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Short Communication

CHORDC1, the novel interacting partner of tau protein

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Abstract. Alzheimer's disease is currently not curable. Almost all attempts to identify disease-modifying drugs failed and the causes of disease etiology are not well understood. Neurofibrillary tangles composed of pathological tau protein belong to the main hallmarks of this disease. Identification of novel physiological and pathological tau interacting proteins may lead to a better understanding of Alzheimer's disease pathology and tau physiology and therefore we performed a screening of the brain library by a yeast two-hybrid system intending to identify new tau interaction partners. We identified CHORDC1 (cysteine and histidine-rich domain-containing protein 1) as a novel tau interaction partner by this approach. The CHORDC1-tau interaction was validated by co-immunoprecipitation from rat brain tissues and by *in vitro* co-localization in the cellular model expressing full-length human tau protein. We believe that our results can be useful for researchers studying tau protein in health and disease.

Key words: Protein-protein interactions — Tau protein — Alzheimer's disease — Yeast two-hybrid system

Worldwide, an increasing number of patients are suffering from Alzheimer's disease (AD), and although several therapeutic approaches have been developed to target this most common type of dementia so far, there is still no reliable treatment (Alzheimer's Association 2023). The main pathological hallmarks of AD are amyloid plaques, consisting of amyloid-β peptides, and the neurofibrillary tangles, composed mainly of pathological tau proteins, whereby tau pathology correlates much better with disease progression (Braak et al. 2006). Tau protein is a multifunctional protein that fulfills several different and important physiological functions. In the axon of the neurons, the tau protein's major role is binding to tubulin and the subsequent induction of nucleation, elongation, and bundling of tubulin into microtubules. Under pathological conditions, tau protein is hyperphosphorylated and truncated leading to cytotoxic tau aggregation and the formation of neurofibrillary tangles resulting in neurodegeneration (Iqbal et al. 1986; Novak et al. 1991). Those changes lead to the loss or impairment of several functions of the tau protein, deteriorating many processes (Lu et al. 2013) resulting in cell death, neuronal loss, cognitive decline, and synaptic dysfunction (Yanamandra et al. 2013).

The tau pathology in AD is a complex issue, which is dependent on many different processes and factors. Since protein-protein interactions are a fundamental part of all molecular pathways and biological processes, the interaction partners of tau may directly influence the formation of pathological tau proteins prone to aggregation. Tau interaction partners could be changed under disease conditions, either the physiological interactions could be impaired, or new, non-physiological interactions can occur. Studying the interactome of a tau protein is an essential tool for a better understanding of the involvement of these proteins in physiological and pathological processes.

To better understand the role of the tau protein in its physiological and pathological conditions, we decided to

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continue our previous study (Sinsky et al. 2020) and searched for novel interacting partners of N-terminal tau protein fragment by yeast two-hybrid system. The N-terminus of tau protein remains flexible upon binding to microtubules and is exposed to protein-protein interactions. Furthermore, the N-terminus of tau interacts with the SH3 domain of many key regulatory proteins (Mandelkow et al. 2012). Our previous findings support the fact that the N-terminus of the tau protein may play a role in tau conformational dynamics since the truncation of tau at its N-terminus resulted in changes in the crosslinking pattern of tau (Hornakova et al. 2022). Moreover, the N-terminus of tau has been used as a therapeutic target to develop drugs for AD (Cummings et al. 2023).

The yeast two-hybrid system can detect real and specific protein-protein interactions (PPIs) since the transcription of the reporter genes requires persistent activation of their promoter by re-assembled transcriptional factor. Although yeast's posttranslational glycosylation system differs from the one in mammals, yeasts have many mammalian posttranslational modifications such as phosphorylation, methylation-demethylation, acetylation and ubiquitination (Hamilton and Gerngross 2007; Separovich et al. 2021). The drawback of the system, in the case of proteins from different organisms, is the possibility of proteins being folded differently compared to their native organism.

