Review article

SH-SY5Y human neuroblastoma cell line: *in vitro* cell model of dopaminergic neurons in Parkinson’s disease

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**Keywords:** Parkinson’s disease; SH-SY5Y cells; cell model; differentiation; dopaminergic neuron

**Objective** To evaluate the human neuroblastoma SH-SY5Y cell line as an *in vitro* model of dopaminergic (DAergic) neurons for Parkinson’s disease (PD) research and to determine the effect of differentiation on this cell model.

**Data sources** The data of this review were selected from the original reports and reviews related to SH-SY5Y cells published in Chinese and foreign journals (Pubmed 1973 to 2009).

**Study selection** After searching the literature, 60 articles were selected to address this review.

**Results** The SH-SY5Y cell line has become a popular cell model for PD research because this cell line possesses many characteristics of DAergic neurons. For example, these cells express tyrosine hydroxylase and dopamine-β-hydroxylase, as well as the dopamine transporter. Moreover, this cell line can be differentiated into a functionally mature neuronal phenotype in the presence of various agents. Upon differentiation, SH-SY5Y cells stop proliferating and a constant cell number is subsequently maintained. However, different differentiating agents induce different neuronal phenotypes and biochemical changes. For example, retinoic acid induces differentiation toward a cholinergic neuronal phenotype and increases the susceptibility of SH-SY5Y cells to neurotoxins and neuroprotective agents, whereas treatment with retinoic acid followed by phorbol ester 12-O-tetradecanoylphorbol-13-acetate results in a DAergic neuronal phenotype and decreases the susceptibility of cells to neurotoxins and neuroprotective agents. Some differentiating agents also alter kinetics of 1-methyl-4-phenyl-pyridinium (MPP⁺) uptake, making SH-SY5Y cells more similar to primary mesencephalic neurons.

**Conclusions** Differentiated and undifferentiated SH-SY5Y cells have been widely used as a cell model of DAergic neurons for PD research. Some differentiating agents afford SH-SY5Y cells with more potential for studying neurotoxicity and neuroprotection and are thus more relevant to experimental PD research.

**Parkinson’s disease (PD)** is an age-related progressive neurodegenerative disorder with a prevalence of 1%-2% in people over the age of 50. Because of its prevalence and lack of effective treatment, PD is a major societal health problem. Recently, significant advances have been made in the experimental studies of PD, especially through the use of cell models. The development of a stable and reliable dopaminergic (DAergic) neuronal cell model is particularly necessary for studying the pathogenesis of PD and developing therapeutic strategies. An ideal *in vitro* PD cell model should be established in post-mitotic human DAergic neuronal cells susceptible to neurotoxins produced during PD so as to address questions regarding the selective loss of DAergic neurons in the substantia nigra. Presently, PD cell models primarily include non-neuronal tumor cell lines such as pheochromocytoma (PC12) cells, neuronal tumor cell lines represented with human neuroblastoma (SH-SY5Y) cells and primary mesencephalic neurons. These cells mimic many aspects of the DAergic neuron death observed in PD when treated by neurotoxins such as 1-methyl-4-phenyl-pyridinium (MPP⁺), 6-hydroxydopamine (6-OHDA), or rotenone. Primary mesencephalic neurons are a good candidate source of DAergic neurons; however, human primary neurons are extraordinarily difficult to obtain, culture, and handle. Moreover the ethical difficulties in obtaining sufficient and appropriate human primary neurons also limit the application of this cell source. The SH-SY5Y cell line provides an unlimited supply of cells of human origin with the similar biochemical characteristics to human DAergic neurons. This paper will review the characteristics of the SH-SY5Y cells and the effect of differentiation on this cell model and the use of this cell line as a model of DAergic neurons for PD research.

