The Gab2 in signal transduction and its potential role in the pathogenesis of Alzheimer's disease

Xiao-Ling PAN^{1,*}, Ru-Jing REN^{1,*}, Gang WANG¹, Hui-Dong TANG¹, Sheng-Di CHEN^{1,2}

¹Department of Neurology and Neuroscience Institute, Ruijin Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai 200025, China

²Laboratory of Neurodegenerative Diseases, Institute of Health Science, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences and Shanghai Jiaotong University School of Medicine, Shanghai 200025, China

© Shanghai Institutes for Biological Sciences, CAS and Springer-Verlag Berlin Heidelberg 2010

Abstract: The growth factor receptor-bound protein 2 (Grb2)-associated binder (Gab) proteins are intracellular scaffolding/docking molecules, and participate in multiple signaling pathways, usually acting as the downstream effector of protein-tyrosine kinases (PTKs)-triggered signal transduction pathway. When phosphorylated by PTKs, Gab proteins can recruit several signaling molecules (p85, SHP2, and Crk), and subsequently activate multiple transmitting signals that are critical for cell growth, survival, differentiation and apoptosis. Recently, it has been reported that *Gab2* polymorphism is associated with the increase in the risk of Alzheimer's disease (AD) and is involved in the pathogenesis of AD. This review mainly focuses on the structure and function of Gab2 protein and its role in the pathogenesis of AD.

Keywords: *Gab2*; signal transduction; Alzheimer's disease; *ApoE*

1 Introduction

The growth factor receptor-bound protein 2 (Grb2)-associated binder (Gab) proteins are a family of intracellular scaffolding/docking molecules, including mammalian Gab1, Gab2, Gab3 and a putative Gab4 protein, *Drosophila* DOS, and *Cawnorhabditis elegans* Soc1 and a putative Gab protein from *Nematostella vectensis* (Ne-Gab)^[1]. They are called scaffolding/docking proteins because they act as the downstream of cell surface receptors and interact with a variety of cytoplasmic signaling proteins, such as Grb2, phosphatidylinositol 3-kinase (PI3K), SHP2, and Crk, subsequently integrating and amplifying different cellular signals, such as PI3K, Ras-MAPK, and Fas signal transmission pathways^[1-7].

In 2007, the first genome-wide association study was conducted. Six single nucleotide polymorphisms (SNPs) from *Gab2* gene have been confirmed to be associated with late-onset Alzheimer's disease (LOAD). Of these SNPs, the SNP rs2373115 may be a strong risk factor in *ApoE*ε4 carriers with an odds ratio of 4.06 (confidence interval 2.81-14.69). Also, interference with *Gab2* gene expression has been found to increase tau phosphorylation *in vitro*, which is a major component of neurofibrillary tangles^[8]. Meanwhile, some studies reveal that the interaction of Gab2 with amyloid precursor protein (APP) may influence phosphor-ERK-1 and -2 signaling, although the meaning of this event in the pathology of AD is not clear^[9,10]. Studies on the association between the polymorphism of *Gab2* gene and the pathogenesis of AD are being conducted in many countries.

2 Gab2 and its role in signal transduction

2.1 Structure, recruitment and physiological function of Gab2 The *Gab2* gene is located on chromosome 11q13.4-

*These authors contributed equally to this work.

Corresponding author: Gang WANG; Sheng-Di CHEN

Tel: +86-21-64457249

E-mail: wgneuron@hotmail.com; chen_sd@medmail.com.cn

Article ID: 1673-7067(2010)03-0241-06

Received date: 2009-11-09; Accepted date: 2010-02-24

Fig. 1 The structure of Gab2 protein. The PH domain mediates phosphatidylinositol-3,4,5-triphosphates (PIP3) binding on the inner surface of plasma membrane; P, the central praline-rich sequences (PXXP), providing binding motifs favored by SH3-containing proteins such as Grb2. Stars indicate the potential binding sites for SH2-containing proteins such as p85. Arrows indicate the potential binding sites for Crk and Ras-GAP. Triangles indicate the potential binding sites for SHP2.

q13.5 in human, and the molecular weight of Gab2 protein is 97-100 kD. Gab2 is expressed ubiquitously, at high levels especially in brain, kidney, lung, heart, testis and ovary^[2]. Like other Gab family members, Gab2 contains an N-terminal Pleckstrin homology (PH) domain, a central praline-rich domain (PRD) and multiple tyrosines within potential binding motifs favored by various Src homology 2 and 3 (SH2, SH3) domain-containing proteins (Fig. 1). All the 3 domains, especially the PH domain, are highly conserved in the process of organic evolution.

