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Viral vector strategy for glial cell line-derived neurotrophic factor therapy for stroke

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1. ABSTRACT

Glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor-beta superfamily. Over the last decade, GDNF has been shown to promote regenerative and restorative effects on dopaminergic neurons. Accumulating evidence also demonstrates that administration of GDNF to areas of ischemic brain injury limits cerebral infarction and reduces damage to motor functions in animal models of stroke. Neurotrophic factor and anti-apoptotic mechanisms, among others, have been proposed to underlie the therapeutic effects of GDNF. A major obstacle for GDNF therapy is the protein delivery to the brain, as well as its sustained bioavailability over time. Gene therapy and the use of viral vectors offer a technique for longevity of GDNF expression within the brain. In this review, we consider the risks and benefits of GDNF gene therapy as it relates to the treatment of stroke.

2. OVERVIEW OF GLIAL CELL-LINE DERIVED NEUROTROPHIC FACTOR

Stroke is the third leading cause of death in the United States, affecting one individual every 45 seconds. Parkinson's disease (PD) is the second most common neurodegenerative disorder and the disease is associated with damage to dopaminergic (DA) neurons in the midbrain. Stroke and PD can produce long-term, debilitating effects to victims, affecting the individual's mobility and behavior. Treatment with glial cell-line derived neurotrophic factor (GDNF), a protein, offers promise for stroke, PD and other neurodegenerative disorders. The distinctive and regenerative effect that GDNF has on DA neurons provokes further research into the protein's effects on the central nervous system (CNS). Furthermore, with the recognition of overlapping etiologies between stroke and PD, therapeutic outcomes with GDNF in either one or both diseases reveal heightened interest in our understanding of neurodegeneration, as well as designing neurotrophic factor-based treatment strategies.

GDNF is a member of the transforming growth factor-beta (TGF-B) superfamily (Table 1). The GDNF gene is found in all major regions of the brain (1). The GDNF protein has garnered special attention for its neuroprotective capacity associated with DA neurons and spinal cord motor neurons. The therapeutic effect that

Table 1. Summary of studies on Glial cell line-Derived Neurotrophic Factor

Milestones	Reference
GDNF is discovered, purified and cloned in 1993. The protein is recognized for promoting dopaminergic (DA) neurons in the midbrain. GDNF is a member of the transforming growth factor-beta superfamily.	27
Parkinson's disease (PD), characterized by progressive damage to DA neurons, treated with GDNF in a rat model leads to regeneration of DA neurons.	28
Phase I clinical trial of GDNF administration to Parkinson patients revealed no serious side effects and a significant increase in DA uptake.	30, 12
Administration of GDNF to areas of ischemic insult reduces cerebral infarction.	29
GDNF protein does not readily traverse the tight junctions of the BBB.	2
Viral vector gene therapy offers longevity of GDNF expression; administration of Ad/GDNF vector in rabbit before ischemia reduces TUNEL labeling and caspase-3 immunoreactivity.	18
Pretreatment with an HSV amplicon/GDNF vector prior to ischemic insult in rat reduces motor asymmetry and bradykinesia; HSV amplicon/GDNF vector reduces tissue loss one month after ischemia.	23
Post stroke administration of Sendai/GDNF vector in gerbils prevents delayed hippocampal neuronal death.	30

GDNF has on ischemic brain injury is correlated with anti-apoptotic signaling. In rat models, GDNF upregulates anti-apoptotic factors, such as Bcl-2 and Bcl-X, thereby reducing caspase activity and the occurrence of apoptosis (2).

The GDNF family ligands (GFL) are comprised of GDNF, neurturin, artemin and persephin. Each GFL has its own glycosylphosphatidlyinositol (GPI)-linked receptor. The GFL protein attaches to a GPI-linked GFR-alpha factor, and this binding event activates GDNF. The formation of GDNF ligand-receptor complex initiates downstream signaling through the proto-oncogene, c-Ret and tyrosine kinase. GDNF binding to GFR-alpha also activates a mitogen-activated protein kinase (MAPK), which results in an enduring change in gene expression (1). Downstream signaling also occurs through activation of cytoplasmic Src family tyrosine kinase when c-Ret is not available (3). This signaling in the absence of c-Ret occurs through contact with the neural cell adhesion molecule (NCAM), a possible alternative signaling receptor for GDNF.