**SH-SY5Y CELL LINE AS A USEFUL NEURONAL CELL MODEL**

The SH-SY5Y cell line is a thrice cloned subline of SK-N-SH cells which were originally established from a bone marrow biopsy of a neuroblastoma patient with sympathetic adrenergic ganglial origin in the early 1970’s. This cell line has been widely used as model of neurons since the early 1980’s as these cells posses many...
biochemical and functional properties of neurons. The SK-N-SH cell line contains cells with three different phenotypes: neuronal (N type), Schwannian (S type), and intermediary (I type). The SH-SY5Y cell line is a comparatively homogeneous neuroblast-like cell (N type). This cell line exhibits neuronal marker enzyme activity (tyrosine and dopamine-β-hydroxylases), specific uptake of norepinephrine (NA), and expresses one or more neurofilament proteins; these cells also express opioid, muscarinic, and nerve growth factor receptors. In addition, SH-SY5Y cells possess the capability of proliferating in culture for long periods without contamination, a prerequisite for the development of an in vitro cell model. SH-SY5Y cells were derived from immature neoplastic neural crest cells that exhibit properties of stem cells and the SH-SY5Y cells are induced to differentiate upon treatment with a variety of agents, including retinoic acid (RA), phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), brain-derived neurotrophic factor (BDNF), dibutyryl cyclic AMP (dBcAMP), purine, or staurosporine. Consequently, the SH-SY5Y cell line has been widely used in experimental neurological studies, including analysis of neuronal differentiation, metabolism, and function related to neurodegenerative and neuroadaptive processes, neurotoxicity, and neuroprotection.

**POTENTIALITY OF SH-SY5Y CELL LINE AS PD CELL MODEL**

Pathologically, PD is characterized by the loss of mesencephalic DAergic neurons. As SH-SY5Y cells possess many characteristics of DAergic neurons, the SH-SY5Y cell line has recently been used as a DAergic neuronal model for PD research. Several characteristics of the SH-SY5Y cell line make it useful as a model for DAergic neurons: firstly, the SH-SY5Y cells possess the ability to synthesize dopamine (DA) and NA because the cells express tyrosine and dopamine-β-hydroxylases. Secondly, SH-SY5Y cells express dopamine transporter (DAT), a protein expressed only in DAergic neurons within the central nervous system; DAT regulates DA homeostasis through specific uptake and sequestration of DA. Because DAT is a prerequisite for MPP⁺ incorporation into neurons, the SH-SY5Y cell line has been widely utilized to study mechanisms of MPP⁺-induced neurotoxicity and the pathogenesis underlying MPP⁺-induced PD mimics. Thirdly, although the SH-SY5Y cells express only low levels of DA receptors, DA agonists, which may be neuroprotective, can be evaluated in SH-SY5Y cells. Finally, the SH-SY5Y cells can be differentiated into a more pronounced DAergic neuronal phenotype in the presence of some agents and some differentiated SH-SY5Y cells are similar to primary mesencephalic neurons. Some agents (for example, RA) that induce differentiation also confer tolerance on SH-SY5Y cells, therefore, the role of toxicity or protection cannot be evaluated in these differentiated cells. This means that undifferentiated SH-SY5Y cells, but not differentiated cells, are an appropriate cell model for studying neurotoxicity or neuroprotection and thus are relevant in experimental PD research.

**LIMITATIONS OF UNDIFFERENTIATED SH-SY5Y CELL LINE AS PD CELL MODEL**

Although undifferentiated and differentiated SH-SY5Y cells have been widely used as a PD cell model, use of undifferentiated SH-SY5Y cells as PD cell model involves some disadvantages. Firstly, as a continuously dividing cell line, the number of undifferentiated SH-SY5Y cells increases during the course of the experiment, so it is difficult to distinguish whether neuroprotective or neurotoxic agents influence the proliferation rate or the rate of cell death. Secondly, SH-SY5Y cells in cultures are unsynchronized and do not always exhibit the typical markers of mature neurons, which leads to uncertainty in experiments. Thirdly, undifferentiated SH-SY5Y cells do not express high levels of DA synthetic enzymes and DAT. Fourthly, undifferentiated SH-SY5Y cells exhibit less sensitivity to neurotoxins and neuroprotective agents than primary mesencephalic neurons. Another problem to be addressed is that MPP⁺ entry into undifferentiated SH-SY5Y cells is different entry into neurons in vivo (discussed in detail below). Moreover, the low expression of DA receptors makes study of the therapeutic effects of DA agonists in PD difficult. Finally, although SH-SY5Y cells express dopamine-β-hydroxylase and tyrosine hydroxylase (TH) activity, catecholamine (DA and NA) synthesis may be limited by a deficiency of dihydroxyphenylalanine decarboxylase activity. Although the SH-SY5Y cell line offers a good cell model for PD research, these weaknesses mean that the cell line is not an ideal PD cell model.

