

# Therapeutic Strategies for Parkinson's Disease: The Ancient Meets the Future—Traditional Chinese Herbal Medicine, Electroacupuncture, Gene Therapy and Stem Cells

Xuan Wang · Xi-Bin Liang · Feng-Qiao Li · Hui-Fang Zhou · Xian-Yu Liu · Jian-Jun Wang · Xiao-Min Wang

Accepted: 28 March 2008 / Published online: 11 April 2008  
© Springer Science+Business Media, LLC 2008

**Abstract** In China, it has been estimated that there are more than 2.0 million people suffering from Parkinson's disease, which is currently becoming one of the most common chronic neurodegenerative disorders during recent years. For many years, scientists have struggled to find new therapeutic approaches for this disease. Since 1994, our

research group led by Drs. Ji-Sheng Han and Xiao-Min Wang of Neuroscience Research Institute, Peking University has developed several prospective treatment strategies for the disease. These studies cover the traditional Chinese medicine—herbal formula or acupuncture, and modern technologies such as gene therapy or stem cell replacement therapy, and have achieved some original results. It hopes that these data may be beneficial for the research development and for the future clinical utility for treatment of Parkinson's disease.

Special issue article in honor of Dr. Ji-Sheng Han.

X. Wang · X.-M. Wang (✉)  
Department of Physiology, Capital Medical University,  
Key Laboratory for Neurodegenerative Disorders of the Ministry  
of Education, Beijing 100069, People's Republic of China  
e-mail: xmwang@ccmu.edu.cn

X.-B. Liang  
Department of Neurology and Neurological Sciences,  
Stanford University, Stanford, CA 94305, USA

F.-Q. Li  
Division of Neurology, Duke University Medical Center,  
Durham, NC 27710, USA

H.-F. Zhou  
Department of Anesthesiology, Washington University School  
of Medicine, St. Louis, MO 63110, USA

X.-Y. Liu  
Department of Anesthesiology and Basic Medical Science,  
University of Missouri-Kansas City School of Medicine,  
Kansas City, MO 64108-2792, USA

J.-J. Wang  
Department of Psychiatry, Emory University School of  
Medicine, 615 Michael Street, Atlanta, GA 30322, USA

X.-M. Wang  
Neuroscience Research Institute and Department of  
Neurobiology, Peking University, Key Laboratory for  
Neuroscience of the Ministry of Education, Beijing 100083,  
People's Republic of China

**Keywords** Parkinson's disease · Triptolide ·  
Electroacupuncture · Gene therapy · Stem cells

## Introduction

Parkinson's disease (PD) is one of the most common chronic neurodegenerative disorders of the central nervous system (CNS), affecting about 3% of the population over the age of 65 worldwide [1]. A recent cross-sectional prevalence investigation in China showed that the prevalence of PD was about 1.7% in male and 1.6% in female in people aged over 65 years old [2]. It has been estimated that about 2.0 million people in China are suffering from this disease. But the actual number might be even higher considering those patients living in the countryside who may never have been diagnosed. Therefore, PD is becoming one of the most critical health problems in China.

The pathological hallmark of PD is a specific degeneration of dopaminergic (DAergic) neurons in substantia nigra of midbrain, followed by the significantly reduced content of neurotransmitter-dopamine (DA) in striatum, and resulting in the clinical symptoms. Firstly described by

Dr. James Parkinson as early as in 1817, the cause of chronic nigral cell death in PD and its underlying mechanisms remain obscure nowadays. In the clinic, treatment with levodopa (L-Dopa), the precursor of DA, is simply symptom-modifying within limited time rather than a cure [3]. Therefore, a more effective therapy is critically needed not only for relief of the symptom but also halt or even reversal of the progress of the disease. For many years, scientists from all over the world have been striving to find new therapeutic approaches for PD and great efforts have been made from pharmacological, neurosurgical approaches to the gene therapy and stem cell transplantation. Alternatively, we envision that the traditional Chinese medicine (TCM), including herbal formula and acupuncture, may provide a unique strategy in combating the devastating neurodegenerative disease.

Starting from 1994, our neurodegenerative disease research group in Neuroscience Research Institute, Peking University, supervised by Drs. Ji-Sheng Han and Xiao-Min Wang, has established and dedicated to explore the utility of TCM in treatment of PD in preclinical studies. We have also been engaged in deciphering the mystery of the ancient clinical practices using modern state-of-art technologies. These studies have achieved some original results and some related articles have been published in recent years, which may shed light upon the old mystery of the basic disease mechanism and its future clinical utility for treatment of PD.

### Traditional Chinese Herbal Medicine

The ancient Chinese medical books had described some characteristic symptoms as trembling of the hands and shaking of the head that resemble Parkinson's-like symptoms today. The treatment of these symptoms with Chinese herbal medicine has been used over centuries. Even nowadays, herbal medicine is still very popular for treatment of PD in Asian countries such as China, Japan and Korea. The most significant characteristic of Chinese herbal formulations is that they are usually composed of multiple crude herb materials. Given that the pathogenesis and causes of most diseases like PD could not be single factor-derived, it is reasonable to use such combined treatment as herbal formulation with multiple biologically active components to address a variety of pathogenesis aspects. However, because of lack of reliable quality control and failure to meet Western criteria, there is still a long way to go for TCM to be widely accepted by Western countries. To simplify the complexity of herbal formulation, we envision that the efficacy of TCM is derived from the combination of single active components. Therefore, we try to identify some potent bioactive compounds from herbal extractions aimed at specific pathological factors.

