UNIVERSITY OF SZEGED

Doctoral School of Theoretical Medicine

The role of syndecans in pathological protein aggregation

PhD dissertation thesis summary

Anett Hudák

Supervisor: Dr. Tamás Letoha

Szeged

2025

Introduction

Neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), represent one of the most serious health challenges of the 21st century [1]. AD is currently the leading cause of dementia worldwide, and the number of affected individuals is steadily increasing [2]. As current treatments only offer symptomatic relief, early diagnosis of AD is essential to enable intervention before severe central nervous system damage occurs [3]. A key element in this process is the identification of reliable, preclinical biomarkers.

A common pathological hallmark of AD and other neurodegenerative diseases is the abnormal accumulation of misfolded, aggregation-prone proteins—such as Aβ, tau, and α-synuclein [4-6]. These aggregates cause intracellular damage and propagate from cell to cell in a prion-like manner [5,7,8]. Endocytosis plays a central role in their propagation, particularly the lipid raft-dependent, non-canonical pathways that these proteins preferentially utilize for cellular entry [6,9,10].

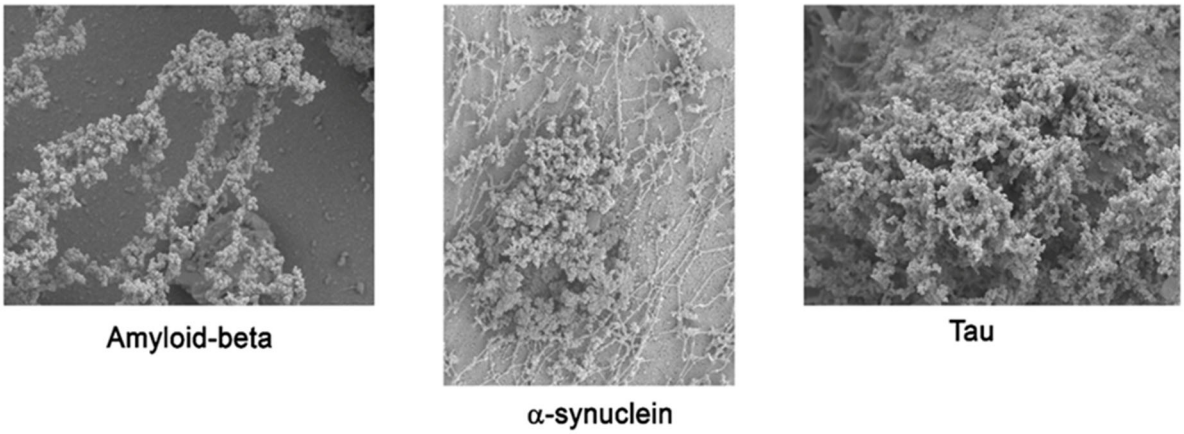

Figure 1. Scanning electron microscopy (SEM) image of toxic protein aggregates that play a major role in neurodegeneration

One of the major regulators of endocytosis is the cell surface heparan sulfate proteoglycan (HSPG) family, in particular the transmembrane syndecans (SDC1-4) [6,11,12]. The glycosaminoglycan (GAG) side chains of these proteins electrostatically interact with aggregating proteins, promoting their uptake and fibrillation [13-15]. Neuronal SDC3 has been shown to be particularly effective in delivering Aβ1-42, α-syn and tau into cells in a lipid raft-dependent manner [5-7,16]. Furthermore, literature data have demonstrated that SDC3 expression is increased in postmortem AD brain tissues, an observation that has led us to investigate this phenomenon in detail in our in vitro and in vivo experimental models,

highlighting the potential biomarker role of SDC3, which may open new perspectives in the early detection of the disease [7,17,18].

Another key player is apolipoprotein E (ApoE), which affects aggregation and uptake by binding to Aβ and HSPGs [19-21]. Our study showed that ApoE2 enhances, whereas ApoE4 inhibits syndecan-mediated Aβ uptake. This interaction is also isoform-specific and affects AD progression.

Our results suggest that syndecans, in particular SDC3, play a key role in the early stages of neurodegenerative diseases by facilitating the entry, intracellular processing and spreading of protein aggregates. Their prominent role makes them promising targets for new diagnostic procedures and therapeutic interventions.

