Analysis of β-Amyloid Peptide -Binding Proteins in Microglial Cells

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Abstract: Alzheimer's disease is the most common form of dementia in the elderly. Although β-amyloid peptide (Aβ) has been considered major cause of Alzheimer pathology, molecular and cellular mechanisms of the disease development of Alzheimer have not been clarified yet. Presently Aβ has been considered to induce neural cell death by direct penetration of aggregated form or indirect cell death by inflammatory responses induced by Aβ-activated microglia. In order to understand Aβ induced microglial activation, we searched the proteins which bind to Aβ in activated microglial cell line. We stimulated Ra2 microglial cell line with Aβ. Activated Ra2 cells were immunoprecipitated with anti- Aβ and run the gel. Membrane was silver stained and bands were cut and digested with enzyme. They were analyzed by LC/MS/MS. We found that several proteins including myosin 9 and actin bound to Aβ. By the addition of Aβ, actin binding was enhanced and other proteins including IQGAP1, Plectin strongly bound to Aβ. These results indicate that Aβ binds to the proteins belonging to cellular cytoskeletal system.

Keywords: Alzheimer, Abeta, LC/MS/MS, microglia, IQGAP1, actin.

INTRODUCTION

Alzheimer's disease is the most common form of dementia. More than 35 million people have Alzheimer's disease worldwide. The principal risk factor for Alzheimer's disease is age [1]. Cerebral plaques laden with β-amyloid peptide (Aβ) have been considered major cause of Alzheimer pathology [2]. Aβ outside the cell spontaneously self-aggregates. Aβ can also grow into fibrils, which arrange themselves into β-pleated sheets to form the insoluble fibers of advanced amyloid plaques. Aβ damages neurons by two ways. One is direct toxic effect of Aβ to neurons. Another is indirect damages to neurons by microglial activation.

Aβ binds to CD36 and other scavenger receptors on microglia, which may induce microglial activation and produce inflammatory cytokines and chemokines, reactive oxygen species (ROS) and reactive nitrogen species (RNS) and other neurotoxins [3,4]. CD36 has established roles in the endocytic uptake of altered self components, including oxidized phospholipids, apoptotic cells and amyloid proteins [5] Recently it has been shown that CD36-TLR4-TLR6 activation as a common molecular mechanism by which atherogenic lipids and Aβ stimulate sterile inflammation [6]. We have shown that Aβ 25-35 induces Akt and IκBα phosphorylation within 15 min after addition to culture medium [7]. Further Aβ 1-42 induces Akt and PI3/Akt phosphorylation within 5 min [8]. These results indicate that monomer or soluble oligomer Aβ but not aggregated Aβ bind to cell membrane and induces immflamatory responses. In these cases Aβ may stay in cell membrane or enter cells by endocytosis. It has been postulated that in neuronal cells Aβ works inside cells and may dampen excitatory transmission and prevent neuronal hyperactivity [9]. In order to understand the Aβ-induced microglial activation, we tried to find out Aβ binding proteins inside microglia by LC/MS/MS methods.

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MATERIALS AND METHODS

Materials

Synthetic human Aβ1-42 was obtained from Peptide Institute Inc (Osaka, Japan). Aβ1-42 was dissolved in 0.1% NH_3 according to the manufacturer's instructions. Mouse monoclonal anti-Aβ antibody (Millipore, MAB1561) was used for Immunoprecipitation. This monoclonal anitibody (IgG2b) binds to human and mouse Aβ. The epitope lies between amino acids 18-22 of Aβ.

Cell Culture

Microglial cell line Ra2 was cultured in MGI medium [Eagle's MEM supplemented with 0.2% glucose, 5 μg/ml bovine Insulin (Sigma-Aldrich), and 10% fetal bovine serum (FBS, Invitrogen)] and 0.8 ng/ml mrGM-CSF (Pharmingen) [10].

Immunoprecipitation

The cells were lysed with RIPA buffer (25 mM Tris-HCl, pH8.0, 150 mM NaCl, 10% glycerol, 2mM EDTA, 5 mM $MgCl_2$, 0.3% NP40 1 mM PMSF). 5 mg of affinity-purified monoclonal antibodies was covalently coupled to 50 mL Protein G beads (GE healthcare) by using 20mM DMP. Cell lysates were pre-incubated with protein G beads for 2 hour at 4 °C and then centrifuged. The supernatant was incubated with antibody-cross-linked protein G beads overnight at 4 °C. The beads were washed five times with RIPA buffer and then suspended in 0.1M glycine (pH 1.7) at 4 °C for 1 hr. The eluted sample was neutralized by the addition of 1M Tris-HCl (pH 9.0). The eluted proteins were mixed with sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10%

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glycerol, 5% 2-mercaptoethanol, and 5% bromophenol blue). A fraction of the eluate was monitored by SDS-PAGE and silver stained.

