THERAPEUTIC POTENTIAL OF MENSTRUAL BLOOD-DERIVED ENDOMETRIUM STEM CELLS ON *IN VITRO* AND *IN VIVO* PARKINSON'S DISEASE MODELS

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by

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LIST OF ABBREVIATIONS

aCSF	Artificial cerebrospinal fluid
α-syn	Alpha-synuclein
AD-MSCs	Adipose-derived mesenchymal stem cells
AEP	Asparagine endopeptidase
AP	Anterior posterior
ARE	Antioxidant response element
ARTN	Artemin
Bad	B-cell lymphoma/leukemia-2 associated agonist of cell death
Bax	B-cell lymphoma/leukemia-2 associated X protein
BCA	Bicinchoninic acid disodium
Bcl-xl	B-cell lymphoma-extra large
BDNF	Brain-derived neurotrophic factor
BM-MSCs	Bone marrow-derived mesenchymal stem cells
BrdU	Bromodeoxyuridine
BSA	Bovine serum albumin
CC	Corpus callosum
cDNA	Complementary deoxyribonucleic acid
CDNF	Conserved dopamine neurotrophic factor
CM	Conditioned medium
c-Myc	Cellular myelocytomatosis
CNS	Central nervous system
COX-2	Cyclooxygenase
COMT	Catechol-O-methyltransferase
DA	Dopamine

DG	Dentate gyrus
DIL	1,1’-dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate
DMEM-HG	Dulbecco’s modified eagle medium-high glucose
DNPH	2,4-dinitrophenylhydrazine
DOPAL	3,4-dihydroxyphenylacetaldehyde
DV	Dorsoventral
EDSCs	Endometrium-derived mesenchymal stem cells
EGFP	Enhanced green fluorescent protein
ELISA	Enzyme-linked immunosorbent assay
Em	Emission
ESCs	Embryonic stem cells
Ex	Excitation
FBS	Fetal bovine serum
$Fe3^{+}$-TPTZ	$Fe3^{+}$-tripyridine triazine
FL-MSCs	Fetal liver-derived mesenchymal stem cell
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GCLC	Glutamate—cysteine ligase catalytic subunit
GCLM	Glutamate-cysteine ligase modifier subunit
GDNF	Glial cell-derived neurotrophic factor
Gpi	Globus pallidus interna
GSH	Glutathione
GSH-Px	Glutathione peroxidase
HGF	Hepatocyte growth factor
HMOX-1/2	Heme oxygenase-1/2
HRP	Horseradish Peroxidase

ICAM-1	Intercellular adhesion molecule-1
IFN-γ	Interferon-γ
IGF-1	Insulin-like growth factor-1
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-2/4/6/8/10/13	Interleukin 2/4/6/8/10/13
iNOS	Inducible nitric oxide synthase
IP	Intraperitoneal injection
iPSCs	Induced pluripotent stem cells
Keap-1	Kelch-like ECH-associated protein 1
KEGG	Kyoto Encyclopedia of Genes and Genomes
Klf-4	Kruppel-like factor 4
LBs	Lewy bodies
LDH	Lactic dehydrogenase
L-DOPA	L-3,4-dihydroxyphenyl-alanine
LV	Lateral ventricle
MAO-A/B	Monoamine oxidase type A/B
MANF	Mesencephalic astrocyte-derived neurotrophic factor
MenSCs	Menstrual blood-derived endometrial stem cells
MenSCs-CM	Conditioned medium derived from MenSCs
MenSCs-EDM	Exosomes deprived MenSCs-CM
MenSCs-Exo	MenSCs-derived exosomes
MFB	Medial forebrain bundle
ML	Mediolateral
MPP^+	Methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine

MSCs	Mesenchymal stem cells
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	Nerve growth factor
Nqo-1	NADPH quinine oxidoreductase 1
Nrf-2	NF-E2-related factor 2
NS	No significance
NSCs	Neural stem cells
NT-3/4/5	Neurotrophin-3/4/5
NTN	Neurturin
Oct3/4	Octamer-binding transcription factor 3/4
O.D.	Optical density
6-OHDA	6-hydroxydopamine
PBS	Phosphate buffer saline
PD	Parkinson's disease
PFA	Paraformaldehyde
PI3K	Phosphoinositide-3 kinase
PIP3	Phosphatidylinositol triphosphate
PRDX-1	Peroxiredoxin-1
PSPN	Persephin
qRT-PCR	Quantative reverse transcription-polymerase chain reaction
ROS	Reactive oxygen species
SD	Standard deviation
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulum

Sox-2	Sex determining region Y-box 2
SPSS	Statistical Product and Service Solutions
Str	Striatum
T-AOC	Total antioxidant compounds
TGF-β	Transforming growth factor-beta
TH	Tyrosine hydroxylase
Th1/17	T helper 1/17
TMB	3,3',5,5'-Tetramethylbenzidine
TNF-α	Tumor necrosis factor-α
T-reg	Regulatory T cells
TXN	Thioredoxin
UC-MSCs	Umbilical cord-derived mesenchymal stem cells
V3	The third ventricle
VEGF	Vascular endothelial growth factor
VTA	Ventral tegmental area

LIST OF SYMBOLS

α	alpha
β	beta
°C	degree Celsius
κ	kappa
g	g-force
g	gram
hrs	hours
Hz	hertz
γ	gammar
KDa	kilodalton
Kg	kilogram
Kv	kilovolt
L	liter
μg	microgram
μL	microliter
μm	micrometer
μM	micromolar
mg	miligram
mL	mililiter
mm	milimeter
mM	milimolar
Δψm	mitochondrial membrane potential
M	molar
ng	nanogram

nm	nanometer
%	percentage
pg	picogram
rpm	revolutions per minute
s	seconds
U	unit

POTENSI TERAPEUTIK SEL TUNJANG ENDOMETRIUM DARIPADA DARAH HAID TERHADAP MODEL PENYAKIT PARKINSON *IN VITRO* DAN *IN VIVO*

ABSTRAK

Sel stem endometrium yang diperolehi daripada darah haid manusia (MenSC) telah menunjuk potensi terapeutik terhadap pelbagai penyakit dari segi imunoregulasi dan regenerasi tisu. Walau bagaimanapun, kesannya terhadap penyakit Parkinson (PD) tidak diketahui. Kajian ini bertujuan untuk menilai fungsi perlindungan MenSC dan derivatifnya terhadap PD model *in vitro* dan *in vivo*. Sel neuroblastoma (SH-SY5Y) dan kepingan tengah otak didedahkan kepada 1-methyl-4-phenylpyridinium (MPP^+) untuk membina PD model *in vitro*. Metil-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) telah digunakan untuk membina model mencit PD melalui suntikan intraperitoneal. Selepas ko-kultur dalam model PD vitro dengan medium MenSCs (MenSCs-CM), daya maju kepingan otak tengah telah diuji dengan menggunakan Prestoblue dan asai lactate dehydrogenase. Ekpresi gen-gen yang berkaitan dengan inflamasi, anti-oksidan dan apoptosis telah dikesan oleh qRT-PCR. Dihydroethidium, Rhodamine123, dan Annexin V/PI digunakan untuk mengesan spesies oksigen reaktif (ROS), potensi membran mitokondria, dan apoptosis sel. Array protein telah digunakan untuk menganalisis faktor yang terdapat dalam MenSCs-CM. Selain itu, MenSC telah dipindahkan ke bahagian striatum (Str) otak mencit PD dengan menggunakan alat stereotaxic. Masa kelangsungan tahap MenSC, dopamin (DA), ekspresi gen keradangan dan gen anti-oksidan telah dinilai. Kyoto Encyclopedia of Genes and Genomes (KEGG) digunakan untuk menganalisis laluannya. Keputusan menunjukkan MenSCs-CM mengurangkan neurotoksisiti MPP^+ dengan mengawal ekspresi

