup> This phenomenon has been widely confirmed by other studies in different neural systems, indicating that the survival effects of BDNF, as well as other growth factors only last so long as the treatment itself.<sup>156</sup> To overcome this limitation or to increase the effectiveness, the usage of several combinations of the BDNF with other growth factors or electrical stimulation (ES) was suggested (Table 2). Some studies have established that a chronic depolarization of the auditory nerve via ES provided a trophic effect on SGCs without the contact of HCs,<sup>157,158</sup> however, these findings turned out to be controversial.<sup>159</sup> The effectiveness of the combination of BDNF with ES on SGCs survival also appeared to be not so universal (Table 2). This inconsistency displayed substantial methodological differences among these studies. The selection of animals can also be crucial for such studies: cats<sup>160</sup> and rats<sup>161</sup> are possibly more sensitive to ES than guinea pigs.<sup>162</sup> For future translation to patients, it is critical to note that continued chronic ES after cessation of BDNF delivery had significantly reduced the rate of SGCs loss.<sup>163</sup> In addition, there was also evidence about the protective effects on the SGCs that employed other neurotrophic factors, such as glial cell line-derived neurotrophic factor (GDNF),<sup>164</sup> fibroblast growth factor (FGF),<sup>165</sup> and ciliary neurotrophic factor (CNTF).<sup>166</sup> The combination of BDNF and any one of the other growth factors has been reported to be more effective than either agent alone in protecting SGCs and in decreasing ABR threshold (Table 2). Thus, the combination of BDNF with other agents may be promising as a potential therapeutic agent to promote the survival of SGCs in the auditory system. However, mini-osmotic pump appears to be inappropriate for use in patients due to fixed delivery period and high risk of bacterial contamination related to the surgical technique of inserting the pump into the cochlea and cannula clogging.<sup>167</sup>

### Viral mediated BDNF delivery

Viral vectors are known to be a potent method for gene delivery, which can be successfully applied for the treatment of the

inner ear. Several types of viruses have been created for inner ear transfection: Ad, AAV, lentivirus, herpes simplex virus (HSV), vaccinia virus, and Sendai virus.<sup>168</sup> It was considered that gene delivery via cochleostomy into the basal turn of the scala media was more effective than via RWM due to the existence of tight junctions between the scala tympani and scala media.<sup>169</sup> HSV, as a gene transfer vector is identified to be a promising carrier because of its ability to infect several cell types, including quiescent and proliferating cells. HSV with BDNF had successfully infected HCs and neurons, and this saved 94.7% of the original population of SGCs after aminoglycoside ototoxic injuries.<sup>170</sup> Additionally, Ad vectors can also be beneficial, basically, due to their possibility to infect different cell types effectively and to provide relatively fast beginning of gene production following infection. The first successful transfection by Ad of retinal Muller cells in vivo, which then were able to secrete BDNF, was made by Di Polo et al.<sup>121</sup> Later, the same methodology was used for gene delivery into the inner ear. Rejali et al.<sup>171</sup> demonstrated that Ad.BDNF transfected guinea pig fibroblast cells could release BDNF in vitro. The administration of Ad.BDNF into the cochlea following aminoglycoside deafening had been reported to enhance SGC survival rate at 21 days,<sup>169</sup> 28 days,<sup>172</sup> and 48 days<sup>171</sup> postinoculation, and to decrease psychophysical as well as ABR thresholds.<sup>173</sup> Recently, it was also found that Ad.BDNF had improved the auditory nerve survival and peripheral sprouting in *Pou4f3* mutant mouse ears.<sup>143</sup> However, adenovirus gene expression appears to be transitory, hence high levels of transgenes cannot be persistent over a long period. Moreover, another limitation of Ad vectors is the activation of immune responses.<sup>174</sup> One more effective viral vector, AAV, had shown different cell tropisms at different serotypes.<sup>175</sup> Among them, AAV2 may easily transduce HCs and SGCs in vitro,<sup>175</sup> although, in vivo, it was revealed that this vector can only infect spiral limbus, spiral ligament, and spiral ganglion cells, following its delivery across the RWM.<sup>176</sup> Besides, a recent paper had demonstrated a successful transfection of the basilar membrane area (epithelial and mesothelial cells) by AAV2 carrying the *BDNF* gene.<sup>177</sup> Moreover, AAV is considered to be one of the most promising gene therapy vectors for human clinical trials.<sup>168</sup> However, there are some limitations for AAV vectors, which include their inability to perform effective gene transduction in patients with antibodies against AAV or those with AAV infection in the past, for instance, in childhood when it could be very common. Additionally, the risk of virus toxicity due to the application of viral vectors continues to be a major restriction for its clinical use.