Identification of Proteins

For LC-MS/MS ion search analysis, protein spots were excised from the gel. The gel pieces were destained and dried by vacuum centrifugation. For carbamidomethyl modification, the dried gel pieces were rehydrated in 100 mM ammonium bicarbonate containing 10 mM DTT. After removal of the solution, the gel pieces were alkylated and then rehydrated in a trypsin digest solution (Trypsin Gold, Mass Spectrometry Grade (Promega Co., Madison, WI)). LC-MS/MS ion search analysis was performed using an LCQ Advantage nanospray ionization iontrap mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA) combined with a MAGIC2002TM HPLC System (Michrom BioResources, Inc., Auburn, CA) that was equipped with a MonoCapR column of 0.1 mm diameter and 50 mm in length (AMR Inc., Tokyo Japan). The MS/MS spectrum data collected repeatedly were submitted to the program Mascot (Matrix Science Inc., Boston, MA).

RESULTS

Several Proteins Bind to Aβ 1-42 in Microglial Cells

We used affinity separation techniques using anti- Aβ bound to beads to fractionate extracts from Ra2 cells (Fig. **1**). In this procedure, we added Aβ 1-42 in lysis of Ra2 cells. The bound proteins were run in SDS PAGE and stained with silver. Several bands were detected. Without Aβ 1-42 we could detect several bands (Fig. **2** midle lane). By the addition of Aβ 1-42, many new bands appeared (Fig. **2**, right). Among them we took four bands. Band No1 and No3 appeared only in the lane of Aβ 1-42 addition. Band No2 and No4 appeared both in the lane with Aβ 1-42 and without Aβ 1-42. However, No4 band is stronger in the lane with Aβ 1-42 than in the lane without Aβ 1-42 (Fig. **2**).

IQGAP1 and Plectin Bind to Aβ 1-42 in Microglial Cells

Gel bands were cut out and purified. Extracted trypsin-digested proteins of the samples were analyzed by LC-MS/MS Band. No2 and No4, which were found both in Ra2 microglia with or without Aβ 1-42, were myosin 9 (No2) (Fig. **3A**) and actin (No4) (Fig. **3B**).

No3, which was strongly expressed in Ra2 microglia with Aβ 1-42, was IQGAP1 (Fig. **3C**). No1, which expressed in Ra2 microglia with Aβ 1-42, was Plectin (Fig. **3D**).

DISCUSSION

Aβ binds to Several Proteins in Microglial Cells

By immunoprecipitation using anti-Aβ antibody, we found four strong bands without addition of Aβ 1-42. Among them No2 was analyzed by LC/MS/MS and found to be myosin 9. This band was not enhanced by the addition of Aβ. However, No4 protein was greatly enhanced by the addition of Aβ. No4 protein was found to be actin. These results indicate that the addition of Aβ enhances actin expression. We speculate that Aβ 1-42 induces cellular migration, which induces F-actin in activated cells. Addition of Aβ 1-42 induces many new proteins in microglial cells. By the addition of Aβ 1-42, we detected several new bands. Among them we determined most strong band (No3). By the analysis of LC/MS/MS, we found that it was IQGAP1.

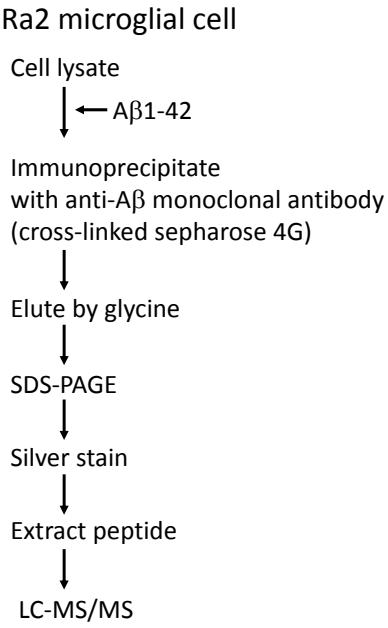

Fig. (1). Flow diagram for proteomic isolation of Aβ binding proteins. Ra2 cells were lysed in RIPA buffer, which were immunoprecipitated with anti-Ab and run the gel. Stained gel bands were cut and digested for LC/MS/MS.

