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Review

Functions of Rhotekin, an Effector of Rho GTPase, and its Binding Partners in Mammals

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Abstract: Rhotekin is an effector protein for small GTPase Rho. This protein consists of a Rho binding domain (RBD), a pleckstrin homology (PH) domain, proline-rich regions and a C-terminal PDZ-binding motif. We and other groups have identified various binding partners for Rhotekin and proposed their possible physiological roles. However, functions of Rhotekin *per se* are largely unknown and information about its physiological roles are fragmentary. In this review, we summarize known features of Rhotekin in neuronal tissues and cancer cells. We also describe characteristics of binding partners for Rhotekin and predicted roles of their interaction.

Keywords: Rho; Rhotekin; PDZ domain; SH3 domain

1. Introduction

Rhotekin was originally identified as a binding partner for small GTPase Rho by yeast two-hybrid analysis using a mouse embryo cDNA library [1]. The name is derived from the Japanese "teki" meaning a target. Expression of Rhotekin mRNA was observed in bran, kidney lung and skeletal muscles. Another family member, Rhotekin-2, has been found in lymphocyte [2]. Rhotekin has a predicted molecular mass of 61 kDa and contains an active Rho-binding domain (RBD), a pleckstrin-homology (PH) domain, two proline-rich motifs and a C-terminally located class 1 PDZ-binding motif (Figure 1). RBD of Rhotekin selectively interacts with GTP-bound activated form of Rho small GTPase and this domain inhibits both intrinsic and GTPase activating protein(GAP)-enhanced GTPase activity of Rho [1]. Using these features of Rhotekin RBD, the recombinant protein has been utilized for monitoring the activation state of Rho [3].

Physiological functions of other effectors of Rho, such as Rho-kinase/ROCK and mDia, have been revealed by intensive investigations. In contrast, the information about the function of Rhotekin is yet very limited. In this review, we will summarize physiological features of Rhotekin and its binding partners.

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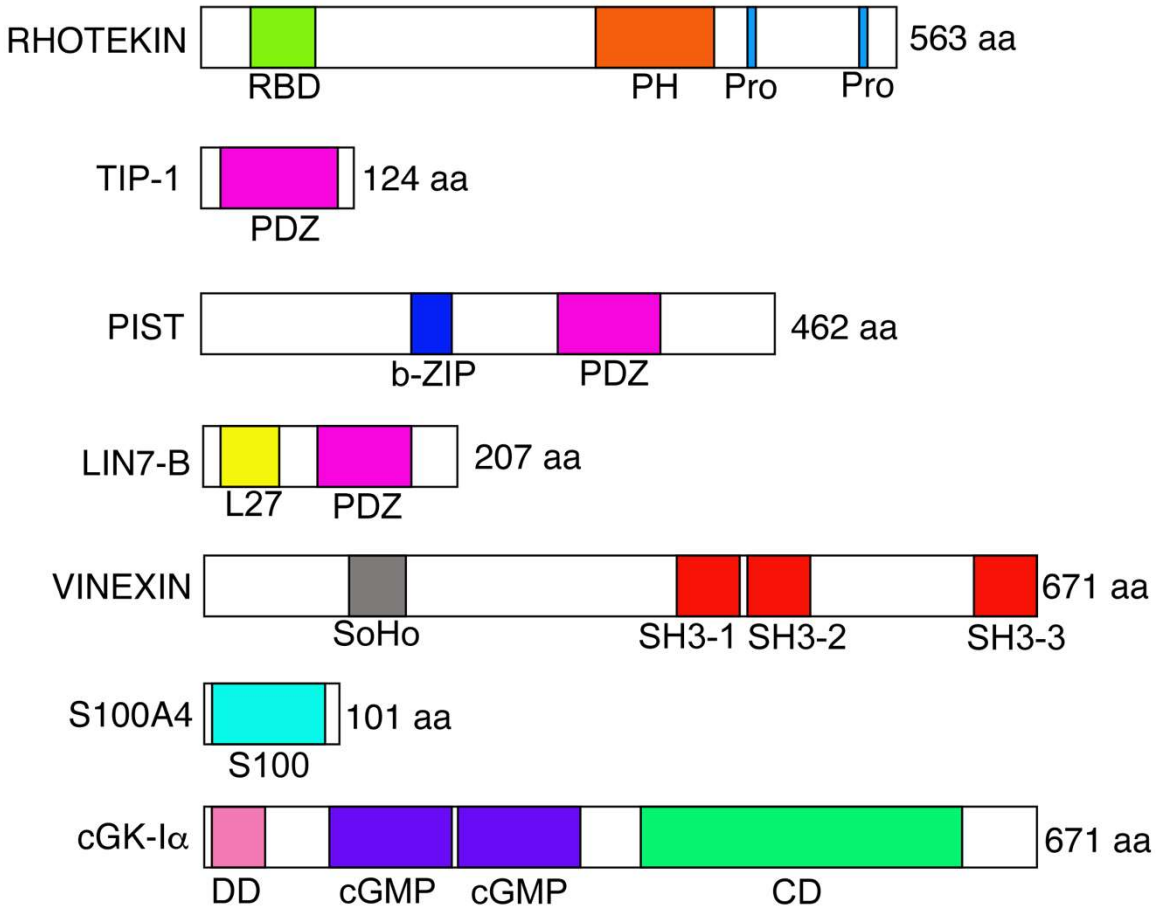