We cloned the N-terminus of human tau protein (amino acids 2–243 of the longest human tau isoform) into the *S. cerevisiae* yeast two-hybrid expression vector (pGBKT7) and used it for the screening of tau interaction partners using the Takara Mate & Plate Library (Normalized human brain cDNA library, with 3.2×10^6 of independent clones). Screening and the removal of false positives were carried out according to the manufacturer's protocol. Strains used in the screening was the Y2H Gold strain (genotype: *MATa, trp1-901, leu2-3,112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1UAS–Gal1 TATA–His3, GAL2UAS–Gal2TATA–Ade2URA3::MEL1 UAS–Mel1 TATA AUR1-C MEL1*) containing the bait protein and Y187 strain (genotype: *MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4Δ, gal80Δ, met–, URA3::GAL1UAS–Gal1TATA–LacZ, MEL1)* carrying the library. From the screening, we obtained 63 positive clones. We found that the removal of false positives is a very important step in the screening because the majority of the identified potential interactions were false positives (96.82%). The removal of false positives after two-hybrid system screening is in some cases not applied, which may lead to the identification of proteins that in reality do not interact.

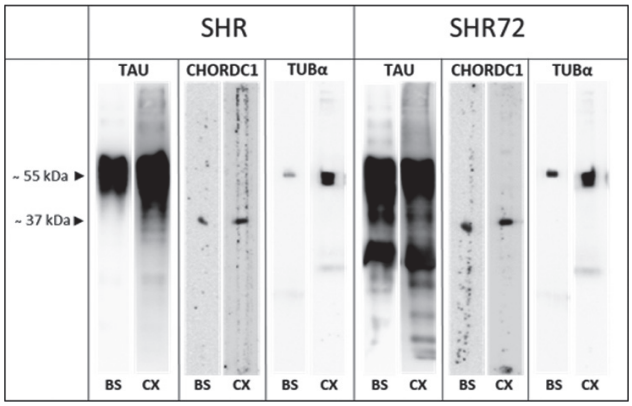

Figure 1. Validation of CHORDC1 interaction with tau protein by co-IP from rat brain tissues. CHORDC1 was co-immunoprecipitated from rat brain tissues by the mixture of anti-tau antibodies (DC25, DC18, and DC190), for detail see Sinsky et al. (2020). BS, brain stem; CX, cortex; SHR, SHR control rat: SHR72, transgenic rat expressing truncated pathological tau protein. As control of co-immunoprecipitation also immunoblot of known interaction partner of tau – tubulin-α is shown. The eluted tau proteins were visualized using DC25 mAb.

We identified only 2 truly tau interacting partners, but one of them we excluded from further validation due to its frameshift in the open reading frame leading to the translation of only a short irrelevant peptide. We identified one tau-interacting protein, the cysteine and histidine-rich domain-containing protein 1 (CHORDC1) that has not been so far known as a tau-interacting partner. Surprisingly, we were not able to identify any known interacting protein with the N-terminus of tau. One of the reasons could be that such proteins were not present in the library, or are present in their truncated forms. Other reasons could be that they may not be properly folded, or they can possess different post-translational modifications in yeast, compared to human proteins.

For *in vitro* validation experiments of the CHORDC1-tau interaction, we performed co-immunoprecipitation (co-IP) and co-localization experiments. The methods of Western blotting and co-localization are carried out as described in Sinsky et al. (2020). Because we were interested in whether CHORDC1-tau interaction is not altered under pathological conditions, we carried out the co-IP experiments using brains from both, the control rat (SHR) and also a transgenic rat model (SHR72). The SHR72 rat model expresses truncated pathological tau protein and possesses a similar pathology comparable to human tauopathies (Zilka et al. 2006). Co-immunoprecipitation from rat cortexes and brain stems (all mixtures of 3 tissues from either SHR or from SHR72) was performed as previously described (Sinsky et al. 2020) using the mixture of anti-tau antibodies (DC25, DC18, and DC190), and detection on Western blot was performed by CHORDC1 antibody (Proteintech Biotechnology) (Fig. 1). Although we have not performed quantification experiments it appears that a slightly higher amount of CHORDC1 was co-IP from SHR72 brain tissues, in both the cortex and brain stem, compared to SHR control rats. This difference could

