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LMD proteomics provides evidence for hippocampus field-specific motor protein abundance changes with relevance to Alzheimer's disease

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ABSTRACT

Background: Human hippocampal area Cornu Ammonis (CA) 1 is one of the first fields in the human telencephalon showing Alzheimer disease (AD)-specific neuropathological changes. In contrast, CA2 and CA3 are far later affected pointing to functional differences, which may be accompanied by differences in proteome endowment and changes.

Methods: Human pyramidal cell layers of hippocampal areas CA1, CA2, and CA3 from neurologically unaffected individuals were excised using laser microdissection. The proteome of each individual sample was analyzed and differentially abundant proteins were validated by immuno-histochemistry.

Results: Comparison of CA1 to CA2 revealed 223, CA1 to CA3 197 proteins with differential abundance, among them we found motor proteins MYO5A and DYNC1H1. Extension of the study to human hippocampus slices from AD patients revealed extensive depletion of these proteins in CA1 area compared to unaffected controls.

Conclusion: High abundance of motor proteins in pyramidal cell layers CA1 compared to CA2 and CA3 points the specific vulnerability of this hippocampal area to transport-associated changes based on microtubule dysfunction and destabilization in AD.

1. Introduction

The human hippocampus is a major component of the mesial temporal lobe region with important functions in memory and spatial navigation. In its transverse lateral to medial direction the hippocampus is subdivided into fields or areas CA1 (CA for Cornu Ammonis) through CA4, and the dentate fascia. The CA fields together with the dentate fascia constitute the hippocampus proper

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, amyloid β; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; C, cysteine; CA, Cornu Ammonis; DAPI, 4′,6-diamidino-2-phenylindole; DTT, dithiothreitol; DYNC1H1, cytoplasmic dynein 1 heavy chain 1; FDR, false discovery rate; GO, gene ontology; IPA, ingenuity pathway analysis; LC-MS/MS, liquid chromatography tandem mass spectrometry; LMD, laser microdissection; M, methionine; *m/z*, mass-to-charge-ratio; MAPRE3, microtubule-associated protein RP/EB family member 3; MAPT, microtubule-associated protein tau; MS/MS, tandem mass spectrometry; MYO5A, unconventional myosin-Va; NFTs, neurofibrillary tangles; NRXN1, neurexin-1; NTs, neuropil threads; PBS, phosphate *buffered* saline; PFA, paraformaldehyde; PSC, processed spectral counts; PSD95, Postsynaptic density protein 95; PSEN1, presenilin-1; PSMs, peptide spectrum matches; RT, room temperature; RTN4, reticulon-4; S, serine; SLC6A3, sodium-dependent dopamine transporter; SPTBN1, spectrin 1 beta chain; T, threonine; Y, tyrosine

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or hippocampus in a narrow sense. Subiculum and entorhinal cortex link the hippocampus to the six-layered isocortex and project to the hippocampus by means of the trisynaptic neuronal circuit. Together, these components constitute the hippocampal formation. Memory loss and disorientation, clinical hallmarks of early Alzheimer's disease (AD), correspond with hippocampal sclerosis [1] and neurofibrillary pathology in the entorhinal cortex, hippocampal CA1 and subiculum [2]. In parallel with progress in immunohistochemical methodology diagnosis of neurodegenerative diseases has changed considerably. Presently, virtually all neurodegenerative diseases are considered as proteinopathies [3] associated with selective layer- or region-specific neuron loss. Alzheimer's disease is characterized by pathological extracellular Aβ and intracellular tau accumulation [4,5]. The role of both proteins in the pathogenesis of AD is still a matter of debate [6,7]. It is even feasible that both proteins are symptomatic and not causes of disease onset and progress [8,9]. In AD, light-microscopically visible neurofibrillary tangles (NFTs) and neuropil threads (NTs) consist of hyper-phosphorylated 3R and 4R tau. Appearance of NFTs and NTs is considered a histological hallmark of AD. Amount and systemic spread of the latter are the basis for neuropathological staging of AD [10–12]. Nerve cell loss, the final and irreversible step in all neurodegenerative diseases, is more difficult to assess and not subject of routine neuropathological diagnosis [13]. Early tau pathology in hippocampal CA1 pyramidal cells and more pronounced cell death in the same region contrast with later affected CA2 and CA3 pyramidal cells. Interestingly, CA2 pyramidal cells are early affected in argyrophilic grain disease [14,15], another tauopathy. Lewy neurites in sporadic Parkinson's diseases first appear in CA2 and later in CA1 and CA3 [16]. These examples clearly show disease-specific selective vulnerability in fields with close topographic relation and anatomical interconnections. So far, regional analysis of genome expression by single cell laser microdissection (LMD) yielded a complex pattern of changes in hippocampal subfields, brainstem and cerebellum in human aging and AD brains [17–19].