**EFFECT OF DIFFERENTIATION ON SH-SY5Y CELL LINE**

Differentiation produces cells with a functionally mature neuronal phenotype

Many lines of evidence indicated that differentiation results in SH-SY5Y cells with a functionally mature neuronal phenotype. Upon differentiation, cells stop proliferating, become a stable population, and show extensive neurite outgrowth with morphological similarity to living neurons in the human brain. Since bioactive TPA was first used to induce differentiation of SH-SY5Y cells in 1981, a large number of differentiation protocols have been published. Although differentiated SH-SY5Y cells differ from mature neurons in the human brain, differentiated SH-SY5Y cells may become an ideal PD cell model.

Upon differentiation, SH-SY5Y cells possess more biochemical, ultrastructural, morphological, and electrophysiogical similarity to neurons. Differentiated SH-SY5Y cells express a variety of neuronal-specific
markers, including NA, growth-associated protein (GAP-43), receptors for neurotrophic factors, neuropeptides, neurosecretory granula, neuron-specific enolase (NSE), neuronal nuclei (NeuN), vesicle proteins such as synaptophysin, and neuronal-specific cytoskeletal proteins, including microtube associated protein (MAP), Tau, and neurofilament proteins.\(^{15,21}\) MAP, GAP-43, NeuN, and synaptophysin are classical markers of mature neurons. However, whether these mature neuron-like cells have the functions of neurons is still unclear. There is evidence that differentiated SH-SY5Y cells are more excitable and their membrane potential increased relative to undifferentiated cells. For example, these cells begin to release NA following stimulation with acetylcholine\(^{22}\) and express functional changes of potassium conductances\(^{23}\) and hormone-induced Ca\(^{2+}\) entry.\(^{24}\) Differentiated cells also differ from undifferentiated cells in levels of neurotransmitter and neuropeptide receptors such as DA\(^{25}\) and opioids.\(^{26}\) High levels of dopamine-\(\beta\)-hydroxylase, TH, and DAT activity in differentiated SH-SY5Y cells are also functional indicators of adrenergic neuron-like differentiation. These characteristics indicate that differentiation induces functional properties resembling those of mature neurons.

DA agonists have been proved to be powerful and potent anti-Parkinsonian agents in both in vitro and in vivo PD models. Because DA agonists exert their effect predominantly via binding to DA receptors,\(^{27}\) the therapeutic effect of DA agonists cannot be elucidated in undifferentiated SH-SY5Y cells that express low levels of DA receptors. Increasing evidence shows that protein levels of G-protein-coupled D2 and D3 dopamine receptors are elevated in differentiated SH-SY5Y cells.\(^{17}\) This has been further substantiated by the findings that D3-prefering agonists, D2-prefering agonists, or non-selective compounds have little or no neuroprotective effects in undifferentiated SH-SY5Y cells,\(^{28}\) whereas these same agents have robust neuroprotective effects in SH-SY5Y cells sequentially differentiated with RA and TPA (RA/TPA).\(^{29}\) As expression of functional DA receptors are increased in differentiated SH-SY5Y cells compared with undifferentiated cells, neuroprotective effects of DA agonists can be studied in the differentiated cells, which is promising for the development of more effective neuroprotective agents for treating PD.

**Various phenotypes induced by different agents**

The SH-SY5Y cells can be driven toward cholinergic, adrenergic, or DAergic phenotype depending on media conditions. For example, treatment with phorbol esters induces differentiation toward an adrenergic phenotype, whereas RA treatment induces a cholinergic neuronal phenotype.\(^{30}\) It is essential that SH-SY5Y cells are differentiated toward the DAergic phenotype for PD research.

