SH-SY5Y-derived neurons: A neuronal model system for investigating TAU sorting mechanisms and neuronal subtype-specific TAU vulnerability

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1. Abstract

The human-derived SH-SY5Y neuroblastoma cell line is widely used for studying TAU physiology and TAU-related pathology in Alzheimer’s disease (AD) and related tauopathies. SH-SY5Y cells can be differentiated into neuron-like cells (SH-SY5Y-derived neurons), which resemble noradrenergic, dopaminergic or cholinergic neurons, by using various substances. This review evaluates whether SH-SY5Y-derived neurons are a suitable model for investigating intracellular TAU sorting mechanisms in general, and with respect to neuron subtype-specific TAU vulnerability. SH-SY5Y-derived neurons show pronounced axodendritic polarity resembling neuronal cell polarity, high TAU protein levels, axonal TAU localization, expression of the six major human brain isoforms, and TAU phosphorylation similar to AD. This enables studying the isoform- and phosphorylation-dependent impact on TAU subcellular distribution and axodendritic trafficking of TAU. As SH-SY5Y cells are accessible for genetic engineering, stable transgene integration and leading-edge genome editing are valuable and often-used tools for TAU-related research in these cells. Furthermore, SH-SY5Y-derived neurons resemble cells of distinct subcortical nuclei, i.e. the Locus coeruleus (LC), Nucleus basalis (NB) and Substantia nigra (SN), depending on the used differentiation procedure. This allows to study neuron-specific TAU isoform expression and intracellular localization in the context of vulnerability to TAU pathology. Limitations are e.g. the lack of mimicking age-related tauopathy risk factors and the difficulty to define the exact neuronal subtype of SH-SY5Y-derived neurons. In brief, this review discusses the suitability of SH-SY5Y-derived neurons for investigating TAU sorting mechanisms and neuron-specific TAU vulnerability in human-like conditions.

Key words: SH-SY5Y-derived neurons, TAU sorting, neuronal identity, tauopathy, Alzheimer’s disease
2. Introduction

Alzheimer’s disease (AD) and related tauopathies constitute a major scourge of modern society healthcare due to their tremendously high and increasing prevalence \(^1\). The microtubule (MT)-associated protein TAU, which is abundant in axons of mature neurons, is a key player for the pathological cascade of these diseases. Missorting into the somatodendritic compartment, site-specific hyperphosphorylation and formation of neurofibrillary tangles (NFT) containing TAU are classical hallmarks of AD and other tauopathies \(^2-4\). In the last decades, a lot of scientific effort has been invested in unravelling the physiological functions and pathomechanisms connected to TAU. Often-used rodent models (primary neuron cultures, animal models) require animal resources and suffer from limitations in translatability, such as different isoform expression ratios, artificial genetic settings due to multiple human transgenes, or species-dependent deviations regarding the cellular machinery and interactions partners \(^5\). Human induced pluripotent stem cell (hiPSC)-derived neurons overcome many of the described obstacles and constitute a powerful tool for TAU-related research \(^6-10\). However, differentiation of hiPSC-derived neurons is expensive, time-consuming and results in cultures with variable homogeneity and differentiation efficiency \(^11,12\).

The neuroblastoma cell line SH-SY5Y, which was subcloned from the SK-N-SH line \(^13\), is an easy-to-handle and proliferative cell line with well-established differentiation methods for stable neuronal cultures. SH-SY5Y-derived neurons have been widely used for TAU-related research, as they yield homogeneous, reproducible human-derived neuronal cultures with robust expression and axonal distribution of TAU, thereby suitable also for addressing axonal TAU sorting. Interestingly, the neuronal identity of SH-SY5Y-derived neurons depends on the used differentiation procedure (reviewed in \(^14\)), which bears great potential for neuronal subtype-specific TAU research.

