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CYTOSKELETON NEWS

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SEPT 2012

this issue

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ASCB 2012
San Francisco, CA, USA
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The Role of Rac1 GTPase in Neurodegeneration

The neurodegenerative disorder Alzheimer's disease (AD) afflicts 13% of the US population over 65 years of age and costs associated with dementia, including AD, are equivalent to 1% of the world's gross domestic product. AD is the most common cause of dementia and characterized by a progressive loss of memory and cognitive abilities correlated with dendritic spine loss and eventual neuron death. The two traditional pathological markers of AD are extracellular aggregation of amyloid beta protein (Aβ) and intracellular aggregation of hyperphosphorylated tau[1,2]. Recent investigations of the pathological time course of AD indicate that soluble Aβ, synaptic dysfunction, and dendritic spine loss are more closely correlated with disease progression than the traditional markers[3-5].

As the primary site of excitatory synapses, dendritic spines are essential for neurotransmission[1,2,6,7]. Spine morphology (see upper fluorescent image), mobility, and stability are controlled by actin cytoskeletal dynamics as filamentous actin (F-actin) is a primary component of spines[6,7]. As a key regulator of actin dynamics, the GTPase Rac1 has a pivotal role in the maintenance and reorganization of dendritic spines[8,9]. Perhaps then it should be no surprise that Rac1, Aβ, and Aβ's precusor protein APP have a complex relationship. Rac1 regulates APP transcriptional expression[10], cleavage of APP into Aβ[11-13], and secretion of APP cleavage products[14] (Fig. 1). Also, Rac1 protein expression is increased in the hippocampi of AD brains compared to control brains[15] and Rac1 immunoreactivity is increased in the cortex of an AD mouse model[16]. *In vitro*, there are conflicting data regarding the effects of Aβ on Rac1 activity with reports of either an increase[17,18] or decrease[19]. The Aβ-induced increase is correlated with increased Rac localization to the plasma membrane and elevated actin polymerization[18]. The *in vitro* decrease was confirmed with a similar observation in an AD animal model[19]. Rac1 is also an essential component of the inflammatory cascade involving Aβ-mediated generation of reactive oxygen species and AD pathogenesis[20]. Besides Aβ, the tau protein also aggregates in AD and Rac1 appears to be involved with this pathology as well. A constitutively active splice variant of Rac1, Rac1b, has recently been linked to tau tangle formation in nucleus basalis neurons of AD subjects. Rac1b accumulation in these dysfunctional neurons increases with the severity of cognitive impairment and is correlated with decreased expression of genes involved in lipid metabolism and cell cycle[21].

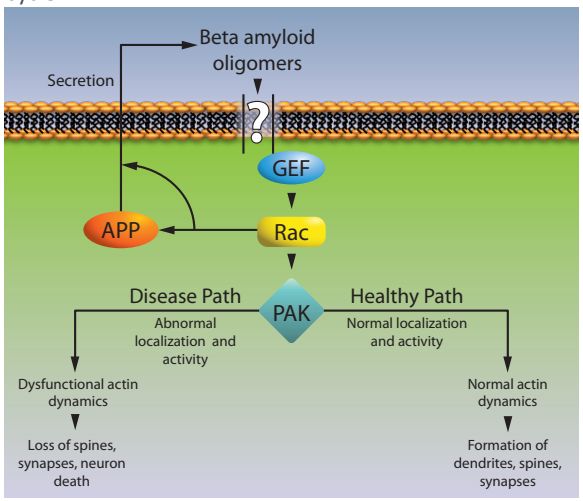

Figure 1: Schematic for functional interactions between Rac1, p21 activated kinase (PAK), amyloid precursor protein (APP), and amyloid beta (Aβ) in the pathogenesis of Alzheimer's disease

As might be expected, Aβ not only affects Rac1, but also Rac1's primary downstream effector, p21-activated kinase (PAK). Studies with AD brains and AD animal models suggest that in early stages of AD, total and active PAK levels are increased while in mid to late stage AD, total and active PAK levels decrease[21-24]. These decreases are accompanied by pathological changes in the expression and activity of actin-binding proteins[1,2,22]. In the brains of AD patients and older AD mice, active PAK in a complex with Rac/Cdc42 localizes to the plasma membrane to a greater extent than observed in control brains[23]. This pattern of PAK localization is also observed in cultured

RHO FAMILY PRODUCTS

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neurons, accompanied by a loss of F-actin from spines and dendrites as well as spine loss[23]. Not surprisingly, pharmacological inhibition of PAK causes memory impairments and pathological changes in actin-binding proteins in adult mice[22] (Fig. 1). It should be noted that PAK changes could also involve the Cdc42 GTPase.

Rac1 is not the only Rho family GTPase implicated in neurodegeneration. Activity of Rho and its downstream effector ROCK are a major focus of AD research[19,25-28] with ROCK considered a pharmaceutical target for AD treatments[29]. Aβ increases Rho activity which is linked to inhibition of neurite outgrowth and synapse formation[2]. Besides AD, both Rho and Rac are likely involved in cellular processes associated with changes in neurite extention and retraction in the neurodegenerative disorder Parkinson's disease[30,31].