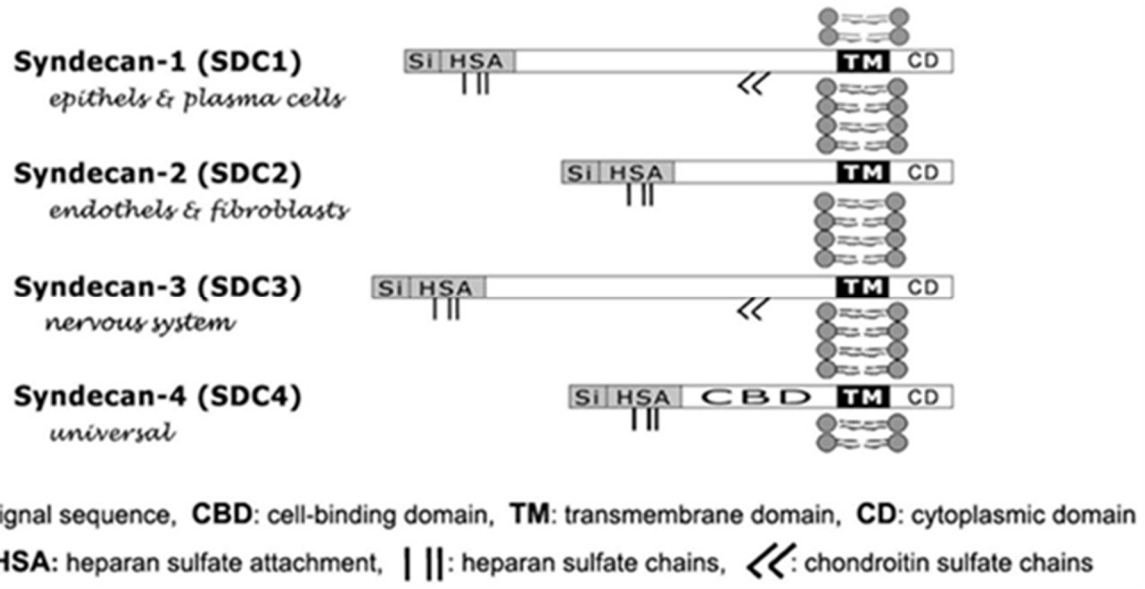

Figure 2. Structural representation of wild-type SDC proteins

Aims

The primary objective of this study is to investigate the role of syndecans (SDCs), a family of heparan sulfate proteoglycans, in the pathogenesis of Alzheimer's disease (AD) and Parkinson's disease (PD). The primary aim of this research was to explore how syndecans mediate the lipid raft-dependent endocytosis of pathologically conformational proteins such as amyloid beta (Aβ), tau and α-synuclein, and how they may contribute to the intracellular processing, prion-like spreading and neurodegeneration of these aggregates.

Furthermore, we aimed to map the isoform-specific interactions between syndecans and aggregation-prone proteins, with a particular focus on their differential roles in the processes of internalisation, intracellular seeding and pathological spreading in the CNS. Our studies have analysed the structural and functional specificities of SDC1-4 with respect to Aβ, tau and α-

synuclein uptake, revealed the mechanisms of endocytosis mediated by SDCs and investigated how ApoE isoforms affect these processes.

Materials and methods

Cell lines and transfections

Human hCMEC/D3 (blood-brain barrier), wild-type K562 (haematopoietic) and SDC overexpressing cell lines (including the mutant SDC4 cell line) and human SH-SY5Y (neuroblastoma) cell lines were used for experiments. Stable expression of the syndecan isoforms (SDC1-4) was established by lentiviral transduction [5,7,21-23]. Cell expression was checked by flow cytometry using APC-labelled antibodies specific for the respective SDC isoforms. The differentiated neuronal phenotype was established in SH-SY5Y cells after 7 days of treatment with retinoic acid (10uM) and 50ng/ml BDNF [5].

Protein uptake and internalization experiments

Aggregation-prone proteins (Aβ1-42, tau and α-synuclein), apolipoproteins (ApoE) and transferrin (Trf) were applied in fluorescent (e.g. FITC-, 5-TAMRA-, or Alexa Fluor 633 and HiLyte Fluor 647-labeled) format. They were added to cells at concentrations of 5 μM, 500 nM or 25 ug/ml at different time points (1h, 3h, 6h, 18h) and their uptake was measured by flow cytometry (FACScan, Becton Dickinson, AMNIS FlowSight) and confocal microscopy (Olympus FV1000) [5,7,21].

Aggregation studies

Thioflavin T (ThT) binding assays were used to evaluate the formation of amyloid fibrils in different cell lines. WT K562, SH-SY5Y and SDC transfected cells were plated in 96-well microplates and incubated with Aβ1-42 (5 μM), α-syn or tau (5 μM) monomers at different time intervals (1, 3, 6 and 18 h) [5,7,21]. Following incubation, ThT (15 μM) was added to the cell cultures 10 min before measurement, and fluorescence intensity was measured using a plate reader (Biotek Cytation 3) at excitation wavelength 440 nm and emission wavelength 480 nm [24]. The multiple change in fluorescence intensity relative to the background ThT signal was calculated to determine the extent of fibril formation in Aβ1-42, α-syn or tau-treated cells. In separate experiments, Aβ1-42 fibrils (5 μM) were incubated with SH-SY5Y and K562 cells, including SDC transfectants and SDC4 mutants, for up to 18 h. The cell surface binding and fibril formation of Aβ1-42 were also analyzed by SEM (scanning electron microscopy) techniques [5].

Lipid raft-dependent endocytosis study

Lipid raft-associated endocytosis was confirmed by colocalisation studies with flotillin-1 and -2 antibodies, including confocal microscopy and co-immunoprecipitation studies. Flotillin-1 and 2 proteins were labeled with Alexa Fluor-546 fluorescent antibody.

Inhibition of GAG chains

The function of cell-surface glucosaminoglycans, in particular heparan sulfate (HS) chains, was inhibited with sodium chlorate (NaClO3) (50 mM, 48 h), which inhibits the sulfation step. The effect of the treatment was checked by flow cytometer.