The current review aims to evaluate the general suitability of SH-SY5Y-derived neurons for research on mechanisms of TAU sorting, focusing on TAU cellular polarity, total TAU and TAU isoform expression, intracellular localization, phosphorylation, and the possibilities of genetic engineering in SH-SY5Y cells. Moreover, the chances and challenges of the procedure-dependent identity of SH-SY5Y-derived neurons will be discussed regarding their utility to mimic neuronal subtypes of brain regions that are early affected in AD.
3. General suitability of SH-SY5Y-derived neurons for TAU sorting research

Neuronal polarity and total TAU expression. A suitable neuronal cell model for TAU sorting research needs to meet certain demands, including resemblance of neuronal morphology in form of pronounced neuronal polarity and axonal outgrowth, neuronal maturation, indicated by common neuronal marker proteins\(^ {15}\) and, strikingly, high expression levels and axonal localization of TAU protein.

Little amounts of TAU protein are detectable in undifferentiated SH-SY5Y cells, in which it is present both in the cytoplasm and the nucleus\(^ {16}\). Differentiation of SH-SY5Y cells, inducible with retinoic acid (RA) and various other substances (reviewed in\(^ {14}\)), results in neurite outgrowth and strong increase of overall TAU protein levels\(^ {17,18}\) with a neuron-like subcellular distribution, i.e. increased axonal and decreased somatic TAU levels (Fig. 1A,B)\(^ {16,17,19}\). The use of combinatorial treatments, e.g. RA and brain-derived neurotrophic factor (BDNF)\(^ {17,19,20}\) or BDNF and neuronal growth factor\(^ {17,19,20}\), further enhances the axonal outgrowth, the total TAU expression to levels comparable with the human brain\(^ {19}\), and the segregation of axonally localized TAU and somatodendritically localized microtubule-associated protein 2 (MAP2) (Fig. 1B)\(^ {17,20}\), indicating a pronounced neuronal maturity and polarity\(^ {21}\). Moreover, differentiation leads to drastic upregulation of other common neuronal maturation markers in SH-SY5Y cells, such as synaptophysin, neuronal nuclei, neuron specific enolase, growth-associated protein or synaptic vesicle protein II\(^ {17,19,22–26}\). Whether SH-SY5Y-derived neurons exhibit the formation of the axon initial segment (AIS), a key player in developing and maintaining cellular and TAU protein polarity\(^ {27–31}\), was not yet addressed. It remains unclear if a detectable AIS precedes or coincides the neuronal maturation in SH-SY5Y-derived neurons, as it is typically seen during neuronal development\(^ {27}\).

The strong expression of TAU, but also the upregulation of other neuronal markers such as MAP2 indicate the neuronal character of SH-SY5Y-derived neurons and make them a valuable system for TAU sorting-related studies. In other words, the neuron-like TAU localization enables the investigation of TAU sorting mechanisms in these cells. In this context, it would be worth to examine if SH-SY5Y-derived neurons properly distribute also transfected TAU, an often-faced challenge when working with neuronal cultures\(^ {32}\). Given the formation of an AIS in fully
differentiated cells, which needs to be evaluated in future studies, this model could also serve for the investigation of TAU-AIS interactions that are responsible for the regulation of retrograde and anterograde TAU trafficking, which takes place at the AIS \cite{28,30,31}. Especially the question of anterograde TAU transit at the proximal axon is striking as the lack of stable microtubule structures within this compartment \cite{33} make continuous MT-directed transport unlikely.

**TAU isoform expression.** Alternative splicing of the exons 2 and 3 (either 0N, 1N, or 2N isoforms) as well as exon 10 (3R or 4R isoforms) results in six major TAU isoforms in the mature human brain \cite{34–36}. The isoform expression pattern is brain region-specific and changes during developmental stages \cite{34,37–39}. The axodendritic distribution is markedly different depending on the TAU isoform \cite{31}. Therefore, a suitable model for studying TAU sorting should display the expression of all human TAU isoforms.

Early studies investigating the TAU isoform expression showed consistently that undifferentiated SH-SY5Y cells express only the shortest TAU isoform 0N3R \cite{16,18,40,41}. More recently, TAU mRNA containing exon 2 (1N) or exon 10 (4R) isoforms was found in untreated SH-SY5Y cells, suggesting at least basal expression of larger isoforms (Fig. 1C) \cite{19}. Reports about differentiated SH-SY5Y-derived neurons vary in their described isoform expression pattern. Former studies detected either no shift in isoform expression upon RA treatment \cite{18}, weak expression of an additional 64 kDa-sized isoform (probably representing the 2N4R isoform) \cite{40}, or low levels of 4R isoform mRNA upon three weeks of RA treatment \cite{16}. More recent findings, however, showed that undifferentiated SH-SY5Y cells already express high amounts of 1N isoforms, and that differentiated cells express all six isoforms found in the human brain (Fig. 1C) \cite{19}.