Accumulating evidences suggest that an inflammatory response in the CNS is involved in the degeneration of DAergic neurons. The inflammatory response in the CNS is characterized by the presence of abnormally activated microglia, the resident immune surveillance cells in the brain, which are functionally similar to peripheral macrophages. Upon various stimuli or brain injury, microglia can be rapidly overactivated followed by subsequent release of a host of cytotoxic substances such as nitric oxide (NO), cytokines, excitatory amino acid, reactive oxygen species (ROS), arachidonic acid and its derivatives. Excessive exposure of neurons, especially DAergic neurons which is very vulnerable, to these cytotoxins can initiate the apoptotic signaling pathways, and thus lead to progressive degeneration or cell death of DAergic neurons [4]. The drugs with inhibitory properties on the activation of microglia or release of neurotoxins may exert neuroprotective effect and prove to be effective in slowing or even halting the progressive neurodegeneration.

In our effort in exploring new herbal drugs, extracts of the traditional Chinese herb *Tripterygium wilfordii* Hook F (TWHF) have drawn our attention (Chinese Patent-The extracts of TWHF on prevention and treatment of nervous system diseases. Patent No.: ZL00107779.1). TWHF (Celastraceae) has been historically used in TCM. The part with pharmacological efficacy is in the root. T<sub>II</sub>, the active anti-inflammatory component of TWHF, has been reported to be effective in treatment of a variety of inflammatory and autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus, and now has been used in clinical trials [5, 6]. Triptolide (designated as T<sub>10</sub>), the most abundant and active component of TWHF, is the principal active diterpenoid further purified from T<sub>II</sub>. T<sub>10</sub> exerts the anti-inflammatory, immunosuppressive and anti-fertility activities, and its potency is about 100–200 times higher than that of T<sub>II</sub> [7]. In 1999, Qiu et al. reported that T<sub>10</sub> inhibited the expression of interleukin (IL)-2 in peripheral lymphocytes induced by phorbol 12-myristate 13-acetate (PMA), CD3 antibody and ionomycin [8].

T<sub>10</sub> can penetrate the blood-brain-barrier (BBB) easily because of its lipophilic character and small molecular size. The brain is one of the organs most abundant in T<sub>10</sub> after systemical administration of T<sub>10</sub>. We therefore hypothesized that T<sub>10</sub> might exert similar anti-inflammatory and immunosuppressive activities in CNS and protect neurons from inflammatory damage. Our group demonstrated for the first time that T<sub>10</sub>, and its analog T<sub>4</sub>, possess potent neuroprotective properties on DAergic neurons both in vitro and in vivo [9, 10]. T<sub>10</sub> can promote the axon growth of rat DAergic neurons in primary culture and inhibited cytotoxic effects of neurotoxins MPP<sup>+</sup> (1-methyl-4-phenyl-pyridinium ion, MPP<sup>+</sup>) and  $\beta$ -amyloid (A $\beta$ ) in PC12 cells. T<sub>10</sub> inhibited microglial activation and

decreased the release of pro-inflammatory cytokines induced by lipopolysaccharide (LPS) in primary neuroglia mixed culture. Most significantly,  $T_{10}$ , at concentration of as low as 0.1 nM, can inhibit the production of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) by activated microglia. At 0.1  $\mu$ M,  $T_{10}$  almost completely inhibited the release of TNF- $\alpha$ . The effect of  $T_{10}$  on different inflammatory cytokines is varied.  $T_{10}$  in low concentration (0.1–1 nM) showed no obvious effect on the release of IL-1 $\beta$  induced by LPS, while only at higher concentration (0.1–1  $\mu$ M), it displayed potent inhibitory activity [11].  $T_{10}$  protected DAergic neurons in substantia nigra and alleviated the abnormal rotational behaviors induced by amphetamine in PD rat model by transection of medial forebrain bundle (MFB) with a wire knife. The mechanism may be due to the improvement of brain derived neurotrophic factor (BDNF) expression and the neurotrophic activity of nerve growth factor (NGF) [12, 13]. The beneficial activities of  $T_{10}$  on DAergic neuronal protect was further confirmed with an inflammatory PD model by injection of LPS into the substantia nigra. Using this inflammatory model,  $T_{10}$  significantly improved the behavior of PD rats, decreased DAergic neuron death and increased DA level in striatum after intraperitoneal injection with  $T_{10}$  (5  $\mu$ g/kg) for 24 days. These results indicate that  $T_{10}$  can reduce the inflammation-mediated the death of DAergic neurons through inhibiting the over-activation of microglia and the excessive release of cytokines induced by LPS [10]. In addition to the anti-inflammatory effect, we postulate that the following mechanisms may also contribute to the neuroprotective effect of  $T_{10}$ . (1) Anti-oxidative stress activity. As evidence, we have found that  $T_{10}$  can inhibit the accumulation of ROS induced by glutamate or MPP<sup>+</sup> in PC12 cells [14, 15]. (2) Inhibiting glutamate toxicity and Ca<sup>2+</sup> overload.  $T_{10}$  inhibited the apoptosis of PC12 cells induced by A $\beta$  by inhibiting the production of ROS and by reversing the decrease of mitochondrial membrane potential [15]. (3) Inhibiting the apoptosis of neurons and activation of NF- $\kappa$ B. It has been reported that  $T_{10}$  inhibited the activation of NF- $\kappa$ B transduction induced by TNF- $\alpha$  [16]. (4) Upregulating the release of neurotrophins. NGF and BDNF in substantia nigra decreased significantly in PD patients, while  $T_{10}$  and its analog  $T_4$  improve the expression of BDNF mRNA in primary midbrain cell culture, and show synergistic effects on the neurotrophic activity of NGF [9]. It is still unknown whether  $T_{10}$  improve the secretion of NGF of glial cells to play its neurotrophic or protective effects on DAergic neurons (Fig. 1).