Figure 1. Structures of Rhotekin and its binding partners. Structural domains of proteins are abbreviated as follows: RBD, Rho-binding domain; PH, pleckstrin-homology; Pro, proline-rich motif; b-ZIP, basic leucine zipper domain; L27, Lin-2 and Lin-7 domain; PDZ, PSD-95/Discs large/Zona occludens-1 domain; SoHo, sorbin homologous domain; SH3, src homology 3 domain; S100, S100 domain; DD, dimerization/docking domain; cGMP, cyclic GMP binding domain; CD, catalytic domain.

2. Rhotekin in neuronal tissues

Although mRNA expression of Rhotekin has been analyzed in the original paper [1], the information about Rhotekin protein in mammalian tissues was lacked because specific antibody for this protein had not been developed. Therefore, we produced a specific antibody against Rhotekin using N-terminus 334 amino acid of this protein as an antigen [4]. Using the antibody, we found the unique pattern of Rhotekin expression in rat brain during embryonic to postnatal development [5]. In western blotting, our antibody detected four major bands (molecular masses about 75 kDa, 68 kDa, 61 kDa and 50 kDa), which were considered as splicing isoforms, in rat brain at various developmental stages (Figure 2). The protein with 75 kDa was detected at embryonic day 12.5 (E12.5) rat brain but this protein disappeared until after birth and re-increased up to postnatal 30 days (P30). Another band with 68 kDa was only observed at P21 and P30 brain. The 61 kDa protein was highly expressed in embryonic brain but gradually decreased during development. The 50 kDa bands was detected at limited periods, E18.5 to P11. Immunofluorescence analysis revealed that Rhotekin is distributed in soma, axon and dendrite of primary cultured rat hippocampal neurons [5,

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6]. In dendrite, localization of Rhotekin partially overlapped with synaptophysin, a representative marker for synapse. These results suggest that Rhotekin may play essential roles in neuronal development with isoform specific manners.

Thereafter, Iwai et al. reported that the participation of Rhotekin in the survival, differentiation and neurite outgrowth of neurons [7]. In this report, the authors also found the inhibitory role of Rhotekin in the proliferation of neural stem cells. Rhotekin may be one of the essential molecules for the development of neuronal tissues whereas the precise molecular mechanism of its function is yet obscure.

