S100B PROTEIN PROMOTES TAU PROTEIN HYPERPHOSPHORILATION THROUGH DKK-1 PROTEIN UP-REGULATION AND RELATIVE WNT PATHWAY DISRUPTION IN CULTURED HUMAN NEURONS

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Neurofibrillary tangles (NFTs) formation is thought to be as the main responsible for the florid exacerbation of Alzheimer's Disease (AD) neurodegeneration, since it is associated with tau protein hyperphosphorilation, collapse of the microtubule network, disturbances of axoplasmic transports, synapse loss, neuritic atrophy, and neuronal death. Recent findings indicate that chronic neuroinflammation in AD brain is a key event prompting NFTs formation and the identification of pro-inflammatory signalling molecules able to promote tau protein hyperphosphorilation is fundamental to better understand the intimate molecular progression of AD.
S100B belongs to a large family of EF-related Ca^{++} and Zn^{++}-binding proteins by low molecular weight produced by astroglial cells. Depending its concentration in the extracellular milieu, S100B promotes both a pro-survival effect on neurons and neurites outgrowth at nM concentrations and a toxic one at μM concentrations (neuronal death via apoptosis). Neuroinflammatory effects of S100B on are mediated its interaction by receptor for advanced glycation end products (RAGE). Over the years, different preclinical and clinical evidence suggest that S100B aberrant release in the CNS is tightly linked to Alzheimer's disease (AD) neuropathology. S100B is in fact increased in post-mortem Alzheimer's disease (AD) brains and its up regulation correlates with brain atrophy occurring in AD patients. No molecular evidence have been yet produced about a possible S100B mediated tau protein hyperphosphorilation have yet been produced. Neuronal progenitor stem cells (NSC) and human neuroblastoma SHSY5Y cells were cultured in neurobasal B27/DMEM 10% FCS, 5% HS supplemented medium and subsequently differentiated in mature neuronal lineage with 10 μg/ml retinoic acid. S100B protein (0.05-5 μM) was added to differentiated cells in the precence or absence of specific RAGE receptor blocking antibody. JNK, DKK-1 protein expression, as well as Wnt pathway proteins, were evaluated both by immunofluorescence and Western blot analysis. In the same experimental condition EMSA for AP-1/cJUN supershift was also performed.
The present study demonstrates that micromolar S100B levels exposure activates trhough RAGE interaction JNK activation and subsequent nuclearAP-1/cJun activation. S100B induced AP-1/cJun activation leads to DKK-1 up-regulation. The specific JNK inhibitor SP600125 potentiates RAGE blocking antibody to mediate this effect. DKK-1, is here indicated as responsible for S100B-mediated Wnt pathway disruption, leading to tau protein hyperphosphorilation in human neurons. To confirm the experimental data obtained in primary cultured neurons the specific siRNA for DKK-1 transfected in human neuroblastoma SHSY5Y cells abolish the S100B induced Wnt pathway disruption and relative tau hyperphosphorilation.