Taken together, a series of investigation has been conducted in our group and have disclosed the neuroprotective effects of  $T_{10}$  from phenomena to mechanisms. Now, we are engaged in identification of the direct target molecule of  $T_{10}$ . Using structure-activity relationship (SAR)

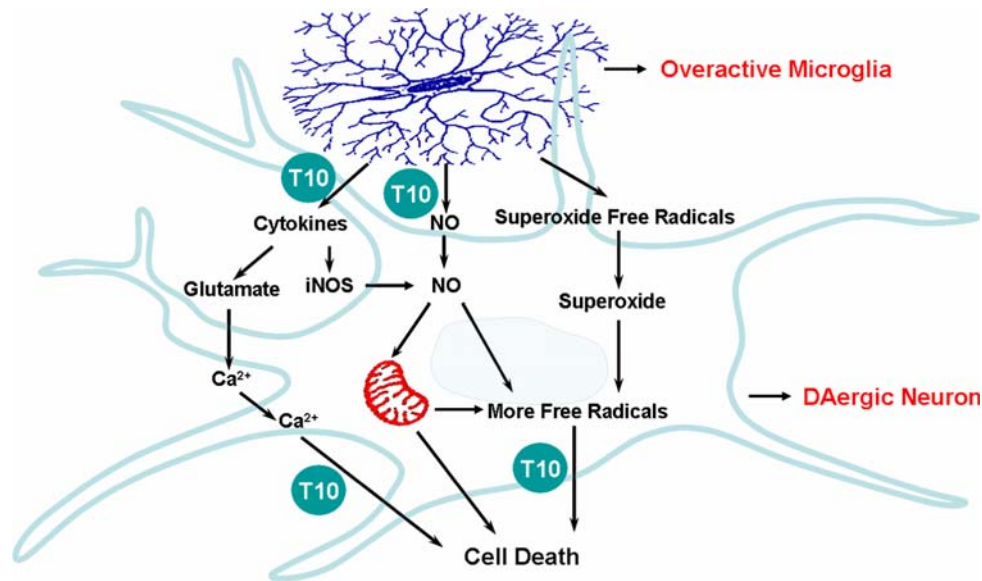
analysis, we are expecting to validate the crucial groups or domains of  $T_{10}$  which are relevant to its favorable bioactivities and toxicities, respectively. Once validated, we will be able to conduct the molecular modification on the structure of  $T_{10}$  to enhance the therapeutic efficacy and reduce toxicity or side effects.

## Electroacupuncture

As a modality for PD treatment, acupuncture is being used widely in the world as a weighty form of alternative medicine. The latest statistical data show that at least 40 percent of patients used alternative therapies to ameliorate the symptoms, and acupuncture is one of the most popular modalities [17–21]. After the acupuncture treatment, 70–80% of patients reported the improvement in subjective symptoms and several motor scores as well as the significant amelioration of their sleep and mood. No side effects were found.

Electroacupuncture (EA) is a significant innovation on the traditional manual acupuncture using the state-of-art technology. In the past 40 years, Dr. Ji-Sheng Han has been actively dedicated to decipher the mechanism of acupuncture. EA is more suitable for the experimental research for its easiness to be objectively and precisely controlled independent of operators compared to traditional acupuncture. The parameters of EA relate to its therapeutic efficacy including point-choosing, frequency of EA stimulation and treatment sessions, intensity, and period of treatment. In experiments of Liang XB et al. [22, 23], MFB-transected parkinsonian rats were received EA stimulation at BAIHUI (GV20, at the mid point of the line connecting the 2 ears) and DAZHUI (GV14, just below the spinous process of the vertebra prominens) for a total of 30 min each day at two frequencies (2 Hz and 100 Hz). The intensity of the stimulation was increased stepwise from 1 mA to 2 mA and then to 3 mA, with each step lasting for 10 min. By systematic comparisons, we claimed that the long-term effects of 100 Hz stimulation were better than that of 2 Hz stimulation, mainly because the former could still attenuate the rotational behavior significantly in the fourth week but the latter could not. 100 Hz EA could significantly increase the survival rate of DAergic neurons in substantia nigra pars compact of the lesioned side of PD rats, which indicated that 100 Hz EA could hinder the progressive degeneration of DAergic neurons and therefore had unambiguous protective effects.

The previous studies pointed out that EA stimulation could affect the DA content in the brain; for example, in rats, EA stimulation at the points on the Governor Vessel (GV, a group points in the middle of the back) could raise striatal DA content. It was also reported that stimulating RENZHONG (GV26, at the dividing line between the



**Fig. 1** Mechanisms of neuroprotective effect of T10. In CNS, overactive microglia can lead to the death of DAergic neurons by releasing inflammatory factors, NO or superoxide free radicals. Inflammatory factors can upregulate the level of iNOS, which aggravates the effects of NO. These cytokines also activate excitable amino acid-glutamine, and then stimulate the Ca<sup>2+</sup> release. Redundant NO entering into the soma of neurons can inhibit the mitochondrial

complex I and lead to the generation of more free radicals. Superoxide free radicals bring on superoxide of neurons and cause cell damage. T10, a Chinese Herb TWHF monomer, may play the neuroprotective role on DAergic neurons by inhibiting the activation of microglia, resulting in the declines of inflammatory cytokines, NO, superoxide free radicals or Ca<sup>2+</sup> overload

upper and middle thirds of the philtrum) and CHENGJI-ANG (CV24, in the middle of the mentolabial groove) at the same time could significantly elevate striatal DA content [24]. Our work testified that 100 Hz EA stimulation at BAIHUI and DAZHUI also could increase striatal DA content in PD rats [25]. We hypothesize that EA stimulation may enhance the activity or content of tyrosine hydroxylase (TH) which is the rate limiting enzyme of DA or the reuptake of DA by DAergic presynaptic terminals (our new data, unpublished).