Analysis of syndecan-3 expression in APPSWE-Tau transgenic animal model

SDC3 expression was measured by immunohistochemical staining and ELISA in brain tissue from 12-month-old transgenic APPSWE-Tau and wild-type C57BL6 mouse models [17]. Samples were derived from paraffin-embedded brain slices fixed in 4% paraformaldehyde. Mouse brain endothelial cells were isolated using the method of Assmann et al. [25]. SDC3 expression of monocytes and primary brain endothelial cells (PBEC) isolated from mice was analyzed using mouse SDC3 antibody and specific monocyte or endothelial markers (CD11b or PECAM-1) by flow cytometry imaging [17].

Statistical analysis

Results are expressed as mean values ± standard error of the mean (SEM). Differences between experimental groups were evaluated by one-way analysis of variance (ANOVA). Values of $p < 0.05$ were accepted as significant. Pearson's correlation coefficient was calculated for the correlation between SDC3 or HS expression and Aβ1-42 uptake and fibrillation values of SH-SY5Y cells. For colocalization analyses, Mander's overlap coefficient (MOC) was calculated as described by Wesen et al. [26]. Pearson's correlation coefficient was used to measure the strength of the linear relationship between the two variables [17].

Results

We investigated the role of syndecans (SDCs) in the uptake and fibrillation of amyloid-β (Aβ1-42). Although heparan sulfate proteoglycans (HSPGs) are known to promote Aβ1-42 binding and internalization, the specific involvement of transmembrane SDCs remains unclear [7]. In order to isolate the contribution of SDCs, we generated transfectants expressing stable SDCs in K562 cells, a cell line with minimal endogenous HSPGs, no syndecan or glypican expression, and lacking caveolin-1, thereby limiting other endocytic pathways [5,7,27,28]. Selected

transfectants with similar levels of heparan sulfate (HS) and wild-type (WT) K562 cells were incubated with FITC-tagged Aβ1-42 or transferrin (Trf, a clathrin-mediated endocytosis marker) for 1, 3, 6 and 18 h. Uptake and binding were quantified by flow cytometry, using trypan blue dye to quench extracellular fluorescence [7,29]. Flow cytometry showed that SDC3 transfectants showed the highest internalization of Aβ1-42 at all time points, followed by SDC4 (which outperformed WT cells after 6 h), and SDC2 showed increased uptake only after 18 h. SDC1 showed lower uptake after 18 h. In contrast, uptake of Trf was higher in WT cells than in most SDC transfectants, suggesting different endocytic pathways for Aβ1-42 and Trf (Figure 3).

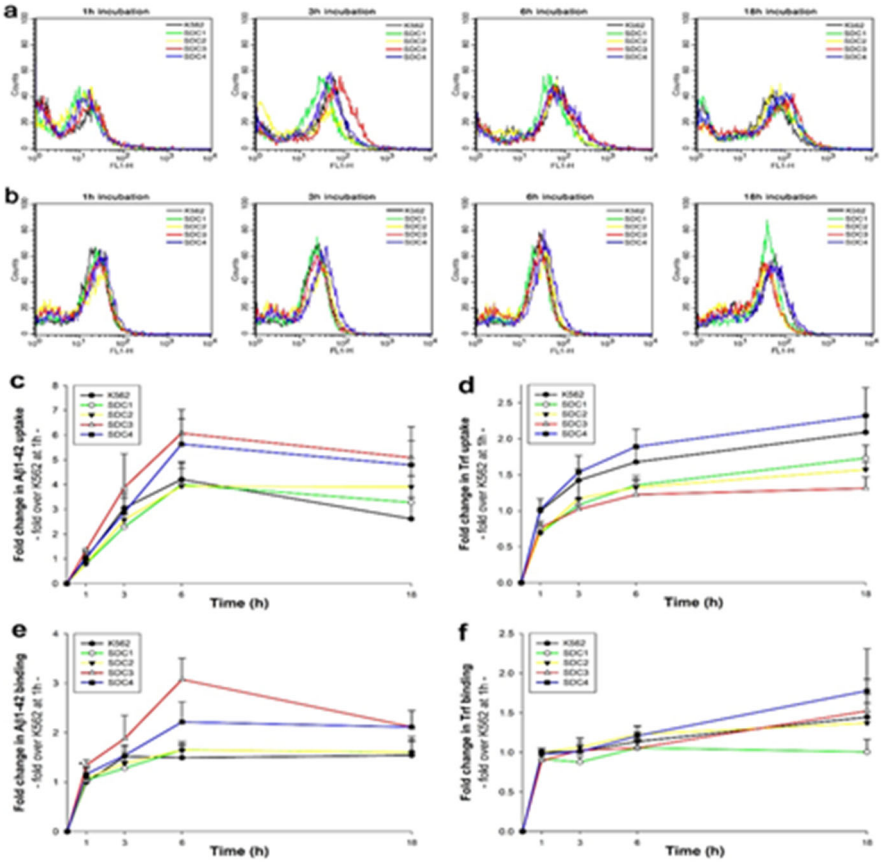

Figure 3. Kinetics of Aβ1-42 and Trf uptake. WT K562 cells and SDC transfectants were FITC-labeled with FITC-labeled Aβ1-42 or Trf for 1, 3, 6 and 18 h at 37 °C. Cell uptake and attachment were analyzed by flow cytometry.