cytokines pro dan anti-radang, memulihkan potensi membran mitokondria, mengurangkan tekanan oksidatif, dan menghalang apoptosis sel. Kesan perlindungan MenSCs-CM kepada PD juga disahkan terhadap kepingan otak. Keputusan menunjukkan bahawa MenSCs-CM telah meningkatkan daya saing hidup sel secara signifikan disebabkan oleh sifat anti-radang dan anti-oksidan. Arus protein menunjukkan terdapat sekurang-kurangnya 12 jenis faktor neurotropik dalam MenSCs-CM, yang boleh menyumbang kepada fungsi perlindungan MenSCs-CM dalam merawat PD. Eksperimen *in vivo* menunjukkan bahawa MenSCs berhidup di dalam bahagian Str otak mencit selama 28 hari. MenSCs yang dipindahkan telah meningkatkan tahap dopamin di bahagian Str dan meninggikan ekspresi gen anti-inflamasi ($p < 0.05$ *vs* kumpulan PD + PBS). Di samping itu, MenSC meningkatkan ekspresi gen anti oksidan *Nrf-2* dan gen aliran bawahnya ($p < 0.05$ *vs* kumpulan PD + PBS). Analisis KEGG menunjukkan faktor-faktor yang dihasilkan oleh MenSCs terlibat dalam mekanisme PI3K/Akt, menerangkan sebahagian fungsi anti-oksidasi MenSC. Kajian ini membuktikan pertama kali bahawa MenSCs-CM dan MenSCs mempunyai kesan perlindungan terhadap model *in vitro* dan *in vivo* penyakit PD, justeru mencadangkan bahawa MenSCs berprospektif digunakan sebagai terapi sel atau tanpa sel bagi penyakit PD.

THERAPEUTIC POTENTIAL OF MENSTRUAL BLOOD-DERIVED ENDOMETRIUM STEM CELLS ON *IN VITRO* AND *IN VIVO* PARKINSON'S DISEASE MODELS

ABSTRACT

Human menstrual blood-derived endometrial stem cells (MenSCs) have shown therapeutic potential on various diseases by immunoregulation and tissue regeneration. However, their effects on Parkinson's disease (PD) remain unknown. The aim of this study was to evaluate the protective function of MenSCs and their derivatives on *in vitro* and *in vivo* PD models. Neuroblastoma cell line (SH-SY5Y) and mouse midbrain slice were exposed to 1-methyl-4-phenylpyridinium (MPP^+) to establish *in vitro* level PD models. Then, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was used to construct PD mouse model by intraperitoneal injection. After co-culture *in vitro* PD models with conditioned medium of MenSCs (MenSCs-CM), the viability of cell and midbrain slice were detected by Prestoblue and lactate dehydrogenase assay. The expression of inflammatory genes, anti-oxidant and apoptosis-related genes were detected by qRT-PCR. Dihydroethidium, Rhodamine123, and Annexin V/PI staining were used to detect reactive oxygen species (ROS), mitochondrial membrane potential, and cell apoptosis, respectively. Protein array was conducted to analyze factors inside MenSCs-CM. Moreover, MenSCs were transplanted to striatum (Str) region of PD mouse brain using a stereotaxic instrument. Survival time of MenSCs, dopamine (DA) level, expression of inflammatory genes and anti-oxidant genes were evaluated. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was used to analyze pathways. Results showed MenSCs-CM attenuated MPP^+-induced neurotoxicity by regulating

pro- and anti-inflammatory cytokines expression, restoring mitochondrial membrane potential, reducing oxidative stress, and inhibiting cell apoptosis. The protective effect of MenSCs-CM on PD was also confirmed on slice level. Results showed MenSCs-CM significantly rescued midbrain slice viability reduction by anti-inflammatory and anti-oxidant properties ($p<0.05$ *vs* MPP^+ + DMEM group). Protein array demonstrated there were at least 12 types of neurotrophic factors in MenSCs-CM, which may contribute to the protective function of MenSCs-CM in treating PD. *In vivo* experiments showed MenSCs survived in Str region of mouse brain for at least 28 days. Transplanted MenSCs significantly increased dopamine (DA) level in Str region and up-regulated the expression of anti-inflammatory genes ($p<0.05$ *vs* PD + PBS group). In addition, MenSCs increased the expression of anti-oxidant gene *Nrf-2* and its down-stream genes ($p<0.05$ *vs* PD + PBS group). KEGG analysis showed factors secreted by MenSCs were involved in PI3K/Akt pathway, which may partly explain the anti-oxidant function of MenSCs. This study provided the first evidence that MenSCs-CM and MenSCs had protective effect on *in vitro* and *in vivo* PD models, suggesting MenSCs is a potential cell source used for cell-based or cell-free therapies in PD.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction of Parkinson's Disease (PD)

1.1.1 Incidence, features, and clinical manifestations of PD

Parkinson's disease (PD) is named after James Parkinson, who first reported this disease in 1817 (Dauer and Przedborski, 2003). It is the second most common age-related neurodegenerative disease, which is developed with mean age of 55 years old (Canet-Aviles *et al.*, 2014). Its prevalence is 1%-2% for seniors above 65 years old and increased to almost 4% in seniors above 85 years old (Canet-Aviles *et al.*, 2014).

The major features of PD are dopaminergic neurons progressive loss in the substantia nigra pars compacta (SNc) of the brain and intracellular accumulation of Lewy bodies (Dezawa *et al.*, 2001, Wolff *et al.*, 2011). Because of dopaminergic neurons loss, SNc pigmentation is normally observed in PD patients (Dauer and Przedborski, 2003). The cell body of dopaminergic neurons is located in the SNc and the axon of dopaminergic neurons mainly project to the putamen area of the striatum (Str) (Dauer and Przedborski, 2003). The loss of dopaminergic neurons causes the decreasing number of dopamine (DA) transporter, and thus lead to DA depletion in putamen of Str (Dauer and Przedborski, 2003). Dopaminergic neurons are also distributed in ventral tegmental area (VTA), which is adjacent to SNc and mainly projects to the caudate of Str (Dauer and Przedborski, 2003). In PD patients, dopaminergic neurons loss is mainly occurred in SNc, while the neurons in VTA region are much less affected (Dauer and Przedborski, 2003). Consistently, DA depletion is not prominent in the caudate (Dauer and Przedborski, 2003).

Clinically, any disease involving Str damage or DA depletion may lead to parkinsonism syndromes. This disease is characterized by postural instability, resting tremor, hypokinesia, altered gait, muscular rigidity, and bradykinesia (Dauer and Przedborski, 2003, Joers and Emborg, 2009, Lindvall and Kokaia, 2010). PD accounts for about 80% parkinsonism cases. 95%-99% cases of PD are sporadic and the etiology is unknown. Usually, the risk factors include old age, head injury and exposure to environmental toxins, like herbicides and pesticides (Billingsley, *et al*., 2018). A small proportion of PD cases are familial, linking to genetic mutations (Billingsley, *et al*., 2018).