The N-terminal PH domain is the most conserved structural element of Gab2 protein. Its binding to PIP3 can promote membrane recruitment and subcellular localization of Gab2. Recent researches have found that the PH domain may also be involved in regulating intracellular signaling (Fas-signaling pathway), not just imply a localization module^[1,11]. The PRD is the central region of Gab2 and contains numerous PXXP motifs, which are binding sites for SH3 domain-containing molecules. The interaction between Gab2 and Grb2 with a PX₃RX₂KP motif is essential for either Gab2 or Grb2 to display their regulatory roles in signal transduction^[1,12]. As shown in Fig. 1, there are multiple sites of tyrosine phosphorylation, which may interact with SH2 domain-containing proteins such as the tyrosine phosphatase SHP2 and the p85 subunit of PI3K. This interaction is critical for the function of Gab2 in mediating intracellular signaling pathways, which are crucial for cell normal growth, differentiation, development and apoptosis^[11-14].

2.2 Gab2 in signal transduction As mentioned above, Gab2 participates in multiple signaling pathways, usually acting as the downstream of protein-tyrosine kinases (PTKs) activation. Here, the role of Gab2 in its network is mainly

discussed. The interactions of Gab2 with other signaling intermediates are dependent on its phosphorylation status. In unstimulated cells, Gab2 is located in the cytoplasm, while upon stimulation by growth hormone (GH), epidermal growth factor (EGF), insulin, G-CSF, interferon, IL-2/3, or T cell receptor^[1-7,11], Gab2 can be recruited to the cell membrane by binding to PIP3 via the PH domain. Then the PRD of Gab2 can interact with Grb2 to form a Gab2-Grb2-Shc complex, which mediates rapid tyrosine phosphorylation. The activated Gab2 possesses a number of docking sites for some key SH2 domain-containing molecules, such as the tyrosine phosphatase SHP2 and the p85 subunit of PI3K, whose recruitment and activation will be induced. The interactions are critical in mediating a variety of cell signaling pathways, such as MAPK, PI3K, and JNK signaling pathways. Firstly, in the Gab2/SHP-2-mediated Ras-MAPK-ERK signal pathway, SHP-2/Gab2 complex has dual effects. On one hand, SHP-2/Gab2 complex may act at the upstream of Ras, although the detailed mechanisms underlying SHP-2/Gab2-mediated regulation of Ras remain to be resolved. Lines of evidence have suggested that in the SHP-2/Gab1 complex, SHP-2 may dephosphorylate a different motif on Gab1, thereby releasing a Ras-negative regulating protein, Ras-GAP. Consequently, it increases Ras activity^[14]. More interestingly, although it is well known that Gab1 plays a critical role in this pathway, research reveals that the inhibitory effect of Gab1 siRNA could be rescued by an exogenous human Gab2^[4,15]. Based on these findings, it is implied that SHP-2/Gab2 complex can activate Ras system in a way like that by SHP-2/Gab1 complex. On the other hand, this complex can sometimes be parallel to or the downstream of ERK activation^[2]. However, the exact role of Gab2 in the Ras-ERK system needs further

investigations. Secondly, for the Gab2/PI3K-mediated signaling pathway, Gab2 is crucial in mast cell functions, such as FcεRI-induced degranulation, which is dependent on PI3K. The Gab2^{-/-} mice are defective in allergic responses^[16,17]. These results imply that Gab2 may act as a principal activator of PI3K in response to FcεRI. Further studies have revealed that Gab2 has some potential binding sites for the SH2 domain of PI3K p85 regulatory subunit, which induces the activation of PI3K. Moreover, the activation of PI3K leads to the production of PIP3, which in turn binds to the PH domain of Gab2, promoting the activation of PI3K. Thus, a positive feedback loop is formed to amplify the PI3K-Akt signaling pathway^[13,18]. Thirdly, in addition to the binding sites for SHP2 and p85, Gab2 also contains numerous YXXP motifs, the potential binding sites for Crk family proteins, which can lead to c-Jun N-terminal kinase (JNK) activation^[13].