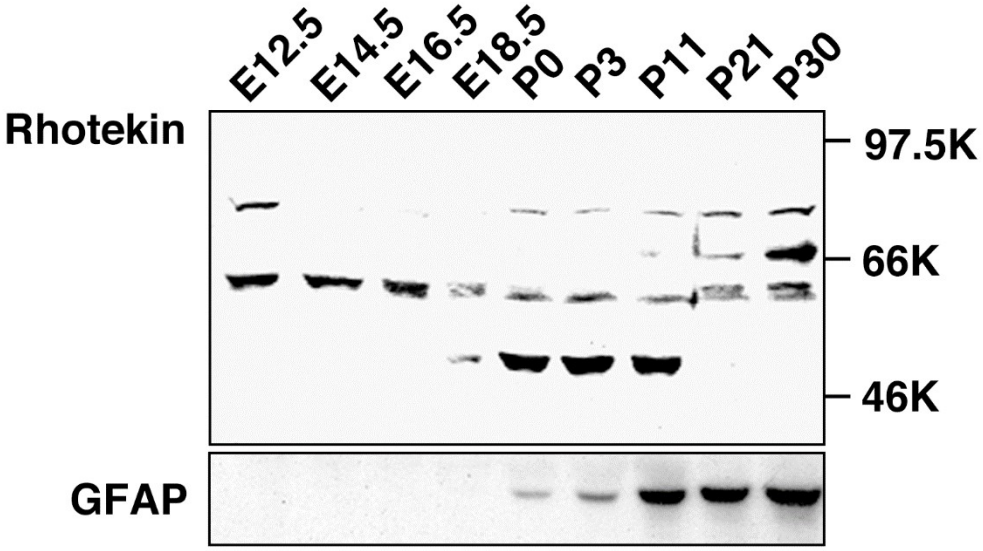

Figure 2. Developmental change of Rhotekin in the rat brain. Cholate extracts of brains from rats at various stages were subjected to SDS-PAGE followed by Western blotting with anti-Rhotekin (upper panel) or anti-GFAP (lower panel). Molecular markers are at right. (Adapted from reference [5].)

3.Rhotekin and cancer cells

There are several reports describing the abnormal expression of Rhotekin in cancer cells. Liu et al. has reported that 71% of gastric cancer examined overexpressed Rhotekin [8]. They also showed that transfection of Rhotekin in AGS gastric cells conferred the resistance to apoptosis induced by serum deprivation and treatment with sodium butyrate [9]. Rhotekin overexpression resulted in the activation of the nuclear kappa B (NF-κB) while inhibitors for NF-κB, curcumin or parthenolide, diminished the anti-apoptotic effect of Rhotekin [9]. Enhanced expression of Rhotekin was also observed in human colorectal carcinoma [10], bladder carcinoma [11] and hepatocellular carcinoma [12]. Recent studies have revealed that micro RNAs (miRNAs) miRNA-152 [12] and let-7a [13] can inhibit tumor cell growth through the downregulation of Rhotekin expression. Rhotekin-mediated signaling pathways may be a novel therapeutic target for cancer.

4. Regulation of the septin cytoskeletal organization by Rhotekin

Septins are a conserved family of cytoskeletal GTP/GDP-binding proteins. They were firstly identified in yeast and 13 genes have been identified in human. Septins are required for cell cycle

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control, cytokinesis and regulation of some signal transduction pathway [14]. They form filamentous structure in mammalian cells [15] but the information about the regulatory mechanisms of septin filaments is limited. Borg, a Cdc42 effector, has been suggested to be a regulator of septin organization [16]. We found that Rho/Rhotekin signaling pathway regulates the structure of septin filaments [4]. Activated form of Rho disrupted the filamentous structure containing Sept9 in mammalian fibroblast cells. Transfection of Rhotekin resulted in similar morphological change of the septin filament. Further analyses revealed that the center region of Rhotekin was essential for the interaction and the transfection of this region caused disruption of the septin filament. These results suggest that Rhotekin is an important linker between Rho signaling and septin complexes.

5. Binding partners for Rhotekin

5.1 TIP-1

TIP-1, Tax-interacting protein 1, was identified as a binding partner for Tax oncoprotein of T-lymphotropic virus type 1 (HTLV-1) by yeast two-hybrid screening [17]. TIP-1 is a 124 aa protein which has single PDZ domain (Figure 1). When Reynaud et al. conducted the yeast two-hybrid analysis using TIP-1 as a bait, they identified Rhotekin as an interacting protein [18]. In the same study, they also revealed that the C-terminus PDZ-binding motif of Rhotekin was responsible for the binding to the PDZ domain in TIP-1. In addition, they found that combinational transfection of TIP-1, Rhotekin and constitutively active form of RhoA, RhoA-V14, caused the strong activation of serum response element (SRE) [18].

5.2 PIST

PIST [PDZ (PSD-95, Discs-large and ZO-1) domain protein interacting specifically with TC10] is a golgi-associated protein that is also called as GOPC (Golgi-associated PDZ and coiled-coil motif-containing protein), FIG (fused in glioblastoma) or CAL [cystic fibrosis transmembrane conductance regulator (CFTR)-associated ligands]. This molecule was originally found as a putative binding partner for TC10 Rho family GTPases [19]. PIST interacts with various proteins such as syntaxin 6 [20], frizzled [21], ClC-3B [22], CALEB/NGC [23], CFTR [24], cadherin 23 [25], protocadherin 15 [26] and regulates localization of these molecules at the plasma membrane. PIST also interacts with beta 1 adrenergic receptor and retains golgi apparatus during biosynthetic pathway, and internalized beta 1 receptor is protected by PIST [27]. The neuronal isoform of PIST (nPIST) forms complex with delta 2 glutamate receptor (GluR ∂2) and Beclin to induce autophagy [28]. nPIST also interacts with stargazin and regulates the synaptic clustering of AMPA receptors [29].