In addition to the therapeutic effect on PD rats, EA stimulation was found to be able to improve behavioral deficits, protect DAergic neurons and augment striatal DA content in subacute MPTP-lesioned mice [26]. This suggested that there was no species difference in point of the effect of EA stimulation on rodent PD model. However, more basic studies and clinical investigations are needed to expand the efficacy of EA, as a common rule, in the treatment of PD.

Nowadays, mechanisms underlying the EA improvement in PD symptoms still remain unclear. In our studies, 100 Hz EA could retard the degeneration of DAergic neurons, increase the gene expression of GDNF in the unlesioned side of substantia nigra reticular and both sides of the global pallidus, of BDNF in the substantia nigra and ventral tegmental area (VTA), and BDNF content in the ventral midbrain [22, 23, 25]. 100 Hz EA could inhibit the activation of microglia and its transformation into

macrophage and prevent the transcription of neurotoxic agents [25]. These results suggested that EA could improve the symptoms of PD through suppressing cellular ROS and inflammation, and increasing the synthesis and release of neurotrophins. Furthermore, EA may play an important role in balancing the basal ganglion circuit and keeping homeostasis. All the current results indicate that the action of EA on the treatment of PD may be versatile by interfering in multiple signaling pathways.

In summary, our group initiated the investigations in the neuroprotective mechanisms of EA, enriched the theories of acupuncture in treating neurodegenerative disease, and found the basis for the further study of EA therapy for PD from the aspects of neuroprotection, anti-inflammation and balancing the nerve circuits. Following our pioneering studies, we are happy to see that a lot of interest has been sparked to explore the molecular mechanism underlying the therapeutic effect of EA on treatment of PD. We believe that these fundamental researches will eventually decipher the mystery of the ancient Chinese clinical practice of acupuncture and may pave an alternative or unique way to the therapy of PD.

## Gene Therapy

With the development of molecular biology technique and the execution of human genome project, PD gene therapy

becomes one of the most active research points. The most common method of gene therapy is to use replication-defective recombinant virus vector for gene delivery, including herpes simplex virus (HSV), retrovirus, adenovirus (Ad) and adeno-associated virus (AAV) [27]. AAV is a single stranded DNA virus of 4.7 kb. It has no immunogenicity, integrates into host chromosome, and amplifies with the division of host cells. Till now, there are no known diseases related to AAV. Recombinant AAV (rAAV) can help exogenous gene express in vivo for long time, and it has been observed that target gene expressed in vivo for 1.5–2 years. All the characteristics above determined that AAV vector was promising in PD gene therapy [28, 29]. However, there are still two critical issues to be addressed before gene therapy moves into clinical application: the long-term, stable, safe expression and manageable expression of target genes. In comparison to glucocorticoid, metallothionein and other inducible system, tetracycline inducible system is relatively sensitive and safe with fewer side effects. It is a drug- and dose-dependent inducible expression system including two parts: tetracycline transactivator (tTA) and a promoter comprised of part of tetracycline operon sequences and key sequences of cytomegalovirus immediate early promoter. It has been reported that putting tetracycline inducible system into human immunodeficiency virus (HIV) or Ad turned out to yield promising results [30, 31].

Recently, there have been tremendous achievements in the study of gene therapy in PD treatment. In 2001, gene therapy was first applied in PD patients [32] with the application of AAV expressing glutamic acid decarboxylase (GAD) gene GAD 65 and GAD 67 that synthesize the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), thereby helping DA system recovery to homeostasis. This breakthrough makes people believe in PD gene therapy. Besides the GAD gene, the major genes studied now include GDNF [33–35] and TH [36, 37]. Our group put human TH/GDNF gene into Ad or AAV vectors, produced Ad or AAV infected neuron-like cell lines and primary cultured neurons, and proved that they could express stably for long time, and had neuroprotective effect [38, 39]. After that, we first innovatively constructed tetracycline inducible rAAV vector expressing both TH and GDNF genes [Patent-Inducible AAV vector expressing two genes for PD therapy. Patent No.: 01136007.0]. The double transgene strategy was introduced to compensate the deficiency of single gene strategy based on the fact that administration of single gene TH can alleviate symptoms but can not stop the progressive loss of neurons. Firstly, we constructed AAV vector carrying reporter genes-red fluorescence protein (RFP) and green fluorescence protein (GFP) genes, and transfected PC12, BHK, HEK293 and MN9D cells. We found that they were highly expressed in

these cell lines, and tetracycline and its derivative doxycycline could effectively control transgene expression. That means we successfully constructed the tetracycline inducible AAV vector [40]. After transfection of AAV vector expressing TH and GDNF genes into PC12, BHK, HEK293 and MN9D cells, TH and GDNF expression in transfected cells was observed, and the production of DA increased effectively [41]. The effectiveness of such a strategy is to be determined in PD animal models and intensive investigations are ongoing.