Further evidence shows a differential uptake in c-Ret and GFR-alpha. Following ischemia and reperfusion, c-Ret mRNA is expressed over a slower, more gradual timescale, whereas GFR-alpha mRNA is expressed immediately after ischemia and sustains a high level through reperfusion. Considering the difference in each receptor's uptake, it is feasible to assume that GFR and c-Ret are up-regulated in different areas of the brain following ischemic insult. The up-regulation of both receptors during the times following immediate ischemic insult and reperfusion allows for the quick response of the GDNF ligand to the area of damage.

GDNF is primed to respond to ischemic brain insult. Excitatory amino acids and their receptors are released in an increased amount during ischemic neuronal insult. GDNF mRNA in the rat brain has been up-regulated to combat excitotoxicity caused by glutamate, kainate and ischemia (2). Cortical neurons in culture show that GDNF limits excitotoxicity via activation of the MAPK pathway. GDNF, therefore, appears to affect neurons in the area of ischemic injury by attenuation of excitotoxicity. Nitric oxide (NO) levels are also increased by ischemic insult. NO release following MCA occlusion in rat brain is virtually blocked by the presence of GDNF (32); GDNF limits the negative effects of NO release following ischemic insult (33).

Following an episode of MCA occlusion, the receptor GFR-alpha is noticeably increased in the areas of ischemic insult (4). Before reperfusion, GFR-alpha is presumably largely up-regulated by the area of ischemic injury. After reperfusion in the same study, a second increment in up-regulation of GFR-alpha is observed, which likely coincides with reperfusion-associated injury.

Unlike adult kidney and brain tissue (5, 6), GDNF can be found in high concentrations within the fetal kidney (FK) (6). GFR-alpha is similarly found at high concentrations within the developing metanephric kidney (7), and both GDNF and GFR-alpha are found to play an important role in the maturation of the renal cortex when observed in animal models (8, 9). Animals with a GFR alpha-1 deficiency displayed complete bilateral renal agenesis and ureteral deficits (10). The elevated levels of GDNF found in FK provide tissue for transplantation to areas of ischemic injury. Cerebral damage caused by MCA occlusion in rats (11) and mice (6) has shown that transplantation of FK tissue to the cortical areas surrounding the area of injury reduced infarction volume and led to significant behavioral improvement. The beneficial effect of FK tissue was not observed when adult kidney tissue was transplanted to the area of cerebral damage, further indicating that FK tissue, but not adult kidney tissue, is a rich source of GDNF.

Diseases to the CNS demand attention, as there are no cures for neurological disorders, including stroke and PD, which affect a sizable amount of the United States population. In additional to medical and health-related problems, a financial burden is experienced by the patients, as well as their families and the community. Gene therapy techniques that incorporate GDNF are becoming increasingly assessed for therapeutic effectiveness. This paper investigates the potential of GDNF to treat stroke and PD, and also considers the possibilities of gene therapy for the treatment of other neurodegenerative disorders.

3. GDNF PROTEIN DELIVERY INTO THE BRAIN

Successful GDNF delivery to a targeted area of injury within the brain is made difficult by the blood brain barrier (BBB). The blood-brain barrier (BBB) consists of tight junctions and forbids the transport of large proteins (less than 600 to 1000 Da) into the brain parenchyma. Although transportof GDNF into the parenchyma may be possible during times of cerebral ischemia, when the tight junctions of the BBB are compromised, the tight junctions are compromised several hours after the stroke onset (2), voiding any therapeutic effects. Thus, successful delivery of GDNF to ischemic brain injury requires a transport-facilitated strategy across the BBB.

In vivo studies have demonstrated that pretreatment with GDNF protein can be administered via intracebroventricularly or intraparenchymally (12) to the cortical surface (13) or hippocampus (14) and such direct brain delivery results in reduced cerebral infarction.

