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Semin Kang March 30, 2022

Investigating LSD1 interaction with tau aggregates by examining LSD1 in rare tauopathy cases

by

Semin Kang

David Katz
Adviser

Neuroscience and Behavioral Biology

David Katz

Adviser

Jie Jiang

Committee Member

William Kelly

Committee Member

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Abstract

Investigating LSD1 interaction with tau aggregates by examining LSD1 in rare tauopathy cases

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Alzheimer's disease (AD), which affects over 10 million people across the world per year, is a secondary tauopathy characterized by abnormal aggregation of β-amyloid plaques and neurofibrillary tangles of hyperphosphorylated tau (NFTs). However, the mechanism underlying tau-mediated neurodegeneration is not well studied. Previously, our lab has shown that the inhibition of the lysine-specific histone demethylase, LSD1 in adult mice induces cortical and hippocampal neurodegeneration, learning and memory deficits, and transcription alternations that match human AD cases. Additionally, we have found that reduction of LSD1 in PS19 tau mice, a transgenic line that overexpresses an aggregation prone version of tau throughout the nervous system, exacerbates neurodegeneration while overexpression of LSD1 rescues hippocampal neurodegeneration. Most importantly, LSD1 colocalizes with cytoplasmic pathological tau in P301S tau mice and human AD cases. Based on this, we hypothesize that after translation, LSD1 interacts with pathological tau in the cytoplasm. This process will prevent LSD1 from entering the nucleus and cause neurodegeneration. AD and other types of rare tauopathy cases, such as Corticobasal Degeneration (CBD), Progressive supranuclear palsy (PSP), Pick's Disease, and Frontotemporal dementia with parkinsonism-17 cases have varying tau pathology. This provides an unique opportunity to interrogate the specificity of LSD1's interaction with pathological tau. To examine this specificity, we perform LSD1 immunohistochemistry in tauopathy and age matched non-demented cases. We recapitulated the pathological tau tangles in AD cases. In addition, we observed some abnormally shaped LSD1 staining in rare tauopathies cases and noted that the LSD1 may be primarily interacting with 4R isoforms of tau rather than the 3R isoform, specifically associating with the tufted astrocytes in the PSP pathology. We believe that this finding of potential LSD1 interaction can provide further insight into how LSD1 can provide a linkage to addressing the neurodegenerative diseases of various tauopathies.

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CHAPTER I

Introduction

Currently more than 55 million people live with dementia worldwide, and there are nearly 10 million new cases every year (World Health Organization, 2021). Alzheimer's Disease (AD), as the most prevalent form of dementia, was shown to be the only cause of death in America's top 10 causes of death that cannot be prevented or cured (alz.org, 2014).

Tauopathy, as a subgroup of neurodegenerative diseases, is characterized by the deposition of pathological tau in the brain. AD, the most common neurodegenerative disease, is a tauopathy characterized by abnormal aggregation of β-amyloid plaques and neurofibrillary tangles of hyperphosphorylated tau (NFTs) (Gamblin et al., 2003). Yet, the spectrum of tau pathologies goes beyond the AD to include Pick disease, progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), etc. (Kovacs, 2017).

Tau is a microtubule binding protein that plays a fundamental role in stabilizing microtubules (MTs) in the axons of neurons (Wang, 2016). By alternative splicing of the MAPT gene, there are six tau isoforms expressed in the adult human brain (Barbier, 2019). Tau protein consists of a N-terminal projection domain, a proline-rich domain and a c-terminal MT binding domain (Barbier, 2019). The positively charged proline-rich domain plays an important role in binding with negatively charged MTs (Barbier, 2019) and the post-translational phosphorylation could mediate the bind affinity of tau against TMs (Barbier, 2019).

A disruption of this microtubule network, which can be caused by tau loss of function, is the bridge to tauopathies (Barbier, 2019). When the tau loses its function, it becomes hyperphosphorylated and accumulates abnormally in the somata and dendrites of affected cells and forms pathological filamentous inclusions (paired helical filaments, or PHFs), which

aggregate to form insoluble neurofibrillary tangles (Hall, 1997). Pathological tau has been associated with neuronal cell death, on top of synaptic loss and increased inflammation (Gendron, 2009), but how pathological tau causes/contributes to neurodegeneration is not well understood. Thus, the objective of this project is to investigate the molecular mechanisms underlying tau-mediated neurodegeneration, which could be used as a potential therapeutic target.

Lysine-specific histone demethylase, LSD1, represses transcription by removing mono- or di-methyl groups from lysine 4 histone 3 (H3K4me1/2) (Shi et al., 2004). Our lab has found that LSD1 specifically colocalized with cytoplasmic tau aggregates in human AD cases, suggesting that pathological tau could sequester LSD1 from the nucleus into cytoplasm and inhibit its function (Figure 1, Christopher et al., 2017). To more clearly determine the role of LSD1, our lab addressed this question by crossing floxed *Lsd1* mice to the *Cagg-Cre* tamoxifen inducible *Cre* transgene to engender inducibly deleted LSD1 in adult mice. Tamoxifen injection resulted in the widespread loss of LSD1 protein in hippocampal and cerebral cortex neurons, with the astrocytes and oligodendrocytes staying intact, revealing that within the brain LSD1 loss is confined to neurons (Christopher et al., 2017). Widespread severe neurodegeneration was accordingly noted in the hippocampus and cerebral cortex within weeks of *Lsd1* deletion and through this, our lab was able to verify that LSD1 is continuously required to prevent neurodegeneration (Christopher et al., 2017). Furthermore, deletion of LSD1 in adult mice induces cortical and hippocampal neurodegeneration implicating learning and memory deficits. We have also found transcription alterations induced by loss of LSD1 that match human AD cases (Christopher et al., 2017). Taken together, LSD1 was able to be concluded as the pathway

that can be studied further, divulging its functional interaction with pathological tau leading to various tauopathies.