### Stem Cells Replacement Therapy

Early in 1979, Perlow et al. transplanted human fetal mesencephalic tissue derived from naturally aborted fetuses, rich in primary DAergic neurons, into the brain of PD rat models, and found that the defect in behavior was significantly improved [42]. In the past twenty years, several meaningful researches on the cell therapy for PD have been carried out [43–46]. However, the method has many problems to be solved before being implemented. The first one is the insufficiency of donor cells. This limitation and others such as poor survival rate of grafted cells have hindered its application in the clinical medicine, and urged scientists to find a new cell source for replacement.

In 1999, stem cell research was appraised one of the most valuable investigations by *Science*. Since 1997, neural stem cells (NSCs) isolated from fetal brains have been cultivated in vitro and transplanted into animal models of PD to test their therapeutic efficacy [47–49]. Meanwhile, more and more researches have focused on the embryonic stem (ES) cell transplantation therapy for PD in recent years, especially after 1998, the human ES cells were cultured successfully in vitro [50, 51]. Although ES cells possess the unique characters absent in other stem cells, it should not to conclude that they are the most appropriate cell type for replacement therapy for PD due to the latent tumor-forming trait.

Now it is well known that stem cell transplantation treatment is not so simple and easy to make progress. The most exerted disputation is whether it is needed for pre-differentiation of stem cells in vitro before cerebral transplantation. Present opinion is that only those stem cells pre-induced in vitro have therapeutic effect after transplantation [52, 53]. From 2000, our research group has begun to study the properties and therapeutic effect of mesencephalic NSCs derived from naturally aborted human fetus [54]. The results showed that these NSCs differentiated into DAergic neurons when incubated with cocktail induction media containing forskolin, FGF8 and GDNF. After being transplanted into the striatum of parkinsonian rats, pre-induced NSCs improved the disordered behaviors,

while those non-induced NSCs had no therapeutic efficacy. The mechanisms for differential effects of induced and non-induced NSCs grafts had been analyzed further. Induced NSCs possessed the greater differentiation capacity, but poorer migration ability. They remained in the graft site-striatum and differentiated into more neurons and astrocytes, and some TH-positive cells. Non-induced NSCs, in contrast, migrated into other brain areas after transplantation and had lower differentiation capacity. These above differences might result in differential therapeutic efficacy. The results further demonstrated that pre-differentiation was the key step for the final successful treatment on PD. Therefore, many researches on the induction methods of DAergic differentiation emerged [55–57]. Our research group found that forskolin, as a coactivator, induced DAergic differentiation when it was treated with other neurotrophic or growth factors together [58]. With the thorough investigations, people find that the committed induction of stem cells is very difficult. It is necessary to understand the developmental process of neurons clearly, which requires the evolvement of developmental neurobiology.

Another issue in our experiment was that even though a higher percentage of DAergic neurons obtained in vitro, the survival ratio of these cells was very low after transplantation. This may be due to the lower number of total survived cells [54, 59]. Although survival of DAergic neurons differentiated from stem cells was still far from satisfaction, these studies resulting in improved behaviors of PD models indicate that such an approach might be feasible. These two results seem to be contradictory, because behavioral improvement depends on the number of surviving DAergic neurons to a great extent. This urges people to find out other facilitatory functions of grafted stem cells on behavioral changes. Nevertheless, the problem of lower survival ratio of DAergic neurons should be resolved first of all. In January of 2005, a paper published

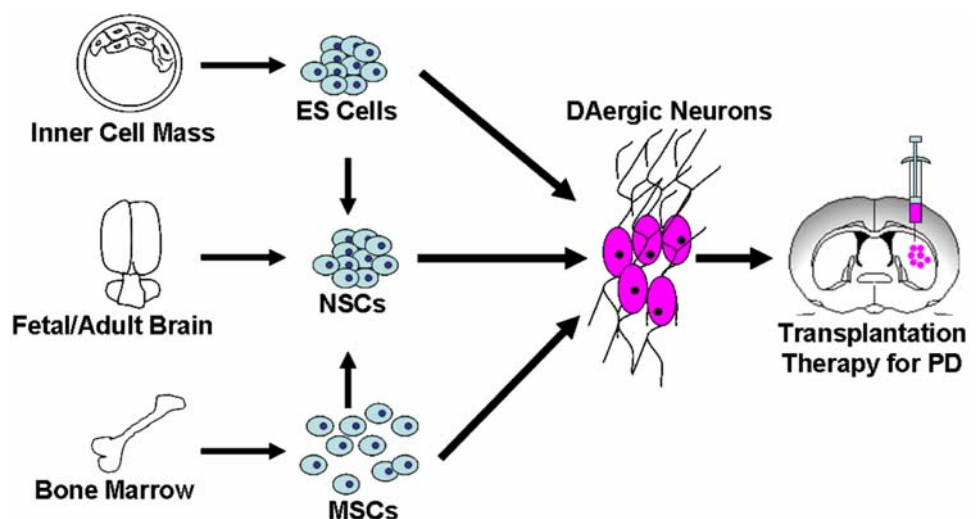
on *Journal of Clinical Investigation* firstly reported the function of ES cells derived from primate at PD therapy [60]. Researchers refined the transplantation method, grafting the progenitors of DAergic neurons, not the mature ones after induction. This change apparently increased the survival ability of grafted cells in brain, and the grafts achieved good therapeutic effect on PD primates, however, long-term cell survival, tumor formation potential and therapeutic effects have not been confirmed.

