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Insulin growth factor binding protein 7 is a novel target to treat dementia

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ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia in the elderly but effective therapeutic strategies to treat AD are not yet available. This is also due to the fact that the pathological mechanisms that drive the pathogenesis of sporadic AD are still not sufficiently understood and may differ on the individual level. Several risk factors such as altered insulin-like peptide (ILP) signaling have been linked to AD and modulating the ILP system has been discussed as a potential therapeutic avenue. Here we show that insulin-like growth factor binding protein 7 (IGFBP7), a protein that attenuates the function of ILPs, is up-regulated in the brains of AD patients and in a mouse model for AD via a process that involves altered DNA-methylation and coincides with decreased ILP signaling. Mimicking the AD-situation in wild type mice, by increasing hippocampal IGFBP7 levels leads to impaired memory consolidation. Consistently, inhibiting IGFBP7 function in mice that develop AD-like memory impairment reinstates associative learning behavior. These data suggest that IGFBP7 is a critical regulator of memory consolidation and might be used as a biomarker for AD. Targeting IGFBP7 could be a novel therapeutic avenue for the treatment of AD patients.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder. It currently affects more than 30 million people worldwide. This number is expected to double until 2025 due to increased life expectancies. AD arises on the pathological background of amyloid-ß-plaques, neurofibrillary tangles and severe neurodegeneration leading to dementia (Haass and Selkoe, 2007). Expect for the few cases that develop an early onset form of AD that is caused by mutations in the amyloid precursor protein (APP) itself or the proteins that process APP (Haass and Selkoe, 2007), aging is the major risk factor for the sporadic form of AD that accounts for 98% of all cases. At present there is no effective treatment for AD and all phase III clinical trials targeting Aβ pathology have failed indicating that treatments targeted towards Aβ pathology are too late to help patients that already suffer from memory disturbances (Miller, 2012; Mullard, 2012). At the same time, such data shows that we still understand very little of the pathological mechanisms that mediate memory decline in sporadic AD. There is now emerging evidence that sporadic AD is driven by various combinations of genetic and environmental risk factors that likely differ on the individual level. One interesting observation has been a link between de-regulated signaling of ILPs – that include insulin and insulin-like growth factors (IGF) 1 and IGF2 – and sporadic AD (Piriz et al., 2011). Several population-based studies have reproducibly shown that the risk to develop cognitive decline in elderly people is increased in individuals suffering from type 2 diabetes (Arvanitakis et al., 2004; Luchsinger, 2012). Such findings inspired subsequent studies and one promising line of research has been the analysis of ILP signaling cascades for their role in AD pathogenesis (Piriz et al., 2011). The role of IGF1 and IGF2 – that are both expressed in the adult brain (Agis-Balboa et al., 2011) – in AD pathogenesis has been studied (Carro et al., 2002). In particular IGF1 has been linked to neuroprotection (Dudek et al., 1997; Russo et al., 2005) and administration of IGF1 or insulin was found to have beneficial effects in humans suffering from ataxia or AD (Arpa et al., 2011; Craft et al., 2011). Such data are in line with findings demonstrating a role of ILPs in memory formation, neuronal and synaptic plasticity as well as adult neurogenesis (Agis-Balboa et al., 2011; Castro-Alamancos and Torres-Aleman, 1994; Chen et al., 2011; Llorens-Martín et al., 2009). Despite such promising results there is also evidence that age-related neurodegenerative processes such as amyloid deposition are promoted by IGFs and IGF1 in particular (Araki et al., 2009; Cohen and Dillin, 2008; Cohen et al., 2009; Freude et al., 2009). As a solution to this paradox it has been suggested that each organ has probably an optimal level of ILP signaling and that both decreased and increased ILP levels could contribute to neurodegenerative diseases depending on the specific context (Cohen and Dillin, 2008). Thus, rather than targeting for example IGF1 it would be desirable to learn more on the mechanisms that mediate fine-tuning of the ILP system in the adult brain. A line of

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research that had remained relatively unexplored in this context is the role of IGF binding proteins (IGFBPs). IGFBPs bind with high affinity to IGFs and thereby attenuate their function by regulating their bioavailability (Baxter, 1991). The mammalian genome encodes seven IGFBPs that are named IGFBP1 to IGFBP7. IGFBPs are expressed in the adult brain and there is evidence that IGFBPs contribute to brain cell homeostasis and play a role in memory formation and AD pathogenesis (Agis-Balboa et al., 2011; Duron et al., 2012; Kerimoglu et al., 2013; Ocrant, 1993) but mechanistic studies are still rare. Thus we decided to analyze the potential role of IGFBPs in AD pathogenesis in greater detail.

In this study we show that the onset of cognitive decline in a mouse model for Alzheimer's disease correlates with an up-regulation of IGFBP7 in the hippocampus. We find that this effect is linked to altered DNA-methylation in the promoter region of the *Igfbp7* gene. Mimicking the AD-situation in wild type mice by increasing hippocampal IGFBP7 levels leads to memory impairment. Conversely, attenuating IGFBP7 function in a mouse model for AD rescues ILP signaling and memory function. In line with these data we observe that in the brains of human AD patients DNA-methylation of the *Igfbp7* gene is decreased which is consistent with the fact that *Igfbp7* gene expression and IGFBP7 protein levels are elevated at a relatively early state of the disease. In conclusion our data provide evidence that IGFBP7 contributes to memory decline in AD and suggest that inhibition of IGFBP7 could be a novel therapeutic approach to treat AD pathogenesis.