## Biodegradable materials for sustained BDNF release

In order to achieve sustained delivery of BDNF, other approaches were tested *in vivo*. A promising advance is the development of extracochlear application systems based on the ability of BDNF to diffuse across the RMW. Biodegradable hydrogel infiltrated with BDNF<sup>178</sup> has been successfully applied as a sustained-release carrier. With that, Ito et al<sup>178</sup> demonstrated significantly higher BDNF concentration in the guinea pigs' cochlear perilymph in the hydrogel group in comparison to either control animals or the group that received an injection through the RWM. These data provided factual evidence for the diffusion of BDNF across the guinea pig's round window. As a result, after 1 week, animals implanted with BDNF-containing collagen hydrogels had lower ABR thresholds and higher density of surviving SGCs from the basal up to the apical cochlear turns, moreover, the perilymph level of BDNF was over 100 times higher than control animals treated with saline.<sup>178</sup> Another option for sustained BDNF release to the cochlea was a gelatin sponge (Gelfoam®, Pharmacia and Upjohn, Bridgewater, NJ, USA) placed onto RMW.<sup>179</sup> Of interest, Havenith et al<sup>179</sup> had used a 10-fold less dose of BDNF than Ito et al.<sup>178</sup> Anyway, both studies are very significant, as they showed the possibility of usage of biodegradable carriers that contributed to low risk of cell toxicity. Nowadays, for the treatment of hearing loss, among all growth factors, only recombinant human insulin-like growth factor-1 (IGF-1) combined with gelatin hydrogel has been approved for clinical trials. It has finished Phase I/IIa clinical trial on the safety and efficacy of local IGF-1 application via the RWM using gelatin hydrogel for patients with acute sensorineural hearing loss.<sup>180</sup>

## Cell-based BDNF delivery

Apart from other approaches, Schwann cells or fibroblasts have been suggested as cell therapy methods. These types of cells have been tested both *in vitro* and *in vivo*.<sup>181</sup> The disadvantages include difficulties with the retrieval of cells and the potential to spread viral particles intracranially.<sup>181</sup> One study had suggested the creation of an electrode, which can secrete BDNF, using the *ex vivo* gene transfer pattern employing Ad containing *BDNF* gene insert. The proposed electrode was coated with fibroblasts transduced with the BDNF transgene. Then, the electrode with encased BDNF was transplanted into the scala tympani in a hydrogel scaffold on a CI to increase the survival of SGCs.<sup>171</sup> Another study that combined cell-based gene transfer with alginate technology, had demonstrated the survival effects on auditory neurons of encapsulated BDNF-expressing Schwann cells.<sup>182</sup> Such

transplanted cells, encapsulated in a biocompatible matrix, would be protected against strong immune responses, and this would allow the patients to avoid using toxic immunodepressants, thus minimizing the associated risk of transplant rejection.

Without a doubt, osmotic minipump, gene therapy, stem cell therapy, and cochleostomy are hopeful steps toward successful treatment of the degeneration of SGCs in the inner ear and the loss of hearing. However, it is difficult to predict how these technologies can be translated into clinical practice. Moreover, BDNF is documented to act in dose-dependent manner, while accurate control of the dose, for some approaches, has remained to be a challenge.

## Nanoparticulate delivery

NP delivery to the inner ear is a new promising strategy for targeting compounds to the cochlea in a sustained and controlled manner. Poly(D,L-lactide-*co*-glycolide acid) PLGA, a biodegradable and nontoxic polymer approved by US Food and Drug Administration for human use, has been established as the best nanoparticulate drug carrier.<sup>183</sup> Furthermore, recent work had demonstrated that systemic application of PLGA nanoparticles loaded with rhodamine resulted in the appearance of NPs in the liver, cochlea, and kidney, however, the placement of NPs into the RMW caused substantially higher accumulation of rhodamine in the scala tympani.<sup>184</sup> Another study had shown a wide distribution of PLGA throughout all turns of chinchilla cochlea when NPs were placed upon the RWM for a duration of 40 minutes.<sup>185</sup> Although delivery of BDNF to the inner ear using NPs has not been studied yet, either for systemic or for intratympanic administration, we assume that PLGA nanoparticles are a very promising approach for the inner ear drug targeting.