Stem cells replacement therapy for PD has achieved many inspiring developments, but we must be aware that there will be a very long time before the use of these cells in clinic. It is needed to explore the possibility of the integration of grafts into host cells to establish normal nervous connections. In cell transplantation treatment for PD, it is very crucial to select the appropriate grafted cell type (Fig. 2). Although a lot of problems need to be resolved, stem cell therapy is still the best hope for exploring new approaches for PD therapy.

## Summary

During more than one century after PD discovery, the mysterious veil of this disease is being uncovered gradually. As effective treatment methods in alleviating the symptoms of PD patients, TCM and acupuncture are being used widely. The underlying mechanism of their efficacy is being recognized gradually. We believe that a combination of ancient TCM and modern techniques will initiate new approaches for PD treatment. No matter what therapeutic methods, the effects need to be confirmed by strict and long-term animal experiments before clinical application. It is likely that, through the endeavors of researchers worldwide, the prospective therapeutic key that benefit for PD patients will be found in the near future.

**Fig. 2** Stem cells selection for PD transplantation therapy. In PD therapy, stem cells for transplantation can be derived from ES cells, fetal/adult NSCs, or bone marrow-derived mesenchymal stem cells (MSCs). Before transplantation, these cells need to be induced to generate more DAergic neurons, which may be crucial and beneficial for the final successful therapeutic effects for PD after transplantation



**Acknowledgments** This study was supported by the National Basic Research Program of China (2006CB500700), National Natural Science Foundation of China (30430280, 30472245, 30571704 and 30500255) and Natural Science Foundation of Beijing (kz200510025014).