1.1.2 Pathogenesis of PD

1.1.2(a) Cellular mechanisms

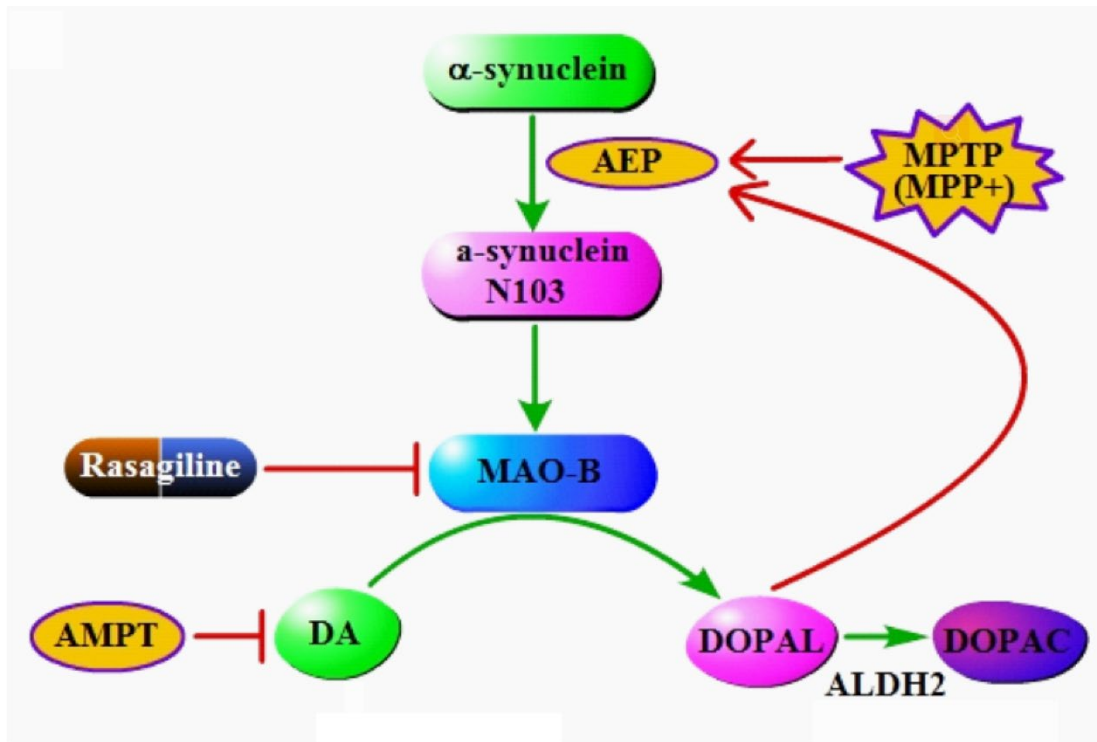
Evidences suggest two major hypotheses related to the pathogenesis of PD (Schapira *et al*., 2007, Billingsley *et al*., 2018). One is misfolded intracellular α-synuclein caused by several reasons. α-synuclein is encoded by gene *SNCA* and its mutation directly induces misfolded α-synuclein protein (Billingsley *et al*., 2018). Besides, mutations in the *PARKIN* or *UCH-L1* genes can damage the ubiquitin-proteasome system to detect and degrade the misfolded proteins (Setsuie and Wada, 2007, Kasten *et al*., 2018). Furthermore, reactive oxygen/hydrogen species may increase the risk of generation abnormal conformation and oxidatively modified proteins (Subramaniam and Chesselet, 2013, Deweerdt *et al*., 2016). Misfolded α-synuclein (α-syn) may directly cause cell damage or may aggregate inside the cells and then forms Lewy bodies (LBs) (Deweerdt, 2016). Controversy exists regarding the meaning of LBs formation (Shults *et al*., 2006, Wakabayashi *et al*., 2013). Some researchers reported LBs caused cell damage perhaps by disturbing substance

transportation in neurons and it may sequester proteins playing a crucial role in cell survival (Katsuse *et al*., 2003). LBs contributed to microtubule regression, mitochondria loss, and nuclear degradation in neurons of PD patients (Power *et al*., 2017). Recasens *et al.* isolated LBs-enriched fractions from postmortem PD patients and transplanted into SN or Str region of wild type mice and monkeys, results showed the LBs fractions induced neurodegeneration on both mice and monkeys (Recasens *et al*., 2014). However, other researchers proposed that there was no correlation between protein deposition and the apoptosis of dopaminergic neurons (Markesbery *et al*., 2009, *Parkkinen et al*., 2011). Clinical study showed the distribution and density of LBs had no relationship with the severity of dopaminergic neurons loss (Parkkinen *et al.*, 2011). Besides, LBs was also found in some elderly people without neurodegenerative disease (Markesbery *et al.*, 2009). The inclusion formation may be a defensive process meant to remove soluble abnormal proteins and to prevent the misfolded protein to be involved in the organized metabolism of cells, which can protect cells from harmful effects of abnormal proteins (Shults *et al.*, 2006, Wakabayashi *et al.*, 2013).

The interaction between α-Syn and monoamine oxidase type B (MAO-B) have been clarified recently (Zhang *et al*., 2017, Kang *et al*., 2018). MAO-B can catalyze the oxidative deamination of monoamine substances, such as DA, and produces aldehydes and H_2O_2 (Finberg and Rabey, 2016). α-Syn selectively combines with MAO-B and activates its enzymatic activity (Zhang *et al*., 2017). MAO-B catalyzes DA and produces metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL), which is highly toxic and can lead to oligomerization of α-Syn through covalent modifications of lysine residues (Plotegher *et al*., 2017). Besides, DOPAL can activates asparagine endopeptidase (AEP) and then AEP cleaves human a-Syn at N103. a-Syn N103 fragment has a stronger affinity with MAO-B than α-Syn and thus causes

abnormal DA metabolism by MAO - B (Kang *et al*., 2018). Furthermore, the oligomerization of α-Syn can cause synaptic vesicle damage and DA leak (Plotegher *et al*., 2017, Post *et al*., 2018). After the released DA is converted to DOPAL by MAO-B in dopaminergic neurons, DOPAL would induce a vicious cycle that further facilitating the oligomerization of α-Syn and causing synaptic impairment (Plotegher *et al*., 2017). The molecular interactions between MAO-B, α-Syn, AEP, and DOPAL were illustrated in Figure 1.1.

Mitochondrial dysfunction is considered as another key event to provoke neurodegeneration (Schapira *et al.*, 2007). It directly causes cell apoptosis or cause ATP depletion and oxidative stress, which may trigger cell apoptosis (Subramaniam and Chesselet, 2013). Mitochondrial respiration can consume almost all oxygen and oxidants are produced as byproduct in this process (Subramaniam and Chesselet, 2013). In PD patients or in drug-induced PD model, the complex I is inhibited and consequently the mitochondrial electron transport chain is blocked, which can largely increase the production of superoxide (Gubellini and Kachidian, 2015). The excess superoxide may react with lipids, proteins, DNA, and mitochondrial resulting in cell damage and cause further mitochondrial dysfunction (Gubellini and Kachidian, 2015). Because the DA metabolism process can produce large amount of superoxide radicals and hydrogen peroxide (H_2O_2), dopaminergic neuron itself is a fertile environment for ROS accumulation (Zeng *et al.*, 2018). The vesicular storage of DA may be disrupted due to mitochondria-related ATP depletion and then DA leaks into cytoplasm and involves in producing ROS (Zeng *et al.*, 2018). Therefore, DA plays a crucial role in increasing the susceptibility of SNc dopaminergic neurons to oxidative attack (Zeng *et al.*, 2018).



Adapted from Kang *et al*., 2018

Figure 1.1 Molecular interactions between MAO-B, α-Syn, AEP, and DOPAL. Schematic model for AEP-cleaved a-Syn N103 triggering MAO-B activation that feeds forward to further activate AEP by DA metabolite DOPAL.

1.1.2(b) Disordered innate and adaptive immunity

Although the pathogenesis of PD is not completely revealed, mounting evidences prove that the dysregulation of immune system including innate and adaptive immunity plays essential roles in the pathogenesis of PD (Chen *et al.*, 2018).