In summary, Gab2 plays a crucial role in controlling cell growth, survival, and proliferation. When overexpressed, Gab2 may also promote oncogenesis, such as melanoma in

the breast and ovarian cancer^[19,20]. However, under normal conditions, oncogenesis is rare, due to the negative feedback of Gab2 activation. Brummer *et al.* have found that both SHP2 and 14-3-3 proteins can terminate signaling of Gab2 activation, by promoting the dissociation of Gab2 from Grb2^[1,21].

3 Gab2 and *Gab2* polymorphism in the pathogenesis of AD

3.1 Gab2 in the pathogenesis of AD AD is a progressive neurodegenerative disorder with a high prevalence in the world, and is the most common form of dementia in the old population. It is characterized by intracellular neurofibrillary tangles (NFTs) containing hyperphosphorylated tau protein, extracellular amyloid plaques containing deposits of beta amyloid (Aβ), and neuronal loss. In recent years, accumulating evidence has indicated that the incidence of AD in some populations is substantially influenced by genetic risk factor^[22]. The role of Gab2 in the pathogenesis of AD via tight association with tau and APP has been reported (Fig.

Fig. 2 Gab2 has tight associations with tau and APP. The solid arrows indicate activation, and the dashed arrows indicate inactivation. Gab2 may suppress Gsk3-dependent tau phosphorylation and promote neural/glial cell proliferation, thus protecting against AD pathogenesis. Meanwhile, the interaction between Gab2-Grb2 and APP is suggested to be involved in the neuropathogenesis of AD, although the meaning of this coupling in AD pathogenesis is still unknown.

2). Studies have demonstrated that the overexpression of glycogen synthase kinase-3 (Gsk3) accounts for tau phosphorylation, increased amyloid production, microglia-mediated inflammation^[23,24], and also the linkage between amyloid and tau pathology^[25]. It has been clear that Gab2 is the principle activator of PI3K signaling pathway, and PI3K activates Akt, which then promotes Gsk3 phosphorylation (inactivation), so we can imply that Gab2 can reduce the occurrence of Tau protein phosphorylation. In the study of Reimain *et al.*, the neuroglioma cells over-expressing wild-type tau protein are transfected with siRNA targeting Gab2. At 4 d after transfection, the level of phosphorylated tau at serine262 is increase by 1.73 fold, although this increase is not attributable to a concomitant increase in total tau^[8]. This finding indicates that Gab2 may suppress Gsk3-dependent tau phosphorylation, and protect against the pathogenesis of AD. Moreover, Gab2 can interact with APP in neurons and this coupling occurs to a higher extent in AD subjects only^[8]. Previous studies have revealed that this coupling may influence phosphor-ERK-1 and -2 signaling, although the meaning of this event in the pathology of AD is not clear^[9,10]. Since the interaction between Gab2-Grb2 and APP is significantly enhanced in AD brain^[8], it is suggested that this coupling is involved in the neuropathogenesis of AD. In deed, Gab2 can interact with GC-GAP, a Rho family GTPase-activating protein that is also predominantly expressed in the brain. Via this coupling, Gab2 may regulate the cellular localization of GC-GAP, thus precisely regulating Rac and Cdc42 activities. This may play a role in dendritic morphogenesis and also in neural/glial cell proliferation during early embryo development^[26]. According to these findings, it is hypothesized that Gab2 plays an important role in preventing neuronal tangle formation and neuron loss, and loss-of-function of Gab2 haplotype would diminish such protection, increasing the susceptibility to AD.