## References

- Lang AE, Lozano AM (1998) Parkinson's disease. First of two parts. *N Engl J Med* 339:1044–1053
- Zhang ZX, Roman GC, Hong Z, Wu CB, Qu QM, Huang JB, Zhou B, Geng ZP, Wu JX, Wen HB, Zhao H, Zahner GE (2005) Parkinson's disease in China: prevalence in Beijing, Xian, and Shanghai. *Lancet* 365:595–597
- Siniscalchi A, Gallelli L, Mercuri NB, Ibbadu GF, De Sarro G (2006) Role of lifestyle factors on plasma homocysteine levels in Parkinson's disease patients treated with levodopa. *Nat Neurosci* 9:11–16
- Gao HM, Jiang J, Wilson B, Zhang W, Hong JS, Liu B (2002) Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. *J Neurochem* 81:1285–1297
- Chen BJ (2001) Triptolide, a novel immunosuppressive and anti-inflammatory agent purified from a Chinese herb *Tripterygium wilfordii* Hook F. *Leuk Lymphoma* 42:253–265
- Qiu D, Kao PN (2003) Immunosuppressive and anti-inflammatory mechanisms of triptolide, the principal active diterpenoid from the Chinese medicinal herb *Tripterygium wilfordii* Hook f. *Drugs R&D* 4:1–18
- Zhao G, Vaszar LT, Qiu D, Shi L, Kao PN (2000) Anti-inflammatory effects of triptolide in human bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 279:L958–L966
- Qiu D, Zhao G, Aoki Y, Shi L, Uyei A, Nazarian S, Ng JC, Kao PN (1999) Immunosuppressant PG490 (triptolide) inhibits T-cell interleukin-2 expression at the level of purine-box/nuclear factor of activated T-cells and NF-kappaB transcriptional activation. *J Biol Chem* 274:13443–13450
- Li FQ, Cheng XX, Liang XB, Wang XH, Xue B, He QH, Wang XM, Han JS (2003) Neurotrophic and neuroprotective effects of triptolide, an extract of Chinese herb *Tripterygium wilfordii* Hook F, on dopaminergic neurons. *Exp Neurol* 179:28–37
- Zhou HF, Liu XY, Niu DB, Li FQ, He QH, Wang XM (2005) Triptolide protects dopaminergic neurons from inflammation-mediated damage induced by lipopolysaccharide intranigral injection. *Neurobiol Dis* 18:441–449
- Zhou HF, Niu DB, Xue B, Li FQ, Liu XY, He QH, Wang XH, Wang XM (2003) Triptolide inhibits TNF-alpha, IL-1 beta and NO production in primary microglial cultures. *Neuroreport* 14:1091–1095
- Li FQ, Lu XZ, Liang XB, Zhou HF, Xue B, Liu XY, Niu DB, Han JS, Wang XM (2004) Triptolide, a Chinese herbal extract, protects dopaminergic neurons from inflammation-mediated damage through inhibition of microglial activation. *J Neuroimmunol* 148:24–31
- Xue B, Jiao J, Zhang L, Li KR, Gong YT, Xie JX, Wang XM (2007) Triptolide upregulates NGF synthesis in rat astrocyte cultures. *Neurochem Res* 32:1113–1119
- He QH, Zhou HF, Xue B, Niu DB, Wang XM (2003) Neuroprotective effects and mechanism of *Tripterygium Wilfordii* Hook F monomer triptolide on glutamate induced PC12 cell line damage. *Beijing Da Xue Xue Bao* 35:252–255
- Gu M, Zhou HF, Xue B, Niu DB, Wang XM (2004) The effect of Chinese Herb *Tripterygium Wilfordii* Hook F. Monomer T<sub>10</sub> on in vitro model of Alzheimer's disease. *Acta Physiol Sin* 56:73–78
- Lee KY, Park JS, Jee YK, Rosen GD (2002) Triptolide sensitizes lung cancer cells to TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis by inhibition of NF-kappaB activation. *Exp Mol Med* 34:462–468
- Rajendran PR, Thompson RE, Reich SG (2001) The use of alternative therapies by patients with Parkinson's disease. *Neurology* 57:790–794
- Shulman LM, Wen X, Weiner WJ, Bateman D, Minagar A, Duncan R, Konefal J (2002) Acupuncture therapy for the symptoms of Parkinson's disease. *Mov Disord* 17:799–802
- Cristian A, Katz M, Cutrone E, Walker RH (2005) Evaluation of acupuncture in the treatment of Parkinson's disease: a double-blind pilot study. *Mov Disord* 20:1185–1188
- Eng ML, Lyons KE, Greene MS, Pahwa R (2006) Open-label trial regarding the use of acupuncture and yin tui na in Parkinson's disease outpatients: a pilot study on efficacy, tolerability, and quality of life. *J Altern Complem Med* 12:395–399
- Tan LC, Lau PN, Jamora RD, Chan ES (2006) Use of complementary therapies in patients with Parkinson's disease in Singapore. *Mov Disord* 21:86–89
- Liang XB, Liu XY, Li FQ, Luo Y, Lu J, Zhang WM, Wang XM, Han JS (2002) Long-term high-frequency electro-acupuncture stimulation prevents neuronal degeneration and up-regulates BDNF mRNA in the substantia nigra and ventral tegmental area following medial forebrain bundle axotomy. *Brain Res Mol Brain Res* 108:51–59
- Liang XB, Luo Y, Liu XY, Lu J, Li FQ, Wang Q, Wang XM, Han JS (2003) Electro-acupuncture improves behavior and upregulates GDNF mRNA in MFB transected rats. *Neuroreport* 14:1177–1181
- Qian ZN, Gu ZL, Pan JX (1985) The effect of acupuncture analgesia on the monoamine transmitters in the rat striatum and spinal cord. *Zhen Ci Yan Jiu* 13:199–121
- Liu XY, Zhou HF, Pan YL, Liang XB, Niu DB, Xue B, Li FQ, He QH, Wang XH, Wang XM (2004) Electro-acupuncture stimulation protects DAergic neurons from inflammation-mediated damage in medial forebrain bundle-transected rats. *Exp Neurol* 189:189–196
- Kang JM, Park HJ, Choi YG, Choe IH, Park JH, Kim YS, Lim S (2007) Acupuncture inhibits microglial activation and inflammatory events in the MPTP-induced mouse model. *Brain Res* 1131:211–219
- Jolly D (1994) Viral vector systems for gene therapy. *Cancer Gene Ther* 1:51–64
- Kotin RM, Siniscalco M, Samulski RJ, Zhu XD, Hunter L, Laughlin CA, McLaughlin S, Muzyczka N, Rocchi M, Berns KI (1990) Site-specific integration by adeno-associated virus. *Proc Natl Acad Sci USA* 87:2211–2215
- Samulski RJ, Zhu X, Xiao X, Brook JD, Housman DE, Epstein N, Hunter LA (1991) Targeted integration of adeno-associated virus (AAV) into human chromosome 19. *EMBO J* 10:3941–3950
- Harding TC, Geddes BJ, Murphy D, Knight D, Uney JB (1998) Switching transgene expression in the brain using an adenoviral tetracycline-regulatable system. *Nat. Biotechnol.* 16:553–555
- Massie B, Couture F, Lamoureux L, Mosser DD, Guilbault C, Jolicoeur P, Belanger F, Langelier Y (1998) Inducible overexpression of a toxic protein by an adenovirus vector with a tetracycline-regulatable expression cassette. *J Virol* 72:2289–2296
- During MJ, Kaplitt MG, Stern MB, Eidelberg D (2001) Subthalamic GAD gene transfer in Parkinson disease patients who are candidates for deep brain stimulation. *Hum Gene Ther* 12:1589–1591
- Mandel RJ, Spratt SK, Snyder RO, Leff SE (1997) Midbrain injection of recombinant adeno-associated virus encoding rat glial cell line-derived neurotrophic factor protects nigral neurons