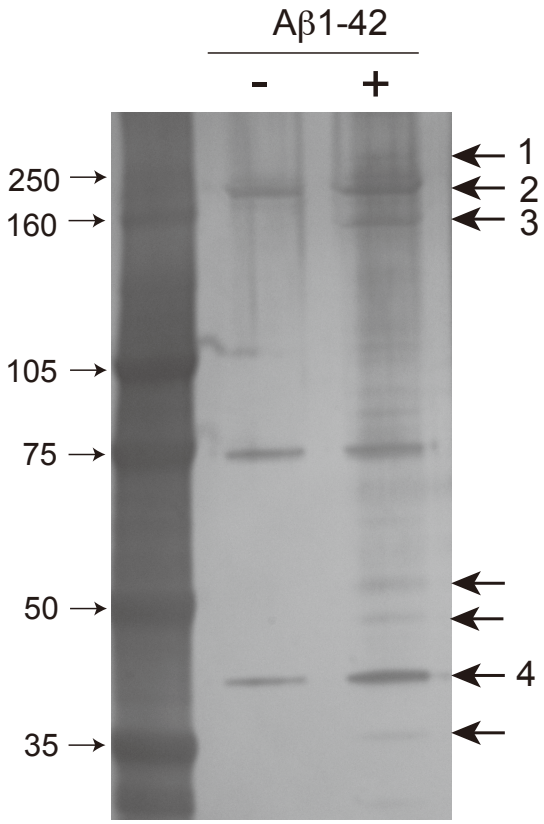

Fig. (2). Several proteins bind to Aβ by immunoprecipitation. Ra2 extracts were treated with anti Aβ antibody cross-linked protein G beads. The bound proteins were stripped from the beads and run on SDS-PAGE gels and stained with silver.

(**A**)

Myosin-9 sequence

MAQQAADKYL YVDKNFINNP LAQADWAAKK LVWVPSSKNG FEPASLKEEV
GEEAIVELVE NGKKVKVNKD DIQKMNPPKF SKVEDMAELT CLNEASVLHN
LKERYYSGLI YTYSGLFCVV INPYKNLPIY SEEIVEMYKG KKRHEMPPHI
YAITDTAYRS MMQDREDQSI LCTGESGAGK TENTKKVIQY LAHVASSHKS
KKDQGELERQ LLQANPILEA FGNAKTVKND NSSRFGKFIR INFDVNGYIV
GANIETYLLE KSRAIRQAKE ERTFHIFYYL LSGAGEHLKT DLLLEPYNKY
RFLSNGHVTI PGQQDKDMFQ ETMEAMRIMG IPEDEQMGLL RVISGVLQLG
NIAFKKERNT DQASMPDNTA AQKVSHLLGI NVTDFTRGIL TPRIKVGRDY
VQKAQTKEQA DFAIEALAKA TYERMFRWLV LRINKALDKT KRQGASFIGI
LDIAGFEIFD LNSFEQLCIN YTNEKLQQLF NHTMFILEQE EYQREGIEWN
FIDFGLDLQP CIDLIEKPAG PPGILALLDE ECWFPKATDK SFVEKVVQEQ
GTHPKFQKPK QLKDKADFCI IHYAGKVDYK ADEWLMKNMD PLNDNIATLL
HQSSDKFVSE LWKDVDRIIG LDQVAGMSET ALPGAFKTRK GMFRTVGQLY
KEQLAKLMAT LRNTNPNFVR CIIPNHEKKA GKLDPHLVLD QLRCNGVLEG
IRICRQGFPN RVVFQEFRQR YEILTPNSIP KGFMDGKQAC VLMIKALELD
SNLYRIGQSK VFFRAGVLAH LEEERDLKIT DVIIGFQACC RGYLARKAFA
KRQQQLTAMK VLQRNCAAYL RLRNWQWWRL FTKVKPLLNS IRHEDELLAK
EAELTKVREK HLAAENRLTE METMQSQLMA EKLQLQEQLQ AETELCAEAE
ELRARLTAKK QELEEICHDL EARVEEEEER CQYLQAEKKK MQQNIQELEE
QLEEEESARQ KLQLEKVTTE AKLKKLEEDQ IIMEDQNCKL AKEKKLLEDR
VAEFTTNLME EEEKSKSLAK LKNKHEAMIT DLEERLRREE KQRQELEKTR
RKLEGDSTDL SDQIAELQAQ IAELKMQLAK KEEELQAALA RVEEEAAQKN
MALKKIRELE TQISELQEDL ESERASRNKA EKQKRDLGEE LEALKTELED
TLDSTAAQQE LRSKREQEVS ILKKTLEDEA KTHEAQIQEM RQKHSQAVEE
LADQLEQTKR VKATLEKAKQ TLENERGELA NEVKALLQGK GDSEHKRKKV
EAQLQELQVK FSEGERVRTE LADKVTKLQV ELDSVTGLLS QSDSKSSKLT
KDFSALESQL QDTQELLQEE NRQKLSLSTK LKQMEDEKNS FREQLEEEEE
AKRNLEKQIA TLHAQVTDMK KKMEDGVGCL ETAEEAKRRL QKDLEGLSQR
LEEKVAAYDK LEKTKTRLQQ ELDDLLVDLD HQRQSVSNLE KKQKKFDQLL
AEEKTISAKY AEERDRAEAE AREKETKALS LARALEEAME QKAELERLNK
QFRTEMEDLM SSKDDVGKSV HELEKSKRAL EQQVEEMKTQ LEELEDELQA

