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Detection of molecular markers of ferroptosis in human Alzheimer's brains

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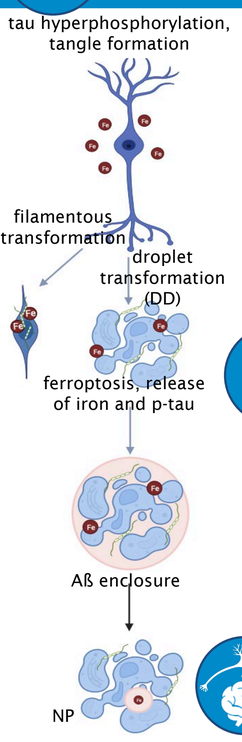
Background



- Alzheimer´s disease **(AD)** reveals typical pathological lesions
→ extracellular deposits of amyloid-beta **(Aß)** peptides
→ intracellular aggregates of hyperphosphorylated tau protein **(p-tau)** visible as **pretangles**, neurofibrillary tangles **(NFT)**, neuropil threads and neuritic plaques **(NP)**
- pretangles can develop into either NFTs via filamentous transformation, or into neuritic plaques via droplet degeneration
- droplet degeneration **(DD)** signifies the beginning of neuritic plaque formation during Alzheimer´s pathogenesis[1]

microglia exhibited strong ferritin expression and Perl´s iron staining showed iron in microglia, droplet spheres, and Aß plaque cores[1]

- ferroptosis is an iron-caused cell death
- transferrin-receptor **(TfR)** is a necessary part of iron import into cells that is physiologically expressed in neurons and endothelial cells of the human brain
- Feng et al. described an extensive expression of TfR as a marker of ferroptosis[2]
- the ferroptosis database FerrDB described different marker genes of ferroptosis → **PTGS2** gene is the most upregulated gene of ferroptotic cell death[3]

Research Questions

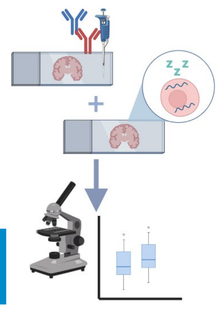
- **Is DD a morphological manifestation of ferroptosis?**

→ Does the TfR and ferritin expression differ quantitatively in the different Braak stages of AD?
→ Do the histological hallmarks of AD express TfR as a ferroptosis marker and ferritin as a key protein of iron metabolism?
→ Can ferroptotic marker genes like PTGS2 be detected at transcriptomic level in hallmarks of AD?

Methods

- evaluation of an antibody against TfR
- immunohistochemical staining with anti-TfR/ferritin antibodies and AT8, as p-tau marker, on prefrontal cortex on high and low Braak stages
- visualization of ferroptotic marker genes (PTGS2 and TFRC) by in situ hybridization in AD brains

→ investigation of spatial correlation of these with histopathological hallmarks of AD, comparison of expression in different Braak stages



Results

1 TfR expression in human brain

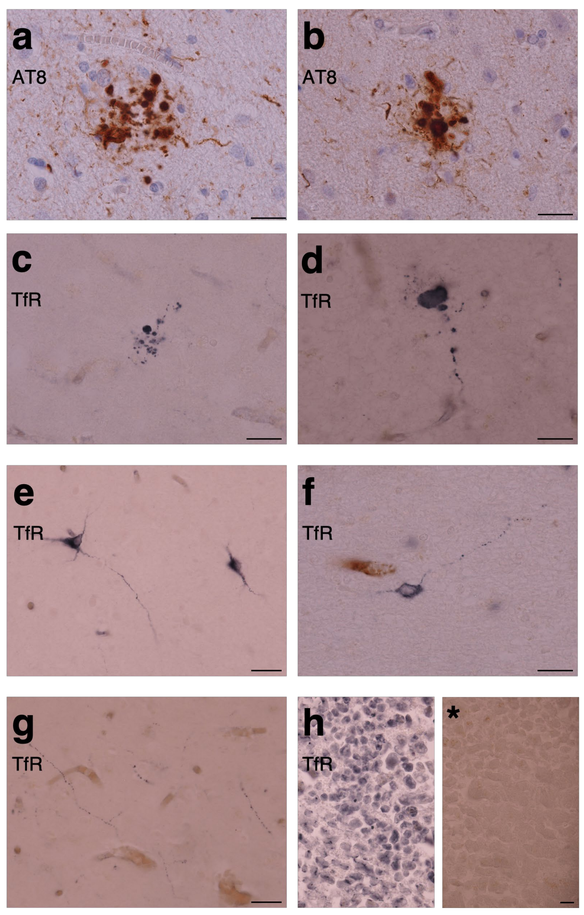


Figure 1 Paraffin-sections with AT8-positive DD are shown **(a,b).** Paraffin-sections of human prefrontal cortex were stained with anti-TfRC in black **(c-g).**

Accumulations of globular TfR-positive structures exhibiting typical features of droplet degeneration are shown **(c,d)**. Cells with stained cell soma and beaded processes that appeared to be degenerating were visible **(e-g)**. Paraffin-section of human liver was stained with anti-TfRC in black as a positive control **(h)**. TfR signals can be seen in hepatocytes and on their membrane.