Administration of GDNF still creates a safety issue due to the risk of GDNF interfering with other receptors and other neurons. The restorative property of GDNF can be noticed in neonatal rats. An intracerebal administration of GDNF to the neonatal rats reduced the damage caused by hypoxic and ischemic brain damage (15). In animal studies, intracerebral introduction of GDNF protein has protected injured DA neurons, promoted regenerative sprouting from axotomized DA axons and stimulated DA turnover and release in rescued neurons (16). Intracerebral introduction of GDNF protein, however, poses the risk of GDNF interaction with other receptors as well as presenting a surgical risk with the chance for brain hemorrhage during or following the procedure.

In view of circumventing the risks associated with intracerebral GDNF delivery, recent studies have also explored peripheral delivery of GDNF; for example, GDNF can effectively bypass the BBB in the form of TAT-GDNF. The application of TAT-GDNF in adult stroke animals resulted in reduced caspase-3 and apoptotic activity, reduced DNA fragmentation and increased neuronal survival (2). This novel systemic approach in GDNF transport is discussed in the accompanying paper by Kilic and colleagues (see FBS paper).

While GDNF delivery to areas of cerebral injury has increased DA levels through DA neurons, as well as benefiting motoneurons (important in the case of PD), GDNF protein application in clinical trials has produced mixed results. A Phase II study with PD patients showed that continuous administration of GDNF directly into the putamen did not alter brain functioning; additionally, monthly injections of GDNF intracerebroventricularly to PD patients resulted in considerable side effects (17). Also, there were no clear detections of GDNF even reaching the targeted areas within the brain. Taken together, these studies emphasize the need for a more reliable delivery of GDNF protein to the brain. *In vivo* and *in vitro* studies verify the regenerative effect GDNF has on DA neurons and motoneurons; a safe and efficient method of delivery must be identified to maximize these effects of GDNF for the treatment of neurodegenerative disorders.

4. THE ADVENT OF GDNF GENE THERAPY

When considering a vehicle for GDNF delivery, the treatment must include a method that will allow for a stable release of GDNF over time. The need for prolonged GDNF bioavailability in the brain is critical especially in the case of neurodegenerative disorders, such as PD, when a specific cell population progressively deteriorates. Notwithstanding, such GDNF secretion in the ischemic brain appears to be equally important for optimal benefits. Although stroke has been traditionally considered an acute neurological disorder, accumulating evidence suggests an on-going cell death cascades even at long delay periods following the initial ischemic insult. These observations categorize stroke, at the very least its secondary pathological symptoms, as a progressive disorder that would likely require chronic therapy. Rather than direct insertion of GDNF protein, a longer-lasting GDNF gene can be administered (and targeted at the area of ischemic insult) in order to create continuous expression and release of GDNF. Gene therapy provides a technique that allows for the longevity of GDNF gene expression.

Studies have utilized viral vectors to supply GDNF. Viral vectors with GDNF gene are considered for ability to cross the BBB, and also because of the longevity of GDNF expression afforded by the viral vector. Viral vectors coupled with gene therapy present a unique option for the delivery of GDNF to an area of recent ischemic injury. Commonly used viral vectors include adeno-associated viral (AAV), herpes simplex viral (HSV), adenoviral (Ad) and Sendai virus vector. Viral vector usage has the benefit of GDNF expression for longer amounts of time compared to other methods of GDNF protein delivery.

Sustained GDNF expression has successfully been observed *in vivo* via application of viral vector/GDNF to the nigrostriatal region of the brain (16). Studies with viral gene transfer of GDNF shows a decrease in ischemic tissue loss, a reduction in caspase activity, decreased terminal deoxynucleotidyl transferase-mediated dUTP biotin *in situ* nick end labeling (TUNEL), a reduction in brain infarction and a restoration of motor behaviors in animals with cerebral ischemia (20, 21, 22). Furthermore, the use of the Sendai virus (19), AAV (20) and adenoviral (21, 22) vectors have resulted in the successful penetration of GDNF gene to ischemic brain areas.

