The interaction between ADAM22 and 14-3-3\(\beta\)

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Abstract ADAM family consists of a number of transmembrane proteins that contain a disintegrin and metallopeptase domain. ADAMs are involved in a highly diverse set of biological processes, including fertilization, neurogenesis, myogenesis and inflammatory response. The ADAM proteins have both cell adhesion and protease activities. \(\text{Adam}^{22}\) is highly expressed in human brain. The \(\text{adam}^{22-/-}\) mice presented severe ataxia and died before weaning, but the function of ADAM22 is still unknown. \(14-3-3\beta\) interacting with ADAM22 was detected by using yeast two-hybrid assay. The specificity of interaction between ADAM22 and \(14-3-3\beta\) was proved by \textit{in vitro} binding assay and immunoprecipitation. The major \(14-3-3\beta\) binding site was located in the last 28 amino acid residues of ADAM22 cytoplasmic tail. Protein \(14-3-3\beta\) is abundant and plays an important role in mediating cell diffusion, migration and cell cycle control. The interaction of ADAM22 and \(14-3-3\beta\) suggests that the ADAM22 may play a crucial role in neural function and development.

Keywords: ADAM22, 14-3-3\(\beta\), yeast two-hybrid assay, immunoprecipitation, cell adhesion.

ADAM (A Disintegrin And Metalloprotease) is a family of cell surface proteins. Approximately 31 members of ADAM family have been identified. A typical ADAM is a multi-domain protein including pro-domain and domain of metallopeptase, disintegrin, cysteine-rich, epidermal growth factor (EGF)-like, transmembrane and cytoplasmic tail domains\(^{[1-4]}\). ADAM22 is abundant in the cerebellum and scarcely can be detected in the spinal cords. It does not contain the RGD sequences, which is critical for integrin binding. A zinc-binding motif, which is essential for protease activity, is disrupted in ADAM22\(^{[5]}\). ADAM22 is a potential cell adhesion molecule. Although emerging data have indicated the distribution, amount and potential function of ADAM22, there is still no report concerning its role in physiological processes and signal transduction. In order to clarify the function of ADAM22 in neural system, the fusion protein of LexA-ADAM22cyt (cytoplasmic tail) was used as a bait to screen a human fetal brain cDNA library by yeast two-hybrid assay. One of the proteins interacting with ADAM22 was screened out, and further work was performed.

1 Materials and methods

1.1 Yeast two-hybrid screen and tests

ADAM22 cytoplasmic tail was amplified by PCR and subcloned into pEG202, resulting in
an in-frame fusion between the LexA DNA binding domain and ADAM22: pEG-ad22cyt. The LexA-ADAM22cyt is used as a bait in yeast two-hybrid assay. The subcloning of cytoplasmic tail of ADAM29 and ADAM10 into pEG202 was carried out similarly. To test the major binding site, a serial deletion of ADAM22 cytoplasmic tail was prepared and subcloned into pEG202. Briefly, yeast strain EGY48 harboring a LexAop-LacZ reporter (a gift from Dr. T. Wang, University of Washington) was transformed with the bait construct pEG-adam22. Expression of LexA-ADAM22cyt was confirmed by Western blotting with the monoclonal antibody against LexA. After confirming lack of auto-activation on selective plate, the selected yeast transformants were transformed with a human fetal brain cDNA library. About one million cells were screened on UHWA galactose plates containing X-gal. Plasmid DNA was purified from the blue colonies and re-tested for ADAM22cyt interaction in yeast and then sequenced. They were then retransformed into yeast with different bait vectors to determine the specificity of interaction. The procedure is according to the protocol of Frederick and colleagues[6].

1.2 Binding assay in vitro

For binding assay, the PCR amplified full-length 14-3-3 β cDNA was ligated in frame into the E. coli expression vector pGEX (Amersham Pharmacia Biotech). pGEX-14-3-3β was transformed into E. coli BL21 and induced with IPTG. GST fusion protein and yeast lysate were extracted[9,10]. GST alone was prepared in parallel with GST-14-3-3β. GST and GST-14-3-3β proteins coupled to glutathione beads (Amersham Pharmacia Biotech) were incubated at 4°C for 2 h with lysates prepared from yeast that had been transfected with pEG-ADAM22cyt. The beads were then washed 3 times in washing buffer (25 mmol/L Tris-HCl, pH 7.5, 1 mmol/L EDTA, 0.5 mmol/L dithiothreitol) and boiled in SDS sample buffer. SDS electrophoresis and proteins transferred from the gel to a nitrocellulose membrane were carried out. Western blotting was performed with monoclonal antibody against LexA. Lysate prepared from the yeast transfected with pEG202 was used as a control.

1.3 Immunoprecipitation and Western blot

To confirm the ADAM cytoplasmic tail binding to 14-3-3β, the mammalian expression constructs of MYC-adam22cyt and HA-14-3-3β were made by subcloning adam22cyt into pCMV-Myc and 14-3-3β into pCMV-HA, respectively. Expression constructs of MYC-adam22cyt and HA14-3-3β were cotransfected into the cell line HEK293. After 2 days, cells were lysed in lysis buffer (50 mmol/L Tris-Cl, pH 7.5, 150 mmol/L NaCl, 1% Triton X-100, 0.5% deoxycholate, and 10 mg/mL each of pepstatin A, leupeptin, and aprotinin). After incubation on ice for 15 min, the lysate was centrifuged at 10000 r/min for 20 min, and the supernatant was recovered. Immunoprecipitation was performed with antibody against HA and a monoclonal antibody against MYC was used for immunoblotting. Immunoprecipitation and Western blot were according to the manufacturer’s instructions (Clontech Laboratories, Inc). pCMV-Myc and pCMV-HA were per-