(h*) omission control. Scale bar = 20μm

1 TfR expression in human brain

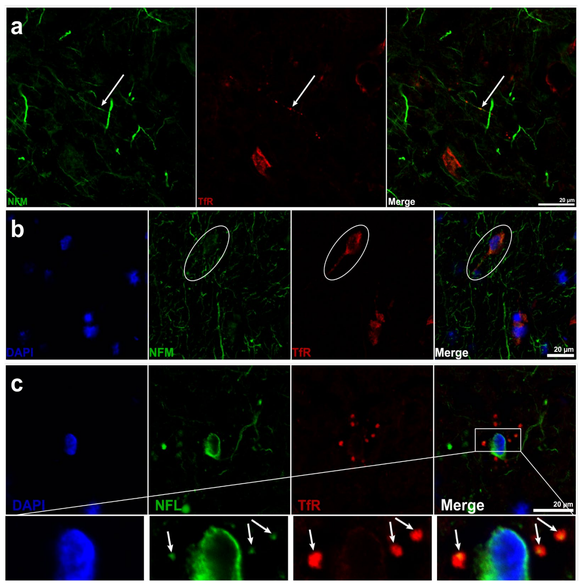


Figure 2 Fresh-frozen human prefrontal cortex sections were stained with anti-TfRC in red, different cell markers (NFL, NFM) in green and cell nuclei with DAPI in blue. A colocalization of TfR-positive structures with NFM **(a, b)** and NFL **(c)** can be seen.

2 Comparison of TfR and ferritin expression in human brain

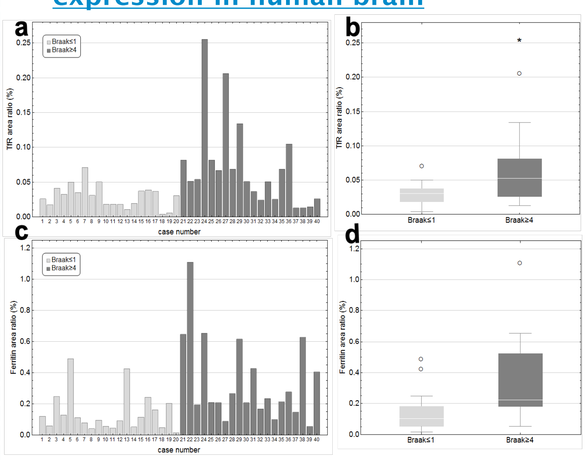


Figure 3 The comparison of TfR and ferritin expression between a group with Braak≥4 and Braak≤1 is shown. There is a significant higher TfR **(a+b; p=0.007)** and ferritin **(c+d; p=0.001)** expression in Braak≥4.

3 Spatial correlation investigations of TfR + AT8

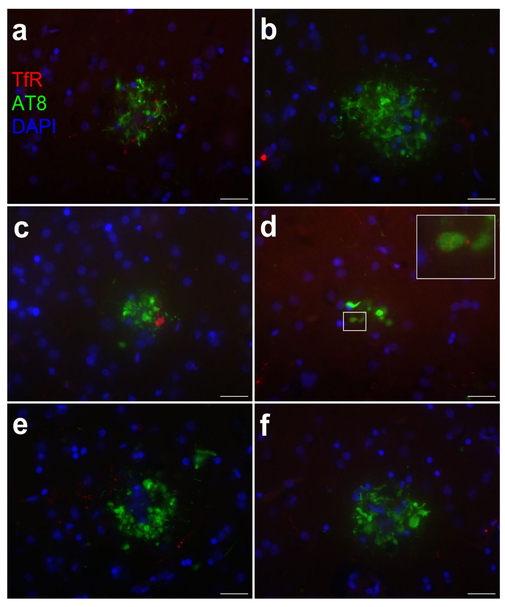


Figure 4 Paraffin-sections of human prefrontal cortex were stained with anti-TfR in red and AT8 in green and cell nuclei with DAPI in blue. Beaded neuritic processes in DD can be seen **(a)**, but more often an adjacency of DD and TfR-positive structures **(b-e)**. In most cases DD completely remain without TfR-positive struc