Thioflavin T (ThT) fluorescence assays showed that SDC3 and SDC4 significantly promoted Aβ1-42 fibrillation ($p < 0.05$). Our scanning electron microscopy (SEM) studies confirmed enhanced fibrillar Aβ1-42 aggregation on SDC3 and SDC4 transfectants after only 1 h of incubation. Confocal microscopy further confirmed these results, showing increased intracellular Aβ1-42 fibrils and colocalization with SDCs, especially SDC3, after 18 h ($p < 0.01$). The Mander's overlap coefficient (MOC > 0.7) indicated strong intracellular

colocalization between SDCs and Aβ1-42, in contrast to reduced Trf internalization in SDC transfectants, confirming different uptake pathways.

Flotillins, markers of lipid rafts, colocalized intracellularly with SDCs and Aβ1-42. Microscopic data confirmed that SDC-mediated Aβ1-42 internalization occurs via flotillin-dependent lipid rafts, independent of clathrin or caveolin pathways. Inhibition of HS sulfation with sodium chlorate significantly reduced Aβ1-42 binding and fibrillation, particularly in SDC3 and SDC4 cells. Using SDC4 structural mutants, we found that the presence of HS chains was crucial for Aβ1-42 uptake - mutants lacking HS chains did not increase uptake, while those retaining HS chains showed uptake similar to WT SDC4.

In neuronal SH-SY5Y cells, SDC3 overexpression enhanced Aβ1-42 uptake and fibrillation, and there was a significant correlation between SDC3 expression and these processes ($r = 0.63$ and $r = 0.80$, respectively), highlighting the key role of SDC3 in neurons.

We extended our analysis to α-synuclein (α-syn) and tau fibrils, proteins that also play a key role in neurodegeneration. Using SDC-overexpressing K562 cells, we found that the predominantly neuronal SDC3 significantly increased the uptake of both α-syn and tau fibrils, while SDC4 had minimal effect. Uptake was lipid raft-dependent, but occurred via clathrin- and caveolin-independent pathways, as shown by flotillin colocalization. Incubation of monomeric α-syn and tau with SDC transfectants showed that SDC3 (and to a lesser extent SDC4) promoted their fibrillation over time. After 18 h, our scanning electron microscopy experiment revealed increased mature fibrils on the surface of cells expressing SDCs, supporting that SDCs induce fibrillation. Remarkably, fibril formation enhanced SDC-mediated uptake, suggesting a feedback mechanism promoting the internalization of aggregated proteins.

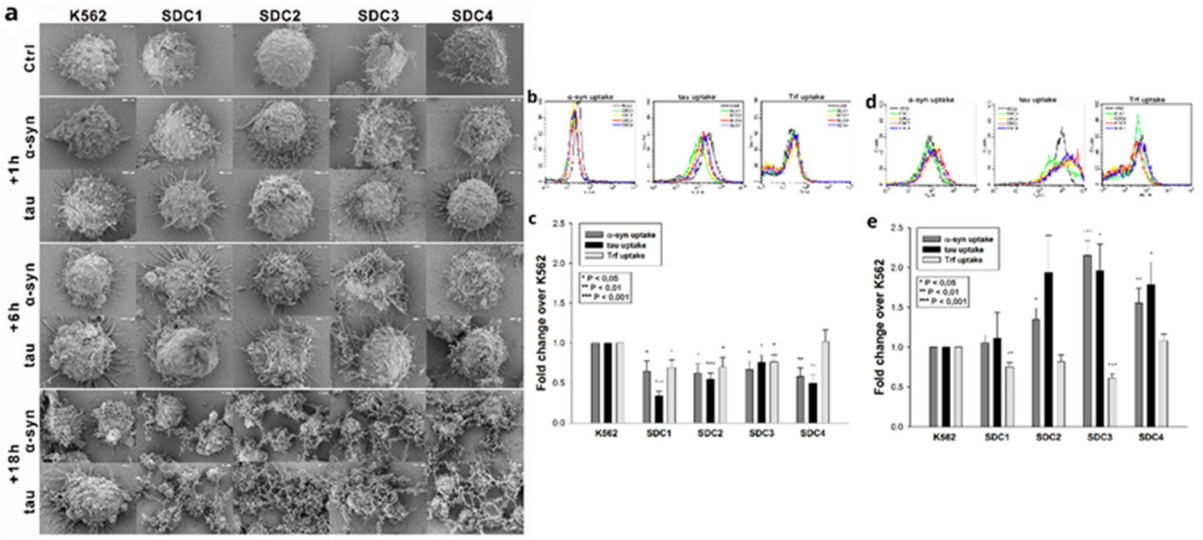

Figure 4. Cellular uptake of α-syn and tau fibrils into SDC transfectants. a) Scanning electron microscopy images of α-syn and tau fibrils and WT K562 cells and SDC transfectants treated with fibrils after 10 min and 3 h of incubation.