Microglia cells are a predominant type of resident immune cells in the brain (Gelders *et al.*, 2018, Song and Colonna, 2018). They account for about 20% of total glia cells and play an important role in immune surveillance and central nervous system homeostasis (Smith *et al.*, 2012). Microglia cells are found in two forms:

resting and activated forms (Smith *et al.*, 2012). The resting microglia cells have long branching processes and small cellular body, and are termed ramified microglia cells (Song and Colonna, 2018). During development, they are involved in synaptic pruning in the thalamus, cerebellum and hippocampus (Smith *et al.*, 2012, Song and Colonna, 2018). They are responsible for removing normally occurring apoptotic neurons by efferocytosis (Song and Colonna, 2018). Furthermore, they can release low level neurotropic factors to support neurons and glia cells survival (Song and Colonna, 2018). When the central nervous system (CNS) is infected by pathogens or in presence of tissue damage, ramified microglia cells undergo a series of changes in morphology, gene expression and function, and become activated (Huang and Halliday, 2012). The cell processes of activated microglial cells disappear and present ameboid morphology (Smith *et al.*, 2012). After activation, microglia can be divided into M1 state and M2 state (Huang and Halliday, 2012, Kannarkat *et al.*, 2013). Microglia in the M2 state can release anti-inflammatory cytokines, such as interleukin-4 (IL-4) and interleukin-13 (IL-13) , engulf damaged neuron fragments, and promote tissue repair and neurons regeneration (Gendelman and Mosley, 2015, Olson and Gendelman, 2016). But when microglia cells are over-activated, they turn to the M1 state and release a large number of neuroinflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) by activating MAPKs/NF-κB/ERK pathway and lead to neurons apoptosis (Gendelman and Mosley, 2015, Olson and Gendelman, 2016). Microglia cells have a close relationship with the progress of PD (Song and Colonna, 2018). Excessive ROS and activated M1 type microglia cells were found in postmortem of PD patients (Song and Colonna, 2018). In 6-hydroxydopamine (6-OHDA) induced PD rats, M2 phenotype microglia increased in the first three days after drug treatment, then gradually decreased and shifted to M1 phenotype at later

time points, when dopaminergic neurons death was manifest (Ambrosi *et al.*, 2017). Therefore, prevention microglia polarization to M1 phenotype may be a target for prevention and treatment of PD.

Accumulating evidences have shown the adaptive immune system especially the various T lymphocytes are closely related with the progression of PD (Gelders *et al.*, 2018). Total lymphocyte numbers are reduced in PD patients compared to healthy people (Bas *et al.*, 2001, Baba *et al.*, 2005). The absolute value and percentage of $CD4^{+}$ $CD25^{+}$ activated T cells are increased in PD patients. Meanwhile the number of naïve subset T cells ($CD4^{+}CD45RA^{+}$), helper T cells ($CD4^{+}$), and memory helper T cells ($CD4^{+}CD29^{+}$) are all decreased (Bas *et al.*, 2001). Suppressor/cytotoxic T cells ($CD8^{+}$) remained unchanged (Bas *et al.*, 2001). In its counterpart, the number of T-reg cells in peripheral blood were reported to be lower in PD patients and in 6-OHDA-induced PD animal model (Baba *et al.*, 2005, Ambrosi *et al.*, 2017). Besides, the number of $CD8^{+}$ cells were reported to be higher in PD patients (Baba *et al.*, 2005). T helper 1/17 (Th1/17) cells are subsets of $CD4^{+}$ T-lymphocytes and are responsible for regulating pro-inflammatory response playing a crucial part in many inflammatory diseases (Liu *et al.*, 2017, Kustrimovic *et al.*, 2018). In the peripheral blood of PD patients, naive T cells ($CD4^{+}$) are inclined to differentiate into Th1 lineage (Kustrimovic *et al.*, 2018). In peripheral blood, the number of Th17 cells of PD patients was much higher compared to healthy people (Chen *et al.*, 2017). In drug-induced acute PD mice, Th17 cells were found to traverse the lesioned brain-blood barrier (BBB) and reach substantial nigra of brain and cause inflammation by promoting glial activation (Liu *et al.*, 2017). However, peripheral immunity also can play a positive action to decrease inflammation. For example, in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced acute PD model, pro-inflammatory Th1 cells were reduced, while

anti-inflammatory T-reg cells were elevated in the lymphoid tissue (Huang *et al.*, 2014). Different stages of PD patients or animal models, different model induction drugs and administration methods may contribute to different results. In summary, the peripheral adaptive immunity is associated with the pathogenesis of PD. Targeting the adaptive immunoregulation especially T cell response maybe a therapeutic strategy in PD (Chen *et al.*, 2018).

1.1.3 Model systems in the study of PD

1.1.3(a) Cell level models

For cell level model, experimental conditions are easy to be controlled and operated and it is economic and efficient to construct a cell level model. Primary mesencephalic neurons, human neuroblastoma (SH-SY5Y) cell line and pheochromocytoma cell line (PC12) are widely used cells for constructing PD models (Falkenburger *et al.*, 2016). When the cells are exposed to neurotoxins such as, 6-OHDA, MPTP, paraquate or rotenone, they can mimic many characters observed in PD, such as reactive oxygen species (ROS) generation, inflammation, and neuron apoptosis (Beal *et al.*, 2001, Xie *et al.*, 2010).

Since one of the typical characters of PD is the loss of mesencephalic DA neurons, human primary mesencephalic neurons are an ideal cell type for model establishment (Falkenburger *et al.*, 2016). However, primary neurons of human source are quite difficult to be obtained, cultured, and handled and there exist ethical issues, which limits the application of this cell type (Beal *et al.*, 2001, Schüle *et al.*, 2011).

SH-SY5Y is a neuronal tumor cell line, which was initially established from a neuroblastoma patient (Schüle *et al.*, 2011). It can be cultured for a long period *in vitro*

without contamination, providing an unlimited supply of human origin cells (Lopes *et al.*, 2010, Xie *et al.*, 2010). SH-SY5Y cells have classic features of DA neurons, such as possessing tyrosine and dopamine-β-hydroxylases enzyme activity to synthesize DA and norepinephrine (Xie *et al.*, 2010, Xicoy *et al.*, 2017). They can express DA transporter (DAT), by which 1-methyl-4-phenylpyridinium (MPP^+) can enter into neurons (Xie *et al.*, 2010). Therefore, SH-SY5Y has been extensively applied to study the pathogenesis and mechanisms of MPP^+-induced cytotoxicity and to study the potential compounds, which can attenuate the cytotoxicity (Xicoy *et al.*, 2017). Additionally, SH-SY5Y cells can pre-differentiated into more pronounced DA neuron phenotype by various agents, such as retinoic acid (Beal *et al.*, 2001, Xie *et al.*, 2010). All these characters of SH-SY5Y cells make it a very useful tool to study the mechanisms of PD.

1.1.3(b) Organotypic slice level models

Cell level and animal level models have been well developed to be used for physiological and pathological studies, but both of them inevitably have defects (Kim *et al.*, 2013, Schommer *et al.*, 2017). Experimental conditions are easy to control *in vitro*, but cells cultured *in vitro* lose connection with other kinds of cells in tissue as well as some related biochemical characteristics (Schommer *et al.*, 2017). Although animal models can obtain the overall pathophysiological response, the manipulation of experimental conditions is very limited, and it is difficult to analyze a single factor due to the influence of multiple factors *in vivo* (Kim *et al.*, 2013). Organotypic slice culture is a platform established between cell culture and animal model and it could remedy disadvantages of single cell culture and animal studies (Mewes *et al.*, 2012, Schommer *et al.*, 2017). The cultured organotypic brain slices preserve structural and

synaptic organization of brain tissue and keep complex three dimensions neuronal network intact (Humpel *et al.*, 2015). Additionally, decades of slices can be obtained from one brain, which allows repetitive experiments and excludes the influences by variations in individual animal (Kim *et al.*, 2013). Thus, organotypic slice cultures provide a more efficient and reliable ex vivo platform to study the molecular and cellular mechanisms underlying the pathology of neurological disease and to evaluate potential therapeutic treatments for such diseases (Humpel *et al.*, 2015).