The reported isoform ratio in SH-SY5Y-derived neurons differs notably from the human brain \cite{42}, with more 3R than 4R TAU, less 2N isoforms and more 0N3R-TAU \cite{19}. This may suggest that a cultivation time of up to three weeks produces SH-SY5Y-derived neurons at an intermediate stage of maturity. Later studies, however, found roughly equal amounts of 3R and 4R isoforms, as typically seen in the adult human brain \cite{43–46}, after RA treatment \cite{47}. Despite this discrepancy regarding the isoform ratio, these studies demonstrate the abundant presence of all six TAU isoforms, which makes SH-SY5Y-derived neurons valuable for further investigation of TAU isoforms in the context of TAU distribution and mislocalization. One bottleneck of tracing the emergence and progression of AD in recent mouse models is, despite harbouring human TAU transgenes, the presence of all six human isoforms. Although recent transgenic mouse lines achieve the expression of all six human isoforms in ratios similar to the human brain \cite{48,49}, they
inevitably lack a human cellular environment, in contrast to SH-SY5Y-derived neurons. In other words, SH-SY5Y-derived neurons can help to clarify if and how different TAU isoforms contribute to cellular TAU functions under physiological conditions and possibly convey tauopathy-related toxicity, e.g. by being more susceptible for mislocalization, hyperphosphorylation or aggregation. The fact that distinct tauopathies show a distinct composition of TAU-NFTs, e.g. consisting only of 3R or 4R isoforms or a combination of both 46,50, strongly indicates a connection between isoform expression and aggregation propensity, which warrants further investigation. Taken together, the TAU isoform ratio of SH-SY5Y-derived neurons differs from that in the mature human brain, but the strong expression of all six major isoforms already upon brief differentiation periods allows investigating isoform-specific localization and mislocalization propensity.

**TAU phosphorylation state.** More than 90 reported phosphorylation sites illustrate the striking importance of these posttranslational modifications for TAU function 46. The phosphorylation state of TAU directly influences the microtubule-binding affinity and thereby its mobility and intracellular localization 51–55. Hyperphosphorylation of the AT8 motif (including Ser-202 & Thr-205) or other motifs strongly correlates with somatodendritic missorting of TAU and is characteristic for NFT-TAU formation 55–61. Pseudo-phosphorylation of the four KXGS motifs was shown to cause TAU missorting and synapse loss 30,62, hinting at the importance of TAU phosphorylation state and its regulation within the pathological cascade in AD and related diseases.