Treatment with HSV amplicon/GDNF for one month following an incident of stroke resulted in a decrease in animal tissue loss (23). Importantly, viral vector transmission confers the protective benefits of GDNF protein to areas with cerebral ischemia. In the same study with HSV amplicon/GDNF, pretreatment injections of the vector into the cerebral cortex, lessened the damage to motor movement. In the case of stroke, for viral vector/GDNF to be beneficial, the viral vector/GDNF must be initiated two to three days before MCA occlusion (19,

21, 23, 24). Vector administration one hour to three days following the reperfusion of the MCA did not result in the same level of protection as the more immediate treatment. GDNF gene therapy has been observed and successful in other neuronal tissues. Application of recombinant AAV (rAAV) with GDNF gene to the retina (25) resulted in a decrease of ischemic injury. In gerbils with transient cochlear ischemia, a pretreatment of Ad/GDNF decreased progressive cell and hearing losses (26).

Currently, there are no studies that have examined viral vectors that are capable of being "switch on" and "shut off" to control GDNF secretion. The creation of a viral vector/GDNF that allows a switch to turn on and off GDNF secretion would be favorable to manipulate expression of GDNF within the brain. The threat of perpetual GDNF expression bears special importance as there is a critical time constraint on viral vector/GDNF administration following ischemic injury. Unregulated expression of GDNF during a time when its therapeutic effects are minimal has the potential to react with other receptors and alter the action of other neurons. Additionally, a decision must be made concerning how much GDNF gene to incorporate into the viral vector, to provide benefit and to avoid adversely affecting surrounding components.

5. IMPROVING THE VIRAL VECTOR STRATEGY FOR GDNF THERAPY

Since the discovery of gene therapy, the technique has been used as a potential cure for disease, from cancer to hearing loss. The main protocol is to insert the therapeutic gene of interest into a virus and administer the recombinant virus to the subject. GDNF has also been used in this manner as mentioned previously in the paper. However, there are drawbacks to utilizing this method as a potential vehicle for transport of GDNF into the brain. The problems with the process itself are all related to the immune system. The short-lived nature of the virus, which is due to its inability to reproduce at an efficient level, reduces the efficacy of its gene product. There is also the host immune system, which actively destroys any foreign material present in the body. In relation to host defenses, the threat of toxicity and associated inflammatory response generated pose a vital concern to the health of the subject. Aside from these general caveats with gene therapy, there are also concerns over the GDNF protein.

As previously discussed, GDNF can provide neuroprotection if given before an ischemic episode and up until an hour after reperfusion. After one-hour (22) to three days (23) following reperfusion, the activity of GDNF delivered via viral vectors was decreased. This gives credence to suggestion that GDNF activity may be inhibited during the later stages of reperfusion (29). However, in a study involving 6-OHDA-lesioned rats (42, 43), in which neuroprotection was achieved by GDNF, the authors suggest that carefully manipulating the dose of the viral vector may overcome the issue of decreased activity. Therefore, decreased GDNF activity may be alleviated by determination of an optimal viral load required for stroke subjects.

Even if the optimal viral concentration of GDNF is identified, the expression of GDNF at high levels can be toxic. This is evidenced by the discontinuation of Amgen GDNF/ PD clinical trials due to concerns over cerebral toxicity in a past rhesus monkey experiment and the development of antibodies to GDNF among the patient pool (39). Low level expression of GDNF has been demonstrated, though, as having beneficial neurological effects in the same rhesus monkeys (39). Due to the sudden onset of stroke, a low level release of GDNF can act as a therapeutic molecule to protect against neuronal damage. In addition to gene therapy, other methods of introducing GDNF protein into the brain have been investigated. A combination of these approaches to deliver the protein and gene into the brain may pavee the way for the optimal dose, timing and route for GDNF therapy.