Different tissue-derived brain slices have been applied in neuroscience research (Tan *et al.*, 2018), such as hippocampus region (Daviaud *et al.*, 2013, Kim *et al.*, 2013, Schommer *et al.*, 2017), mesencephalon (Shen *et al.*, 2010, McCaughey-Chapman and Connor, 2017, de Araujo *et al.*, 2018), Str (Kress and Reynolds, 2005, Sin *et al.*, 2008, Shen *et al.*, 2010), and cortex (Kress and Reynolds, 2005). Among all cultures, organotypic cultures from the ventral mesencephalon (VM) area are widely used to model PD by adding various neurotoxins in the medium to induce dopaminergic neurons death (Stahl *et al.*, 2009). Keiko *et al* used the neurotoxin paraquat to establish a PD model at mesencephalon slice level and studied the protective function of several substances, such as inhibitors of nitric oxide synthase and DA D2/3 agonists (Shimizu *et al.*, 2003). Dopaminergic neurons in mesencephalon-striatum slices co-culture was demonstrated to be more resistant to cytotoxicity than dopaminergic neurons in single mesencephalon slice cultures (Katsuki *et al.*, 2001). Stahl *et al* devised a new technology to introduce neurotoxins 6-OHDA to SN region of unilateral mesencephalon slice by means of microelectrode, which could mimic *in vivo* stereotactic models (Stahl *et al.*, 2009). Furthermore, the contralateral side of slice serves as internal controls thus avoiding variation among different slices (Stahl *et al.*, 2009). Worthy to mention, Daviaud and his group have

developed a sagittal organotypic slice containing complete nigrostriatal pathway, which was composed of SN, medial forebrain bundle (MFB) and Str areas. Subsequently, progressive nigrostriatal degeneration was induced without using any neurotoxins, only by mechanical transection of MFB while preparing the slices (Daviaud *et al.*, 2014).

In conclusion, *ex vivo* organotypic slice cultures provide a promising platform for neuroscience research and can contribute to neurodevelopment (McCaughey-Chapman and Connor, 2017), electrophysiology (Wang *et al.*, 2015), pathology mechanisms of brain disorders, such as neurodegenerative disease (McCaughey-Chapman and Connor, 2017), demyelination (Tan *et al.*, 2018) and ischemia (Humpel *et al.*, 2015), and to study potential therapeutic compounds/cells for diseases (Qi *et al.*, 2019).

1.1.3(c) Toxin-induced animal models

Although PD animal models can not accurately recapitulate all aspects of human diseases, they have become quite useful tools to assess the effects of new pharmacological therapies, gene therapies and cell transplantation. In this chapter, several widely used neurotoxins to generate PD models are introduced. These neurotoxins include MPTP, 6-OHDA, paraquat (N, N-dimethyl-4-4-bipiridinium) and rotenone, which have received the most attention (Terzioglu and Galter, 2008).

MPTP was discovered in 1982 and it is the only neurotoxin that can mimic human parkinsonism (Gubellini and Kachidian, 2015). Thus, it became the most common used drug to induce PD model (Gubellini and Kachidian, 2015). It is highly lipophilic and can penetrate the BBB within minutes (Dauer and Przedborski, 2003,

Blesa *et al.*, 2012). For the ability to become toxic, MPTP must be converted to MPP^+ in glia cells and serotonergic neurons by MAO-B (Gubellini and Kachidian, 2015). After released into the extracellular compartment by unknown mechanisms, MPP^+ can enter dopaminergic neurons with DAT-dependent manner and accumulate in the mitochondria (Gubellini and Kachidian, 2015). It can block electron transport chain by inhibiting complex I and impair oxidative phosphorylation process, thereby causing energy crisis and ROS accumulation, finally inducing apoptosis of dopaminergic neurons (Schober *et al.*, 2004, Gubellini and Kachidian, 2015, Yun *et al.*, 2015, Schirinzi *et al.*, 2016). MPTP induced PD model is already successfully done in primates, rodents, sheep, dogs, frogs, and invertebrates such as leech and planarian (Shimohama *et al.*, 2003, Yun *et al.*, 2015). Primates are more sensitive to MPTP than rodents (Zeng *et al.*, 2018). However, the pathology is different from human PD because there is no typical LBs generation and there is no consistent neurons lost from other monoaminergic nuclei in MPTP-induced PD models (Gubellini and Kachidian, 2015). Although the MPTP induced models are not as perfect to mimic human PD pathologies, they are now the gold standard for assessment of novel strategies and agents for the treatment of PD (Schirinzi *et al.*, 2016, Zeng *et al.*, 2018).

The agent of 6-OHDA is the first drug applied to construct PD models since late 1960s (Emborg *et al.*, 2004, Uversky *et al.*, 2004, Gubellini and Kachidian, 2015). Different from MPTP, this neurotoxin cannot pass through the BBB and it should be administered into brain by stereotaxic apparatus whilst the preferred injection sites are substantia nigra, median forebrain bundle or Str (Emborg *et al.*, 2004, Schober *et al.*, 2004, Gubellini and Kachidian, 2015, Yun *et al.*, 2015, Schirinzi *et al.*, 2016). Only neonatal animals whose blood BBB is immature can be affected by systemic administration of 6-OHDA (Emborg *et al.*, 2004). It has been proven to be an effective

toxin in primates, rodents, dogs and cats (Shimohama *et al.*, 2003, Tieu *et al.*, 2011, Gubellini and Kachidian, 2015). After injected with 6-OHDA, animals manifest with an asymmetric circling behavior and the dose of drug decides the extent of the nigrostriatal lesion and the magnitude of asymmetric circling behavior. Because the lesion size and abnormal motor function can be quantitatively assayed, this kind of models is quite useful to assess the function of new drugs or new cell and gene therapy (Shimohama *et al.*, 2003, Tieu *et al.*, 2011). However, different from human PD, none of these animal models have LBs formation in the SNc region and the damage of 6-OHDA to dopaminergic neurons is nonspecific (Tieu *et al.*, 2011). Besides, like many other drug-based PD models, acute PD models induced by 6-OHDA lack age-related and progressive characters of PD patients (Tieu *et al.*, 2011).

Paraquat is an organic compound widely used in agriculture as herbicide and is also called N, N′-dimethyl-4,4′-bipyridinium dichloride (Cheng *et al.*, 2018). It is not only poisonous to green plants but also hypertoxic to human and animals and there is no antidotes against paraquat poisoning (Suntres *et al.*, 2018). Although similar structure to MPP^+, paraquat is not as easy to pass through BBB as MPP^+ and rely on neutral amino acid transporter (Dauer and Przedborski, 2003, Zeng *et al.*, 2018). The toxicity mechanisms between paraquat and MPP^+ are dissimilar (Tieu *et al.*, 2011). Firstly, paraquat is not substrate of DAT and how paraquat enter dopaminergic neurons is unknown (Tieu *et al.*, 2011). Besides, paraquat induces ROS mainly mediated by redox cycling with nitric oxide synthase and NADPH oxidase (Tieu *et al.*, 2011). Furthermore, different from MPTP, paraquat is not an inhibitor of complex I of the electron transport chain inside mitochondrial (Zeng *et al.*, 2018). It is closely related to the development of PD, because it can cause dopaminergic neuron loss and α-synuclein contained inclusions after administration to mice (Vaccari *et al.*, 2017).

Paraquat is useful to clarify the relationship between α-synuclein aggregation and the development of PD. However, paraquat cannot significantly induce DA depletion in striatal and motor dysfunction, which may limit the use of this drug (Tieu *et al.*, 2011).

Rotenone is extracted from the roots of tropical plants as a kind of natural pesticide (Gubellini and Kachidian, 2015). It can readily enter into all cells because of highly lipophilic character (Gubellini and Kachidian, 2015). After entering mitochondrial, rotenone blocks mitochondrial respiration chain by specifically inhibition the activity of complex I and the binding site with mitochondrial complex I is the same with MPP^+ (Dauer and Przedborski, 2003, Gubellini and Kachidian, 2015, Yun *et al.*, 2015, Schirinzi *et al.*, 2016). When rats were systemically and chronically exposed to rotenone by intravenous injection, it resulted in LBs formation, nigrostriatal pathway damage, and clinical manifest of PD, such as postural instability and tremor (Zeng *et al.*, 2018). Therefore, this neurotoxin may enable researchers to clarify the relationship between apoptosis of dopaminergic neurons and LBs formation (Gubellini and Kachidian, 2015). However, the nigrostriatal pathway damages induced by rotenone are not consistent and this drug can cause high mortality rate of animals (Betarbet *et al.*, 2000, Gubellini and Kachidian, 2015).