Materials and methods

Animals

Three month-old male wild type (C57Bl/6J) mice were obtained from Janvier and housed with free access to food and water under standard light/dark conditions (12 h–12 h). Male APPPS1-21 mice (Radde et al., 2006) containing the Thy1-APP (KM670/671NL) and Thy1-PS1 (L166)) transgenes were employed as a model for Alzheimer's disease. To detect the presence of the transgene DNA was isolated from tails and subjected to a polymerase chain reaction (PCR) as described previously (Radde et al., 2006). Mice that did not contain the transgenes were used as control littermates. All experiments were carried out in accordance with the animal protection law and were approved by the District Government of Germany.

Human tissue samples

Human brain tissue was obtained from Boston University Medical Center (protocol number H-24454) Massachusetts Alzheimer Diseases Research Center (protocol number 2004-P-001613/4; BWH). Use of this tissue was approved by the local ethical committees.

Behavioral experiments

Mice were single housed and habituated to the testing room at least 7 days before behavior testing. For the open field test mice were placed in the center of a plastic arena (length: 1 m; width: 1 m; side walls (height): 20 cm) for 5 min. The explorative behavior was recorded by a camera and analyzed using the VideoMot2 software (TSE Systems). Fear conditioning training was performed using the fear conditioning system from Med-Associates. The procedure consisted of exposing the mice to the conditioning context (3 min) followed by a single electric foot shock (0.7 mA, constant current, 2 s). Afterwards the mice were left in the conditioning box for 15 s before being returned to their home cage. Freezing was analyzed 24 h later during re-exposure to the conditioning context. Water maze training was performed in a circular tank (diameter: 1.2 m) filled with opaque water. A platform (11 × 11 cm) was submerged below the water's surface in the center of the target quadrant. The swimming path of the mice was recorded by a video camera and analyzed by the VideoMot2 software (TSE). For each training session, the mice were placed into the maze subsequently from four random points of the tank and were allowed to search for the platform for 60 s. If the mice did not find the platform within 60 s, they were gently guided to it. The mice were allowed to remain on the platform for 15 s. The mice were subjected to a memory test (probe trial) 24 h after the last training session. During the probe test the platform was removed from the tank and the mice were allowed to swim in the maze for 60s.

Implantation of microcannulae and intra-hippocampal injections

Intra-hippocampal injections were performed as described previously (Peleg et al., 2010). In brief, the mice were anesthetized and microcannulae were stereotactically implanted to the hippocampus (1.7 mm posterior to the Bregma; 1.0 mm lateral from midline; and 1.5 mm ventral). After recovery from surgery, the mice received bilateral injections (1 μl, at a rate of 0.25 μl min^{-1}) of IGFBP7 (R&D Systems, 0.5μg/μl), IGFBP7 blocking antibody (R&D Systems, 1μg/μl) or IgG (dissolved in sterile 0.1% BSA in sterile PBS) at the indicated time points shown in experimental designs of the respective figures. For hippocampal injections a 1.5 mm-gauge needle that extended 0.5 mm beyond the tip of the guide cannula was used.

Methylated DNA immunoprecipitation (MeDIP)

MeDIP was performed using 6 μg of DNA (dissolved in TE buffer) isolated from the mouse hippocampus or human prefrontal cortex (Brodmann area 9) using phenol–chloroform. Immuno-selection was carried out with 5 μl of mC specific antibody (Eurogentec) after denaturation at 95 °C and then incubated for 2 h. PBS–BSA-pre-washed magnetic beads were then added and again incubated for 3 h at 4 °C. Unbound DNA was washed away with the aid of magnetic block. The complex was washed three times with IP buffer and then re-suspended in digestion buffer (250 μl) containing 3.5 μl of proteinase K and then incubated for 3 h at 50 °C. DNA was then subjected to phenol/chloroform extraction and ethanol precipitation and subjected to qPCR analysis.

Chromatin immunoprecipitation (ChIP)

ChIP was performed following the modification of the Invitrogen ChIP kit protocol. Tissue samples from the hippocampus were fixed in 1% formaldehyde and cross-linked cell lysates were sheared by sonication in 1% SDS lysis buffer to generate chromatin fragments with an average length of 100–200 bp. The chromatin was then immunoprecipitated using an antibody specific to DNA methyltransferase 1 (DNMT1) (cell signaling) and DNA methyltransferase 3a (DNMT3a) (cell signaling) overnight at 4 °C. Protein–DNA–antibody complexes were precipitated with protein G-magnetic beads for 1 h at 4 °C, followed by two washes in low salt buffer, two washes in high salt buffer, and three washes with 1× Tris–EDTA buffer. The precipitated protein–DNA complexes were eluted from the antibody with 1% SDS and 0.1 M $NaHCO_3$, and then incubated overnight at 65 °C in 200 mM NaCl to reverse formaldehyde cross-links. Following proteinase K digestion, phenol–chloroform extraction and ethanol precipitation, samples were subjected to qPCR.

Quantitative real-time PCR (qPCR)

Total RNA was extracted from hippocampal mouse tissue and the human prefrontal cortex (Brodmann area 9) using the Tri-Reagent (Sigma-Aldrich, Deisenhofen) according to the manufacturer's recommendation and the concentration of RNA was determined using a Nano-Drop® ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and a bioanalyzer (Stratagene). RNA samples (1 μg) were reverse-transcribed into first-strand cDNA with the