## Similarities, controversies, and future prospects

Hearing or vision loss is not only a prominent burden to the health care system, but also a ground for the worse quality of life associated with isolation and decreased socializing and, as a consequence, leads to an uprising of symptoms of depression on individuals.<sup>186,187</sup> In both cases, the neurodegenerative disorder of neurons of sensory organs is a crucial causal factor. Together with this, BDNF is considered to be a promising and an effective protein that is able to protect and increase the survival of RGCs as well as SGCs.

Besides, systemic delivery with oral, intravenous, and intramuscular routes are still thought as the most appropriate methods of drug administration, and they are currently recognized as the first line tactic in the treatment of the



inner ear or eye posterior segment disorders in clinic. On the other hand, the availability of drug in the posterior eye segment or scala tympani following systemic administration is limited by the presence of BRB or BCB, which are almost nonpermeable to large proteins, like BDNF (14 kDa). A variety of efforts related to the delivery of pure BDNF to the target organs has been documented in many research papers. For retinal diseases, single or multiple intravitreal injections are known as the most convenient methods, while for the inner ear, implantation of the osmotic minipump is the most applicable. It turned out that establishing the dose could be a challenging task which, fairly frequently, was not so universal. As mentioned earlier, 5  $\mu\text{g}$  of BDNF for one rat's eye was as an optimal dose, whereas 30  $\mu\text{g}$  BDNF was chosen as the optimal dose for cats. As for the ear, studies have shown that the total effective dose range for guinea pigs was 10–16.8  $\mu\text{g}/\text{ear}$ , for rats was 1.35  $\mu\text{g}$ , and for cats was 37.5  $\mu\text{g}$ . Even if the doses look comparable, it would be incorrect to compare them for some obvious reasons, for instance, intravitreal injection is mostly single dosing, whereas osmotic minipump delivers the drug over a span of 2 weeks. Also, of note is the fact that the volume of human eye is  $\sim 4$  mL, while the volume of cochlea is lesser,  $\sim 70$ –80  $\mu\text{L}$ .<sup>188</sup> Overall, it has been explicitly clear that for both eye and ear, the BDNF has a dose-dependent manner of action. Eventually, it has been shown, that increasing the dose may lead to lack of effectiveness of BDNF neuroprotection,<sup>97</sup> which is possibly due to negative feedback-mediated down-regulation of TrkB receptors.<sup>189</sup> Additionally, overdosing of BDNF enhances the excitability of the neurons that contribute to the initiation of seizures,<sup>190</sup> thus, it may be epileptogenic.<sup>191</sup> Furthermore, BDNF is found to cause an increase in the formation of free radicals, specifically nitric oxide (NO), hence boosting necrotic cell death.<sup>116</sup> To improve its effectiveness, reduce adverse effects, or to overcome the limitations, like transient action, it has been suggested that BDNF should be used in combinations. Mixtures of BDNF with other growth factors led to the enhancement of neuronal survival. Of interest, CNTF was exploited for both retina and inner ear, and it showed positive effect for immediate and delayed after-deafness treatment,<sup>192</sup> while for retina treatment, no additive effect was observed.<sup>114</sup> Another study had proposed the usage of LINGO-1 antagonist in combination with BDNF in rats with progressive neuropathy secondary to high intraocular pressure. LINGO-1 antagonist prevented the negative regulation of activity for TrkB receptors during interaction with BDNF and showed long-term protection for RGCs.<sup>112</sup> Besides, the combination with free radical scavenger S-PBN<sup>101,116</sup> or NO synthase inhibitor L-NAME