6. UNDERSTANDING THE STROKE PATHOLOGY FOR GDNF THERAPY

Production of an embolism or thrombosis which affects blood flow to the brain results in a stroke. The resulting hypoxic environment triggers an ischemic cascade resulting in irreversible neuronal damage. GDNF induces the inhibition of NMDA-induced calcium influx and stimulates the MAPK pathway in vitro (34). Experiments utilizing hippocampal brain slice (35) and cortical cultures (34) have indicated that pretreatment with GDNF decreases the level of NMDA-induced neuronal toxicity. In addition to NMDA-mediated neurotoxicity, ischemia cell death cascades also include the production of reactive oxygen species, as well as apoptosis-like processes (23, 36, 37). Of note, exposure of primary cortical cultureswith the HSV amplicon-based vector containing the gene for GDNF, alleviated hydrogen peroxide-induced neuronal damage (23). In vivo experiments revealed GDNF reduced caspase immunoreactivity and TUNEL labeling (36, 37), indicating the protein's anti-apoptotic property of GDNF. Similarly, decreased caspase-3 expression and DNA fragmentation was achieved by the TAT-GNDF protein in stroke adult mice (2). As noted above, the initial cell death inherent in stroke is abrupt and devastating, thus requiring immediate therapeutic intervention. However, equally destructive to the CNS is the secondary ischemia-associated cell death which progresses over time, and without subsequent treatment, is likely to exacerbate the stroke pathology. For example, edema formation becomes apparent after stroke and needs to be addressed. Accordingly, when contemplating with GDNF therapy, the timing of administration after stroke is critical. Interestingly, GDNF has been shown to relieve brain edema during ischemia (38).

Although there have been numerous in vitro and in vivo studies identifying the effects of GDNF in response to stroke, no clinical trials have been carried

out to date. As mentioned above, GDNF has been used in clinical trials for PD (39, 40, 41). In designing clinical trials of GDNF in stroke patients, one needs to consider an in-depth understanding of the stroke pathology, in addition to optimizing the viral vector delivery system if pursuing gene therapy.

7. FUTURE STUDIES/INSIGHTS

Transplantation of cells, which secrete a high level of GDNF, has been utilized as a method of neuroprotection. Stem cells have also been chosen as a viable source of GDNF due to their plastic state of gene expression and totipotent nature. Neural stem cells have been identified with an ability to secrete neurotrophic factors (45), such as. mesenchymal stem cells, which have also been shown to reduce stroke deficits in transplanted rats (46). Alternative GDNF-secreting cells, such as pineal gland and carotid body have also been transplanted and demonstrated to exert benefits in stroke models. Stem cells and novel tissues that secrete neurotrophic factors, especially GDNF, pose as efficacious graft sources for transplantation therapy in stroke.

Recent advances in gene therapy focusing on gene regulation should facilitate overcoming the obstacle of regulating GDNF expression. For example, the hypoxia response element found within the sequence for the erythropoietin gene promotes transcription of this gene only during hypoxic conditions (47). The design of recombinant hypoxic response element/GDNF sequence for use in gene therapy would aid in stable and efficient GDNF expression during and after an ischemic episode.

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9. REFERENCES

1. Ali C, A. Buisson, F. Docagne, E. MacKenzie, O. Nicole, L. Plawinski & D. Vivien: Neuroprotection mediated by glial cell line-derived neurotrophic factor: involvement of a reduction of NMDA-induced calcium influx by the mitogen-activated protein kinase pathway. *J Neurosci* 21(9), 3024-3033 (2001)

2. Kilic U, E. Kilic, G. P. Dietz & M. Bahr: Intravenous TAT-GDNF is protective after focal cerebral ischemia in mice. *Stroke* 34, 1304-1310 (2003)

3. Porteryaev D, A. Titievsky, Y. F. Sun, J. Thomas-Crusells, M. Lindahl, M. Billaud, U. Arumae & M. Saarma: GDNF triggers a novel ret-independent Src kinase family-coupled signaling via a GPI-linked GDNF receptor alpha1. *FEBS Lett* 463, 63-66 (1999)

4. Sarabi A, C. F. Chang, Y. Wang, B. J. Hoffer, & M. Morales: Time course study of GFR alpha-1 expression in an animal model of stroke. *Exp Neurol* 170, 283-289 (2001)

5. Stromberg I, L. Bjorklund, M. Johansson, A. Tomac, F. Collins, L. Olson, B. Hoffer & C. Humpel: Glial cell line-derived neurotrophic factor is expressed in the developing but not adult striatum and stimulates developing dopamine neurons *in vivo*. *Exp Neurol* 124, 401-412 (1993)

6. Tomac A. C, A. Grinberg, S. P. Huang, C. Nosrat, Y. Wang, C. V. Borlongan, S. Z. Lin, Y. H. Chang, L. Olson, H. Westphal & B. J. Hoffer: GFR alpha 1 availability regulates GDNF signaling: evidence from mice carrying one or two mutated alleles. *J Neurosci* 95, 1011-1023 (1999)

