neural stem cells (NSCs), neurons and DA neurons. Mouse embryonic stem cells-derived NSCs, or fully differentiated neurons and dopamine neurons have been shown to have neuroprotective effects for the treatment of PD [62–64].

Human embryonic stem cells (hESCs) were first isolated by culturing inner cell mass cells with feeder cells of mouse embryonic fibroblasts (MEFs) [65]. In the past two decades, strategies have been developed to direct the hESC differentiation into the neural stem cells and neurons, in particular dopamine neurons for PD. Studies have shown the differentiation of hESCs into midbrain dopamine (DA) neurons by the application of specific patterning molecules that regulate midbrain development in vivo [66, 67]. Transplantation of hESCs-derived neural precursor cells to the PD rats showed that grafted cells differentiated DA neurons in vivo and development of protocol for producing more DA neurons in vitro is required [68]. Moreover, Yan et al. developed protocols for generating specifically midbrain-like DA neurons from hESC-derived neuroepithelial cells by applying growth factors SHH and FGF8 in a specific sequence [69]. Their study suggested that early exposure to growth factor FGF8 and SHH instructs early precursors to adopt a region identity leading to differentiation of midbrain neuroepithelial cells. These hESC-derived dopamine neurons were able to improve the locomotive deficits of PD rat models, provided that grafted hESC-derived dopamine neurons functioned in vivo [70]. In order to increase the efficiency of DA production from pluripotent stem cells, Chamber et al. developed a protocol by inhibiting SMAD signalling to enhance proliferation and survival of midbrain DA neurons from hESCs [71]. They reported that addition of Noggin and SB431542 for inhibiting SMAD signalling induces complete neural conversion of >80 % of hESCs under adherent culture conditions. Fasano et al. have reported that neurons in developmental default towards anterior regionalization, but may be shifted towards a midbrain-like identity by FGF8 or Wnt1 treatment [72]. In order to further improve complete conversion of hESCs to the dopamine neurons and decrease the teratoma potential in vivo, the same group developed a floor-plate-based method for generating hESCs-derived DA neurons in a differentiation medium containing activators of sonic hedgehog (SHH) and canonical WNT signalling in vitro. They found that these DA neurons efficiently grew for more than 18 weeks and restored the amphetamine-induced rotation dysfunctions in vivo after being transplanted into 6-OHDA-lesioned rats and MPTP-lesioned rhesus monkeys [73]. Muramatsu et al. implanted NSCs derived from cynomolgus ES cells unilaterally in the putamen of neurotoxin- lesioned cynomolgus monkeys. They found that transplantation of NSCs derived from cynomolgus monkey ES cells can restore DA function in a primate model of PD [74]. Another group reported that using lentiviral vectors to express the key DA neuron-regulating gene, *LMX1A*, in hESCs produced ventral midbrain DA neurons of the A9 subtype which account for more than 60 % of all neurons generated from *LMX1A*-transfected hESCs [75]. To determine the functional properties of hESC-derived DA neurons in vivo, hESC-derived midbrain dopamine neurons and fetal brain DA neurons were engrafted into rat models of PD. MRI and PET imaging analysis showed that grafted hESC-DA neurons survived, projected long neural branches, and played similar functions to improve the locomotive deficits of PD rats as fetal brain DA neurons, providing further preclinical evidence of hESC-derived dopamine neurons for treatment of PD [76].

The major concerns to use stromal cell as feeder cells for culturing hESCs-derived cells for clinical purpose are that hESCs-derived cells contain some rodent cells and may increase the risk of immune rejections. To overcome this problem, some studies developed feeder-free culture system to use matrigels to replace the feeder cells [36, 77]. Schulz et al. moved towards clinical applicability by generating the neurons in a serum-free suspension system [78]. Vazin et al. were successful in replacing the PA6 stromal cells with growth factors SDF-1, PTN, IGF2, and EFNB1, which induced the differentiation of hESCs directly into TH-positive DA neurons [79]. Growth factors SHH and FGF8 were reported to substitute for PA6 stromal cells in generating DA cells after an initial induction step of differentiating hESCs into NSCs. They endeavoured to develop a scalable process applicable to the clinic and easily brought to Good Manufacturing Protocol (GMP) standards. Their culture protocols did not involve serum, but they made the important discovery that cells could be stored at each of the intermediate stages in their four-step process (propagation of ESC → generation of neural stem cells (NSC) → induction of dopaminergic precursors maturation of dopaminergic neurons) without loss of functional ability, allowing cells to be transplanted at an appropriate time point in neural development [80].

Although hESCs can be efficiently differentiated into a large amount of DA neurons in vitro and showed solid functions to restore the motor dysfunctions in PD animal models, including mice, rats and non-human primates, clinical trials have not been performed for treating PD patients. The main problems with hESCs are: i) the phenotypic stability of hESC-derived dopamine neurons after transplantation, and ii) the worry about residual undifferentiated hESCs within the large numbers of cells that need to be injected for human therapy. The residual undifferentiated hESCs might indeed lead to tumor formation