Consequently, early TAU studies with SH-SY5Y cells put great effort into analysing the phosphorylation state of TAU in SH-SY5Y cells. They revealed that large portions of TAU residues, including Ser-199, Ser-202 (AT8 epitope), Thr-231/Ser-235 (AT180 epitope), Ser-262 (12E8 epitope) and Ser-396/Ser-404 (PHF1 epitope) are phosphorylated in undifferentiated SH-SY5Y cells (Fig. 1D) 18,41,63. As these residues largely overlap with those, which appear hyperphosphorylated in NFT-containing TAU, the TAU protein of SH-SY5Y cells was considered as abnormally phosphorylated in an AD-like manner 41. However, the high degree of phosphorylation can be explained by the notably high levels of 0N3R-TAU in undifferentiated SH-SY5Y cells. In early developmental stages, when 0N3R-TAU is the predominant isoform, TAU typically shows an increased phosphorylation level 34,60. Interestingly, no substantial change in TAU phosphorylation was seen upon differentiation with RA 18 despite the expression of larger TAU isoforms 19,47. This might be, at least partially, due to the fact that 0N3R-TAU appears as the major isoform also in differentiating SH-SY5Y cells 19.
Okadaic acid and other phosphatase inhibitors were able to evoke AD-like TAU hyperphosphorylation, disassembly of microtubules and increased degeneration of undifferentiated SH-SY5Y cells\(^{40,64,65}\). This coincides with an inactivation of PP1 and PP2A phosphatases and an increased activity of MAPK, CDC2 and CDK5 kinases\(^{64}\), thereby confirming their regulating role for TAU modifications, as it was postulated from previous \textit{in vitro} interaction assays (reviewed in \cite{46}), and directly linking phosphorylation state, MT stability and cell death. In other words, SH-SY5Y cells – either undifferentiated or differentiated – turned out as a highly valuable system for addressing how TAU phosphorylation is modulated and how this modulation can be compromised in disease conditions. Thus, many recent TAU studies in SH-SY5Y cells focused on TAU(hyper)phosphorylation, including the role of kinases/phosphatases and cellular pathways in misbalancing the TAU phosphorylation state \cite{63,66–69}, the influence of microglia-mediated neuroinflammation on TAU expression levels and phosphorylation \cite{47,70–72}, the effects of diabetes-associated hyperglycaemia on the phosphorylation state and TAU metabolism \cite{73–76}, the connection of TAU phosphorylation and sleep disorders in AD patients \cite{77}.

In brief, the phosphorylation state of TAU influences its subcellular distribution and plays an important role in the TAU mislocalization in disease conditions \cite{51–55}. The TAU phosphorylation state in SH-SY5Y cells displays that of healthy human brains and even more that of AD patients \cite{41,46}, and its regulation involves known TAU-interacting kinases/phosphatases \cite{64,66,67}. This comparability makes SH-SY5Y cells not only a valid model for further investigation of TAU phosphorylation, but also provides options for examining the interplay of TAU phosphorylation and intracellular (mis)sorting.
Figure 1: Suitability of SH-SY5Y-derived neurons for TAU sorting research. A) Representative images of undifferentiated SH-SY5Y cells (left, top panel) and SH-SY5Y-derived neurons (left, bottom) in culture (cultures were grown on Poly-D-Lysine (20 µg/ml)-coated glass coverslips in DMEM/F12 (#10565018, TFS) and 10 % fetal bovine serum (BioChrom AG); for differentiation, cells were grown for 7 days in DMEM/F12, 10 % fetal bovine serum and 10 µM retinoic acid (RA), followed by 7 days in serum-free DMEM/F12 and 10 ng/ml brain-derived neurotrophic factor (BDNF). Note the altered morphology and pronounced neurite outgrowth upon differentiation. Scale bar: 50 µm. B) Immunostainings of SH-SY5Y-derived neurons (cells were fixed with 3.7 % FA for 1 h, blocked with 5 % BSA and 0.1 % Triton X-100 for 5 minutes, immunostained with polyclonal anti-TAU (K9JA, 1:1000 in PBS, A0024, DAKO, 2nd AB: donkey anti-rabbit + AlexaFluor488, 1:1000 in PBS, A21202, TFS) and chicken anti-MAP2 (1:2000, ab5392, Abcam, 2nd AB: goat anti-chicken AF647, 1:1000 in PBS, A21449, TFS) antibodies, and mounted (PolyMount, Polysciences), procedure adapted from 78) demonstrate the strong expression and polarized distribution of neuronal maturation markers TAU (green, mainly axonal) and MAP2 (red, mainly somatic). Scale bar (top): 50 µm, scale bar (bottom): 20 µm. C) Western blot analysis of TAU isoform expression (de-phosphorylated lysates) in undifferentiated SH-SY5Y cells (lane 1), differently treated SH-SY5Y-derived neurons (lanes 2 – 5) and human brain lysate (lane 6). The very left lane shows a recombinant TAU standard. Note the abundance of all six isoforms upon differentiation with varying ratios compared to the human brain. The blot was adapted and modified from 18. D) Overview of the TAU protein (grey bar, colored sections indicate distinct TAU domains) and common tauopathy-associated TAU hyperphosphorylation sites (yellow boxes, corresponding epitopes that are recognized by specific antibodies are indicated dashed boxes) that appear highly phosphorylated in SH-SY5Y cells.