Syndecans (SDCs) enhance cellular internalization of apolipoprotein E (ApoE) isoforms, and therefore the uptake of ApoE isoforms was investigated in SDC-overexpressing cell lines. The low expression of heparan sulfate proteoglycan (HSPG) in the K562 cell line and the lack of caveloae ability to induce caveloae endocytosis make this model ideal for isolated studies of the effect of SDCs overexpression on ApoE uptake without the influence of other HSPGs or caveloae-mediated endocytosis. Taking into account the heparan sulfate chains involved in ApoE binding, SDC transfectants were selected with similar HS expression and treated with FITC-tagged ApoE2, ApoE3 and ApoE4 isoforms, and wild-type (WT) K562 cells. It is important to note that SDC overexpression did not affect LRP1 receptor expression, so LRP1 levels in SDC transfectants and WT K562 cells were identical. After 3 h of incubation, fluorescence of extracellularly bound ApoE was quenched by the addition of trypan blue and the amount of ApoE internalized into the cells was measured by flow cytometry. Our results showed that the overexpression of SDCs increased cellular uptake of ApoE in an isoform-specific manner, with neuronal SDC3 showing the most significant effect. Among the SDCs, ApoE2 and ApoE3 uptake is enhanced the most, while ApoE4 had a more modest effect. The overall fluorescence intensity of SDC transfectant cells was significantly increased by ApoE treatment, indicating a concomitant increase in ApoE binding and internalization.

Colocalization studies measured by Manders' overlap coefficient (MOC) and Pearson's correlation coefficient (PCC) demonstrated that SDC3 and SDC4 showed the strongest association with ApoE2 and ApoE3 isoforms (MOC ~0.5; PCC ~0.4), while the association of SDC1 with ApoE was slightly weaker (MOC ~0.4; PCC ~0.3) and was lowest for SDC2. In immunoprecipitation assays, ApoE2 showed the strongest binding to SDC3, followed by ApoE3 and ApoE4, supporting the isoform-specific differences observed in the uptake.

We then further investigated the interaction between ApoE and SDC3 in the SH-SY5Y cell line, widely used as a neuronal model, both in the undifferentiated and differentiated state. Here again, similar colocalization values were measured (MOC ~0.5; PCC ~0.4 for ApoE2 and ApoE3), confirming our preliminary observations. Overexpression of SDC3 in differentiated SH-SY5Y cells increased the uptake of all ApoE isoforms, most notably ApoE2, while ApoE4 internalization was lowest. The increase in ApoE uptake was proportional to the increase in SDC3 expression.

Our study also included the aggregation of amyloid-β (Aβ1-42) in SDC3-overexpressing SH-SY5Y cells. Overexpression of SDC3 increased Aβ1-42 fibrillation after 18 h of incubation, whereas there was no significant effect at 3 h. ApoE isoforms differentially modulated Aβ1-42 aggregation: ApoE2 reduced aggregation even in SDC3-overexpressing cells, whereas ApoE4 significantly enhanced it. Our electron microscopy studies confirmed these results. The ApoE isoforms also affected the cellular uptake of Aβ1-42 differently: at 3 h, ApoE2 increased, whereas ApoE4 decreased, the internalization of Aβ1-42; however, at 18 h, this effect was reversed, with ApoE4 increasing the uptake of Aβ1-42, whereas ApoE2 and ApoE3 decreased it.

ApoE4 was also capable of fibrillation independently, as confirmed by fluorescence and electron microscopy studies, especially after 18 h of treatment, while this phenomenon was less pronounced with ApoE3 and no fibril formation was found with ApoE2. Further studies have shown that overexpression of SDC3 in differentiated SH-SY5Y cells significantly enhances the cellular uptake of Aβ1-42, which is differentially modified by ApoE isoforms. ApoE2 decreased and ApoE4 increased the presence of Aβ1-42 aggregates after 18 h of incubation as confirmed by confocal microscopy. The mutual colocalization of ApoE4 and Aβ1-42 fibrils was also confirmed by Mander and Pearson coefficients (~0.5 and ~0.45), consistent with the previous association of AD plaques with ApoE4.

Taken together, the results show that SDCs, in particular SDC3, enhance the internalization of ApoE isoforms in a cell type- and isoform-dependent manner, and that ApoE isoforms differentially regulate the aggregation and cellular uptake of Aβ1-42, shedding new light on the role of ApoE in amyloid pathology. These findings highlight the potential use of SDC3 as a therapeutic target to manipulate ApoE-mediated cellular processes in neurodegenerative diseases.

The role of SDC3 in endothelial cells is also gaining prominence in inflammatory responses, where SDC3 expression in inflamed vascular endothelial cells plays a role in the binding of leukocytes and chemokines, contributing to the development of neuroinflammation and neurodegeneration in Alzheimer's disease [17,30]. In light of this, SDC3 may even be used as a blood-based biomarker in disease diagnosis and monitoring.