To address this interaction, our lab utilized PS19 tau mice, a tauopathy mice model which expresses P301S mutated form of tau from human familial frontotemporal dementia patients throughout the central nerve system (Engstrom et al., 2019, Yoshiyama et al., 2007). First, we found LSD1 is sequestered into the cytoplasm of hippocampal and cortical neurons from PS19 tau mice (Figure 2), which is very similar to what we observed in human AD cases (Engstrom et al., 2019). Therefore, it was possible to study the functional interaction between LSD1 and pathological tau by manipulating the level of LSD1 in PS19 tau mice. This was achieved by deleting the LSD1 manually and enabling the deletion to proceed through the germline, removing one copy of *Lsd1* in PS19 tau mice to produce PS19 tau mice heterozygous for LSD1 (PS19; Lsd1Δ/+). Through this step, we found that there was a significant decrease in the nuclear localization of LSD1 compared to PS19 tau mice, suggesting that the lowered levels of LSD1 allows tau to accelerate the depletion of LSD1 (Engstrom et al., 2019). Additionally, the result has also shown PS19 tau mice to exacerbate neurodegeneration in the brain and the recorded survival was significantly exacerbated along with symptoms of paralysis on PS19; Lsd1Δ/+. This included hind limb clasping that led to inability to feed itself (Engstrom et al., 2019). Finally, the reduction of LSD1 worsens the transcriptional alteration in PS19 tau mice, suggesting the specificity of tau functioning through LSD1. Logically, if reducing the level of LSD1 exacerbates tauopathy phenotypes such as neurodegeneration, overexpression of LSD1 could counter hippocampal neurodegeneration, serving as a protection mechanism against neurodegeneration in PS19 tau mice. We found that viral overexpression of LSD1 at the

hippocampus of PS19 tau mice ameliorates tau-mediated neurodegeneration and transcription alternation, further supporting the specificity of tau functioning through LSD1 (Engstrom et al., 2019). Based on this, we hypothesize that after translation, LSD1 interacts with pathological tau in the cytoplasm. This process will prevent LSD1 from entering the nucleus and cause neurodegeneration.

As was mentioned earlier, pathological tau is a hallmark of neurodegenerative diseases. However, AD (including AD-TDP43) and other types of rare tauopathy cases, such as CBD, PSP, Pick's disease, and Frontotemporal dementia with parkinsonism-17 cases have varying tau pathology and symptoms. AD expresses both tau isoforms containing 3 microtubule binding repeats (3R) and 4 microtubule binding repeats (4R) (Iqbal et al., 2010). The tau pathology of AD follows neuroanatomical pathways and can reflect transmission of abnormal tau proteins from cell to cell in a "prion-like" manner (Ando, 2021). At the early stage, pathological tau is associated with neurodegeneration in hippocampus and entorhinal cortex, leading to learning and memory deficits (Wolfe, 2012). Then at the late stage, pathological tau is transmitted to cortical regions (Vogel et al., 2020). The disease symptoms are also related to deficits in language, reasoning, and social behavior (Kumar et al., 2021).

In this project, I am also including a subset condition of AD pathology that involves DNA binding protein of 43 kDa, with the common name TDP-43. Known for having its inclusions up to 57% of AD cases, TDP-43 deposits can be also located in neurons with neurofibrillary tangles (Meneses, 2021). TDP-43 pathology is also synergistic with AD, worsening the severity of pathological symptoms commonly observed in patients struggling with

neurodegenerative diseases (Meneses, 2021). AD-TDP43 follows a similar pattern of AD tauopathy in that the pathology also exhibits both 3R and 4R isoforms.

One of the most prominent examples of 3R tauopathy is Pick's disease, which is unique in that it is a form of cortical atrophy that has neuronal lesions that are balloon-shaped, hence giving the name "Pick bodies." Commonly, Pick-body-like inclusions can be detected in neurons. This disease features similar phenotypes to FTLD-tau in patients, such as deterioration of language, personality and memory (Chung et al., 2020). On the other hand, PSP and CBD both tend to have prominent accumulation of 4R tau in neurons and glial cells (Chung et al., 2020). CBD and PSP are alike in that they exhibit symptoms of parkinsonism and postural instability (Chung et al., 2020). However, these two diseases vary in locations of pathological tau. While cerebral cortex and basal ganglia are preferentially affected by the CBD, the PSP tends to show neuronal loss in globus pallidus, subthalamic nucleus, and substantia nigra, along with astrocytes (Chung et al., 2020). Because these rare tauopathies are so different in terms of pathology, it provides an unique opportunity to interrogate the specificity of LSD1's interaction with pathological tau. Thus, my project is to investigate how the tau aggregates interact with LSD1 to inhibit the required function of LSD1 in these rare tauopathies. Because we have shown that the LSD1 colocalizes with pathological tau in the human AD cases, we hypothesize that LSD1 will also colocalize with both the 3R and 4R isoforms of pathological tau in other tauopathies.

A. Figures

Figure 1

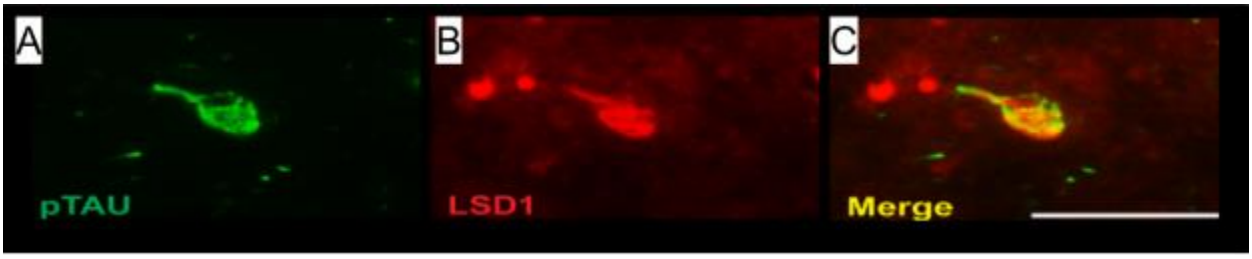

Figure 1. *LSD1 is colocalized with pathological tau in human AD cases. Figure 1A-C are immunofluorescence images of LSD1 (red), pathological tau(green), and merged immunofluorescence in AD cases (Christopher et al., 2017).*