Genetic engineering of SH-SY5Y cells. SH-SY5Y-derived neurons display many features of matured neuronal cells, including the post-mitotic character. This makes SH-SY5Y-derived neurons, similarly to primary rodent cultures, inaccessible for stable genetic engineering approaches. However, in the undifferentiated state, SH-SY5Y cells are rapidly dividing and can be used for the stable integration of transgenes, including variants of the TAU-encoding MAPT gene. In the past, transfection and stable integration of linearized 1N3R- and 1N4R-MAPT cDNA into SH-SY5Y cells was used to mimic the misbalance of 3R/4R isoform ratios 79,80, which is...
caused by MAPT variants that affect alternative splicing in several tauopathies (reviewed in 81). The stable overexpression of 4R isoforms was also used to assay effects of proteasomal inhibition on TAU protein levels 82, while integration of a recombinant TAU isoform containing exon 6 unravelled the distinct cellular distribution of this non-canonical TAU isoform similar, which resembles that of MAP2 83. By using site-directed recombination assays, recent studies generated SH-SY5Y cells with stable pro-aggregant TAU versions 72,84. All these approaches have the notable limitation that they are based on artificial overexpression of transgenic TAU isoforms or mutants. The application of recent genome editing techniques, such as CRISPR/Cas9, was shown to work also for SH-SY5Y cells in general 85–89. The use of CRISPR/Cas9 allows to directly target the endogenous MAPT and generate complete knock-out lines or induce mutations on a single base level that are thought to be involved in TAU (mis)sorting, e.g. by using the improved CRISPR/Cas9 prime editing method 90.

However, one has to consider the genetic predispositions of SH-SY5Y cells, as large-scale chromosomal abnormalities and imbalances are reported for neuroblastoma cell lines in general 91–95. Accordingly, SH-SY5Y cells show trisomy of chromosome 7 (chr7), a duplication of the q-arm of chr1, and further complex rearrangements on the majority of chromosomes leading to both copy number gains and losses 96. Besides other loci of neurobiological interest, a copy number gain of the MAPT locus on chr17 was confirmed in different studies 96–100. This genetic arrangement of SH-SY5Y cells complicates the generation of homozygous MAPT mutant knock-out (KO) or knock-in cell lines, as it requires editing of presumably three MAPT gene copies, and also impedes heterozygous edits, as it might be difficult to achieve a 50 % decrease of mutant gene expression. However, the successful generation of a MAPT-KO SH-SY5Y cell line recently demonstrated that the SH-SY5Y cells are accessible for CRISPR/Cas9-based homozygous MAPT editing 101.

The susceptibility to TAU pathology varies drastically among different brain regions, neuronal subtypes and depending on the type of disease\textsuperscript{102–104}. Given the fact, that TAU expression, TAU sorting, AIS formation or TAU isoform ratios also differ among neuronal tissues\textsuperscript{37}, there might be causal connections between these variations and tissue-specific vulnerability to tauopathies like AD. Therefore, the neuronal identity of a cell model for studies addressing intracellular TAU sorting and localization mechanisms is of certain relevance.

Undifferentiated SH-SY5Y cells are considered immature catecholaminergic neurons since they express markers of immature neurons\textsuperscript{23,24} and key proteins of the catecholaminergic metabolism\textsuperscript{14,23,24,105,106}. Interestingly, the reports about the neuronal identity of mature SH-SY5Y-derived neurons vary depending on the substances used for differentiation protocols (Fig. 2A)\textsuperscript{14}. The most common and often-used substance, the vitamin A derivative retinoic acid (RA), was shown to elevate the levels of activated choline acetyltransferase, which is typical for cholinergic neurons\textsuperscript{23,107,108}. However, the cholinergic character of RA-treated cells is under debate, as the expression of noradrenaline\textsuperscript{25} and of the vesicular monoamine transporter, a key enzyme of catecholaminergic neurons, was reported in some studies\textsuperscript{23,107}, but not in others\textsuperscript{17}. Another common procedure, the combinatorial treatment of RA and the brain-derived neurotrophic factor (BDNF) results in extensively branched neurons, which are categorized based on the expression of marker proteins either as noradrenergic\textsuperscript{17}, dopaminergic\textsuperscript{109} or cholinergic\textsuperscript{110}. Besides RA and BDNF, phorbol esters (e.g. Phorbol-12-myristate-13-acetate (TPA))\textsuperscript{25,111–113}, dibutyryl-cAMP (db-cAMP)\textsuperscript{114–116} or other drugs are used alone or in combination\textsuperscript{25,117} to generate SH-SY5Y-derived neurons with varying neuronal identity, e.g. noradrenergic (TPA, db-cAMP) or dopaminergic (RA+TPA).