7. Yu T, S. Scully, Y. B. Yu, G. M. Fox, S. Q. Jing & R. P. Zhou: Expression of GDNF family receptor components during development: implications in the mechanisms of interaction. *J Neurosci* 18, 4684-4696 (1998)

8. Moore M. W, R. D. Klein, I. Farinas, H. Sauer, M. Armanini, H. Phillips, L. F. Reichardt, A. M. Ryan, K. Carver-Moore & A. Rosenthal: Renal and neuronal abnormalities in mice lacking GDNF. *Nature* 382, 76-79 (1996)

9. Pichel J. G, L. Y. Shen, H. Z. Sheng, A. C. Granholm, J. Drago, A. Grinberg, E. J. Lee, S. P. Huang, M. Saarma, B. J. Hoffer, H. Sariola & H. Westphal: Defects in enteric innervation and kidney development in mice lacking GDNF. *Nature* 382, 73-76 (1996)

10. Cacalano G, I. Farinas, L. C. Wang, K. Hagler, A. Forgie, M. Moore, M. Armanini, H. Phillips, A. M. Ryan, L. F. Reichardt, M. Haynes, A. Davies & A. Rosenthal: GFR alpha 1 is an essential receptor component for GDNF in the developing nervous system and kidney. *Neuron* 21, 53-62 (1998)

11. Chiang Y. H, S. Z. Lin, C. V. Borlongan, B. J. Hoffer, M. F. Morales & Y. Wang: Transplantation of fetal kidney tissue reduces cerebral infarction induced by middle cerebral artery ligation. *J Cerebral Blood Flow Metab* 19, 1329-1335 (1999)

12. Wang Y, S. Z. Lin, A. L. Chiou, L. R. Williams & B. J. Hoffer: GDNF protects against ischemia-induced injury in the cerebral cortex. *J Neurosci* 17, 4341-4348 (1997)

13. Abe K & T. Hayashi: Expression of the glial cell line-derived neurotrophic factor gene in rat brain after transient MCA occlusion. *Brain Res* 776, 230-234 (1997)

14. Miyazaki H, Y. Okuma, Y. Fujii, K. Nagashima & Y. Nomura: Glial cell line-derived neurotrophic factor protects against delayed neuronal death after transient forebrain ischemia in rats. *Neuroscience* 89, 643-647 (1999)

15. Ikeda T, X. Y. Xia, Y. X. Xia, T. Ikenoue, B. Han & B. H. Choi: Glial cell line-derived neurotrophic factor protects against ischemia/hypoxia-induced brain injury in neonatal rat. *Acta Neuropathol (Berl)* 100, 161-167 (2001)

16. Bjorklund A, B. Georgievska & D. Kirik: Overexpression of glial cell line-derived neurotrophic factor using a lentiviral vector induces time- and dose-dependent downregulation of tyrosine hydroxylase in the intact nigrostriatal dopamine system. *J Neurosci* 24(29), 6437-6445 (2004)

17. Annett L, H. F. Baker, C. Burger, A. Eslamboli, B. Georgievska, D. Kirik, R. J. Mandel, N. Muzyczka & R. M. Ridley: Continuous low-level glial cell line-derived neurotrophic factor delivery using recombinant adeno-associated viral vectors provies neuroprotection and induces behavioral recovery in a primate model of parkinson's disease. *J Neurosci* 25(4), 769-777 (2005)