1.2 Current Treatment for PD

Pharmacological and surgery are two common treatments to relieve symptoms of PD (Bezard *et al.*, 2001, Yasuhara *et al.*, 2015). Because the impaired motor function of PD is related to the DA depletion in the Str region, symptomatic treatment helps to restore neurotransmitter DA level by administration medicines, such as L-3,4-dihydroxyphenyl-alanine (L-DOPA) and DA agonists (Braak *et al.*, 2003). Although PD symptoms are relieved by L-DOPA increasing DA administration, it brings drug-

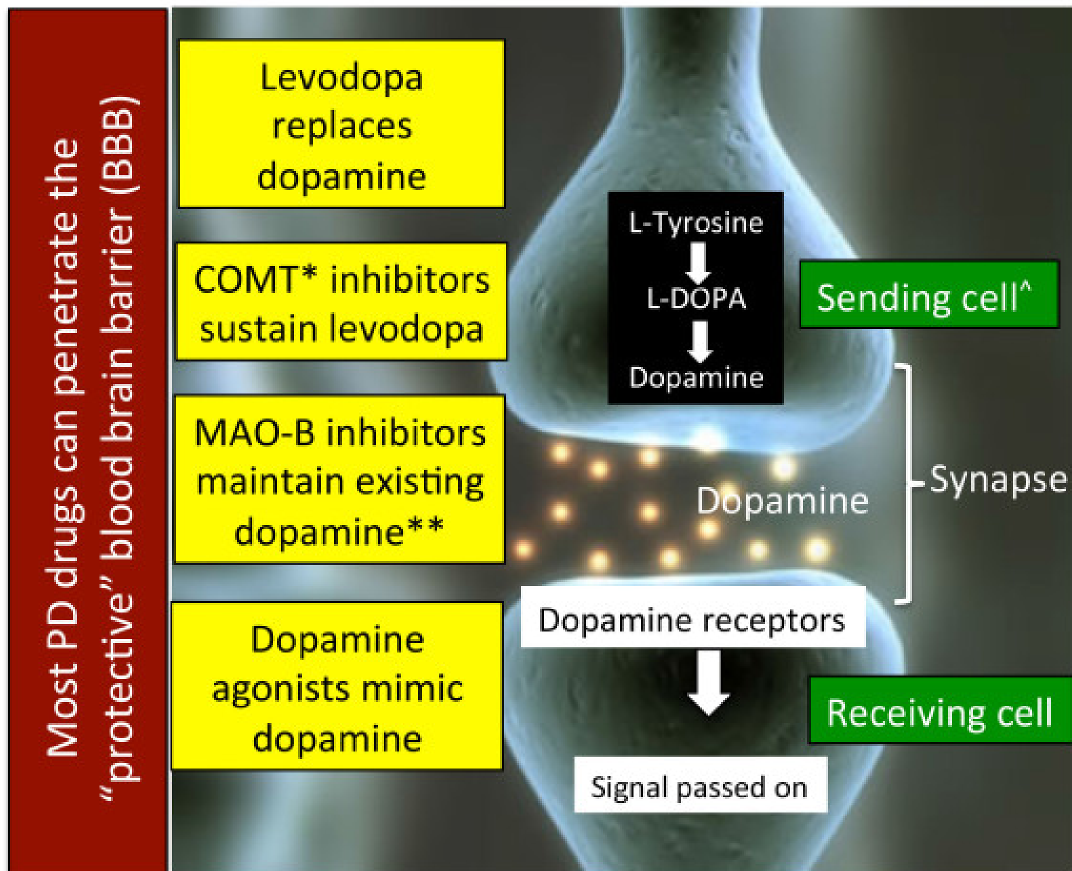
induced side effects, such as motor fluctuations and dyskinesia and it cannot retard or reverse the progressive degeneration of dopaminergic neurons (Bezard *et al.*, 2001, Yasuhara *et al.*, 2015). Furthermore, the efficacy of treatment become lower and lower as the disease progresses and new clinical manifests occur, among which some symptoms even fail to respond to medicines (Braak *et al.*, 2003, Langston *et al.*, 2006). DA agonists, such as rotigotine, pramipexole, and ropinirole, which can activate DA receptors and relieve motor dysfunction (Ceravolo *et al.*, 2016). The adverse effects of DA agonist include both peripheral and central events such as orthostatic hypotension, gastrointestinal disease, psychosis, and hallucinations, which limit their long-term using (Bonuccelli and Ceravolo, 2008).

In addition to increasing DA level, two types of medications have been administrated to utilize existing DA more effectively. One type is catechol-O-methyltransferase (COMT) inhibitors (Finberg and Rabey, 2016). COMT inhibitors, such as tolcapone and entacapone, can block the breakdown of levodopa and thus more levodopa can reach brain and convert to DA by dopa decarboxylase (Waters, 2000). Another type is MAO-B inhibitors (Finberg and Rabey, 2016). Monoamine oxidase (MAO) is a kind of enzyme, which catalyzes the oxidative deamination of monoamine substances, such as DA, and produces aldehydes and H_2O_2 (Finberg and Rabey, 2016). MAO has two isoforms MAO-A (monoamine oxidase type A) and MAO-B, which can be differentiated by their inhibitor specificities and substrate (Riederer and Laux, 2011). Clorgyline and moclobemide are inhibitors of MAO-A. The inhibition of MAO-A increases noradrenaline level in noradrenergic neurons and serotonin level in serotonergic neurons, which has antidepressant function (Finberg, 2014, Finberg and Rabey, 2016). MAO-B inhibitors, such as selegiline, rasagiline, and safinamide can improve dyskinesia, reduce off-time and delay the need for levodopa, which are used

as anti-Parkinson drug (Cereda *et al*., 2017, Dézsi and Vécsei, 2017). Some compounds combining symptomatic DA treatments with COMT or MAO-B inhibitors have been investigated aiming to enhance the long-term effect for PD patients (Nomoto *et al.*, 2003, Schapira and Olanow, 2004). However, none has fulfilled the requirements of preventing PD progression (Nomoto *et al.*, 2003, Schapira and Olanow, 2004). The pharmocological treatment of PD was summarized in Figure 1.2.

Surgery includes deep brain stimulation of subthalamic nucleus, mutilation of globus pallidum interna or ventral intermediate thalamic, which are performed when medications do not work and have been proved to improve motor function and quality of life (Videnovic and Metman, 2008, Rowland *et al*., 2015). However, complications would occur after surgery, such as worsening verbal fluency, cerebral hemorrhage, sensory disturbance, visual field defect, and dysphagia, which may bring another big burden to patients (Højlund *et al*., 2016, Weinkle *et al*., 2018).

Medical Management of Parkinson's Disease#



#Not discussed here are anticholinergics, selective serotonin inverse agonists (SSIA), NMDA antagonists, and acetylcholinesterase inhibitors. *COMT =Catechol-O -methyl transferase inhibitors act either extracerebrally or cross the BBB. **MAO-B = monoamine oxidase B. ^Dopaminergic neurons.

Cited from Church F. 2017

Figure 1.2 Pharmocological treatment of PD. This figure is cited from (Church F. 2017, March 20). In dopaminergic neurons, L-tyrosine is converted to L-dopa under catalization of tyrosine hydroxylase. Then L-dopa is converted to dopamine by dopa decarboxylase. After dopamine is reseased from presynaptic membrane, it enters receiving cells by dopamine receptors or is metabolized by MAO-B and COMT. So, four kinds of drugs can be used to increase dopamine level: L-dopa, MAO-B inhibitors, COMT inhibitors, and dopamine agonists.