Figure 2

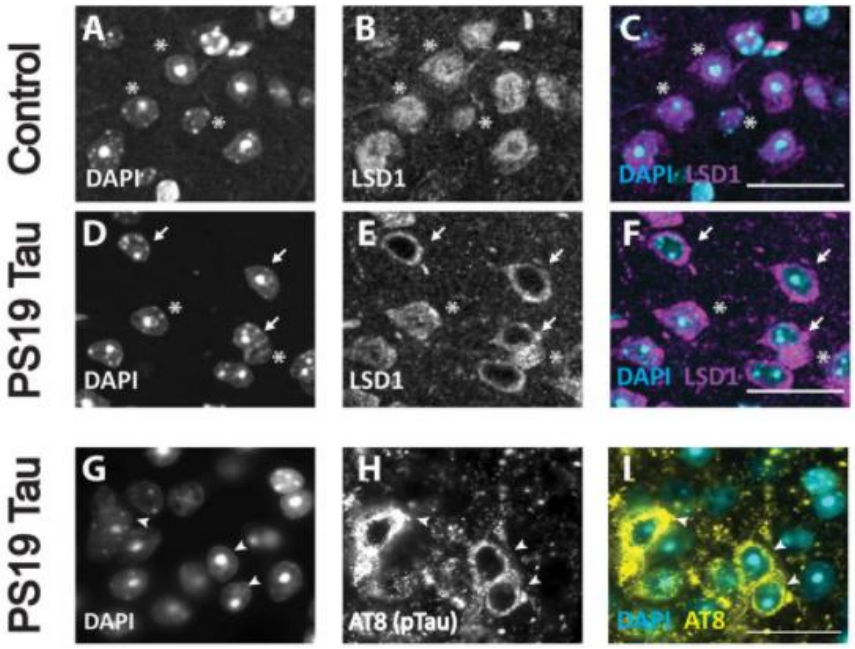

Figure 2*. This set of nine images displays the LSD1 sequestration and tau accumulation in the presence of pathological tau. The images 2A, B, and C are representative immunofluorescence of 12-month-old control wild-type mice showing the nuclear marker DAPI (4',6-diamidino-2-phenylindole) (2A), LSD1 (2B), and merged (2C) in the cerebral cortex where LSD1 is localized specifically to DAPI-positive nuclei. The images 2D, E, and F are representative images of the cerebral cortex in 12-mo-old PS19 Tau mice. Staining for DAPI (2D), LSD1 (2E), and merged (2F) shows that LSD1 is localized outside the nucleus and depleted from the DAPI-positive nucleus. Arrows denote cells where LSD1 is localized outside of the nucleus, and asterisks denote LSD1 localized specifically to the nucleus. The images 2G, H, and I are representative immunofluorescence of 12-mo-old PS19 Tau mouse with staining for DAPI (2G), AT8-positive hyperphosphorylated tau (2H), and merge (2I) where hyperphosphorylated tau accumulates in the cytoplasm of the cell body. Arrowheads denote hyperphosphorylated tau (Engstrom et al., 2019).*

CHAPTER II

Methodology

To examine the co-localization of LSD1 with pathological tau in tau-mediated neurodegeneration, we perform LSD1/pTau AT8 immunohistochemistry in tauopathy and age matched non-demented cases. We analyze the human brain tissue from various brain regions, including frontal cortex, basal ganglia, and hippocampus, which are highly affected by different tauopathies. The brain samples are provided by the Emory University Alzheimer's Disease Research Center Brain Bank.

1. LSD1/pTau AT8 Immunohistochemistry:

Immunohistochemistry is a method commonly used in research, yet we have developed a revised protocol that best works with our brain tissue slides. This two-day staining protocol begins with the deparaffinization of the brain tissue slides utilizing xylenes. Once the antigen is retrieved through microwaving in the citrate buffer, the edges of the tissue in each slide is marked with a PAP Pen, which is a pen that creates a thin film-like hydrophobic barrier when a circle is drawn around a specimen on a slide. This helps the amount of staining solutions to remain on the slides, maximizing the interaction of the tissues with the solutions. Then, hydrogen peroxide solution is applied to the slides to block the endogenous peroxidase activity. In the following step, Triton X is used to increase the lipid membrane permeabilization which will improve the antibody-antigen bonding. Once the permeabilization is done, the blocking solution made with animal serum (goat) will prevent non-specific binding. As the last part of the first day of staining, the slides are incubated with the primary polyclonal rabbit antibody against LSD1 (abcam 17721) overnight at 4 degrees Celsius.

The second day of staining begins with applying the biotinylated secondary antibody (goat anti-rabbit) which will specifically bind with the primary antibody. Solution created with Vectastain ABC HRP Kit is applied afterwards to form the avidin-biotin complex. Once the incubation is done, the DAB working solution (Vector SK-4110 -Impact DAB) is applied to generate color. The slides are then dried, and coverslip is then placed on top of the slides for imaging analysis.

The protocol is maintained for the pTau AT8 immunohistochemistry, which is necessary in order to compare with the LSD1 immunohistochemistry. However, instead of using the polyclonal rabbit antibody, the monoclonal mice antibody (ThermoFisher MN 1020) is used, with the secondary antibody being the horse anti-mice in order to bind with the primary antibody accordingly. Additionally, the blocking solution of goat serum is replaced with horse serum when carrying out the pTau AT8 immunohistochemistry.

1. Information about cases

The information about human brain tissue slides (donated from Emory Alzheimer's Disease Center Brain Bank) are shown on the table below:

	AD-TDP43	AD	FTDP	CBD	Pick's	PSP	Ctrl
Cases	7	7	5	7	5	7	7
Brain regions	Hippocampus, Frontal Cortex	Hippocampus, Frontal Cortex	Frontal Cortex	Frontal Cortex	Frontal Cortex	Basal Ganglia	Hippocampus, Frontal Cortex, Basal Ganglia

CHAPTER III

Results

Age-matched non dementia cases: LSD1 staining has a normal nuclear shape.