TEDAKLRLEV NLQAMKAQFE RDLQGRDEQS EEKKKQLVRQ VREMEAELED
ERKQRSMAMA ARKKLEMDLK DLEAHIDTAN KNREEAIKQL RKLQAQMKDC
MRELDDTRAS REEILAQAKE NEKKLKSMEA EMIQLQEELA AAERAKRQAQ
QERDELADEI ANSSGKGALA LEEKRRLEAR IAQLEEELEE EQGNTELIND
RLKKANLQID QINTDLNLER SHAQKNENAR QQLERQNKEL KAKLQEMESA
VKSKYKASIA ALEAKIAQLE EQLDNETKER QAASKQVRRT EKKLKDVLLQ
VEDERRNAEQ FKDQADKAST RLKQLKRQLE EAEEEAQRAN ASRRKLQREL
EDATETADAM NREVSSLKNK LRRGDLPFVV TRRIVRKGTG DCSDEEVDGK
ADGADAKAAE

(**B**)

Actin sequence

MEEEIAALVI DNGSGMCKAG FAGDDAPRAV FPSIVGRPRH QGVMVGMGQK
DSYVGDEAQS KRGILTLKYP IEHGIVTNWD DMEKIWHHTF YNELRVAPEE
HPVLLTEAPL NPKANREKMT QIMFETFNTP AMYVAIQAVL SLYASGRTTG
IVMDSGDGVT HTVPIYEGYA LPHAILRLDL AGRDLTDYLM KILTERGYSF
TTTAEREIVR DIKEKLCYVA LDFEQEMATA ASSSSLEKSY ELPDGQVITI
GNERFRCPEA LFQPSFLGME SCGIHETTFN SIMKCDVDIR KDLYANTVLS
GGTTMYPGIA DRMQKEITAL APSTMKIKII APPERKYSVW IGGSILASLS
TFQQMWISKQ EYDESGPSIV HRKCF

(Fig. 3) contd…..

(C)

IQGAP1 sequence

MSAAEEVDGL GVVRPHYGSV LDNERLTAEE MDERRRQNVA YEYLCHLEEA
KRWMEACLGE DLPPTTELEE GLRNGVYLAK LGNFFSPKVV SLKKIYDREQ
TRYKATGLHF RHTDNVIQWL NAMDEIGLPK IFYPETTDIY DRKNMPRCIY
CIHALSLYLF KLGLAPQIQD LYGKVDFTEE EINNMKIELE KYGIQMPAFS
KIGGILANEL SVDEAALHAA VIAINEAIDR RVAADTFTAL KNPNAMLVNL
EEGLAPTYQD VLYQAKQDKM TNAKNRTENS DRERDVYEEL LTQAEIQGNV
NKVNTSSALA NISLALEQGC AVTLLKALQS LALGLRGLQT QNSDWYMKQL
QSDLQQKRQS GQTDPLQKEE VQAGVDAANS AAQQYQRRLA AVAAINAAIQ
KGIAEKTVLE LMNPEAQLPQ VYPFAADLYQ KELATLQQQS PEHSLTHPEL
TVAVEMLSSV ALINRALESG DMTTVWKQLS SSVTGLTNIE EENCQRYLDE
LMKLKAQAHA ENNAFITWND IQACVDHVNL VVHEEHERIL AIGLINEALD
EGDAQKTLQA LQIPAAKLEG VLAEVAQHYQ DTLIRAKREK AQETQDESAV
LWLDEIQGGI WQSNKDTQEA QRFALGISAI NEAVDSGDVG RTLSALRSPD
VGLYGVIPEC GETYQSDLAE AKKKRLAAGD NNSKWVKHWV KGGYHYYHNL
ETQAGGWAEP PDFVQNSVQL SREEIQSSIS GVTAAYNREQ LWLANEGLIT
KLQACCRGYL VRQEFRSRMN FLKKQIPAIT CIQSQWRGYK QKKAYQDRLA
YLHSHKDEVV KIQSLARMHQ ARKRYRDRLQ YFRDHINDII KIQAFIRANK
ARDDYKTLIN AEDPPMIVVR KFVHLLDQSD QDFQEELDLM KMREEVITLI
RSNQQLENDL NLMDIKIGLL VKNKITLQDV VSHSKKLTKK NKEQLSDMMM
INKQKGGLKA LSKEKREKLE AYQHLFYLLQ TNPTYLAKLI FQMPQNKSTK
FMDSVIFTLY NYASNQREEY LLLRLFQTAL QEEIKSKVDQ IQEIVTGNPT
VIKMVVSFNR GARGQNALRQ ILAPVVKEIM DDKSLNIKTD PVDIYKSWVN
QMESQTGEAS KLPYDVTPEQ ALSHEEVKTR LDNSIRNMRA VTDKFLSAIV
SSVDKIPYGM RFIAKVLKDS LHEKFPDAGE DELLKIIGNL LYYRYMNPAI
VAPDAFDIID LSAGGQLTTD QRRNLGSIAK MLQHAASNKM FLGDNAHLSI
INEYLSQSYQ KFRRFFQLAC DVPELQDKFN VDEYSDLVTL TKPVIYISIG
EIINTHTLLL DHQDAIAPEH NDPIHELLDD LGEVPTIESL IGESCGNSND
PNKEALAKTE VSLTLTNKFD VPGDENAEMD ARTILLNTKR LIVDVIRFQP
GETLTEILET PATNEQEAEH QRAMQRRAIR DAKTPDKMKK SKPMKEDNNL
SLQEKKEKIQ TGLKKLTELG TVDPKNRYQE LINDIAKDIR NQRRYRQRRK
AELVKLQQTY SALNSKATFY GEQVDYYKSY IKTCLDNLAS KGKVSKKPRE
MKGKKSKKIS LKYTAARLHE KGVLLEIEDL QANQFKNVIF EIGPTEEVGD
FEVKAKFMGV QMETFMLHYQ DLLQLQYEGV AVMKLFDRAK VNVNLLIFLL
NKKFYGK