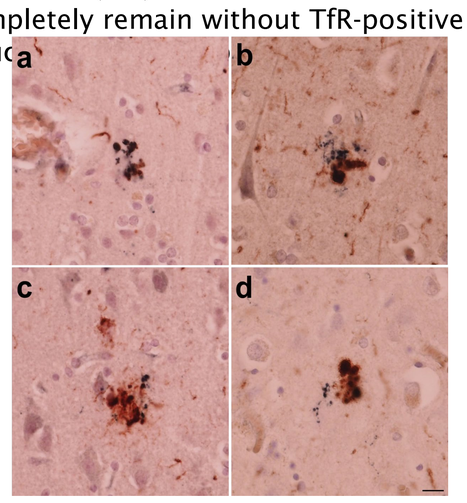


Figure 5 Paraffin-sections of human prefrontal cortex were stained with anti-TfRC (black) and AT8 (brown). A direct overlap of TfR-positive structures and p-tau+ DD can be seen **(b)**, but more often an adjacency of DD and TfR-positive structures **(c-e)**. Scale bar = 20μm

4 Detection of ferroptosis at transcriptomic level

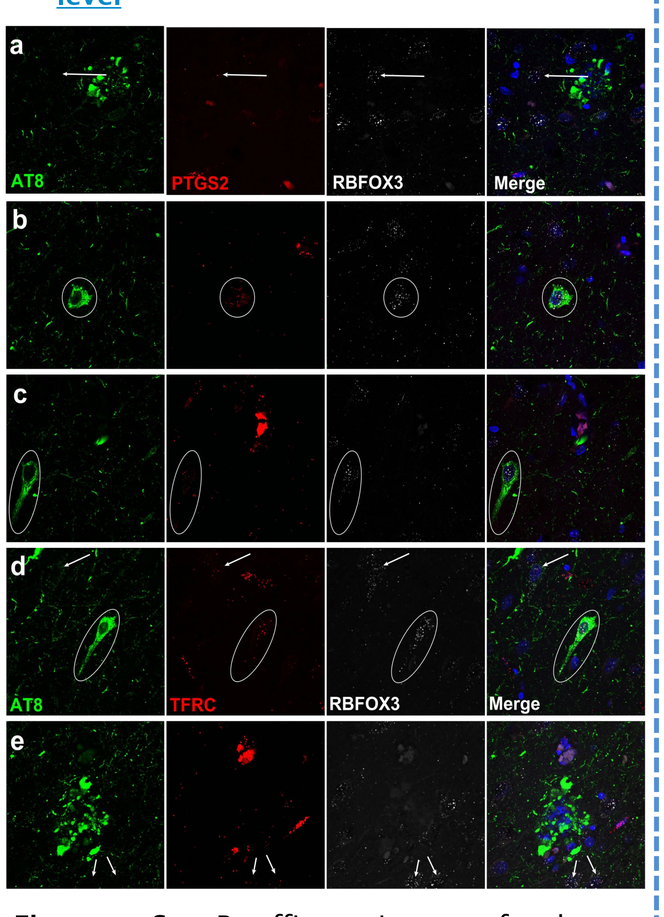


Figure 6 Paraffin-sections of human hippocampus were hybridized with PTGS2 (a-c)/TFRC (d-e) in red and RBFOX3 as a neuron marker in white, stained with AT8 in green and DAPI in blue for nuclear staining. Signals of the mRNA of PTGS2 **(circle in b, c)** and TFRC **(circles in d)** can be seen in pretangles and in neurons around p-tau+ structures **(arrows in a,d,e)**, but less in DD **(a, e)**. Scale bar = 20 μm

Summary/Discussion

1. **TfR** was found on **neurons** with beaded neuritic processes that appeared to be **degenerating** and are **exhibiting typical features of DD**
2. **TfR-positive neurons increased** with **Braak stages** as did **ferritin expression** in microglia
3. Direct **colocalization of TfR-positive and p-tau-positive structures** was a **rare event**, a **spatial proximity was more often**
4. **mRNA** of signature genes of ferroptosis were **detected** in **pretangles and p-tau negative neurons, but less in DD**

Is DD a morphological manifestation of ferroptosis?

With the increased expression of TfR and ferritin in high Braak cases and the demonstration of mRNA of ferroptotic marker genes in AD pathologies, the **hypothesis that DD is ferroptotic is strengthened.**

Due to the strong morphological similiarity of TfR-positive structures to DD, **TfR might be considered as an early ferroptosis marker, which is only expressed transient in AD pathogenesis.**

References:

1: Streit WJ, Rotter J, Winter K, Müller W, Khoshbouei H, Bechmann I (2022) Droplet Degeneration of Hippocampal and Cortical Neurons Signifies the Beginning of Neuritic Plaque Formation. J Alzheimers Dis 85, 1701-1720.
2: Feng H, Schorpp K, Jin J, Yozwiak CE, Hoffstrom BG, Decker AM, Rajbhandari P, Stokes ME, Bender HG, Csuka JM, Upadhyayula PS, Canoll P, Uchida K, Soni RK, Hadian K, Stockwell BR (2020) Transferrin Receptor Is a Specific Ferroptosis Marker. Cell Rep 30, 3411-3423.e7.
3: FerrDb

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