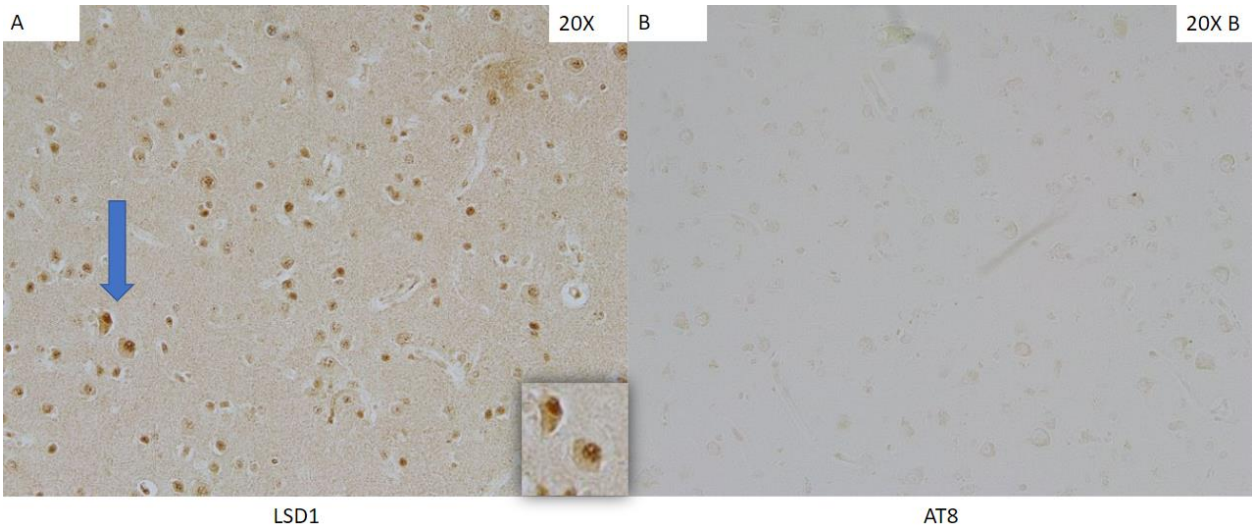

Figure 3*. LSD1 staining has normal nuclear shape while pathological tau is minimally presented in the human non dementia case (O3O3_390). LSD1 Immunohistochemistry (IHC) showing expression of LSD1 in the frontal cortex (A, blue arrow). pTau AT8 IHC showing expression of tau in the frontal cortex (B). The insert is the zoomed-in version of LSD1 staining labeled by blue arrow. Images were taken using a 20X scope.*

To determine the accuracy of IHC protocol and set up a matched case to compare with different tauopathies, LSD1 and pTau AT8 IHC was performed on age-matched human non-dementia cases. The results were as expected; LSD1 was observed to a certain degree, as can be seen on Figure 3A. It is noted by its round nuclear-shaped structure specifically. AT8 IHC, on the contrary, showed minimal amount of pathological tau, revealing that it is normally not present in human cases (Figure 3B). Overall, it suggests that LSD1 is localized in nuclei when pathological tau is not present.

AD cases: the shape of LSD1 IHC staining is similar to that of tau tangle aggregates.

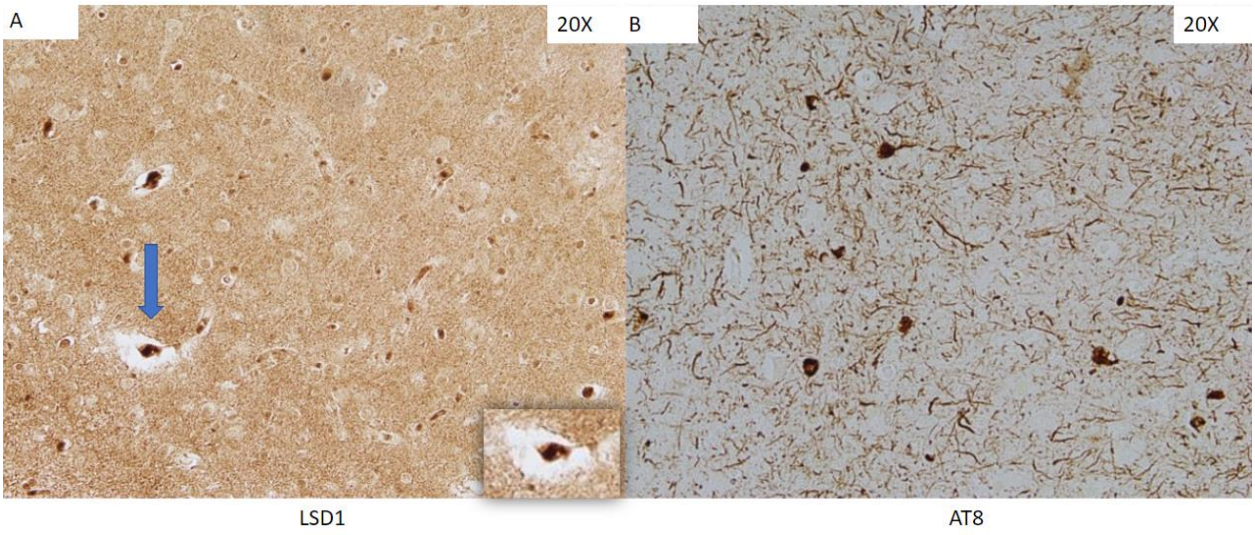

Figure 4*. LSD1 IHC staining is reminiscent of tangle-shaped aggregates in the AD case (E16_66). LSD1 IHC staining (A) has a similar shape to the pTau AT8 IHC staining (B) in the frontal cortex of AD cases. The insert is the zoomed-in version of LSD1 staining (tangle-shaped aggregate) labeled by blue arrow. Images were taken using a 20X scope.*

Notably, LSD1 IHC and pTau AT8 IHC reveal different results from the control non-dementia case. Besides showing a round, nuclear-like shape of staining in the LSD1 IHC, there is a tangle-shaped structure of LSD1 staining (as shown by the arrow, Figure 4A). This is similar to the shape of neurofibrillary tangles of hyperphosphorylated tau, which is also tangle-shaped, caused by the formation of aggregates, shown from the pTau AT8 IHC of the same case (Figure 4B). One important detail is that in the AT8 IHC, there is a presence of neuropil threads throughout the entire staining, whereas this is not to be seen in the LSD1 IHC. This leads to the idea that LSD1 may colocalize itself with neurons, exhibiting its neuronal-specific characteristic. Therefore, we are able to determine that in human AD cases, LSD1 is not only in neuronal nuclei but also associated with cytoplasmic pathological tau. Overall, our observations are similar to the

finding from our previous publication (Christopher et al., 2017) that LSD1 is present in neurofibrillary tangles.