(D)

Plectin sequence

MVAGMLMPLD RLRAIYEVLF REGVMVAKKD RRPRSLHPHV PGVTNLQVMR
AMASLKARGL VRETFAWCHF YWYLTNEGID HLRQYLHLPP EIVPASLQRV
RRPVAMVIPA RRRSPHVQTM QGPLGCPPKR GPLPAEDPAR EERQVYRRKE
REEGAPETPV VSATTVGTLA RPGPEPAPAT DERDRVQKKT FTKWVNKHLI
KHWRAEAQRH ISDLYEDLRD GHNLISLLEV LSGDSLPREK GRMRFHKLQN
VQIALDYLRH RQVKLVNIRN DDIADGNPKL TLGLIWTIIL HFQISDIQVS
GQSEDMTAKE KLLLWSQRMV EGYQGLRCDN FTTSWRDGRL FNAIIHRHKP
MLIDMNKVYR QTNLENLDQA FSVAERDLGV TRLLDPEDVD VPQPDEKSII
TYVSSLYDAM PRVPGAQDGV RANELQLRWQ EYRELVLLLL QWIRHHTAAF
EERKFPSSFE EIEILWCQFL KFKETELPAK EADKNRSKVI YQSLEGAVQA
GQLKIPPGYH PLDVEKEWGK LHVAILEREK QLRSEFERLE CLQRIVSKLQ
MEAGLCEEQL NQADALLQSD IRLLASGKVA QRAGEVERDL DKADGMIRLL
FNDVQTLKDG RHPQGEQMYR RVYRLHERLV AIRTEYNLRL KAGVGAPVTQ
VTLQSTQRRP ELEDSTLRYL QDLLAWVEEN QRRIDSAEWG VDLPSVEAQL
GSHRGMHQSI EEFRAKIERA RNDESQLSPA TRGAYRDCLG RLDLQYAKLL
NSSKARLRSL ESLHGFVAAA TKELMWLNEK EEEEVGFDWS DRNTNMAAKK
ESYSALMREL EMKEKKIKEI QNTGDRLLRE DHPARPTVES FQAALQTQWS
WMLQLCCCIE AHLKENTAYF QFFSDVREAE EQLQKLQETL RRKYSCDRTI
TVTRLEDLLQ DAQDEKEQLN EYKGHLSGLA KRAKAIVQLK PRNPAHPVRG
HVPLIAVCDY KQVEVTVHKG DQCQLVGPAQ PSHWKVLSGS SSEAAVPSVC
FLVPPPNQEA QEAVARLEAQ HQALVTLWHQ LHVDMKSLLA WQSLSRDIQL
IRSWSLVTFR TLKPEEQRQA LRNLELHYQA FLRDSQDAGG FGPEDRLVAE
REYGSCSRHY QQLLQSLEQG EQEESRCQRC ISELKDIRLQ LEACETRTVH
RLRLPLDKDP ARECAQRIAE QQKAQAEVEG LGKGVARLSA EAEKVLALPE
PSPAAPTLRS ELELTLGKLE QVRSLSAIYL EKLKTISLVI RSTQGAEEVL
KTHEEQLKEA QAVPATLQEL EATKASLKKL RAQAEAQQPV FNTLRDELRG
AQEVGERLQQ RHGERDVEVE RWRERVTQLL ERWQAVLAQT DVRQRELEQL
GRQLRYYRES ADPLSAWLQD AKRRQEQIQA VPIANCQAAR EQLRQEKALL
EEIERHGEKV EECQKFAKQY INAIKDYELQ LITYKAQLEP VASPAKKPKV
QSGSESVIQE YVDLRTRYSE LTTLTSQYIK FISETLRRME EEERLAEQQR
AEERERLAEV EAALEKQRQL AEAHAQAKAQ AELEAQELQR RMQEEVARRE

Fig. 3) contd…..