**SH-SY5Y cell differentiation induced by RA**

The effects of RA on SH-SY5Y cells are well characterized. RA exerts its effects by binding to two classes of non-steroid nuclear hormone receptors, the retinoic acid receptors (RARs) and the retinoic X receptors (RXRs). Though RA can bind only to the RARs, activated RAR heterodimerizes with RXR and RAR/RXR heterodimers bind to RA response element (RARE), resulting in transcriptional activation.\(^{5}\) RA induces differentiation through regulation of the transcription of neurotrophin receptor genes.\(^{31}\) the Wnt signaling pathway,\(^{32}\) pathways involving type II protein kinase A (PKA).\(^{33}\) The phenotype of RA-treated SH-SY5Y cells is the subject of some controversy in the literature. RA treatment causes slight accumulation\(^{40}\) and release of NA after carbachol stimulation,\(^{34}\) subsequently inducing SH-SY5Y cells to differentiate to the adrenergic phenotype. However, other reports showed that RA-differentiated SH-SY5Y cells are of a mature cholinergic phenotype.\(^{30}\) With one exception,\(^{35}\) all reports indicate that there are no significant differences in the expression of the DAT and TH between undifferentiated and RA-differentiated SH-SY5Y cells.\(^{15,17}\) Moreover, higher expression of choline acetyl transferase (ChAT) activity\(^{36}\) and vesicular monoamine transporter (VMAT)\(^{17}\) also indicate that differentiated cells are cholinergic. RA-induced decreases in expression of neuropeptide tyrosine (NPY) in SH-SY5Y cells\(^{37}\) may be also relevant to conversion of cholinergic phenotype. Some of the DAergic/adrenergic characteristics of SH-SY5Y cell line were reduced by RA treatment, whereas the cholinergic properties were increased. Although RA alone produces cholinergic neuronal differentiation phenotype, RA together with other agents, such as cholesterol\(^{38}\) or TPA,\(^{17}\) can induce the DAergic neuronal phenotype.

**Phorbol esters as differentiating agents**

Biologically active phorbol esters such as TPA have drastic effects on cell growth and differentiation and nanomolar concentrations of TPA promote differentiation of SH-SY5Y cells. TPA-differentiated SH-SY5Y cells are partially growth inhibited and have a low ornithine decarboxylase (ODC) activity, which cannot be stimulated by a change to fresh medium.\(^{39}\) The lack of ODC, which is the rate-limiting enzyme in the biosynthesis of polyamines,\(^{40}\) in TPA-treated cells may result in the loss of response to the growth-promoting activity of serum. TPA also abolishes the mitogenic response of SH-SY5Y cells to both human insulin-like growth factor I and II (IGF-I and IGF-II); this may be the mechanism for growth control in differentiating SH-SY5Y cells.\(^{41}\) The differentiating effects of phorbol esters are primarily mediated by protein kinase C (PKC) isoforms. PKC might enhance NA release in SH-SY5Y cells by enhancing Ca\(^{2+}\) channel activity.\(^{30}\) TPA, at the optimal concentration, induces a 200-fold increase in NA whereas only a four-fold increase is observed with RA.\(^{40}\) TPA can also enhance carbachol- and K\(^{+}\)-evoked NA secretion.\(^{33}\) In addition, TPA-differentiated SH-SY5Y...
cells exhibit a number of characteristics of the adrenergic neuronal phenotype, including expression of TH, NPY, as well as NSE. NA and NPY, which are co-localized in sympathetic neurons, are two important neurotransmitters produced in adrenergic neurons of the sympathetic nervous system.

Available data suggest that the phenotype of TPA-treated SH-SY5Y cells is adrenergic. RA and TPA may induce SH-SY5Y cells differentiate to cholinergic and adrenergic phenotypes, respectively, via different effects on transforming growth factor β1 (TGF-β1) and bone morphogenetic protein 2 (BMP-2), because TGF-β1 and BMP-2 contribute, in opposite ways, to regulation of the TH expression and neuronal phenotype. TGF-β increases TH expression and this effect is counteracted by BMP-2, suggesting that the prevalence of one or the other of these effects may be important for the final acquisition of adrenergic or cholinergic phenotype. However, SH-SY5Y cells treated first with RA and then TPA develop a DAergic phenotype and have higher levels of TH and DAT, but lower levels of VMAT than undifferentiated cells. Importantly, RA/TPA differentiated cells have a high density of D2 and D3 receptors as compared with undifferentiated, RA-differentiated, or TPA-differentiated cells.