18. Sakurai M, K. Abe, T. Hayashi, Y. Setoguchi, G. Yaginuma, T. Meguro & K. Tabayashi: Adenovirus - mediated glial cell line-derived neurotrophic factor gene delivery reduces motor neuron injury after transient spinal cord ischemia in rabbits. *J Thorac Cardiovasc Surg* 120, 1148-1157 (2000)
19. Shirakura M, M. Inoue, S. Fujikawa, K. Washizawa, S. Komaba, M. Maeda, K. Watabe, Y. Yoshikawa & M. Hasegawa: Postischemic administration of Sendai virus vector carrying neurotrophic factor genes prevents delayed neuronal death in gerbils. *Gene Ther* 11, 784-790 (2004)
20. Tsai T. H, S. L. Chen, X. Xiao, D. W. Liu & Y. P. Tsao: Gene therapy for treatment of cerebral ischemia using defective recombinant adeno-associated virus vectors. *Methods* 28, 253-258 (2002)
21. Yagi T, I. Jikihara, M. Fukumura, K. Watabe, T. Ohashi, Y. Eto, M. Hara & M. Maeda: Rescue of ischemic brain injury by adenoviral gene transfer of glial cell line-deriived neurotrophic factor after transient global ischemia in gerbils. *Brain Res* 885, 273-282 (2000)
22. Zhang W. R, K. Sato, M. Iwai, I. Nagano, Y. Manabe, & K. Abe: Therapeutic time window of adenovirus-mediated GDNF gene transfer after transient middle cerebral artery occlusion in rat. *Brain Res* 947, 140-145 (2002)
23. Harvey B. K, C. F. Chang, Y. H. Chiang, W. J. Bowers, M. Morales, H. J. Federoff, B. J. Hoffer & Y. Wang: HSV amplicon delivery of glial cell line-derived neurotrophic factor is neuroprotective against ischemic injury. *Exp Neurol* 183, 47-55 (2003)
24. Shirakura M, M. Fukumura, M. Inoue, S. Fujikawa, M. Maeda, K. Watabe, S. Kyuwa, Y. Yoshikawa & M. Hasegawa: Sendai virus vector-mediated gene transfer of glial cell line-derived neurotrophic factor prevents delayed neuronal death after transient global ischemia in gerbils. *Exp Anim* 52, 119-127 (2003)
25. Wu W. C, C. C. Lai, S. L. Chen, M. H. Sun, X. Xiao, T. L. Chen, R. J. Tsai, S. W. Kuo & Y. P. Tsao: GDNF gene therapy attenuates retinal ischemic injuries in rats. *Mol Vis* 10, 93-102 (2004)
26. Hakuba N, K. Watabe, J. Hyodo, T. Ohashi, Y. Eto, M. Taniguchi, L. Yang, J. Tanaka, R. Hata & K. Gyo: Adenovirus-mediated overexpression of a gene prevents hearing loss and progressive inner hair cell los after transient cochlear ischemia in gerbils. *Gene Ther* 10, 426-433 (2003)
27. Lin L. F, D. H. Doherty, J. D. Lile, S. Bektesh & F. Collins: GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* 260(5111), 1130-1132 (1993)
28. Espejo E. F, M. C. Gonzalez-Albo, J. Moraes, F. El Banoua, J. A. Flores & I. Caraballo: Functional regeneration in a rat Parkinson's model after intrastriatal grafts of glial cell line-derived neurotrophic factor and transforming growth factor beta-expressing extra-adrenal chromaffin cells of the zuckerkandl's organ. *J Neurosci* 21(24), 9888-9895 (2001)
29. Zhang W. R, T. Hayashi, M. Iwai, I. Nagano, K. Sato, Y. Manabe & K. Abe: Time dependent amelioration against ischemic brain damage by glial cell line=derived neurotrophic factor after transient middle cerebral artery occlusion in rat. *Brain Res* 903, 253-256 (2001)
30. Shirakura M, M. Inoue, S. Fujikawa, K. Washizawa, S. Komaba, M. Maeda, K. Watabe, Y. Yoshikawa & M. Hasegawa: Postischemic administration of Sendai virus vector carrying neurotrophic factor genes prevents delayed neuronal death in gerbils. *Gene Ther* 11, 784-790 (2004)
31. Gill S. S, N. K. Patel, G. R. Hotton, K. O'Sullivan, R. McCarter, M. Bunnage, D. J. Brooks, O. N. Svendsen & P. Heywood: Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nature Med* 9, 589-595 (2003)
32. Wang Y, S. Lin, A. Chiou, L. R. Williams & B. J. Hoffer: Glial cell line-derived neurotrophic factor protects against ischemia-induced injury in the cerebral cortex. *J Neurosci* 17, 4341-4348 (1997)
33. Lingor P, K. Unsicker & K. Krieglstein: GDNF and NT-4 protect midbrain dopaminergic neurons from toxic damage by iron and nitric oxide. *Exp Neurol* 163, 55-62 (2000)
34. Nicole O, C. Ali, F. Docagne, L. Plawinski, E. T. MacKenzie, D. Vivien & A. Buisson: Neuroprotection mediated by glial cell line-derived neurotrophic factor: involvement of a reduction of NMDA-induced calcium influx by themitogen-activated protein kinase pathway. *J Neurosci* 21(9), 3024-33 (2001)
35. Bonde C, B. W. Kristensen, M. Blaabjerg, T. E. Johansen, J. Zimmer & M. Meyer: GDNF and neublastin protect against NMDA-induced excitotoxicity in hippocampalslice cultures. *Neuroreport* 11(18), 4069-73 (2000)
36. Kitagawa H, T. Hayashi, Y. Mitsumoto, N. Koga, Y. Itoyama & K. Abe: Reduction of ischemic brain injury by topical application of glial cell line-derived neurotrophic factor after permanent middle cerebral artery occlusion in rats. *Stroke.* 29(7), 1417-22 (1998)
37. Jin G, N. Omori, F. Li, I. Nagano, Y. Manabe, M. Shoji & K. Abe: Protection against ischemic brain damage by GDNF affecting cell survival and death signals. *Neurol Res*.25(3), 249-53 (2003)
38. Abe K, T. Hayashi & Y. Itoyama: Amelioration of brain edema by topical application of glial cell line-derived neurotrophic factor in reperfused rat brain. *Neurosci Lett* 231(1), 37-40 (1997)
39. Grondin R, Z. Zhang, Y. Ai, D. M. Gash & G. A. Gerhardt: Intracranial delivery of proteins and peptides as a therapy for neurodegenerative diseases. *Prog Drug Res* 61,101-23 (2003)
40. Patel N.K, M. Bunnage, P. Plaha, C. N. Svendsen, P. Heywood & S. S. Gill: Intraputamenal infusion of glial cell line-derived neurotrophic factor in PD: a two-year outcome study. *Ann Neurol* 57(2), 298-302 (2005)
41. Nutt J.G, K. J. Burchiel, C. L. Comella, J. Jankovic, A. E. Lang, E. R. Laws Jr, A. M. Lozano, R. D. Penn, R. K. Simpson Jr, M. Stacy & G. F. Wooten: Implanted intracerebroventricular. Glial cell line-derived neurotrophic factor. Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. *Neurology*60(1), 69-73 (2003)
42. Georgievska B, D. Kirik & A. Bjorklund: Aberrant sprouting and downregulation of tyrosine hydroxylase in lesioned nigrostriatal dopamine neurons induced by long-lasting overexpression of glial cell line derived neurotrophic factor in the striatum by lentiviral gene transfer. *Exp Neurol.* 177(2), 461-74 (2002)