AD-TDP43 cases: the shape of LSD1 IHC staining is similar to that of tau tangle and coil-shaped aggregates

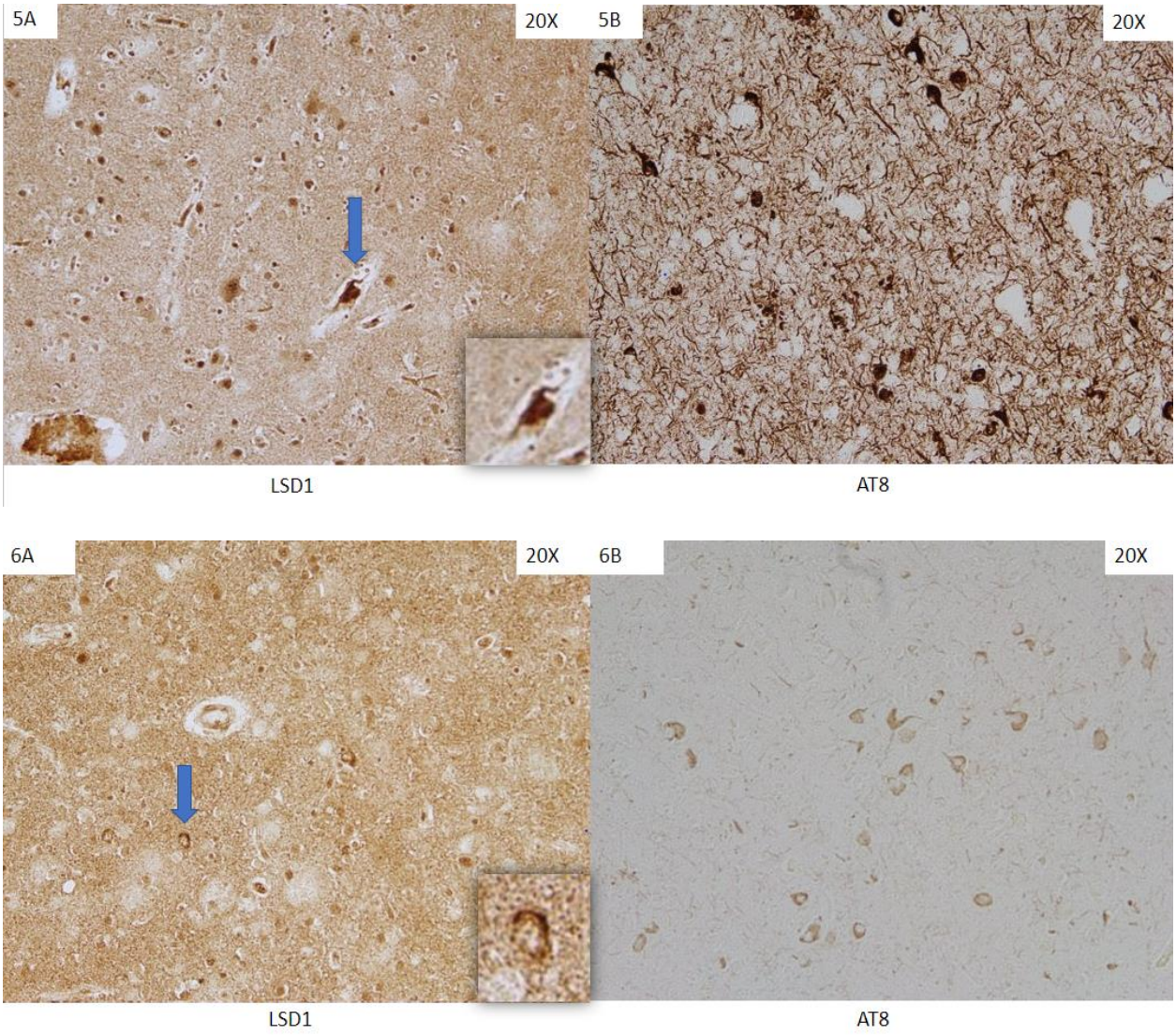

Figure 5-6*. LSD1 IHC staining is reminiscent of tangle and coil-shaped aggregates in the AD-TDP43 cases of E06_154 (Figure 5) and E11_07 (Figure 6). LSD1 IHC (Figure 5A) reveals tangle-shaped staining that is similar to the shapes of staining shown on the pTau AT8 IHC*

(Figure 5B), both stained from the frontal cortex region. The insert in Figure 5A is the zoomed-in version of LSD1 staining (tangle-shaped aggregate) labeled by blue arrow. Images were taken using a 20X scope. LSD1 IHC (Figure 6A) reveals a coil-shaped staining similar to the shapes of staining on the pTau AT8 IHC (Figure 6B), both stained from the frontal cortex region. The insert in Figure 6A is the zoomed-in version of LSD1 staining (coil-shaped aggregate) labeled by blue arrow. The insert in Figure 6B is the zoomed-in version of AT8 staining (oligodendroglial coiled bodies) labeled by blue arrow. Images were taken using a 20X scope.

The LSD1 IHC from AD-TDP43 cases displays similar results to that of AD cases. The LSD1 staining shown on Figure 5A has a clear tangle shape that resembles the tangle shapes of hyperphosphorylated tau aggregates staining from the same case (Figure 5B). This adds to the evidence that LSD1 could colocalize with pathological tau aggregates in the frontal cortex of AD-TDP43 cases. Figure 6A represents an example that is slightly different, which has coil-shaped LSD1 staining similar to AT8 staining of oligodendroglial coiled bodies (Figure 6B) from the same case. This further provides the evidence that there could be a presence of colocalization between LSD1 and pathological tau in neurons (neurofibrillary tangles) and oligodendrocytes (oligodendroglial coiled bodies) of AD-TDP43 cases.