Neurotrophins and SH-SY5Y cell differentiation

Neurotrophins have been shown to modulate survival, differentiation, synaptogenesis, and activity of neurons. The family of neurotrophins is composed mainly of nerve growth factor (NGF), BDNF, neurotrophin-4 (NT-4/5), and neurotrophin-3 (NT-3). These neurotrophins can bind to two types of cell surface receptors, the tyrosine kinase (TRK) receptors and the neurotrophin receptor p75 (NTR). These receptors mediate almost all of their survival-promoting activities, with NGF preferentially activating TrkA and BDNF, NT-4/5 activating TrkB, and NT-3 activating TrkC sites. In addition, several other growth factors, including basic fibroblast growth factor (bFGF), insulin-like growth factor I (IGF1), glial cell line-derived neurotrophic factor (GDNF), and ciliary neurotrophic factor (CNTF) also participate in the induction of cell differentiation. Of these, bFGF and IGF1 effectively induce differentiation of SH-SY5Y cells. SH-SY5Y cell lines generally lack functional neurotrophin receptors and do not differentiate when stimulated with neurotrophins. However, RA treatment causes the upregulation of the neurotrophin receptors making SH-SY5Y cells responsive to neurotrophins. The survival of treated cells is dependent on the continuous co-existence of neurotrophins in the culture media. Once neurotrophins are removed from the culture medium, cells enter apoptotic cell death accompanied by an attempt to reenter the cell cycle.

This review mainly addresses the effect of BDNF on the SH-SY5Y cell line. Because addition of BDNF can avoid the accumulation of S-type in RA-treated SH-SY5Y cells, sequential differentiation with RA and BDNF (RA/BDNF) yields a nearly homogeneous population of human neuron-like differentiated cells. Edsjö and coworkers provided evidence that RA/BDNF treatment led to an increased expression of general neuronal marker genes and the cholinergic marker genes vesicular acetylcholine transporter (VChAT) and choline acetyl transferase (ChAT), suggesting that RA/BDNF treatment of SH-SY5Y cells induces a shift from a sympathetic noradrenergic phenotype toward a cholinergic neuronal phenotype. They also found that RA/BDNF treated SH-SY5Y cells have low NA levels and lack TH induction but NPY expression is increased, which might suggest that the RA/BDNF treated SH-SY5Y cells develop into a sympathetic cholinergic neuronal phenotype. However, another group found that RA/BDNF treated SH-SY5Y cells exhibit many characteristics of primary DAergic neurons, including TH and the DAT activity, uptake of DA, and release of DA after K+ stimulation. Further study of RA/BDNF treated SH-SY5Y cells will be required to determine their phenotype.

Other differentiating agents

Stauorosporine, a non-selective PKC inhibitor, is a strong inducer of differentiation in the SH-SY5Y cell line. Stauorosporine induces a mature adrenergic neuronal phenotype characterized by up-regulation of TH, DAT, and NPY activities and a 30-fold increase in NA content. Guanosine and guanosine-5′-triphosphate each induce cell-cycle arrest in SH-SY5Y cells and a strong increase in TH and DAT levels. This phenomenon supports the view that these purines induce a DAergic/adrenergic phenotype. DBCAMP also induces differentiation of SH-SY5Y cells into an adrenergic phenotype and increases NA production and TH expression through both PKA activation and butyrate.

Hypoxia can also induce phenotypical changes in neuroblastoma cells. Under hypoxic conditions, these cells resemble immature stem cells. As reviewed by Edsjö and coworkers, hypoxia-induced differentiation seems to be a process of de-differentiation and the molecular mechanisms underlying the hypoxia-driven de-differentiation are most likely complex. The mechanism seems to be the reverse of the molecular steps activated during the differentiation of sympathetic precursor cells into neuroblasts and eventually ganglion cells.

Differentiation changes susceptibility of SH-SY5Y cells to neurotoxic and neuroprotective agents

Increasing evidence suggests that differentiation alters the susceptibility of SH-SY5Y cells to neurotoxins and neuroprotective agents. The change of susceptibility appears to be dependent upon the differentiating agents used and not upon differentiation per se. Even though different agents induce almost identical morphological differentiation, SH-SY5Y cells exhibit different...
susceptibilities depending on the inducer. Many reports suggest that RA-differentiated SH-SY5Y cells are less susceptible to PD mimetics and certain harmful agents than undifferentiated cells. The reduced susceptibility is in line with the data on RA-differentiated cells in Alzheimer’s disease (AD) research; these cells are more resistant than undifferentiated cells when exposed to amyloid. There is evidence that chemoresistance does not result from decreased proliferation induced by RA. Available data indicates that RA-differentiated SH-SY5Y cells are not an appropriate cell model for studying neurotoxicity or neuroprotection in experimental PD research. However, due to the insensitivity of SH-SY5Y cells compared with primary mesencephalic neurons, undifferentiated SH-SY5Y cells are also not the optimal PD cell model.