43.Georgievska B, Kirik, D. & A. Bjorklund: Overexpression of glial cell line-derived neurotrophic factor using a lentiviral vector induces time- and dose-dependent downregulation of tyrosine hydroxylase in the intact nigrostriatal dopamine system. *J Neurosci* 24(29), 6437-45 (2004)
44. Johnston R.E, O. Dillon-Carter, W. J. Freed & C. V. Borlongan: Trophic factor secreting kidney cell lines: *in vitro* characterization and functional effects following transplantation in ischemic rats. *Brain Res* 900(2), 268-76 (2001)
45. Llado J, C. Haenggeli, N. J. Maragakis, E. Y. Snyder & J. D. Rothstein: Neural stem cells protect against glutamate-induced excitotoxicity and promote survival of injured motor neurons through the secretion of neurotrophic factors. *Mol Cell Neurosci* 27(3), 322-31 (2004)
46. Kurozumi K, K. Nakamura, T. Tamiya, Y. Kawano, K. Ishii, M. Kobune, S. Hirai, H. Uchida, K. Sasaki, Y. Ito, K. Kato, O. Honmou, K. Houkin, I. Date & H. Hamada: Mesenchymal stem cells that produce neurotrophic factors reduce ischemic damagein the rat middle cerebral artery occlusion model. *Mol Ther* 11(1), 96-104 (2005)
47. Semenza G.L: HIF-1, O(2), and the 3 PHDs: how animal cells signal hypoxia to the nucleus. *Cell* 107(1), 1-3 (2001)

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