Several studies used mass spectrometry to study the human hippocampus, especially comparing tissues from AD-affected individuals and controls [20–25]. However, no studies have been undertaken to analyze the layer-specific hippocampal proteome using LMD. Using this strategy, our objective was to contrast molecular differences between the pyramidal cell layers of CA1 through CA3 that could explain early selective CA1 pyramidal neuron degeneration in AD. To gain insights into the differences of molecular composition of areal and laminar hippocampal architecture, we isolated the pyramidal cell layers of fields CA1, CA2, and CA3 using LMD. LC-MS/MS-based proteomics was used to disclose a spatial and laminar proteome signature of the samples from human neurologically unaffected individuals. The study was further extended by the comparison of AD hippocampal and control slices using immunohistochemistry. Our results reveal novel proteins and pathways that point to the different functional role of the hippocampal areas CA1-CA3, which may play a role in AD pathogenesis.

2. Experimental procedures

2.1. Human brain tissues

The human hippocampal brain tissue samples, extracted *post-mortem* from both control and Alzheimer's disease (Braak stages V/VI) patients, were kindly provided by Prof. Heinsen (Department of Psychiatry, University Würzburg, Germany). Ethical votes for the work with human brain samples are available (no. 2875, ethical commission at Ruhr-University Bochum, 034/12 of the Ludwig-Maximialians-University Munich, and 234/11 of the Julius-Maximilians-University Würzburg).

Sample	Age	Gender	*Post-mortem* time
Control 1	66 years	Male	< 30 h
Control 2	68 years	Male	< 30 h
Control 3	61 years	Male	< 30 h
Control 4	62 years	Female	< 30 h
Control 5	89 years	Female	< 30 h
Control 6	66 years	Male	< 30 h
AD 1	89 years	Female	< 30 h

2.2. LMD of brain sections

Freshly frozen 10 µm sections through the human hippocampal body (hippocampal region between the caudal tip of the uncus and the plane of the rostral parts of the lateral geniculate body) were mounted on polyethylene naphthalate-membrane slides (Leica, Herborn, DE). After fixation in 100% ethanol for 5 min, the slides were stained with 0.1% Cresyl Violet for 10 min. After staining, they were processed for laser microdissection (Leica CTR 6500, Leica Microsystems, Wetzlar, Germany), where the same amount of rectangles with an area of 70.000 μm^2 from all the hippocampal regions were collected in microfuge tubes and stored in − 80 °C for further analysis. LMD preparation was performed for CA1, CA2, and CA3 from six different neurologically unaffected individuals.

2.3. In-solution digestion

The collected tissue material of each individual sample was diluted to a final volume of 74.25 µl in 50 mM ammonium bicarbonate (pH 7.8). After reduction with DTT for 20 min at 56 °C, the samples were alkylated with 0.55 M iodoacetamide for 15 min in the dark at RT. Before adding 1% Trypsin RapiGest SF Surfactant (Waters, Eschborn, Germany) in 50 mM NH_4HCO_3 (pH 7.8) the pH was adjusted to 7.4. To initiate the digestion, 1.8 µl trypsin (Serva Electrophoresis GmbH, Heidelberg, Germany) solution (1 µg/µl 50 mM acetic acid) was added and the samples were incubated overnight at 37 °C. Digestion was stopped by addition of 5.25 µl 10% TFA. The samples were purified with OMIX C18 tips (Varian, Agilent Technologies, Boeblingen, Germany) and after being concentrated in-vacuo, the final volume was adjusted to 17 µl with 1% TFA.

2.4. Mass spectrometry analysis

For LC-MS/MS analysis, 16 µl of the sample was loaded onto an UltiMate 3000 RSLCnano nano-liquid chromatography system (Thermo Scientific, Dreieich, Germany). First, the samples were loaded on a trap column (Thermo Scientific, 75 µm × 2 cm, particle size = 3 µm, pore size 100 Å) with 0.1% TFA (flow rate 10 µl/min). After washing, the trap column was connected with an analytical C18 column (Thermo Scientific, 75 µm × 25 cm, particle size = 2 µm, pore size 100 Å) and the peptides were separated by using a gradient which can be found in our previous publication [26]. For electrospray ionization, eluted peptides were transferred into the nano-electrospray source of a Q-Exactive mass spectrometer (Thermo Scientific), which was directly coupled to the HPLC system. The mass spectrometry device operated in the range between 300 and 2000 *m/z* with a resolution of 30,000 and a maximum acquisition time of 500 ms. For MS/MS fragmentation in the HCD (higher-energy collisional induced dissociation) cell and identification in the orbitrap, the 10 most intensive ions (charge > 1) were selected. Fragments were generated by HCD on isolated ions with a collision energy of 35% and a maximum acquisition time of 50 ms. The *m/z* values initiating MS/MS were set on a dynamic exclusion list for 35 s.