SH-SY5Y cells differentiated with certain agents do exhibit similar susceptibility to primary mesencephalic neurons and in vivo PD models. For example, staurosporine and RA/TPA greatly increase the vulnerability of the cells to many toxic agents with different mechanisms of action, which emphasizes the similarity between differentiated SH-SY5Y cells and primary neurons. These changes of vulnerability might be attributed to various biochemical changes evoked by different differentiating agents, such as overexpression and down-regulation of anti- and pro-apoptotic proteins, or altered signaling in pathways, including the Akt and TrkB pathways. For example, RA increased expression of Bel-2 (an anti-apoptotic protein) and decreased expression of p53 (pro-apoptotic), whereas staurosporine decreased levels of Bel-2 and increased expression of p53. It is worth noting that the low expression of DAT and the presence of VMAT2 also contribute to the insensitivity to MPP⁺ in RA-differentiated cells. The changes in susceptibility are also found in the studies of neuroprotective agents (e.g., eicosapentaenoic acid) that increase cell viability in RA/BDNF differentiated SH-SY5Y cells, the same effect observed in vivo, but that have no effect in undifferentiated cells. So, some differentiated SH-SY5Y cells are more appropriate for studying neurotoxicity or neuroprotection in experimental PD research than others. Moreover, it is still an open question as to whether it is necessary for SH-SY5Y cell line to be differentiated with RA in order to induce the DAergic phenotype required as a neuron model for PD research.

**Differentiation alters kinetics of MPP⁺ uptake**

The neurotoxin MPP⁺, the active neurotoxic metabolite of MPTP, has been shown to induce a syndrome clinically and pathologically similar to PD. MPP⁺ is actively transported into DAergic neurons through the plasma membrane in a DAT-dependent fashion. There is evidence that DAT is absolutely required for MPP⁺ neurotoxicity in vivo. Although SH-SY5Y cells have been widely employed in an MPP⁺ mimicking PD cell model, little is known about whether intracellular accumulation of MPP⁺ is via DAT in this cell line. There is a report suggesting that MPP⁺ accumulation in undifferentiated and RA-differentiated cells via non-DAT mechanisms; a DAT antagonist did not significantly block the toxicity of MPP⁺ in these cells. The low expression of DAT and toxicity of MPP⁺ in the absence of DAT in undifferentiated and RA-differentiated SH-SY5Y cells may invalidate the use of these cells in MPP⁺ mimicking PD model. Fortunately, Steven and associates have provided valuable evidence that kinetics of MPP⁺ uptake in RA/TPA differentiated SH-SY5Y cells is almost completely dependent on DAT. In their experiments, treatment with a DAT antagonist resulted in an almost complete blockade of the toxicity of MPP⁺, as it does in vivo and primary mesencephalic neurons. Therefore, RA/TPA differentiated SH-SY5Y cells are appropriate for use in a MPP⁺-induced PD cell model.

**CONCLUSIONS**

Differentiated and undifferentiated SH-SY5Y cells have gained broad acceptance as models of DAergic neurons for PD research. Some differentiated SH-SY5Y cells are more suitable PD cell models than others, because they stop proliferating, have more DAergic neuronal properties, and have similar susceptibility to neurotoxins and neuroprotective agents as primary neurons. Although differentiated SH-SY5Y cells described to date fall slightly short of the ideal PD cell model, differentiation serves to make SH-SY5Y cells more analogous to DAergic neurons and thus, a reasonable model for exploration of the pathogenesis of PD and evaluation of therapies. In the past two decades, a variety of methods to differentiate SH-SY5Y cells have been evaluated; however, the development of an optimal differentiated SH-SY5Y DAergic cell model for PD requires further researches.

**REFERENCES**


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