3.2 The polymorphism of *Gab2* and the risk of AD In 2007, Reiman *et al.* conducted a genome-wide association study concerning AD risk factors, and they have found that *Gab2* might modify the risk of LOAD in *ApoE* $\epsilon 4$ carriers, primarily due to the SNP rs2373115 (OR=4.06, 95%CI=2.8-14.7, $P=9 \times 10^{-11}$)^[8]. Furthermore, they also conducted a neuronal

microarray study, and found that in LOAD brains, *Gab2* is detected in highly dystrophic neuritis, neurons, and corpora amylacea^[8]. However, although a series of studies concerning the association between *Gab2* and AD have been conducted, the results are inconsistent. Three similar studies from USA, Netherlands and Belgian in Caucasian population all independently confirm an association between Gab2 and LOAD. Moreover, this association appears to be limited to *ApoE* $\epsilon 4$ carriers^[27-29]. And Benedetta *et al.* have found that in Italian population, the involvement of *Gab2* polymorphism in the risk of AD is confined to *ApoE* $\epsilon 4$ non-carriers, suggesting a possible role of *Gab2* as an independent risk factor for AD^[30]. However, some population-based studies in UK, Japan and Spain all fail to confirm this finding^[31-33], and show that *Gab2* does not modify the risk of AD, or at most a minor genetic determinant of AD. In 2009, a case-control study in Chinese Han population also revealed no association between two tested SNPs of *Gab2* and AD^[34]. Therefore, further studies are needed to better clarify and evaluate the potential roles of Gab2 in the pathogenesis of AD.

4 Conclusion

Gab2 is a scaffolding protein and plays a crucial role in multiple signaling pathways that control cell growth, survival, differentiation and apoptosis. In the central nervous system, Gab2 can suppress the activation of Gsk3 and decrease the phosphorylation of tau, which in turn decrease the formation of NFTs. Moreover, Gab2 can interact with A β formation-involved APP. Gab2 may play an important role in preventing neuronal tangle formation and neuron loss, and the loss-of-function of Gab2 haplotype would impair or even eliminate such protection, increasing the risk of AD. However, present research results concerning the role of *Gab2* polymorphism in AD are inconsistent. Further studies are needed to better elucidate the potential contributions of Gab2 to the susceptibility to AD.

Acknowledgements: This work was supported by the National Basic Research Development Program of China (No. 2006CB500706), the National Natural Science Foundation of

China (No. 30700251, 30872729, 30971031), Shanghai Key Discipline Program (No. S30202), and the Program for Outstanding Medical Academic Leader (No. LJ 06003). We thank Dr. Yue HUANG at Prince of Wales Medical Research Institute & School of Medical Sciences, University of New South Wales, for critical reading of the manuscript.

References:

- [1] Wöhrle FU, Daly RJ, Brummer T. Function, regulation and pathological roles of the Gab/DOS docking proteins. *Cell Commun Signal* 2009, 7: 22.
- [2] Gu HH, Neel BG. The 'Gab' in signal transduction. *TRENDS Cell Biol* 2003, 13(3): 122-130.
- [3] Nishida K, Hirano T. The role of Gab family scaffolding adapter proteins in the signal transduction of cytokine and growth factor receptors. *Cancer Sci* 2003, 94(12): 1029-1033.
- [4] Meng SS, Chen ZM, Munoz-Antonia T, Wu J. Participation of both Gab1 and Gab2 in the activation of the ERK/MAPK pathway by epidermal growth factor. *Biochem J* 2005, 391(Pt1): 143-151.
- [5] Baychelier F, Nardeux PC, Cajean-Feroldi C, Ermonval M, Guymarho J, Tovey MG, *et al.* Involvement of the Gab2 scaffolding adapter in type I interferon signalling. *Cell Signal* 2007, 19(10): 2080-2087.
- [6] Pyarajan S, Matejovic G, C.Pratt G, Baksh S, Burakoff SJ. Interleukin-3(IL-3)-induced c-fos activation is modulated by Gab2-Calcineurin interaction. *J Biol Chem.* 2008, 283(35): 23505-23509.
- [7] Tripathi A, Sodhi A. Growth hormone-induced production of cytokines in murine peritoneal macrophages *in vitro*: Role of JAK/STAT, PI3K, PKC and MAP kinases. *Immunobiology* 2009, 214(6): 430-440.
- [8] Reiman EM, Webster JA, Myers AJ, Hardy J, Dunckley T, Zismann VL, *et al.* GAB2 alleles modify Alzheimer's risk in APOE ε4 carriers. *Neuron* 2007, 54(5): 713-720.
- [9] Venezia V, Nizzari M, Repetto E, Violani E, Corsaro A, Thellung S, *et al.* Amyloid precursor protein modulates ERK-1 and -2 signaling. *Ann N Y Acad Sci* 2006, 1090(1): 455-465.
- [10] Russo C, Venezia V, Repetto E, Nizzari M, Violani E, Carlo P, *et al.* The amyloid precursor protein and its network of interacting proteins: physiological and pathological implications. *Brain Res Rev* 2005, 48(2): 257-264.
- [11] Maus M, Medgyesi D, Kövesdi D, Csuka D, Koncz G, Sármay G. Grb2 associated binder 2 couples B-cell receptor to cell survival. *Cell Signal* 2009, 21(2): 220-227.
- [12] Liu Y, Rohrschneider LR. The gift of Gab. *FEBS Lett* 2002, 515 (1-3): 1-7.
- [13] Sármay G, Angyal A, Kertész A, Maus M, Medgyesi D. The multiple function of Grb2 associated binder (Gab) adaptor/scaffolding protein in immune cell signaling. *Immunol Lett* 2006, 104(1-2): 76-82.
- [14] Montagner A, Yart A, Dance M, Perret B, Salles JP, Raynal P. A novel role for Gab1 and SHP2 in epidermal growth factor-induced Ras activation. *J Biol Chem* 2005, 280(7): 5350-5360.
- [15] Caron C, Spring K, Laramée M, Chabot C, Cloutier M, Gu HH, *et al.* Non-redundant roles of the Gab1 and Gab2 scaffolding adaptors in VEGF-mediated signalling, migration, and survival of endothelial cells. *Cell Signal* 2009, 21(6): 943-953.
- [16] Sattler M, Mohi MG, Pride YB, Quinnan LR, Malouf NA, Podar K, *et al.* Critical role for Gab2 in transformation by BCR/ABL. *Cancer Cell* 2002, 1(5): 479-492.
- [17] Huang F, Tong XY, Deng HM, Fu LQ, Zhang RH. Inhibition of the antigen-induced activation of RBL-2H3 cells by Gab2 siRNA. *Cell Mol Immunol* 2008, 5(6): 433-438.
- [18] Sun JM, Pedersen M, Ronnstrand L. Gab2 is involved in differential Phosphoinositide 3-Kinase signaling by two splice forms of c-Kit. *J Biol Chem* 2007, 283(41): 27444-27451.
- [19] Daly RJ, Gu H, Parmar J, Malaney S, Lyons RJ, Kairouz R, *et al.* The docking protein Gab2 is overexpressed and estrogen regulated in human breast cancer. *Oncogene* 2002, 21(33): 5175-5181.
- [20] Horst B, Gruvberger-Saal SK, Hopkins BD, Bordone L, Yang Y, Chernoff KA, *et al.* Gab2-mediated signaling promotes melanoma metastasis. *Am J Pathol* 2009, 174(4): 1524-1533.
- [21] Brummer T, Larance M, Herrera Abreu MT, Lyons M, Timpson P, Emmerich CH, *et al.* Phosphorylation-dependent binding of 14-3-3 terminates signalling by the Gab2 docking protein. *EMBO J* 2008, 27(17): 2305-2316.
- [22] Wan Y, Wang G, Chen SD. Genetic predisposition to inflammation: a new risk factor of Alzheimer's Disease. *Neurosci Bull* 2008, 24: 314-322.
- [23] Hernández F, Avila J. The role of glycogen synthase kinase 3 in the early stages of Alzheimers' disease. *FEBS Lett* 2008, 582: 3848-3854.
- [24] Hernández F, de Barreda EG, Fuster-Matanzo A, Goñi-Oliver P, Lucas JJ, Avila J. The role of Gsk3 in Alzheimer disease. *Brain Res Bull* 2009, 88: 248-250.
- [25] Hernández F, de Barreda EG, Fuster-Matanzo A, Goñi-Oliver P, Lucas JJ, Avila J. Gsk3: A possible link between beta amyloid peptide and tau protein. *Exp Neurol* 2009, doi:10.1016/j.expneurol.2009.09.011.
- [26] Zhao CM, Ma H, Bossy-Wetzel E, Lipton SA, Zhang ZH, Feng GS. GC-GAP, a Rho family GTPase-activating protein that in-