- in a progressive 6-hydroxydopamine-induced degeneration model of Parkinson's disease in rats. *Proc Natl Acad Sci USA* 94:14083–14088
34. Ma DD, Wang XM, Han JS (2000) NIH 3T3 cells or engineered NIH 3T3 cells stably expressing GDNF can protect primary dopaminergic neurons. *Neurol Res* 22:538–544
  35. Ma DD, Wang XM, Jing XJ, Wang J, Xiong L, Song H, Wan Y, Xu GH, Sun QL, Dong HW, Wang XH, Han JS (1997) GDNF cDNA engineered NIH 3T3 cells protect primary dopaminergic neurons. *Chinese Sci Bull* 42:1921–1924
  36. Ledley FD, Grenett HE, Bartos DP, van Tuinen P, Ledbetter DH, Woo SL (1987) Assignment of human tryptophan hydroxylase locus to chromosome 11: gene duplication and translocation in evolution of aromatic amino acid hydroxylases. *Somat Cell Mol Genet* 13:575–580
  37. Flotte TR, Ferkol TW (1997) Genetic therapy. Past, present, and future. *Pediatr Clin North Am* 44:153–178
  38. Xu GH, Ling Y, Wan Y, Wang XM, Han JS (1998) Construction of recombinant adenovirus expressing human tyrosine hydroxylase gene and study of its activities in vitro. *Chinese J Neuroanatomy* 14:313–317
  39. Xu GH, Ling Y, Wan Y, Wang XM, Han JS (1999) Development of the recombinant GDNF-adenovirus and observation of its protective effects on primary cultured dopaminergic neurons against neurotoxin MPP<sup>+</sup>. *Chinese J Biochem Mol Biol* 15:42–47
  40. Wang JJ, Niu DB, Zhang T, Wang K, Xue B, Wang XM (2005) A tetracycline-regulatable adeno-associated virus vector for double-gene transfer. *Neurosci Lett* 378:106–110
  41. Wang JJ, Zhang T, Niu DB, Wang K, Li KR, Xue B, Wang XM (2006) Doxycycline-regulated co-expression of GDNF and TH in PC12 cells. *Neurosci Lett* 401:142–145
  42. Perlow MJ, Freed WJ, Hoffer BJ, Seiger A, Olson L, Wyatt RJ (1979) Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. *Science* 204:643–647
  43. Lindvall O, Brundin P, Widner H, Rehnström S, Gustavii B, Frackowiak R, Leenders KL, Sawle G, Rothwell JC, Marsden CD, Björklund A (1990) Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science* 247:574–577
  44. Widner H, Tetud J, Rehnström S, Snow B, Brundin P, Gustavii B, Björklund A, Lindvall O, Langston JW (1992) Bilateral fetal mesencephalic grafting in two patients with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *N Engl J Med* 327:1556–1563
  45. Freed CR, Breeze RE, Rosenberg NL, Schneck SA, Kriek E, Qi JX, Lone T, Zhang YB, Snyder JA, Wells TH (1992) Survival of implanted fetal dopamine cells and neurologic improvement 12–46 months after transplantation for Parkinson's disease. *N Engl J Med* 327:1549–1555
  46. Freed CR, Greene PE, Breeze RE, Tsai WY, DuMouchel W, Kao R, Dillon S, Winfield H, Culver S, Trojanowski JQ, Eidelberg D, Fahn S (2001) Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 334:710–719
  47. Vescovi AL, Parati EA, Gritti A, Poulin P, Ferrario M, Wanke E, Frölichsthal-Schoeller P, Cova L, Arcellana-Panlilio M, Colombo A, Galli A (1999) Isolation and cloning of multipotential stem cells from the embryonic human CNS and establishment of transplantable human neural stem cell lines by epigenetic stimulation. *Exp Neurol* 156:71–88
  48. Svendsen CN, Clarke DJ, Rosser AE, Dunnett SB (1996) Survival and differentiation of rat and human epidermal growth factor-responsive precursor cells following grafting into the lesioned adult central nervous system. *Exp Neurol* 137:376–388
  49. Uchida N, Buck DW, He DP, Reitsma MJ, Masek M, Phan TV, Tsukamoto AS, Gage FH, Weissman IL (2000) Direct isolation of human central nervous system stem cells. *Proc Natl Acad Sci USA* 97:14720–14725
  50. Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 255:1707–1710
  51. Richards LJ, Kilpatrick TJ, Bartlett PF (1992) De novo generation of neuronal cells from the adult mouse brain. *Proc Natl Acad Sci USA* 89:8591–8595
  52. Studer L, Tabar V, McKay RD (1998) Transplantation of expanded mesencephalic precursors leads to recovery in parkinsonian rats. *Nat Neurosci* 1:290–295
  53. Carvey PM, Ling ZD, Sortwell CE, Pitzer MR, McGuire SO, Storch A, Collier TJ (2001) A clonal line of mesencephalic progenitor cells converted to dopamine neurons by hematopoietic cytokines: a source of cells for transplantation in Parkinson's disease. *Exp Neurol* 171:98–108
  54. Wang X, Lu YY, Zhang HQ, Wang K, He QH, Wang Y, Liu XY, Li LS, Wang XM (2004) Distinct efficacy of pre-differentiated versus intact fetal mesencephalon-derived human neural progenitor cells in alleviating rat model of Parkinson's disease. *Int J Dev Neurosci* 22:175–183
  55. Ling ZD, Potter ED, Lipton JW, Carvey PM (1998) Differentiation of mesencephalic progenitor cells into dopaminergic neurons by cytokines. *Exp Neurol* 149:411–423
  56. Studer L, Csete M, Lee SH, Kabbani N, Walikonis J, Wold B, McKay RD (2000) Enhanced proliferation, survival, and dopaminergic differentiation of CNS precursors in lowered oxygen. *J Neurosci* 20:7377–7383
  57. Kawasaki H, Mizuseki K, Nishikawa S, Kaneko S, Kuwana Y, Nakanishi S, Nishikawa SI, Sasai Y (2000) Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. *Neuron* 28:31–40
  58. Wang X, Li XX, Wang K, Zhou HF, Xue B, Li LS, Wang XM (2004) Forskolin cooperating with growth factor on generation of dopaminergic neurons from human fetal mesencephalic neural progenitor cells. *Neurosci Lett* 362:117–121
  59. Langston JW (2005) The promise of stem cells in Parkinson disease. *J Clin Invest* 115:23–25
  60. Takagi Y, Takahashi J, Saiki H, Morizane A, Hayashi T, Kishi Y, Fukuda H, Okamoto Y, Koyanagi M, Ideguchi M, Hayashi H, Imazato T, Kawasaki H, Suemori H, Omachi S, Iida H, Itoh N, Nakatsuji N, Sasai Y, Hashimoto N (2005) Dopaminergic neurons generated from monkey embryonic stem cells function in a Parkinson primate model. *J Clin Invest* 115:102–109