PSP cases: the shape of LSD1 IHC staining is similar to that of tufted astrocytes

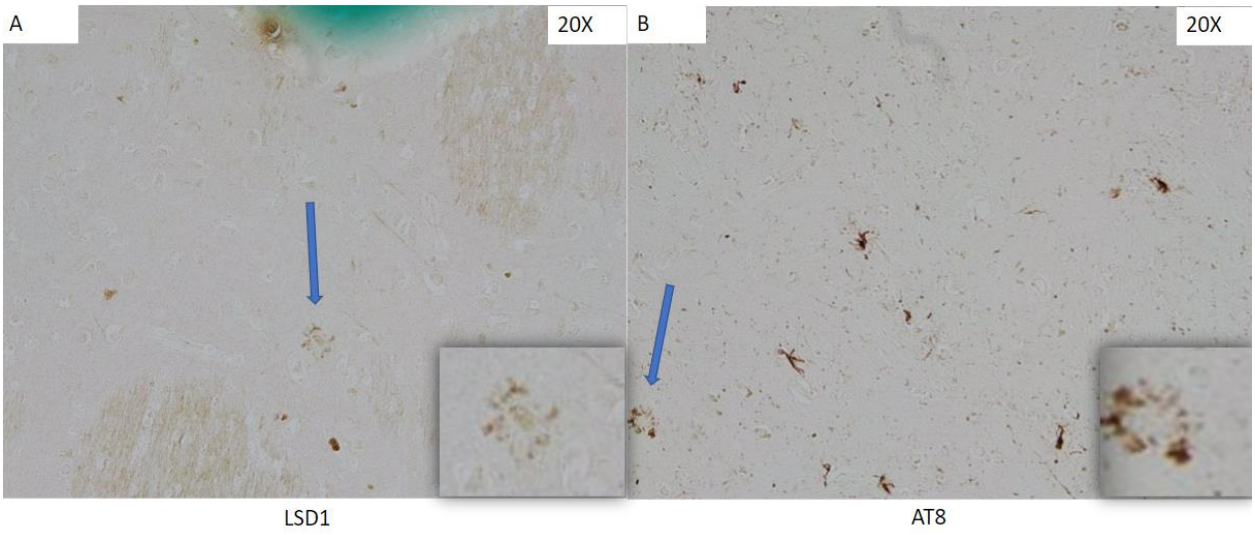

Figure 7. *LSD1 IHC staining is reminiscent of tufted astrocytes in the PSP case, OS90_8. LSD1 IHC (Figure 7A) is showing a similar shape to that of the pTau AT8 IHC (Figure 7B) in the basal ganglia of PSP cases. The insert in Figure 7A is the zoomed-in version of LSD1 staining (tufted astrocytes-shaped aggregate) labeled by blue arrow. The insert in Figure 7B is the zoomed-in version of AT8 staining (tufted astrocytes) labeled by blue arrow. Images were taken using a 20X scope.*

PSP neuropathology typically includes neurofibrillary tangles, neuropil threads, and glial lesions, such as tufted astrocytes (Chung et al., 2020). This can be distinctly seen in the AT8 IHC stained images shown above on the case OS90_8 (Figure 7B). Interestingly, LSD1 IHC has a lesion in the shape that resembles that of the AT8 stained tufted astrocytes (Figure 7A). This provides the first evidence that LSD1 could be specifically colocalized to pathological tau in astrocytes.

Pick's Disease: the shape of LSD1 IHC staining is similar to that of aggregates placed on the edge of Pick bodies.

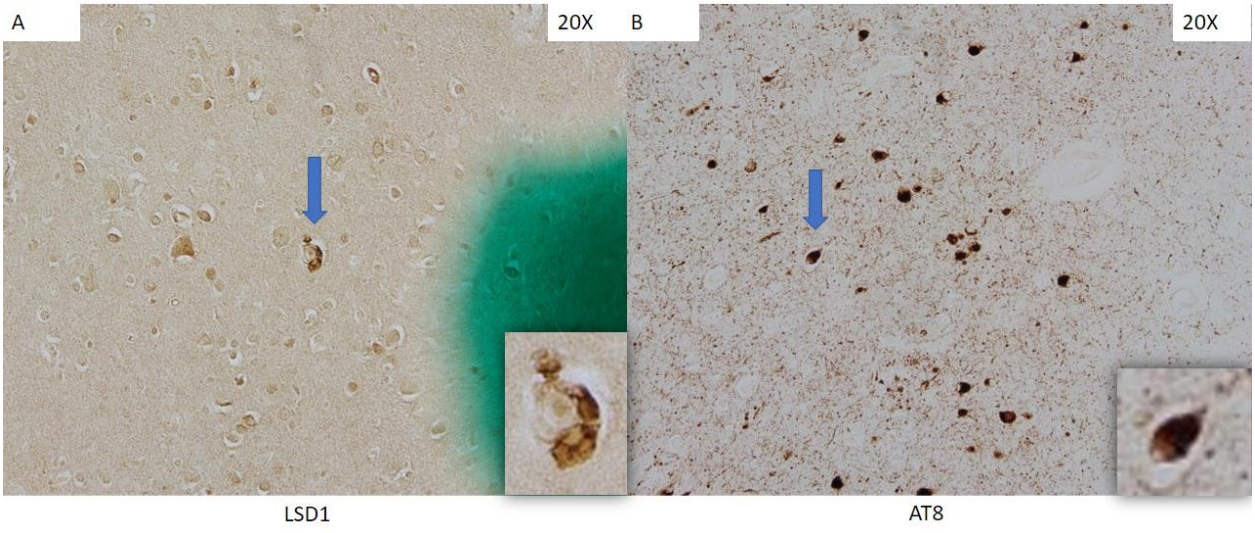

Figure 8. *LSD1 IHC staining is reminiscent of aggregates placing themselves separate from the Pick's bodies in the frontal cortex of Pick's Disease case OS99_21. LSD1 IHC (Figure 8A) does not show much presence of Pick's bodies, and even when it shows, the LSD1 is placed on the edge, not overlapping with the Pick's body, along with the pTau AT8 IHC stained Pick bodies (Figure 8B). The insert in Figure 8A is the zoomed-in version of LSD1 staining labeled by blue arrow. The insert in Figure 7B is the zoomed-in version of AT8 staining (Pick's body) labeled by blue arrow. Images were taken using a 20X scope.*

The darker spot of LSD1 staining on the periphery of the neuron marks the LSD1 placement (Figure 8A), while the structure of the Pick's bodies is showing a round shape on the pTau AT8 IHC (Figure 8B). Overall, we do not see much of Pick's bodies in the LSD1 staining. This adds to the evidence that the LSD1 might not be colocalized with pathological tau in Pick's disease. Pick's Disease has neuronal Pick bodies that are usually similar to the shapes seen from the typical AD cases, but it is critical to note that this similar shape does not convey information about colocalization; rather, it shows the division between the LSD1 and Pick's bodies. Because

Pick's disease exhibits 3R tau isoforms, it suggests that the interaction between LSD1 and pathological tau could be isoform-specific.