When we performed yeast two-hybrid analysis with a human heart cDNA library to find novel interacting partners for Rhotekin, PIST was identified as a binding partner [30]. Mapping analysis revealed that Ser-Pro-Val residues at C-terminus of Rhotekin, which is consistent with a class 1 PDZ-binding motif, interacts with the PDZ domain in PIST. Expression of RhoA-V14 inhibited the interaction of Rhotekin and PIST whereas a constitutively active form of TC10 did not affect the binding. Rhotekin and PIST colocalized at golgi apparatus in non-polarized epithelial Madin-Darby canine kidney (MDCK) cells. When MDCK cells became polarized, Rhotekin and PIST were recruited from the cytosol to adherence junctions (AJ). Expression of RhoA-V14 caused diffused cytosolic localization of Rhotekin in polarized MDCK cells although PIST was still localized at AJ. In addition, expression of the PDZ domain of PIST perturbed the localization of Rhotekin at AJ in

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polarized MDCK cells. The localization of PIST at the cell-cell contact site was prior to that of Rhotekin. These results indicate that PIST recruits Rhotekin to AJ and these two molecules plays essential roles in cell polarity formation and/or maintenance.

5.3 LIN-7

LIN-7 was originally identified as a cell junction protein that regulates the localization of LET-23 receptor during C. elegans vulval induction [31]. Three mammalian homologs of LIN-7 (also called as Veli and MALS) were identified as LIN7-A, -B and -C [32, 33]. LIN-7 is a small scaffold protein possessing an L27 and a PDZ domain (Figure 1). This protein is abundantly expressed in neural tissues and is associated with the PSD-95/NMDA receptor complex [6, 32, 33]. We have identified LIN-7B as a binding partner for Rhotekin by yeast two-hybrid analysis and investigated the interaction between these two molecules [6]. The PDZ-binding motif at C-terminus of Rhotekin was responsible for the binding to LIN7. The interaction between Rhotekin and LIN7 was affected by RhoA-V14. LIN7 and Rhotekin partially colocalized at synapses of primary cultured rat hippocampal neurons and cell-cell contact in polarized MDCK cells. We and other group have reported the involvement of LIN7 in neurodevelopmental disease such as attention deficit hyperactivity disorder (ADHD) [34, 35] and autism [36]. Although the physiological meaning of interaction of Rhotekin with LIN7 remains to be elucidated, Rhotekin may play important roles in the regulation of synaptic transmission in concert with LIN7.

5.4 Vinexin

Vinexin is a member of a multi-domain adaptor family, which was originally identified as a binding partner for vinculin [37]. There are three isoforms in mammals, vinexin-α, -β and -γ. α and γ isoforms are larger variants and have sorbin-homology (SoHo) domain and three SH3 domains (Figure 1) while β isoform is a short variant that contains only three SH3 domains. Vinexin is thought to be involved in the actin cytoskeletal organization and cellular processes such as migration, spreading and proliferation [38]. Vinexin is localized at synapses of primary cultured hippocampal neurons and is phosphorylated by ERK [39]. We have identified vinexin as a binding partner for Rhotkein using yeast two-hybrid analysis with a human heart cDNA library [40]. The proline-rich motif at C-terminus of Rhotekin interacts with the third SH3 domain of vinexin. This interaction was independent of Rho activity. Rhotekin and vinexin were at least partially colocalized at focal adhesions of REF52 fibroblast cells.

Recently, Chang et al. reported the role of Rhotekin and vinexin in the cell cycle progression [41]. They showed the colocalization of Rhotekin and vinexin at the midbody during cell division. Overexpression of vinexin mutant lacking rhotekin-binding motif and knockdown of vinexin or Rhotekin in HeLa cells caused the increase of cells arrested at the midbody stage. They concluded that vinexin localized at the midbody recruits Rhotekin to facilitate the cytokinesis.

5.5 S100 calcium binding protein A4 (S100A4)

S100A4 is a member of S100 calcium-binding protein family. This protein contains two EF-hands calcium binding motifs and is considered to associate with progression of colon and breast cancers [42, 43]. S100A4 selectively interacts with Rhotekin-RBD but not with RBDs of other Rho effectors such as Citron, mDia and ROCK [44]. S100A4 and Rhotekin are co-localized at leading

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edge of migrating cells. The binding between S100A4 and Rhotekin is calcium-dependent. S100A4, Rhotekin and active RhoA form a tripartite complex. From the RNA interference experiments, it is suggested that S100A4 and Rhotekin co-operate to inhibit actin stress fiber formation and promote membrane ruffle formation by EGF stimulation. These results indicate that S100A4 and Rhotekin contribute to confer invasive phenotypes of cancer cells.