- teracts with signaling adapters Gab1 and Gab2. *J Biol Chem* 2003, 278(36): 34641-34653.
- [27] Slegers K, Bettens K, Brouwers N, Engelborghs S, Miegroet H, De Deyn PP, *et al.* Common variation in GRB-associated Binding Protein 2 (*GAB2*) and increased risk for Alzheimer Dementia. *Hum Mutat* 2008, 1036(29): 338-344.
- [28] Schjeide BM, Hooli B, Parkinson M, Hogan MF, DiVito J, Mullin K, *et al.* *GAB2* as an Alzheimer Disease susceptibility gene: follow-up of genomewide association results. *Arch Neurol* 2009, 66(2): 250-254.
- [29] Ikram MA, Liu F, Oostra BA, Hofman A, van Duijn CM, Breteler MM. The *GAB2* gene and the risk of Alzheimer's disease: replication and meta-analysis. *Biol Psychiatry* 2009, 65(11): 995-999.
- [30] Nacmias B, Tedde A, Bagnoli S, Cellini E, Guarnieri BM, Piacentini S, *et al.* Implication of *GAB2* gene polymorphism in Italian patients with Alzheimer's disease. *J Alzheimers Dis* 2009, 16(3): 513-515.
- [31] Miyashita A, Arai H, Asada T, Imagawa M, Shoji M, Higuchi S, *et al.* *GAB2* is not associated with late-onset Alzheimer's disease in Japanese. *Eur J Hum Genet* 2009, 17(5): 682-686.
- [32] Ramirez Lorca R, Boada M, Saez ME, Hernandez I, Mauleon A, Rosende Roca M, *et al.* *GAB2* gene does not modify the risk of Alzheimer's disease in Spanish *APOE* e4 carriers. *J Nutr Health Aging* 2009, 13(3): 214-219.
- [33] Chapuis J, Hannequin D, Pasquier F, Benthay P, Brice A, Leber I, *et al.* Association study of the *GAB2* gene with the risk of developing Alzheimer's disease. *Neurobiol Dis* 2008, 30(1): 103-106.
- [34] Lin K, Tang M, Han H, Guo Y, Lin Y, Ma C. *GAB2* is not associated with late on-set Alzheimer's disease in Chinese Han. *Neurobiol Dis* 2009, 30(1): 103-106.

Gab2 信号转导通路与阿尔茨海默病

潘小玲¹, 任汝静¹, 王刚¹, 汤荟冬¹, 陈生弟^{1,2}

¹上海交通大学医学院附属瑞金医院神经内科, 上海交通大学医学院神经病学研究所, 上海 200025

²中国科学院上海生命科学研究院 / 上海交通大学医学院健康科学研究所, 上海 200025

摘要 作为一类骨架/接头蛋白, 生长因子受体结合蛋白2 (Grb2) 的相关结合蛋白家族(Grb2-associated binder protein) 参与了细胞内多种信号转导通路, 是酪氨酸激酶(PTKs)激活的下游信号转导通路的关键分子。Gab2是这个家族的重要成员, 可被 PTKs 磷酸化激活, 进而招募多种信号分子(如 p85、SHP2 和 Crk), 在细胞增殖、存活、分化和凋亡等过程中发挥重要作用。最近研究表明, *Gab2* 基因多态性可增加阿尔茨海默病(Alzheimer's disease, AD)的患病风险, 从而与 AD 的发病密切相关。本文拟对 *Gab2* 的结构特点、相关信号转导通路及其在 AD 发生过程中可能扮演的角色作一系统综述。

关键词: *Gab2* 基因; 信号转导通路; 阿尔茨海默病; 载脂蛋白 E