CBD: the shape of LSD1 IHC staining is similar to that of tau tangle aggregates

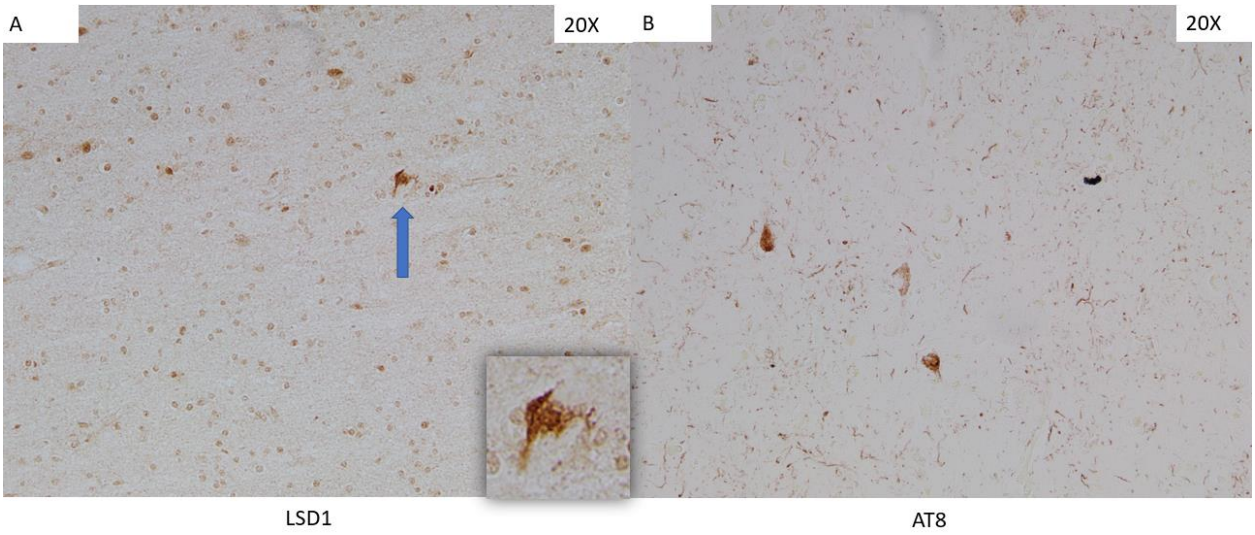

Figure 9*. LSD1 IHC staining is reminiscent of tangle-shaped aggregates in the CBD case (OS99_14). LSD1 IHC staining (A) has a similar shape to the pTau AT8 IHC staining (B) in the frontal cortex of CBD cases. The insert in Figure 9A is the zoomed-in version of LSD1 staining labeled by blue arrow. Images were taken using a 20X scope.*

From the CBD cases, we have found the shape of LSD1 staining (Figure 9A) that is very similar to the tangle-shaped aggregates seen in the pTau AT8 IHC staining (Figure 9B). However, from the CBD cases we have checked, this is rarely happening. We reason that this could potentially be due to the structural difference between 4R isoforms in PSP and CBD, marked by different residue levels (Shi et al., 2021).

CHAPTER IV

Discussion and Future Directions

From our previous research, we have known that pathological tau colocalizes with the LSD1 in AD cases. This led us to question how LSD1 may interact with pathological tau in other tauopathies, which have various pathologies and symptoms. Results from IHC have shown varying results for the different tauopathies in this project, but there is a clear distinction between the isoforms in that the presence of LSD1 is observed with aggregated tau that has 4R domains, but less so in 3R domains. As was mentioned before, AD and AD-TDP43 are tauopathies that express both of the isoforms, hence explaining the reason why the stained images from IHC displays tangle-shaped LSD1 staining, markedly different from the IHC images of non-dementia control cases. The staining of both tauopathies have revealed that LSD1 could place itself on tangle-shaped and coil-shaped tau aggregates, adding credibility to the possibility that LSD1 is colocalizing with pathological tau of 4R isoform in the frontal cortex regions of the human brain.

Observing the tauopathy with 3R isoforms, Pick's Disease yields contrasting staining results. In general, we did not observe a lot of Pick's bodies in the LSD1 IHC. Additionally, the images (Figure 8) show that LSD1 staining is similar to that of aggregates not associating itself with the Pick's bodies; it is placed adjacent to it, separate from the Pick's bodies. Thus, LSD1 might not be interacting with pathological tau of 3R isoform in the frontal cortex. On the other hand, PSP and CBD are prominent tauopathies that exemplify the 4R isoforms of tau. The stained images from PSP (Figure 7) have shown remarkable evidence of LSD1 potentially interacting with tufted astrocytes. LSD1 could be mislocalized to tufted astrocytes, as shown by the lesion in LSD1 IHC shaped similarly to the AT8 IHC stained images of PSP cases, which suggests the possibility of a cell-type (astrocyte) specificity for LSD1 interacting with pathological tau in PSP cases, but more studies need to be done to confirm this. The stained

images from CBD (Figure 9) have also shown results supporting a possible linkage between 4R isoforms of tau and LSD1 interaction. The LSD1 in CBD cases have also displayed similar tangle shape of aggregates on IHC staining compared to the shape shown on the AT8 IHC stained images. However, this was rarely happening in the stained images, indicating that there must be more analysis done to fully uncover the LSD1 interaction with CBD. Overall, we believe that LSD1 might be specifically interacting with 4R tau isoforms, instead of the 3R tau isoforms, across the various tauopathies that were examined. For each type of tauopathy, there might be additional cell type specificity for pathological tau interaction with LSD1.