(**D**)

EAAVDAQQQK RSIQEELQHL RQSSEAEIQA KAQQVEAAER SRMRIEEEIR
VVRLQLETTE RQRGGAEGEL QALRARAEEA EAQKRQAQEE AERLRRQVQD
ESQRKRQAEA ELALRVKAEA EAAREKQRAL QALDELRLQA EEAERRLRQA
EAERARQVQV ALETAQRSAE VELQSKRASF AEKTAQLERT LQEEHVTVAQ
LREEAERRAQ QQAEAERARE EAERELERWQ LKANEALRLR LQAEEVAQQK
SLAQADAEKQ KEEAEREARR RGKAEEQAVR QRELAEQELE KQRQLAEGTA
QQRLAAEQEL IRLRAETEQG EQQRQLLEEE LARLQHEATA ATQKRQELEA
ELAKVRAEME VLLASKARAE EESRSTSEKS KQRLEAEAGR FRELAEEAAR
LRALAEEAKR QRQLAEEDAA RQRAEAERVL TEKLAAISEA TRLKTEAEIA
LKEKEAENER LRRLAEDEAF QRRRLEEQAA LHKADIEERL AQLRKASESE
LERQKGLVED TLRQRRQVEE EIMALKVSFE KAAAGKAELE LELGRIRSNA
EDTMRSKEQA ELEAARQRQL AAEEEQRRRE AEERVQRSLA AEEEAARQRK
VALEEVERLK AKVEEARRLR ERAEQESARQ LQLAQEAAQK RLQAEEKAHA
FVVQQREEEL QQTLQQEQNM LDRLRSEAEA ARRAAEEAEE AREQAEREAA
QSRKQVEEAE RLKQSAEEQA QAQAQAQAAA EKLRKEAEQE AARRAQAEQA
ALKQKQAADA EMEKHKKFAE QTLRQKAQVE QELTTLRLQL EETDHQKSIL
DEELQRLKAE VTEAARQRSQ VEEELFSVRV QMEELGKLKA RIEAENRALI
LRDKDNTQRF LEEEAEKMKQ VAEEAARLSV AAQEAARLRQ LAEEDLAQQR
ALAEKMLKEK MQAVQEATRL KAEAELLQQQ KELAQEQARR LQEDKEQMAQ
QLVEETQGFQ RTLEAERQRQ LEMSAEAERL KLRMVEMSRA QARAEEDAQR
FRKQAEEIGE KLHRTELATQ EKVTLVQTLE IQRQQSDHDA ERLREAIAEL
EREKEKLKQE AKLLQLKSEE MQTVQQEQIL QETQALQKSF LSEKDSLLQR
ERFIEQEKAK LEQLFQDEVA KAKQLREEQQ RQQQQMEQEK QELMASMEEA
RRRQREAEEG VRRKQEELQH LEQQRQQQEK LLAEENQRLR ERLQRLEEEH
RAALAHSEIA TTQAASTKAL PNGRDAPDGP SVEAEPEYTF EGLRQKVPAQ
QLQEAGILSQ EELQRLAQGH TTVAELTQRE DVYRYLKGRS SIAGLLLKPT
NEKLSVYTAL QRQLLSPGTA LILLEAQAAS GFLLDPVRNR RLTVNEAVKE
GVVGPELHHK LLSAERAVTG YKDPYTGEQI SLFQAMKKDL IVRDHGVRLL
EAQIATGGII DPVHSHRVPV DVAYKRGYFD EEMNRILSDP SDDTKGFFDP
NTHENLTYLQ LLERCVEDPE TGLRLLPLTD KAAKGGELVY TDTEARDVFE
KATVSAPFGK FQGRTVTIWE IINSEYFTAE QRRDLLQQFR TGHITVEKII
KIVITVVEEH ERKGQLCFEG LRALVPAAEL LDSGVISHEL YQQLQRGERS
VREVAEADSV RQALRGTNVI AGVWLEEAGQ KLSIYEALKK DLLQPEVAVA
LLEAQAGTGH IIDPATSARL TVDEAVRAGL VGPELHEKLL SAEKAVTGYR
DPYSGQSVSL FQALKKGLIP REQGLRLLDA QLSTGGIVDP SKSHRVPLDV
AYARGYLDKE TNRALTSPRD DARVYHDPST QEPVTYSQLQ QRCRSDQLTG
LSLLPLSEKA VRARQEEVYS ELQARETLEQ AKVEVPVGSF KGRAMTVWEL
ISSEYFTEEQ RQELLRQFRT GKVTVEKVIK IVITIVEEVE TRRQERLSFS
GLRAPVPASE LLDAKILSRA QFDQLKDGKT SVKELSEVGS VRTLLQGSGC
LAGIYLEDSK EKVTIYEAMR RGLLRPSTAT LLLEAQAATG FLVDPVRNQR
LYVHEAVKAG VVGPELHEKL LSAEKAVTGY KDPYSGNTIS LFQAMKKGLV
LRDHAIRLLE AQVATGGIID PVHSHRLPVD VAYQRGYFDE EMNRVLADPS
DDTKGFFDPN THENLTYLQL LERCVEDPET GLRLLPLKGA EKTEVVETTQ
VYTEEETRRA FEETQIDIPG GGSHGGSSMS LWEVMQSNMI PEDQRARLMA
DFQAGRVTKE RMIIIIIEII EKTEIIRQQN LASYDYVRRR LTAEDLYEAR
IISLETYNLF REGTKNLREV LEMESAWRYL YGTGAVAGVY LPGSRQTLTI
YQALKKGLLS AEVARLLLEA QAATGFLLDP VKGERLTVDE AVRKGLVGPE
LHDRLLSAER AVTGYRDPYT EQTISLFQAM KKELIPAEEA LRLLDAQLAT
GGIVDPRLGF HLPLEVAYQR GYLNKDTHDQ LSEPSEVRSY VDPSTDERLS
YTQLLKRCRR DDPSGQMLLL LSDARKLTFR GLRKQITVEE LVRSQVMDEA
TALQLQEGLT SIEEVTKNLQ KFLEGTSCIA GVFVDATKER LSVYQAMKKG
IIRPGTAFEL LEAQAATGYV IDPIKGLKLT VEEAVRMGIV GPEFKDKLLS
AERAVTGYKD PYSGKLISLF QAMKKGLILK DHGIRLLEAQ IATGGIIDPE
ESHRLPVEVA YKRGLFDEEM NEILTDPSDD TKGFFDPNTE ENLTYLQLME
RCITDPQTGL CLLPLKEKKR ERKTSSKSSV RKRRVVIVDP ETGKEMSVYE
AYRKGLIDHQ TYLELSEQEC EWEEITISSS DGVVKSMIID RRSGRQYDID
DAITKNLIDR SALDQYRAGT LSITEFADML SGNAGGFRSR SSSVGSSSSY
PISSAGPRTQ LASWSDPTEE TGPVAGILDT ETLEKVSITE AMHRNLVDNI
TGQRLLEAQA CTGGIIDPST GERFPVTEAV NKGLVDKIMV DRINLAQKAF
CGFEDPRTKT KMSAAQALKK GWLYYEAGQR FLEVQYLTGG LIEPDTPGRV
SLDEALQRGT VDARTAQKLR DVSAYSKYLT CPKTKLKISY KDALDRSMVE
EGTGLRLLEA AAQSSKGYYS PYSVSGSGST AGSRTGSRTG SRAGSRRGSF
DATGSGFSMT FSSSSYSSSG YGRRYASGPS ASLGGPESAV A