Taken together, the classification of SH-SY5Y-derived neurons may depend on the applied substances and be influenced by the focuses of the actual study. It is, however, undisputed that SH-SY5Y-derived neurons resemble key features of noradrenergic, dopaminergic and cholinergic neurons. This gives rise to both i) the great potential of SH-SY5Y-derived neurons for studies on neuronal subtype-specific AD/tauopathy susceptibility and ii) the accompanying challenges, as summarized and discussed below (Fig. 2B).
**Chances.** Neuronal loss occurs within several subcortical nuclei in AD patients’ brains and, depending on the nuclei, already in early disease stages. These subcortical nuclei are, amongst others, the Locus coeruleus (LC, harbouring noradrenergic neurons), the Nucleus basalis (NB, cholinergic neurons) and the Substantia nigra (SN, dopaminergic neurons) (reviewed in 118). TAU-NFT formation in NB neurons and massive depletion of acetylcholine within cortical and hippocampal regions, resulting from loss of NB cholinergic projections, coincide with early clinical symptoms of AD 118–124. Also the noradrenergic neurons of the LC complex are early affected by NFT formation and degeneration in AD patients 125–130, and seem to become compromised even in young adults without any clinical phenotype 103. Within the SN, TAU-NFT formation, pigmented neuronal loss and other pathological alterations are found in AD 131–136 and other tauopathies 137,138.

While comprehensive descriptions of TAU-NFT formation and neuronal loss in these subcortical nuclei are available, the pathomechanisms underlying their vulnerability are elusive 139. If SH-SY5Y-derived neurons properly resemble the neuronal subtypes of LC, NB or SN neurons, they may be a powerful tool for the evaluation of subtype-dependent vulnerability. As described above, several aspects mimicking neuronal TAU physiology are available in SH-SH5Y-derived neurons: subcellular distribution, phosphorylation state, isoform expression levels and ratios, isoform-specific intracellular localization of TAU and cell-stress induced development of NFT formation or, at least, NFT-like hyperphosphorylation.

**Challenges.** As for all cellular models of AD or related tauopathies, which are largely ageing-dependent disorders 140–143, it is questionable whether up to three-week-old neuronal cultures can resemble the cellular properties of subcortical neurons in the brain of aged AD patients. Furthermore, there are the mentioned inconsistencies of expression profiles, e.g. of RA- and RA/BDNF-treated cells, and it remains uncertain whether the neuronal subtype can be clearly defined. Especially, this is a considerable issue, since a more comprehensive biochemical characterization of the generated neurons would be expensive and time-consuming, without the guarantee of a conclusive outcome. Indeed, the available data rather suggest that SH-SY5Y-derived neurons do not resemble clearly segregated and distinct neuronal subtypes, which can be separated by protein expression or transmitter release, but rather exhibit different manifestations of a gradual neuronal entity.

The biggest obstacle of using SH-SY5Y-derived neurons may be that major features of LC, NB and SN neurons, which may contribute to their susceptibility for early TAU pathology in disease,
appear to be difficult to recapitulate in cell culture. For LC neurons (reviewed in \textsuperscript{139}), these features include i) the up to several cm-long, thin and poorly myelinated, heavily branched axons spanning throughout the cortex without relay, which leads to high energy costs and oxidative stress \textsuperscript{144–146} (SH-SY5Y-derived neurons: axons range roughly between 50-150 µm for RA, TPA or db-cAMP treatment \textsuperscript{107,111,114,117} and up 200 µm and more for RA/BDNF \textsuperscript{17,20}, and show only moderate branching), ii) increased energy demand and ROS production due to the tonic activity \textsuperscript{147} (SH-SY5Y-derived neurons: increased excitability and membrane potentials \textsuperscript{148–150} but no tonic activity) and iii) elevated exposure to toxins and pathogens as LC neurons innervate the CNS capillary system and associated astrocyte end feet \textsuperscript{151–153}. As all subcortical nuclei share great similarities regarding morphology and innervation \textsuperscript{154,155}, the mentioned risk factors may be largely true for NB and SN neurons, as well \textsuperscript{144,145,156}.