substantially reduced level of NO and improved RGCs survival in comparison to BDNF alone.<sup>93,116</sup> As for deafened guinea pigs, ES was used in combination with BDNF to prolong survival of SGCs.<sup>163,193</sup> Further, medicinal forms with sustained release appear to be another approach directed to overcome the transient effect of BDNF. Biodegradable polymer drug-delivery systems, like biodegradable hydrogel or Gelfoam<sup>®</sup> with BDNF, have been successfully applied for sustained delivery into the inner ear.<sup>179</sup> For the treatment of retinal disease, there is no documentation about BDNF delivery into the eye using biodegradable polymers. However, one such application had been tested in models of glaucoma, but by exploiting NGF. This showed that biodegradable PLGA microspheres, mixed with bioactive molecules with subsequent degradation of the material *in vivo*, had resulted in slow, sustained NGF release.<sup>134</sup> Alternatively, local drug delivery method, which employs viral gene, has been widely used for both eye and inner ear treatments. Furthermore, first, Ad vector was established by Dr Adriana Di Polo for BDNF therapy of axotomized rats.<sup>121</sup> Of interest, this adenoviral vector was later given by Dr Di Polo as a gift to the researchers who investigated the possibility of using gene therapy in deaf guinea pigs.<sup>171,172</sup> Among the different viral vectors, AAV is the most promising to be translated to patients due to its safety,<sup>194</sup> different cell tropisms, and sustained expression of BDNF.<sup>118</sup> Recent advances in cell-based therapy have met the needs in long-term stable delivery of BDNF. Schwann cells, which are capable of expressing BDNF, have been effectively transplanted into the subretinal space of RCS rat strains<sup>105</sup> and to the guinea pig's cochlea.<sup>182</sup> However, viral vector and cell-based therapy are still limited by surgical technique and human immune system. As a promising solution to protect cells from rejection and tumorigenicity, cell transplantation and gene transfer, combined with encapsulation technologies, has been suggested.<sup>182</sup> BDNF-expressing Schwann cells with alginate encapsulation technology have been applied to deaf guinea pigs and this showed survival effect on auditory neurons. Although BDNF has not been used with such strategy for the treatment of posterior eye segment, data about a Phase I clinical trial exploiting immortalized human retinal pigmented epithelial cells overexpressing CNTF for the treatment of retinitis pigmentosa have been published.<sup>195</sup>

A major challenge that has been raised is how to translate to patients the delivery of BDNF into these organs. Some drawbacks may be associated with the technique of administration itself. For instance, it was demonstrated that surgical manipulation on the patient's ear like cochlear implantation has led to a significant risk of deafness.<sup>196</sup> At the same time,

although repeated intravitreal injections of growth factors has become usual in many retinal clinics, other less invasive routes of administration capable of stable long-term delivery are of interest.<sup>134</sup> In future, the delivery of BDNF to the inner ear or to the posterior segment of the eye via nanotechnology-based products should be investigated more extensively. To date, some successes in the delivery of various therapeutics into the CNS have been widely recognized. Many studies on the different approaches to overcome the BBB are based on the knowledge of transport mechanisms of diverse biological molecules across the BBB. In fact, lots of techniques have been established to facilitate the penetration of NPs into the brain, namely carrier-mediated transport, receptor-mediated transcytosis, adsorptive-mediated transcytosis and cell-mediated transcytosis.<sup>15</sup> A comprehensive understanding of BRB and BCB physiology and matching them with the accumulated knowledge on BBB function should play a key role in the search for new strategies and will elucidate the potential of using NPs for the treatment of degenerative disorders of the retina and inner ear. It is possible to expect that targeted BDNF delivery that employs NPs should assist to bypass the limitations related to surgical intrusion into the sensory organ and simultaneously provide therapeutically effective concentration of molecule at the site of action and avoid unwanted systemic effects.

## Conclusion

Currently, various therapeutic strategies to treat inner ear and posterior eye segment diseases are limited by poor systemic delivery of BDNF. Although local delivery into the eye or inner ear provides a high concentration of BDNF with reduced systemic adverse reactions, anatomic features of these sensory organs require surgical intervention to achieve this delivery, which may lead to severe complications when translated to patients. It would be excellent to apply knowledge about BBB permeation to create a clinically feasible technique for BDNF delivery into the inner ear or posterior eye segment exploiting systemic administration. We assume that promising BDNF-containing drug for the treatment of inner ear and retina disorders should be designed as safe and effective medicine based on nanoparticulate drug delivery systems. This can make possible the long-lasting rescue of auditory and optic neurons from degenerative effects, substantially improving the quality of life of deaf or blind people.

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## Disclosure

The authors report no conflicts of interest in this work.

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