Under inflammatory conditions modeled by TNF-α treatment (7 days, 5 ng/ml), endogenous SDC3 expression was significantly increased in both SH-SY5Y and hCMEC/D3 cells, further confirming the key role of SDC3 in the neuroinflammatory processes of AD. In the brains of APPSWE-Tau transgenic mice, increased Aβ plaque deposition was accompanied by significantly elevated TNF-α concentrations, as confirmed by ELISA. TNF-α levels measured

in whole blood samples from APPSWE-Tau mice were also significantly higher compared to wild-type (WT) controls. TNF-α levels in the blood and brain showed a strong positive correlation with a covariance of 0.80, indicating the co-presence of systemic and CNS inflammation.

Furthermore, our ELISA assays showed that SDC3 levels were elevated not only in the brain but also in peripheral organs such as the liver, supporting our preliminary in vitro results that TNF-α induces SDC3 expression and suggesting that the inflammatory cytokine induces increased SDC3 expression in both central and peripheral tissues.

Considering the increasing importance of the role of endothelial cell SDC3 in inflammatory processes and our previous in vitro data showing that TNF-α induces SDC3 expression in endothelial cells of the human blood-brain barrier, we examined SDC3 expression in primary blood-brain barrier endothelial cells (PBEC) isolated from APPSWE-Tau mice. The SDC3 expression of PBECs from APPSWE-Tau mice was significantly higher than that of cells isolated from WT controls, suggesting that the inflammatory environment also enhances SDC3 levels in the brain endothelium.

Furthermore, as the role of monocytes in the pathogenesis of AD is becoming increasingly prominent, we also examined the SDC3 expression of monocytes isolated from the blood of APPSWE-Tau mice [17,31]. SDC3 expression was significantly increased in monocytes from APPSWE-Tau mice compared to WT mice. Interestingly, the level of SDC3 expression in monocytes showed a strong positive correlation with brain Aβ plaque deposition (r = 0.81), suggesting that SDC3 levels in monocytes may serve as a potential biomarker for assessing the severity of amyloid pathology.

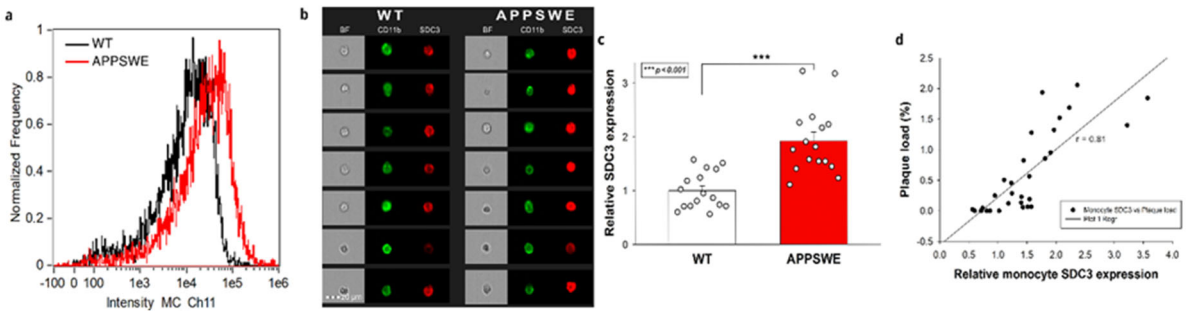

Figure 5. Monocytes isolated from APPSWE-Tau mice show increased SDC3 expression.

Taken together, these results confirm that TNF-α-induced SDC3 expression not only in the nervous system but also on peripheral immune cells and endothelial cells plays a significant role in the progression of AD, and that SDC3 as a molecular switch may mediate inflammatory and amyloid pathological processes between the brain and the peripheral system.

Conclusions and new scientific findings for the future

Among the neurodegenerative diseases, Alzheimer's disease (AD) represents a major societal and health challenge, as it severely impairs cognitive function and quality of life [32,33]. In the present study, we focused on the molecular pathogenesis of AD, with a particular focus on the role of the syndecan proteoglycan family. Our results provide clear evidence that SDC3 plays a prominent role in the formation and intracellular spread of abnormal protein aggregations. SDC3-mediated lipid raft-dependent endocytosis is particularly efficient in the neuronal uptake of Aβ1-42 oligomers, a key mechanism of intraneuronal amyloid accumulation and fibrillation. In addition, SDC3 promotes the intercellular spread of fibrillar tau and α-synuclein forms, which contributes to the progression of neurodegenerative processes. Furthermore, our study demonstrated that ApoE isoforms modulate SDC3-mediated Aβ uptake, with ApoE2 being protective and ApoE4 being risk-increasing. The increased presence of SDC3 further enhances ApoE4-induced amyloid fibrillation, thus influencing amyloid pathology in a complex manner. In terms of inflammatory mechanisms, we show that TNF-α cytokine significantly increases SDC3 expression in the nervous system and blood-brain barrier, which is strongly associated with amyloid plaques and systemic inflammation. Increased SDC3 expression in peripheral monocytes may serve as a potential biomarker for early detection of AD and disease monitoring. Increased endothelial expression of SDC3 may promote transendothelial migration of monocytes, linking neuroinflammation to amyloid pathology.

Our future research will aim to elucidate in detail the molecular mechanisms mediated by SDC3 and to investigate the efficacy of potential therapeutic interventions that may slow disease progression. Overall, our work will enrich the current knowledge of the molecular pathology of Alzheimer's disease and contribute to the development of innovative diagnostic and therapeutic strategies for the treatment of this serious neurodegenerative disease.