It is evident that the role of Rho family GTPases in neurodegenerative diseases merits further study with an emphasis on measuring GTPase concentration and activation levels. To assist your research, Cytoskeleton offers Rac and Rho activation assays, as well as activators, inhibitors, and antibodies .

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Rho Family Small G-protein Tools

Small G-protein Activation Assays	Method	Cat. #	Amount
Rac1,2,3 G-LISA® Activation Assay, colorimetric	G-LISA®	BK125	96 assays
Rac1 G-LISA® Activation Assay, colorimetric	G-LISA®	BK128	96 assays
Rac1 Pull-down Activation Assay Biochem Kit™	Pull-down	BK035 BK035-S	50 assays 20 assays
RhoA / Rac1 / Cdc42 Activation Assay Combo Kit	Pull-down	BK030	3 x 10 assays
RhoA G-LISA® Activation Assay, colorimetric	G-LISA®	BK124	96 assays
RhoA G-LISA® Activation Assay, luminescence	G-LISA®	BK121	96 assays
RhoA Pull-down Activation Assay Biochem Kit™	Pull-down	BK036 BK036-S	80 assays 20 assays

Rhotekin-RBD and PAK-PBD	Purity	Cat. #	Amount
PAK-PBD Protein Binds specifically to active (GTP-bound) Cdc42 and Rac	>80%	PAK01-A PAK01-B	1 x 250 µg 4 x 250 µg
PAK-PBD Beads Binds specifically to active (GTP-bound) Cdc42 and Rac	>80%	PAK02-A PAK02-B	1 x 500 µg 4 x 500 µg
Rhotekin-RBD Protein Binds specifically to active (GTP-bound) Rho	>90%	RT01-A RT01-B	1 x 500 µg 3 x 500 µg
Rhotekin-RBD Beads Binds specifically to active (GTP-bound) Rho	>85%	RT02-A RT02-B	2 x 2 mg 6 x 2 mg

G-protein Modulator	Cell Entry Mechanism	Protein Modulation	Cat. #	Amount
Rho Activator II Deamidation of Rho Gln-63	Cell permeable	Direct	CN03-A CN03-B	3 x 20 µg 9 x 20 µg
Rho Inhibitor I ADP ribosylation of Rho Asn-41	Cell permeable	Direct	CT04-A CT04-B	1 x 20 µg 5 x 20 µg
Rho/Rac/Cdc42 Activator I Deamidation of Rho Gln-63 & Rac/Cdc42 Gln-61	Cell permeable	Direct	CN04-A CN04-B	3 x 20 µg 9 x 20 µg
Rho Pathway Inhibitor I Rho kinase (ROCK) inhibitor Y-27632	Cell permeable	Direct	CN06-A CN06-B	5 x 10 units 20 x 10 units
Rho Activator I SHP-2 phosphatase-mediated Rho activation	Cell permeable	Indirect	CN01-A CN01-B	5 x 10 units 20 x 10 units
Rac/Cdc42 Activator II EGF receptor-mediated Rac/Cdc42 activation	Receptor mediated	Indirect	CN02-A CN02-B	5 x 10 units 20 x 10 units

Purified G-proteins	Purity	Cat. #	Amount
Rac1 His Protein, constitutively-active (Q61L)	>90%	R6101-A	1 x 10 µg
Rac1 GST Protein, dominant-negative (T17N)	>90%	R17G01-A	1 x 25 µg
Rac1 His Protein, wild-type	>90%	RC01-A	1 x 100 µg
RhoA His Protein, constitutively-active (Q63L)	>90%	R6301-A	1 x 10 µg
RhoA His Protein, wild-type	>80%	RH01-A	1 x 100 µg

Small G-protein Antibodies	Host/Type	Species Reactivity	Cat. #	Amount
Rac1 Specific Antibody Human C-terminal Peptide	Mouse/mAb	Hu, Ms, Rt, other extracts	ARC03-A ARC03-B	2 x 50 µg 6 x 50 µg
RhoA Specific Antibody Human RhoA Peptide	Mouse/mAb	Hu, Ms, Rt, other extracts	ARH03-A ARH03-B	1 x 100 µg 3 x 100 µg

Actin Biochem Kits™	Cat. #	Amount
Actin Polymerization Biochem Kit™	BK003	30-100 assays
G-actin/F-actin *in vivo* Biochem Kit™	BK037	30-100 assays

Phalloidin	Excitation/ Emmission	Signal stability * ($T_{1/2}$ in secs)	Cat. #	Amount
Acti-stain™ 488 phalloidin	480/535 nm	57	PHDG1-A	300 Slides
Acti-stain™ 535 phalloidin (Rhodamine phalloidin)	535/585 nm	27	PHDR1	300 Slides
Acti-stain™ 555 phalloidin	535/585 nm	46	PHDH1-A	300 Slides
Acti-stain™ 670 phalloidin	640/670 nm	8	PHDN1-A	300 Slides