1.3 Cell-based Therapies for PD

1.3.1 Stem cell classification

Stem cells are undifferentiated or unspecialized cells, which can differentiate into various specialized cell types and self-renew (Lazic and Barker, 2003). According to their differentiation ability, they can be classified into totipotent, pluripotent and multipotent stem cells and different cell types have specific sources (Lazic and Barker, 2003, Wenker *et al.*, 2015). Totipotent stem cells refer to fertilized zygote and all cells within 16-cell stage, which has the ability to form all cell types. Pluripotent stem cells include two types: embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) (Wenker *et al.*, 2015). ESCs can be collected from inner cell mass at blastocyst stage of embryonic development. They can differentiate into nearly all cell lineages except for trophoblast cells which can form fetal membrane and placenta later (Lazic and Barker, 2003, Parish and Arenas, 2007, Fu *et al.*, 2015). iPSCs, also called ES-like cell, are derived from somatic cells such as fibroblasts by transfecting some self-renewal and pluripotent factors to make somatic cells de-differentiation (Fu *et al.*, 2015, Goodarzi *et al.*, 2015, Wenker *et al.*, 2015). The successful generation of iPSCs was first reported by Kazutoshi Takahashi and Shinya Yamanaka in 2006 by lentiviral expression of four transcription factors in mouse embryonic fibroblasts including cellular myelocytomatosis (c-Myc), kruppel-like factor 4 (Klf 4), octamer-binding transcription factor 3/4 (Oct3/4), and sex determining region Y-box 2 (Sox2) (Takahashi and Yamanaka, 2006). Multipotent stem cells are derived from fetal or adult tissues and they can differentiate into tissue-specific progeny. Neural stem cells (NSCs) and mesenchymal stem cells (MSCs) both belong to this type of cells (Lazic and Barker, 2003, Parish and Arenas, 2007, Fu *et al.*, 2015). Human NSCs were first reported in 1965 and they can be isolated from fetal brain, hippocampus,

subventricular zone of adult brain, and ESCs (Han *et al.*, 2015, Zhu *et al.*, 2016). They have potential to differentiate into different kinds of neurons, astrocytes and oligodendrocytes (Parish and Arenas, 2007, Han *et al.*, 2015, Zhu *et al.*, 2016). Bone marrow-derived mesenchymal stem cells (BM-MSCs) were the first kind of MSCs to be reported and so far, MSCs have been shown also exist in umbilical cord, umbilical cord blood, Wharton's jelly, adipose, placenta, endometrium, menstrual blood, dental tissues, amniotic fluid and etc. (Kögler *et al.*, 2004, Patel *et al.*, 2008, Ullah *et al.*, 2015). They are negative for HLA-DR, CD11b, CD14, CD19, CD34, and CD 45 and meanwhile positive for CD73, CD 90 and CD105 (Horwitz *et al.*, 2005, Salem and Thiemermann, 2010, Ullah *et al.*, 2015). They have multi-differentiation ability *in vitro*, such as chondrogenesis, adipogenesis, osteogenesis, neurogenesis, and cardiogenesis (Patel *et al.*, 2008, Salem and Thiemermann, 2010, Zemelko *et al.*, 2013, Ullah *et al.*, 2015).

1.3.2 Menstrual blood-derived endometrium stem cells (MenSCs)

Menstrual blood-derived endometrium stem cells (MenSCs) were firstly reported in 2007, which were isolated from menstrual blood (Meng *et al*., 2007, Cui *et al*., 2007). Until now, there is still no uniform name for this kind of stem cells. Chen *et al*. summarized all the existing 12 nomenclatures in their review paper (Chen *et al*., 2019). In this thesis, MenSCs was used throughout.

The doubling time of MenSCs is about 19.5 hrs, which is less than umbilical cord-derived MSCs (UC-MSCs, 36 hrs-48 hrs) (Meng *et al*., 2007) and bone marrow-derived MSCs (BM-MSCs, 40 hrs-45 hrs) (Wu *et al*., 2014). They were reported to expand up to 68 doublings with normal karyotype and sustained surface markers (Meng *et al*., 2007). While other researchers showed they would stop proliferation

before 30 doublings (Cui *et al*., 2007). The proliferation speed of MenSCs is negatively correlated with the age of the donors and the cell passage (Liu *et al*., 2018). Because Meng and Cui did not mention the donors age of the menstrual blood samples, the different result of the ability of doubling times maybe was due to different age of donors. Besides, the different components of medium they used maybe another important reason. MenSCs were able to differentiate into three germ layers: ectoderm (neurons), mesoderm (adipocyte, osteocyte, myocyte, cardiomyocyte endothelium) and endoderm (hepatocyte, pancreatic cells, respiratory epithelium) *in vitro* (Meng *et al*., 2007). MenSCs share similar morphology and some surface markers with other sources MSCs, such as positive for CD29, CD73, CD90, CD105 and negative for CD14, CD19, CD34, and CD45. The unique characters of MenSCs are the expression of ESCs marker OCT-4, positive for human telomerase reverse transcriptase, lack of STRO-1 expression, and different secreted factors (Meng *et al*., 2007).

Since the discovery of MenSCs, they have been used for therapy of various diseases in lab, such as stroke (Borlongan *et al*., 2010), type I diabetes (Wu *et al*., 2014), hepatic failure (Chen *et al*., 2017), premature ovarian failure (Wang *et al*., 2017), liver fibrosis (Chen *et al*., 2017), lung injury (Xiang *et al*., 2017), and cardiac diseases (Liu *et al*., 2019). One clinical study showed that MenSCs were intravenously and intrathecally administrated to multiple sclerosis patients and no adverse effects or immune rejection were observed in one-year follow-up, which indicated the feasibility of clinical use (Zhong *et al*., 2009). After co-culture MenSCs with rat primary neurons exposed to oxygen and glucose deprivation, MenSCs significantly promoted neurons survival by releasing vascular endothelial growth factor, brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) (Borlongan *et al*., 2010). MenSCs improved liver function after transplanted to liver fibrosis mouse model by inhibiting

activated hepatic stellate cells. Moreover, *in vitro* co-culture experiment showed MenSCs inhibited the proliferation of activated hepatic stellate cells by secretion of monocyte chemoattractant protein-1, IL-6, interleukin-8 (IL-8), hepatocyte growth factor (HGF) and so on (Chen *et al*., 2017). The transplantation of MenSCs decreased the expression of pro-inflammatory cytokines IL-1β and IL-6 and increased the expression of anti-inflammatory cytokines interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) in lipopolysaccharide-induced acute lung injury mouse model (Xiang *et al*., 2017). MenSCs-derived exosomes (MenSCs-Exo) was shown to inhibit apoptosis of hepatocyte in fulminant hepatic failure mouse model and decrease the expression of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α after co-culture with lipopolysaccharide-induced AML12 hepatocyte cell line (Chen *et al*., 2017). Neuroinflammation and dopaminergic neurons loss played an important role in the pathogenesis of PD (Zeng *et al*., 2018). Although MenSCs and MenSCs-Exo showed protective function in lung or liver disease by anti-apoptosis and immunomodulation, it is unknown whether they have therapeutic effect on PD by promoting neurons survival and regulating inflammatory cytokines expression. This question will be answered in this study.

1.3.3 Cell types used in the treatment of PD

Since the clinical manifests of PD are mainly caused by malfunction of dopaminergic neurons, theoretically, stem cells which can differentiate into dopaminergic neurons can be used for transplantation to provide exogenous dopaminergic neurons to restore the DA abnormalities (Politis and Lindvall, 2012, Yue and Jing, 2015). There are four kinds of stem cell types commonly used for cell therapy to treat PD: ESCs, iPSCs, NSCs, and MSCs (Lindvall *et al.*, 2004, Politis and Lindvall,

2012, Fu *et al.*, 2015, Goodarzi *et al.*, 2015).