Acknowledgements

I would like to thank my thesis supervisor, Dr. Tamás Letoha for the theoretical and practical guidance and the opportunity to carry out the experiments in the laboratory of Pharmacoidea Ltd. in Szeged. I would also like to thank the Doctoral School of Theoretical Medicine of the University of Szeged for the scientific framework and the opportunity to pursue my PhD studies as an individual student.

References

1. Qian, X.; Hamad, B.; Dias-Lalcaca, G. The Alzheimer disease market. *Nat Rev Drug Discov* **2015**, *14*, 675-676, doi:10.1038/nrd4749.

2. Nussbaum, R.L.; Ellis, C.E. Alzheimer's disease and Parkinson's disease. *N Engl J Med* **2003**, *348*, 1356-1364, doi:10.1056/NEJM2003ra020003.
3. Yiannopoulou, K.G.; Papageorgiou, S.G. Current and Future Treatments in Alzheimer Disease: An Update. *J Cent Nerv Syst Dis* **2020**, *12*, 1179573520907397, doi:10.1177/1179573520907397.
4. Guo, F.; Liu, X.; Cai, H.; Le, W. Autophagy in neurodegenerative diseases: pathogenesis and therapy. *Brain Pathol* **2018**, *28*, 3-13, doi:10.1111/bpa.12545.
5. Hudak, A.; Kusz, E.; Domonkos, I.; Josvay, K.; Kodamullil, A.T.; Szilak, L.; Hofmann-Apitius, M.; Letoha, T. Contribution of syndecans to cellular uptake and fibrillation of alpha-synuclein and tau. *Sci Rep* **2019**, *9*, 16543, doi:10.1038/s41598-019-53038-z.
6. Hudák Anett, L.T. Endocytic Pathways Unveil the Role of Syndecans in the Seeding and Spreading of Pathological Protein Aggregates: Insights into Neurodegenerative Disorders. *International Journal of Molecular Sciences* **2025**, doi:10.3390/ijms26094037.
7. Letoha, T.; Hudak, A.; Kusz, E.; Pettko-Szandtner, A.; Domonkos, I.; Josvay, K.; Hofmann-Apitius, M.; Szilak, L. Contribution of syndecans to cellular internalization and fibrillation of amyloid-beta(1-42). *Sci Rep* **2019**, *9*, 1393, doi:10.1038/s41598-018-37476-9.
8. Davis, A.A.; Leyns, C.E.G.; Holtzman, D.M. Intercellular Spread of Protein Aggregates in Neurodegenerative Disease. *Annu Rev Cell Dev Biol* **2018**, *34*, 545-568, doi:10.1146/annurev-cellbio-100617-062636.
9. Steiner, J.A.; Angot, E.; Brundin, P. A deadly spread: cellular mechanisms of alpha-synuclein transfer. *Cell Death Differ* **2011**, *18*, 1425-1433, doi:10.1038/cdd.2011.53.
10. El-Sayed, A.; Harashima, H. Endocytosis of gene delivery vectors: from clathrin-dependent to lipid raft-mediated endocytosis. *Mol Ther* **2013**, *21*, 1118-1130, doi:10.1038/mt.2013.54.
11. Christianson, H.C.; Belting, M. Heparan sulfate proteoglycan as a cell-surface endocytosis receptor. *Matrix Biol* **2014**, *35*, 51-55, doi:10.1016/j.matbio.2013.10.004.
12. Shi, D.; Sheng, A.; Chi, L. Glycosaminoglycan-Protein Interactions and Their Roles in Human Disease. *Front Mol Biosci* **2021**, *8*, 639666, doi:10.3389/fmolb.2021.639666.
13. Maiza, A.; Chantepie, S.; Vera, C.; Fifre, A.; Huynh, M.B.; Stettler, O.; Ouidja, M.O.; Papy-Garcia, D. The role of heparan sulfates in protein aggregation and their potential impact on neurodegeneration. *FEBS Lett* **2018**, *592*, 3806-3818, doi:10.1002/1873-3468.13082.
14. Sarrazin, S.; Lamanna, W.C.; Esko, J.D. Heparan sulfate proteoglycans. *Cold Spring Harb Perspect Biol* **2011**, *3*, doi:10.1101/cshperspect.a004952.
15. Costanzo, M.; Zurzolo, C. The cell biology of prion-like spread of protein aggregates: mechanisms and implication in neurodegeneration. *Biochem J* **2013**, *452*, 1-17, doi:10.1042/BJ20121898.
16. Asundi, V.K.; Carey, D.J. Self-association of N-syndecan (syndecan-3) core protein is mediated by a novel structural motif in the transmembrane domain and ectodomain flanking region. *J Biol Chem* **1995**, *270*, 26404-26410, doi:10.1074/jbc.270.44.26404.
17. Hudak, A.; Letoha, A.; Vizler, C.; Letoha, T. Syndecan-3 as a Novel Biomarker in Alzheimer's Disease. *Int J Mol Sci* **2022**, *23*, doi:10.3390/ijms23063407.
18. Liu, C.C.; Zhao, N.; Yamaguchi, Y.; Cirrito, J.R.; Kanekiyo, T.; Holtzman, D.M.; Bu, G. Neuronal heparan sulfates promote amyloid pathology by modulating brain amyloid-beta clearance and aggregation in Alzheimer's disease. *Sci Transl Med* **2016**, *8*, 332ra344, doi:10.1126/scitranslmed.aad3650.
19. Kim, J.; Basak, J.M.; Holtzman, D.M. The role of apolipoprotein E in Alzheimer's disease. *Neuron* **2009**, *63*, 287-303, doi:10.1016/j.neuron.2009.06.026.
20. Fortea, J.; Pegueroles, J.; Alcolea, D.; Belbin, O.; Dols-Icardo, O.; Vaque-Alcazar, L.; Videla, L.; Gispert, J.D.; Suarez-Calvet, M.; Johnson, S.C.; et al. APOE4 homozygozity represents a distinct genetic form of Alzheimer's disease. *Nat Med* **2024**, *30*, 1284-1291, doi:10.1038/s41591-024-02931-w.