5.6 Cyclic GMP-dependent kinase I alpha (cGK-Iα, PKGIα)

Cyclic GMP-dependent kinase (cGK or PKG) is a serine/threonine kinase that is widely expressed in vertebrate. One of the cGK isoform, cGK-Iα, was identified as a binding partner for Rhotekin [45]. cGK-Iα mediates the phosphorylation of Rhotekin. These two proteins are localized at the plasma membrane and extended neurites in Neuro2A neuroblast cells. Treatment with cGMP caused translocation of Rhotekin to cytosol. Rhotekin and cGK-Iα may contribute to the Rho-mediated neurite retraction.

Concluding remarks

Possible roles of Rhotekin and its binding partners are summarized in Figure 3. We have reported possible involvement of Rhotekin in cell polarity, neuronal synapse formation and focal adhesion [5, 6, 30, 39, 40]. However, precise molecular mechanisms underlying these processes are currently largely unknown. Further intensive studies with various experimental approaches are required to address this issue. Since Rhotekin is abundantly expressed in brain tissues and several kinds of cancer cells, investigation of Rhotekin function may also contribute to the understanding of pathophysiology of neurodevelopmental disorder and cancer.

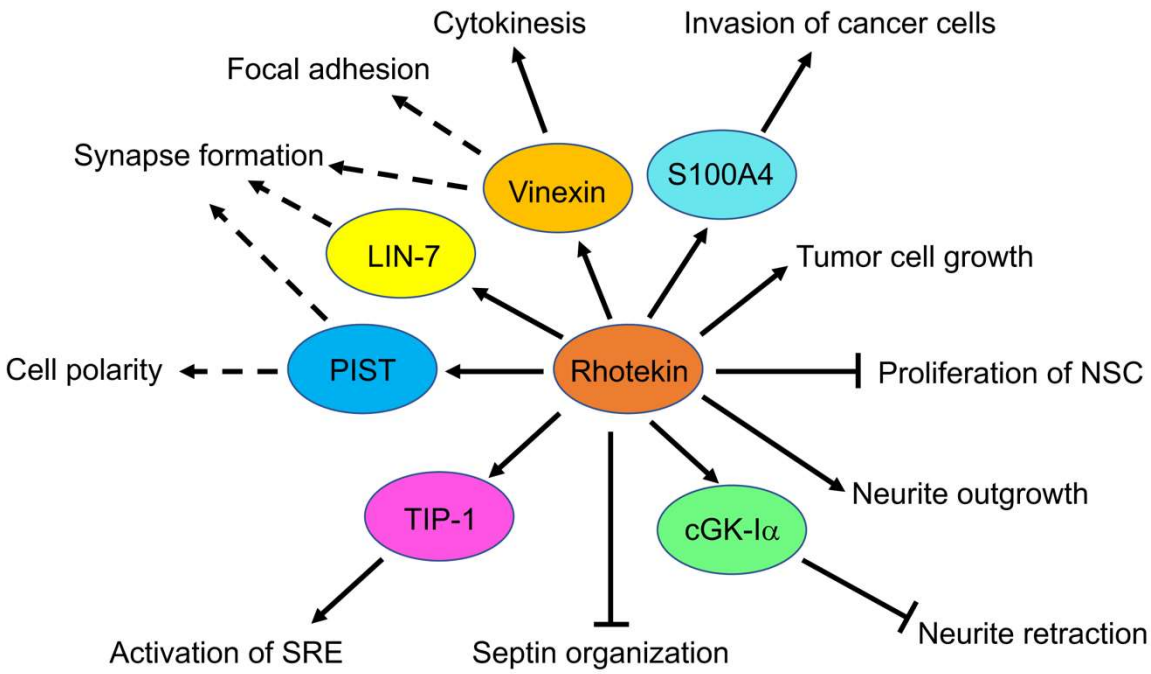

Figure 3. Physiological roles of Rhotekin and its binding partners. Solid lines represent events that are fully understood. Broken lines represent predicted roles that remain to be clarified. Abbreviation are used as follows; NSC, neural stem cells; SRE, serum response element.

Conflicts of interest: The authors declare no conflict of interest

Abbreviations

RBD	Rho binding domain
PH	pleckstrin homology
GAP	GTPase activating protein
NF-κB	nuclear kappa B
MDCK	Madin-Darby canine kidney
AJ	adherence junctions
cGK	cyclic GMP dependent kinase
SH3	Src homology 3
NSC	neural stem cells
SRE	serum response element

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