Fig. (3). LC/MS/MS analysis revealed the binding of IQGAP1 and Plectin to Aβ. The stained bands were extracted and digested by trypsin. The samples were run on LC/MS/MS. Red color showed the detected amino acid sequence. **A**. myosin-9, **B**. actin, **C**. IQGAP1, **D**. Plectin.

Possible Biological and Pathological Meaning of the Binding of IQGAP1 and Aβ

We have shown in this paper that Aβ binds to IQGAP1, an effector of the Rho-family small GTPases Rac1 and Cdc42 [11,12]. Aβ 1-42 originally penetrates cell membrane or ER membrane as a part of amyloid precursor protein (APP). Aβ is produced by the sequential enzymatic actions of beta-site amyloid precursor protein-cleaving enzyme 1 (BACE-1), a β-secretase, and γ-secretase and exist inside cells and outside cells.

What the meaning of Aβ and IQGAP1 binding in microglia? Aβ could induce a local inflammatory reaction associated with regenerative changes in the surrounding neurons. Microglia produce multiple pro-inflammatory factors, including cytokines (tumor necrosis factor-α (TNFα), interleukin (IL)-1, and IL-6), chemokines, reactive oxygen species [8,13] Aβ also produces M-CSF and induces proliferation of microglia [8]. These results indicate that Aβ induces microglial migration by chemokines and proliferation by M-CSF. The Rho-like GTPase, Rac1, induces cytoskeletal rearrangements required for cell migration and proliferation [14]. Vasiliev showed that an intact microtubule cytoskeleton was required to maintain the polarized distribution of actin-dependent protrusions at the leading edge of a migrating fibroblast [15]. RhoA mediates formation of contractile actin structures, such as stress fibres [16], and at the same time promotes stabilization of a sub-population of microtubules [17]. Rac kinase, which exists downstream of Rho kinase promotes contractility by increasing phosphorylation of the regulatory light chain of myosin-2 [18]. IQGAP1 binds to many proteins related to Rho kinase- microtubule cytoskeleton pathway including Rac1 and Cdc42 [19], myosin essential light chain [20]. Taken together, Aβ binds to IQGAP1 and activates Rho kinase- microtubule cytoskeleton pathway to induce cellular migration and proliferation.

Plectin is the most versatile cytoskeletal linker protein known, which was first isolated nealy 30 years ago [21]. Plectin plays important roles in a number of cell functions including migration and wound healing [22]. Plectin has been shown to activate RhoA GTPase [23].