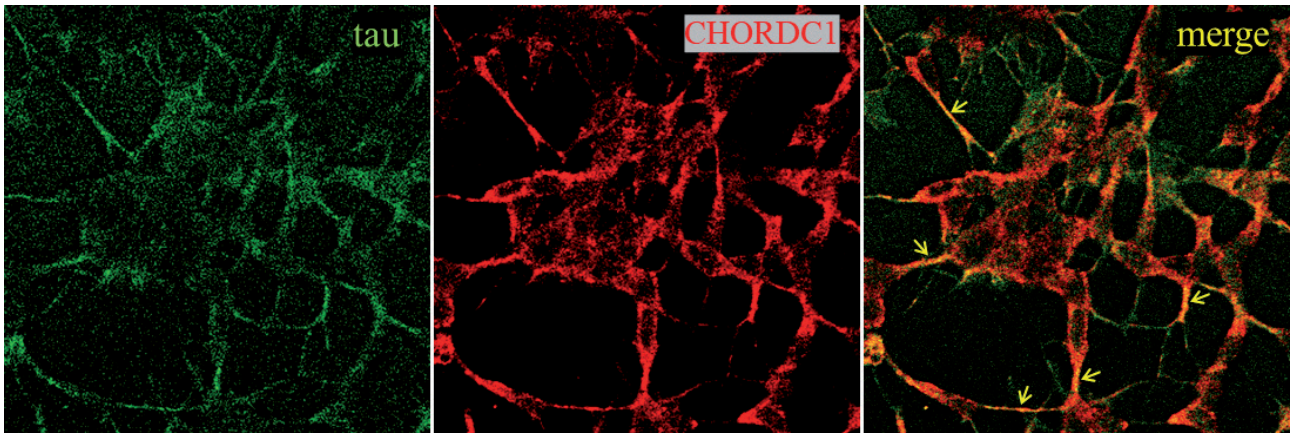

Figure 2. Colocalization of CHORDC1 interaction with tau protein in SH-SY5Y-2N4Rtau cell line. Expression of tau protein was detected by anti-tau Tau1 antibody/Alexa Fluor® 488 goat anti-mouse pAb (green) and CHORDC1 expression by anti-CHORDC1 pAb/Alexa-546 goat anti-rabbit pAb. Yellow arrows highlight the colocalization signal, as a result of green and red emission signal overlay, observed predominantly on dendritic microtubules. (See online version for color figure.)

be due to the higher expression of tau proteins in SHR72 models. Because the SHR72 rat model expresses truncated human tau, amino acids 151-391, and CHORDC1 was identified as an interacting protein with the N-terminus of tau (aa2-243), the interaction of tau with CHORDC1 may take place in the region aa151-243 of tau protein.

For the colocalization experiments, we used the SH-SY5Y-2N4Rtau cell line, the cellular model expressing 2N4R tau, the longest human isoform of tau protein (Zilkova et al. 2011). Interaction of tau with CHORDC1 was detected using anti-tau Tau1 mAb and anti-CHORDC1 pAB. Although both proteins were expressed in the soma and dendrites of cells, with the expression of CHORDC1 being the highest in dendrites, we found that the tau protein interacted with CHORDC1 predominantly on dendritic microtubules (Fig. 2). This co-localization result was not surprising, because the tau protein is known to bind and stabilize microtubules and is present there in high concentration (Binder et al. 1985).

CHORDC1 plays several different roles in several different pathways. CHORDC1 is a component of the IKK complex, a central regulator of NF-κB activation. It was shown that CHORDC1 is required to induce IκBα phosphorylation by IKK resulting in the activation of NF-κB signaling molecules in the cytoplasm. As a consequence, the NF-κB enters the nucleus and activates transcription of a variety of genes participating in the immune and inflammatory response, cell adhesion, and protection against apoptosis (Fusella et al. 2017). CHORDC1 protein is a known interaction partner of Hsp90, and it is proposed to act as its co-chaperone, enabling its protein binding activity. Hsp90 is a vital part of the chaperone system, which is responsible for folding nascent polypeptides, refolding of denatured proteins, and directing of damaged proteins to the ubiquitin-proteasome pathway for degradation. In AD, this mechanism of clearance seems to be impaired or overloaded leading to the accumulation and aggregation of misfolded tau proteins. The mature Hsp90 refolding complex is also inhibiting the degradation of phosphorylated tau proteins. The inhibition of Hsp90 has been shown to lead to decreased levels of phosphorylated tau protein. Several co-chaperones are participating in this removal of phosphorylated tau (Dickey et al. 2007).

The interactions between tau protein, CHORDC1 and Hsp90 suggest a link that CHORDC1 may play a regulatory role or be directly involved in the folding or removal of misfolded and/or pathological tau proteins. CHORDC1 is also involved in multiple stress responses and is involved in centrosome duplication by a different mechanism. Dysregulation of CHORDC1 has been shown in several other diseases such as cancer or cell cycle disorders (Ferretti et al. 2010). Its activity has not yet been associated with any tauopathies. The confirmation of the direct participation of CHORDC1 in the removal of tau protein in pathological conditions, and also the proper folding in physiological conditions by the Hsp90 complex would make it an interesting potential drug target.

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