Previously, the research field has elucidated the idea that tau is intrinsically disordered, exhibiting behaviors that protrude itself as an unstructured protein (Mukrasch et al., 2009). It has prion-like properties that can cause misfolding of other intrinsically disordered tau proteins, eventually inducing propagation of aggregates (Holmes and Diamond, 2014). This led to the discovery of tau's interaction with RNA binding proteins, which also have disordered regions in their domains (Lester et al., 2021; Montalbano et al., 2020). Interestingly, LSD1 is a histone demethylase that has a N-terminal intrinsically disordered domain containing a nuclear localization signal (NLS). Therefore, we proposed a model: in tauopathy, after LSD1 is translated in the cytoplasm, it will interact with pathological tau through the N-terminal disordered domain. This will mask LSD1’s NLS and prevent it from entering the nucleus, eventually inhibiting its normal function and inducing cortical neurodegeneration in mice.

Adult human brain has six identified tau isoforms, with 3R or 4R (repeated sequences) of microtubule-binding regions from exclusion and inclusion of exon 10, respectively through alternative RNA splicing (Buee et al., 2000; D’Souza et al., 2004; Cherry et al., 2021). Each

isoform supposedly has distinct physiological functions. For instance, different isoforms are associated with different interactions with distinct cellular binding partners (Bhaskar et al., 2005, Liu et al., 2016). Additionally, the phosphorylation patterns of the two isoforms are distinct, as is their ability to induce oxidative stress (Sealey et al., 2017). Both isoforms have been known to be capable of manifesting pathology, including higher 3R levels correlating to more severe extracellular tau (ghost tangles) pathology and higher 4R levels exacerbating hippocampal tau hyperphosphorylation (Cherry et al., 2021; Barron et al., 2020). The mechanism of pathology is accordingly different between the two tau isoforms, since each develops a distinct morphology and intramolecular folding interactions (Lo et al., 1993; Goode et al., 2000). Previous data have noted that these different intramolecular interactions between the core microtubule binding domains and flanking regions could differentially influence microtubule behavior. (Goode et al., 2000).

Based on our results, we propose a model that LSD1 is interacting more with the 4R tau isoform than the 3R tau isoform. Most importantly, this might be due to the structural differences between the 3R and 4R tau that lead to possible diversities in LSD1 interaction. 3R tau isoform possesses a core of microtubule binding activity residing in first two repeats and the intervening inter-repeat, with the C-terminal sequences downstream of the repeat region making a strong enhancement to microtubule binding activity in 3R tau isoform (Goode et al., 2000). This is not the case in the 4R isoform because the same sequences in the 4R tau isoform do not reach the same effect, denoting the difference in the strength of microtubule binding affinity between 3R and 4R tau isoforms (Goode et al., 2000). In addition, the structural folds and domain regions are different in the N-terminal extensions (Shi et al., 2021). For instance, 4R tauopathies comprise

all of R2 domain and and one or two residues of R1 domain, whereas this is not the case in the 3R tauopathies, such as the Pick's Disease, where the Pick fold comprises more than half of R1 domain (Shi et al., 2021).

We believe that these dissimilarities may play a key role in directing the LSD1 interaction with various tauopathies. Further studies must be done, however, in order to better understand how structural differences in tau isoforms may be causing LSD1 to associate differently in these tauopathies.

When we look at the various tauopathies that can be observed from the human cases, categorized by its specificity with tau interaction as either the 3R or 4R tau isoforms, LSD1's interaction with tau seems to be unique and subspecific. Most clearly, we have seen a tau interaction with 4R isoforms in the example of PSP cases, where we see LSD1 possibly colocalizing with the tufted astrocytes. Another tauopathy with 4R isoform, CBD has also shown slight evidence of LSD1 interaction with tau, but the results have not been consistent in the IHC staining results. There are a variety of factors as to why CBD has not been yielding similar results, despite the fact that it is also a 4R isoform. One possible reasoning is that the tauopathy of CBD may be better represented in other brain regions. There is a possibility that the pathology is more evidently presented in brain regions such as basal ganglia (Sakakibara, 2004). Unfortunately, we were limited in the aspect of obtaining brain slides; we were unable to obtain the other brain regions of CBD cases. Therefore, we conjecture that we might be able to see more improved IHC staining of basal ganglia regions of CBD cases.

Another analysis is the idea that LSD1 interaction with the 4R tau isoform may be PSP-specific. Previous data have argued that PSP is defined by neurofibrillary tangles and neuropil threads, with tufted astrocytes and oligodendroglial coiled bodies (Shi et al., 2021). According to the structural-based classification of tauopathies, CBD and PSP share a common molecular structure in that tau bands of 64 and 68 kDa, but PSP has a strong C-terminal tau band of 33 kDa, whereas CBD has a strong doublet of 37 kDa (Shi et al., 2021). They also have differences at the residue level in that PSP comprises three-layered core regions, whereas the CBD has four-layered folds (Shi et al., 2021). Thus, despite the same categorization as 4R tauopathies, they have distinct pathways in that tau filament folds are not the same. We believe that these differences in arrangement of bands may play a role in influencing the LSD1 association with tau being more specific to PSP, more so than the CBD.

There are numerous possibilities that leave room for future research. What can be done to ameliorate the findings from IHC is the LSD1-pathological tau dual immunofluorescence, which uses fluorescent-tagged antibodies to label the antigen presence in tissues. Utilizing this, we can study if colocalization is occurring between the proteins. However, limitations exist in that LSD1 and pTau AT8 have minimal compatibility when they are observed using dual immunofluorescence. Thus, there must be an improved protocol or alternative antibodies, such as against 3R and 4R tau isoforms, that can allow the two stainings to exist together. Afterwards, we can perform quantification analysis using ANOVA with post hoc Sidak’s multiple comparison test to determine the percentage of time we find aggregates of LSD1 colocalizing with pathological tau.

Additionally, Human embryonic kidney 293 cells (HEK cells) can be used to test LSD1 interaction with different forms of tau. We have generated a HEK cell line expressing the endogenous LSD1 labeled with fluorescent tag. The cells will be transfected with different forms of tau with respective mutations and can reveal data as to how LSD1 associates with each form of tau. The overarching objective is screening to see if this can contribute to a creation of a drug that can potentially block the LSD1 -tau interaction.

Future research can also utilize viral overexpression to test different forms of LSD1 in the hippocampus of PS19 tau mice. Injecting virus to overexpress HA tagged LSD1 in different versions (full length LSD1, N-terminal deleted LSD1, and LSD1 N-terminal alone), we can study the role of LSD1 N-terminal disordered domain in LSD1 and tau interaction.

CHAPTER V

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