We have shown in this paper that Aβ binds to several proteins inside cells. Aβ may exist inside cells. Already it has been shown that monomeric synaptic Aβ, which stays at inside and outside cells may dampen excitatory transmission and prevent neuronal hyperactivity [23]. Early activation of signaling [7,8] indicates that Aβ may work as a monomer or soluble oligomer, which may be inserted into cell membrane and endocytosed into cytoplasm. Aβ, which enter cells may bind to IQGAP1, Plectin and actin and work to migrate microglia by Rho-Rac1 signaling.

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CONFLICT OF INTEREST

None declared.

REFERENCES

[1] Querfurth HW, LaFerla FM. Alzheimer's disease. N Engl J Med 2010; 362: 329-44.
[2] Akiyama H, Barger S, Barnum S, *et al.* Inflammation and Alzheimer's disease. Neurobiol Aging 2000; 21: 383-421.
[3] Itagaki S, McGeer PL, Akiyama H, Zhu S, Selkoe D. Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. J Neuroimmunol 1989; 24: 173-82.
[4] El Khoury JB, Moore KJ, Means TK, *et al.* CD36 mediates the innate host response to beta-amyloid. J Exp Med 2003; 197: 1657-66.
[5] Moore KJ, Freeman MW. Scavenger receptors in atherosclerosis: beyond lipid uptake. Arterioscler Thromb Vasc Biol 2006; 26: 1702-11.
[6] Stewart CR, Stuart LM, Wilkinson K, *et al.* CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. Nat Immunol 2010; 11: 155-61.
[7] Ito S, Sawada M, Haneda M, *et al.* Amyloid-beta peptides induce cell proliferation and macrophage colony-stimulating factor expression *via* the PI3-kinase/Akt pathway in cultured Ra2 microglial cells. FEBS Lett 2005; 579: 1995-2000.
[8] Ito S, Sawada M, Haneda M, Ishida Y, Isobe K. Amyloid-beta peptides induce several chemokine mRNA expressions in the primary microglia and Ra2 cell line *via* the PI3K/Akt and/or ERK pathway. Neurosci Res 2006; 56: 294-9.
[9] Walsh DM, Selkoe DJ. A beta oligomers - a decade of discovery. J Neurochem 2007; 101: 1172-84.
[10] Sawada M, Imai F, Suzuki H, Hayakawa M, Kanno T, Nagatsu T. Brain-specific gene expression by immortalized microglial cell-mediated gene transfer in the mammalian brain. FEBS Lett 1998; 433: 37-40.
[11] Fukata M, Kuroda S, Fujii K, *et al.* Regulation of cross-linking of actin filament by IQGAP1, a target for Cdc42. J Biol Chem 1997; 272: 29579-83.
[12] Noritake J, Watanabe T, Sato K, Wang S, Kaibuchi K. IQGAP1: a key regulator of adhesion and migration. J Cell Sci 2005; 118: 2085-92.
[13] Heneka MT, O'Banion MK. Inflammatory processes in Alzheimer's disease. J Neuroimmunol 2007; 184: 69-91.
[14] Rodriguez OC, Schaefer AW, Mandato CA, Forscher P, Bement WM, Waterman-Storer CM. Conserved microtubule-actin interactions in cell movement and morphogenesis. Nat Cell Biol 2003; 5: 599-609.
[15] Vasiliev JM, Gelfand IM, Domnina LV, Ivanova OY, Komm SG, Olshevskaja LV. Effect of colcemid on the locomotory behaviour of fibroblasts. J Embryol Exp Morphol 1970; 24: 625-40.
[16] Etienne-Manneville S, Hall A. Rho GTPases in cell biology. Nature 2002; 420: 629-35.
[17] Cook TA, Nagasaki T, Gundersen GG. Rho guanosine triphosphatase mediates the selective stabilization of microtubules induced by lysophosphatidic acid. J Cell Biol 1998; 141: 175-85.
[18] Ridley AJ. Rho GTPases and cell migration. J Cell Sci 2001; 114: 2713-22.
[19] Kuroda S, Fukata M, Kobayashi K, *et al.* Identification of IQGAP as a putative target for the small GTPases, Cdc42 and Rac1. J Biol Chem 1996; 271: 23363-7.
[20] Weissbach L, Bernards A, Herion DW. Binding of myosin essential light chain to the cytoskeleton-associated protein IQGAP1. Biochem Biophys Res Commun 1998; 251: 269-76.
[21] Pytela R, Wiche G. High molecular weight polypeptides (270,000-340,000) from cultured cells are related to hog brain microtubule-associated proteins but copurify with intermediate filaments. Proc Natl Acad Sci USA 1980; 77: 4808-12.
[22] Andrä K, Nikolic B, Stöcher M, Drenckhahn D, Wiche G. Not just scaffolding: plectin regulates actin dynamics in cultured cells. Genes Dev 1998; 12: 3442-51.
[23] Na S, Chowdhury F, Tay B, *et al.* Plectin contributes to mechanical properties of living cells. Am J Physiol Cell Physiol 2009; 296: C868-77.

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