Compared to the limited differentiation potential of NSCs and MSCs, ESCs and iPSCs can differentiate into unlimited DA neurons (Fu *et al.*, 2015, Goodarzi *et al.*, 2015). However, there are many disadvantages of using ESCs and iPSCs. ESCs are prone to generate into tumors, major ethic issues are involved, and it is difficult to obtain quality oocytes (Fu *et al.*, 2015, Goodarzi *et al.*, 2015). As for iPSCs, the genomic stability is still questionable and some factors used for reprogramming or viral vectors integrated into genome can increase the risk of tumorigenesis (Fu *et al.*, 2015, Goodarzi *et al.*, 2015, Yasuhara *et al.*, 2015, Zhu *et al.*, 2016). NSCs can be derived from fetal brain and adult brain mainly in subventricular zone and hippocampus (Zhu *et al.*, 2016). Although the tumorigenesis risk of NSCs is lower than ESCs and iPSCs, the source is quite limited and there exists ethical issues. Besides, it cannot realize autologous transplantation (Goodarzi *et al.*, 2015). Compared to ESCs and iPSCs, which allows almost all lineages differentiation, the differentiation and re-newal ability of the MSCs is much lower, but they are genetically more stable, readily available from extensive sources, more easily isolated, cultured and expanded and no major ethical issues (Levy *et al.*, 2015, Ullah *et al.*, 2015). Besides, MSCs have low immunogenic properties due to the lack of MHC-II, so that they can undergo allograft with low immunological rejection risks (Fu *et al.*, 2015, Goodarzi *et al.*, 2015, Zhu *et al.*, 2016).

1.3.4 The therapeutic potential of MSCs on PD animal models

Ove the last decade, bone marrow-derived MSCs (BM-MSCs) have become the most widely used cell to treat PD animals induced by neurotoxins, such as MPTP and 6-OHDA (Li *et al.*, 2001, Lu *et al.*, 2005, Hellmann *et al.*, 2006, Ye *et al.*, 2007,

Levy *et al.*, 2008, Campeau *et al.*, 2014). BM-MSCs were shown to survive in Str region of PD mice brain, differentiate to dopaminergic neurons expressing tyrosine hydroxylase (TH) and improve animal motor function (Li *et al.*, 2001). The similar results were observed in umbilical cord-derived MSCs (UC-MSCs) transplanted PD mice induced by 6-OHDA (Kang *et al.*, 2013). Hellmann *et al.* constructed a PD model by unilateral 6-OHDA stereotaxic injection and the same number of BM-MSCs were transplanted into both sides of brain. Results showed that there were more cells survived in 6-OHDA lesioned side of hemisphere than contralateral unlesioned side, which indicated that different microenvironment would affect the survival of engrafted MSCs (Hellmann *et al.*, 2006). Besides, after transplanted to healthy side of cerebral hemisphere, BM-MSCs can migrate to the lesioned side (Hellmann *et al.*, 2006). TH or neuturin overexpressed BM-MSCs were also tried to achieve better effect than naïve cells. Results showed they could significantly increase DA level in the damaged Str region and improve motor function of PD rat model (Lu *et al.*, 2005, Ye *et al.*, 2007). Additionally, Ye *et al.* showed that gene modified BM-MSCs achieved better therapeutic effect than naïve cells (Ye *et al.*, 2007). Although MSCs can be pre-differentiated into neuron-like cells *in vitro* detected by surface markers of neurons, it's largely unknown whether the differentiated MSCs can interact with local neurons and establish neuron network after graft and whether neuron differentiation is necessary to achieve better therapeutic effect (Jeong *et al.*, 2004, Ning *et al.*, 2006, Levy *et al.*, 2008, Barzilay *et al.*, 2009, Wolff *et al.*, 2011, Guan *et al.*, 2014). *In vitro* neuron-induced and naïve adipose-derived MSCs (AD-MSCs) were transplanted into SN region of PD rat model separately (McCoy *et al.*, 2008). Results demonstrated these two kinds of grafts can protect dopaminergic neurons survival, which indicates that *in vitro* neural induction before transplantation are not necessary for AD-MSCs to

exert neuroprotective effects (McCoy *et al.*, 2008). Furthermore, AD-MSCs derived neurotrophic factors and cytokines known to promote dopaminergic neurons survival at the lesion site, which may have contributed to the therapeutic effect of AD-MSCs transplantation (McCoy *et al.*, 2008). On the contrary, Levy *et al.* reported that grafting BM-MSCs derived neuron-like cells can ameliorate rotation behavior while naïve BM-MSCs or PBS injected rats did not show a statistically significant reduction in rotations, which proved neural induction of the BM-MSC prior to transplantation had a significant advantage over naive BM-MSCs (Levy *et al.*, 2008). Whether pre-transplanting differentiation is necessary need further study and more evidence. Endometrium-derived stem cells (EDSCs), which can be obtained from endometrium, were used to generate dopaminergic cells for transplantation. Wolff *et al* showed that EDSCs can be induced into dopaminergic neurons *in vitro* and can also differentiate into neuron-like cells *in vivo* (Wolff *et al.*, 2011, Wolff *et al.*, 2015). EDSCs could also migrate to the site of lesion, significantly increase DA and DA metabolite concentrations in the Str, and increase the numbers of TH positive cells in immunocompetent PD mouse or primate model (Wolff *et al.*, 2011, Wolff *et al.*, 2015). Most studies were focused on detecting DA or TH level and rotation function after naïve or induced cell transplantation, while Campeau *et al.* showed that the voiding function of PD rat was also improved after BM-MSCs graft. Non-microencapsulated group improved urodynamic pressure more markedly than the microencapsulated group and there were more TH positive neurons in non-microencapsulated group, which suggested functional improvement required the juxtracrine effect (Campeau *et al.*, 2014). Kumar *et al.* investigated the therapeutic effect of fetal liver MSCs (FL-MSCs) derived dopaminergic neuronal cells on PD. The results showed they can integrate into the Str of PD mice and improve PD symptoms, suggesting FL-MSCs is

also a promising tool for cell therapy (Kumar *et al.*, 2016). Findings of research about MSCs for treatment of PD is summarized in Table 1.1.

Naïve MSCs, modified MSCs and their derivatives as potential treatments for PD have received satisfactory results by cellular replacement or paracrine secretion (Sadan *et al.*, 2009, Lindvall and Kokaia, 2010). However, some major obstacles need to be overcome before these findings can be translated into novel therapies for patients.

Firstly, further studies need to be done about mechanisms to control stem cell migration, survival, proliferation, and differentiation in the pathological environment after grafting (Lindvall and Kokaia, 2010). Stereotaxic intracerebral transplantation is the only method to directly transplant MSCs into different regions of brain. The surgery is time-consuming, expensive, and cause some untoward effects. For example, there are hundreds of PD patients worldwide who had undergone human fetal mesencephalic stem cells striatal transplantation, and more than half show graft-induced dyskinesias with only minimal cells that can survive and differentiate in the injected region (Lindvall and Björklund, 2004, Politis and Lindvall, 2012). Many groups have reported different tissue derived MSCs can survive in the brain, but the duration of survival and methods to increase their survival time needs further study (Li *et al.*, 2001, Wu *et al.*, 2007, Ye *et al.*, 2007, Levy *et al.*, 2008, McCoy *et al.*, 2008, Salem *et al.*, 2014). The survival time is closely related to the transplantation times and dose for patients. For cell replacement therapy, different cell types will be needed for different diseases. For example, PD requires dopaminergic neurons, amyotrophic lateral sclerosis (ALS) needs motor neurons, while Alzheimer's disease (AD) demands several cell types to be effective (Lindvall and Kokaia, 2010). It's difficult to control which direction the cell will differentiate to after transplantation. If the grafted cells