The targeted differentiation of SH-SY5Y cells into neurons with features of noradrenergic, dopaminergic or cholinergic neurons bears great potential for research on AD-selective vulnerability since the mimicked subcortical nuclei are early affected in AD patients. However, the generation of distinct neuronal subtypes does not appear clearly defined with current differentiation procedures, and SH-SY5Y-derived neurons lack major characteristics of their \textit{in vivo} correlates, that might be crucial for tauopathy-related vulnerability.
Figure 2: Treatment-dependent neuronal identity of SH-SY5Y-derived neurons. A) Overview of the reported neuronal subtypes for undifferentiated SH-SY5Y cells and SH-SY5Y-derived neurons with respect to commonly used substances (grey arrows) for differentiation. B) Chances (green) and challenges (orange) of the dirigible differentiation of SH-SY5Y-derived neurons and the reported neuronal subtypes.
5. Conclusion

Human-derived SH-SY5Y neuroblastoma cells are robust, cheap, highly proliferative, and can be differentiated into neuronal cells with straightforward protocols. Differentiated SH-SY5Y-derived neurons meet several requirements of an suitable neuronal cell model for TAU and tauopathy basic research: They exhibit i) fast neuronal maturation and polarity, ii) high levels of total TAU protein, iii) expression of all major human isoforms, iv) efficient axonal targeting of TAU protein, and v) an human brain-like TAU phosphorylation state. Importantly, SH-SY5Y cells are accessible for genetic manipulation, i.e. stable integration of recombinant TAU transgenes and editing of the MAPT locus by means of recent CRISPR/Cas9-based methods prior to neuronal differentiation.

Moreover, SH-SY5Y-derived neurons resemble, depending on the used treatment, neurons of distinct subcortical LC, NB and SN nuclei that are severely affected in AD and other tauopathies. This dirigible differentiation bears great potential for comparative studies of neuron-specific TAU expression patterns, intracellular localization and vulnerability to TAU pathology. However, there are inherent limitations regarding the translatability to human disease, e.g. the lack of age-dependent risk factors, the difficulty of defining the exact neuronal subtype or the lack of brain-spanning projections (on a cm-scale) leading to high energetic costs and oxidative stress. These caveats have to be taken into account when addressing cell type-specific vulnerability in SH-SY5Y-derived neurons.

Nonetheless, the properties of SH-SY5Y-derived neurons discussed in this review make them a powerful neuronal cell model for investigating the mechanisms of and requirements for axonal TAU sorting under human-like conditions.
6. Acknowledgements

We thank Sarah Bachmann and others for critical manuscript revision. This work was funded by Else-Kröner-Fresenius Stiftung and supported by a doctoral fellowship of the Studienstiftung des deutschen Volkes. The authors declare that they have no competing interests.

7. List of Abbreviations

AD, Alzheimer's Disease; AIS, axon initial segment; BDNF, brain-derived neurotrophic factor; CDC2, cell division cycle kinase 2; CDK5, cyclin-dependent kinase 5; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; db-cAMP, dibutyryl-cAMP; hiPSC, human-derived induced pluripotent stem cells; LC, locus coeruleus; MAP2, microtubule-associated protein 2; MAPK, mitogen-activated protein kinase; MAPT, microtubule-associated protein TAU-encoding gene; MT, microtubule, NB, nucleus basalis, NFT, neurofibrillary tangles; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; RA, retinoic acid; Ser, Serine; SN, substantia nigra; Thr, Threonine; TPA, Phorbol-12-myristate-13-acetate.
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