21. Hudak, A.; Josvay, K.; Domonkos, I.; Letoha, A.; Szilak, L.; Letoha, T. The Interplay of Apoes with Syndecans in Influencing Key Cellular Events of Amyloid Pathology. *Int J Mol Sci* **2021**, *22*, doi:10.3390/ijms22137070.
22. Letoha, T.; Keller-Pinter, A.; Kusz, E.; Kolozsi, C.; Bozso, Z.; Toth, G.; Vizler, C.; Olah, Z.; Szilak, L. Cell-penetrating peptide exploited syndecans. *Biochim Biophys Acta* **2010**, *1798*, 2258-2265, doi:10.1016/j.bbamem.2010.01.022.
23. Letoha, T.; Kolozsi, C.; Ekes, C.; Keller-pinter, A.; Kusz, E.; Szakonyi, G.; Duda, E.; Szilak, L. Contribution of syndecans to lipoplex-mediated gene delivery. *Eur J Pharm Sci* **2013**, *49*, 550-555, doi:10.1016/j.ejps.2013.05.022.
24. Xue, C.; Lin, T.Y.; Chang, D.; Guo, Z. Thioflavin T as an amyloid dye: fibril quantification, optimal concentration and effect on aggregation. *R Soc Open Sci* **2017**, *4*, 160696, doi:10.1098/rsos.160696.
25. Assmann, J.C.; Muller, K.; Wenzel, J.; Walther, T.; Brands, J.; Thornton, P.; Allan, S.M.; Schwaninger, M. Isolation and Cultivation of Primary Brain Endothelial Cells from Adult Mice. *Bio Protoc* **2017**, *7*, doi:10.21769/BioProtoc.2294.
26. Wesen, E.; Jeffries, G.D.M.; Matson Dzebo, M.; Esbjorner, E.K. Endocytic uptake of monomeric amyloid-beta peptides is clathrin- and dynamin-independent and results in selective accumulation of Abeta(1-42) compared to Abeta(1-40). *Sci Rep* **2017**, *7*, 2021, doi:10.1038/s41598-017-02227-9.
27. Attisano, L.; Wrana, J.L.; Lopez-Casillas, F.; Massague, J. TGF-beta receptors and actions. *Biochim Biophys Acta* **1994**, *1222*, 71-80, doi:10.1016/0167-4889(94)90026-4.
28. Shi, W.; Bartlett, J.S. RGD inclusion in VP3 provides adeno-associated virus type 2 (AAV2)-based vectors with a heparan sulfate-independent cell entry mechanism. *Mol Ther* **2003**, *7*, 515-525, doi:10.1016/s1525-0016(03)00042-x.
29. Busetto, S.; Trevisan, E.; Patriarca, P.; Menegazzi, R. A single-step, sensitive flow cytofluorometric assay for the simultaneous assessment of membrane-bound and ingested Candida albicans in phagocytosing neutrophils. *Cytometry A* **2004**, *58*, 201-206, doi:10.1002/cyto.a.20014.
30. Arokiasamy, S.; Balderstone, M.J.M.; De Rossi, G.; Whiteford, J.R. Syndecan-3 in Inflammation and Angiogenesis. *Front Immunol* **2019**, *10*, 3031, doi:10.3389/fimmu.2019.03031.
31. Floris, S.; van den Born, J.; van der Pol, S.M.; Dijkstra, C.D.; De Vries, H.E. Heparan sulfate proteoglycans modulate monocyte migration across cerebral endothelium. *J Neuropathol Exp Neurol* **2003**, *62*, 780-790, doi:10.1093/jnen/62.7.780.
32. Wang, S.; Jiang, Y.; Yang, A.; Meng, F.; Zhang, J. The Expanding Burden of Neurodegenerative Diseases: An Unmet Medical and Social Need. *Aging Dis* **2024**, doi:10.14336/AD.2024.1071.
33. Wortmann, M. Dementia: a global health priority - highlights from an ADI and World Health Organization report. *Alzheimers Res Ther* **2012**, *4*, 40, doi:10.1186/alzrt143.