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The expression of ErbB4 in the human hippocampus and in murine primary astrocyte culture

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Morbus Alzheimer is characterized by Aβ plaques, neurofibrillary tangles and different inflammatory conditions that finally lead to neuronal death and neuro- logical symptoms of dementia. Monocausal therapeutic approaches that target amyloid or NFT could not improve clinical symptoms nor the overall outcome and progression of the disease. Accumulation of protein is common for ageing and is not regarded to be necessarily linked to symptoms of dementia. Furthermore, glial response mechanisms to proteopathic load are suspected to have a higher correlation to final clinical stages than load of NFT or Amyloid-β. Therefore, it is crucial to characterize changes of receptor protein expression in glial cells which are able to interact between different pathways of inflammation, cell death and brain circuitry. Specifically, the ErbB4 receptor, a member of the epider- mal growth factor receptor family, is suspected to be involved in AD associated pathways. For the characterization of ErbB4 expression in astrocytes, brain tissue from hu- man (hippocampus, Glioblastoma) and murine samples (whole brain) in control and disease were investigated. Additionally, cultures from primary murine astrocytes were treated with receptor ligands and inflammatory molecule TNFα for 24 h. Subsequently, immunohistochemistry on coverslips, Western Blot and qPCR were performed to detect changes of expression. Immunofluorescent analysis of ErbB4, GFAP, Cleaved-Caspase 3, COX2, C1q and interneuronal markers was performed on postmortem PFA-fixed human samples of hippocampus in AD (n=17) and controls (n=14). The spatial distribution and morphological characteristics of ErbB4 was then analyzed and quantified. In addition a digital segmentation and measurement of fluorescence of ErbB4 and GFAP was performed with ImageJ. Immunofluorescence analysis of astrocytes in the CA1 sector of the human hippocampi indicated that the ErbB4 expression correlated with GFAP throughout all samples. Higher intensities for ErbB4/GFAP in AD condition compared to controls were measured on qualitative and quantitative analyses. Astrocytes in resting state showed ErbB4 expression limited to the nucleus with no expression in the somatic cytoplasm or processes, in contrast to ErbB4 expression found in astroglial processes in AD samples. Different pathological conditions (Glioblastoma, dementia with Lewy bodies) showed similar ErbB4 expression patterns in somata and proximal astroglial processes. In addition, analysis of whole human hippocampal sections showed ErbB4 expression in the dentate gyrus and CA4 sector with triangular shaped cell bodies for ErbB4 and Phospho-ErbB4 in the nuclei and somata of neurons and interneurons. The observation of neuronal ErbB4 expression could be reproduced by NeuN/ErbB4 double positive stainings from controls (rat and mouse). Immunofluorescence on primary and immortalized astrocyte cultures showed higher intensities for ErbB4 and GFAP after TNFα treatment. Preliminary results from total RNA extraction/RT/qPCR of primary astrocyte cultures after TNFα administration (24 h) demonstrated upregulation of the ErbB4 ligand NRG1. Unexpectedly, the Western Blot showed bands for ErbB4 at 50 kDa and 230 kDa, reproducible with Phospho- and C-terminal ErbB4 antibodies, not reported in previous studies. Accordingly, these bands were excluded from further quantification. The current thesis also documents a codistribution of ErbB4 with inflammatory markers C1q, Cleaved-Caspase 3 and COX2, indicating a possible induction of inflammatory pathways. This putative neuroinflammatory response may lead to the release of cell growth and neuroprotective molecules suggestive for an anti- inflammatory response. For future experiments, analyses at RNA and protein levels of different anti-/proinflammatory molecules after treatments would help to further understand the effects of ErbB4 activation. Finally, more comprehensive image analysis including multiple scans from all hippocampal areas could provide a more detailed pattern of ErbB4 expression. Taken together, the increased ErbB4 expression revealed in the current thesis is in agreement with previous studies, highlighting the potential relevance of ErbB4 signalling pathway as a potential therapeutic target in AD.