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ROCK and PRK-2 mediate the inhibitory effect of Y-27632 on polyglutamine aggregation

Jieya Shao[a,b], William J. Welch[c], Marc I. Diamond[a,b,*]

[a] Department of Neurology, UCSF, GH-S572B, 600 16th Street, San Francisco, CA 94143, United States
[b] Department of Cellular and Molecular Pharmacology, UCSF, San Francisco, CA 94143, United States
[c] Department of Surgery, UCSF, San Francisco, CA 94143, United States

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Abstract Polyglutamine expansion in huntingtin (Htt) and the androgen receptor (AR) causes untreatable neurodegenerative diseases. Y-27632, a therapeutic lead, reduces Htt and AR aggregation in cultured cells, and Htt-induced neurodegeneration in *Drosophila*. Y-27632 inhibits both Rho-associated kinases ROCK and PRK-2, making its precise intracellular target uncertain. Over-expression of either kinase increases Htt and AR aggregation. Three ROCK inhibitors (Y-27632, HA-1077, and H-1152P), and a specific ROCK inhibitory peptide reduce polyglutamine protein aggregation, as does knockdown of ROCK or PRK-2 by RNAi. RNAi also indicates that each kinase is required for the inhibitory effects of Y-27632 to manifest fully. These two actin regulatory kinases are thus involved in polyglutamine aggregation, and their simultaneous inhibition may be an important therapeutic goal.
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Keywords: Polyglutamine; Neurodegeneration; ROCK; PRK-2; Huntingtin; Androgen receptor

1. Introduction

Huntington's disease (HD) and x-linked spinobulbar muscular atrophy (SBMA) cause devastating neurodegeneration. HD derives from an expanded CAG codon repeat that produces an elongated polyglutamine tract in the huntingtin (Htt) protein, and SBMA from an elongated tract in the androgen receptor (AR) [1,2]. There is no effective therapy for any polyglutamine disease, and the basic mechanisms that influence intracellular polyglutamine protein aggregation and toxicity are not well understood. Few intracellular targets amenable to mechanism-based therapies have been identified. To improve quantification of intracellular aggregation, we previously developed an intracellular aggregation assay based on fluorescence resonance energy transfer (FRET) [3]. In this system, the first 127 amino acids of AR (ARN127) or the first exon of Htt (Htt exon 1) are fused to cyan fluorescent protein (CFP) as an energy donor, or yellow fluorescent protein (YFP) as an energy acceptor and expressed in cultured cells. Even subtle changes in aggregation may thus be quantitatively measured by FRET [3]. In a screen for biologically active small molecules that inhibit polyglutamine aggregation, we identified Y-27632. Y-27632 reduces polyglutamine aggregation in cultured cells, and when administered to *Drosophila* it reduces Htt-induced neurodegeneration [3].

Y-27632 inhibits ROCK, a Rho-associated serine-threonine protein kinase mainly involved in actin cytoskeleton rearrangement [4–6]. Although Y-27632 has been commonly used to study ROCK functions, like all pharmacological inhibitors it exhibits off-target effects. In vitro studies have shown that Y-27632 also inhibits protein kinase C-related protein kinase (PRK-2) with a similar IC_{50} [7]. Like ROCK, PRK-2 is an effector of the small GTPases Rho and Rac, and mediates actin cytoskeleton rearrangements [8,9]. Thus, the molecular mechanism by which Y-27632 inhibits polyglutamine aggregation is uncertain. It is of fundamental importance to address this issue, as a precise molecular target (or targets) would enable development of a mechanism-based therapy for HD and SBMA. We have combined chemical and genetic approaches to test whether ROCK inhibition is the primary cause of the inhibitory effect of Y-27632 on polyglutamine aggregation. In doing so, we have found that ROCK is only partially responsible for the effect of Y-27632 on aggregation, which is also mediated by PRK-2. Our data suggest that these two kinases together mediate the full anti-aggregation effect of Y-27632.

2. Materials and methods

2.1. Plasmid construction

cDNAs encoding ARN127(Q65)CFP/YFP or Htt exon 1(Q72)CFP/YFP were subcloned from p6R vector into the backbone of pECFP.N1 to drive the expression under the CMV promoter [3]. pCAG-ROCK 1 was kindly provided by Dr. Shuh Narumiya [10], and pXJ40-ROCK 2 by Dr. Thomas Leung [11]. The RBPH domain of rat ROCK 2 [11] was PCR amplified with an amino-terminal myc-tag and cloned into pcDNA3.1. Mutations (N1036T/K1037T) were introduced by Quickchange.

2.2. Cell culture and transfection

HEK293 cells were cultured in DMEM containing 5% FBS and penicillin/streptomycin. Transfection was performed using Plus reagent and Lipofectamine (Invitrogen). For FRET assays, 0.6 μg plasmids encoding ARN127(Q65)CFP/YFP or Htt exon 1(Q72)CFP/YFP were co-transfected (at a 1:3 ratio of CFP:YFP) with or without other plasmids (e.g. ROCK, PRK-2, or RBPH(TT)) into 12-well dishes, grown for 24 h, plated in 96-well black, clear-bottom plates (Costar™ 3603), grown for 24 h, and fixed with 4% paraformaldehyde. ROCK inhibitors (Calbiochem) were added 24 h post-transfection, and cells

*Corresponding author. Address: Department of Neurology, UCSF, GH-S572B, 600 16th Street, San Francisco, CA 94143-2280, United States. Fax: +1 415 514 4112.
E-mail address: marc.diamond@ucsf.edu (M.I. Diamond).

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were treated for 24 h prior to fixing. More details have been described previously [3,12]. For RNAi experiments, negative control siRNA (Santa Cruz, sc-44230), siRNAs against ROCK 1 (Santa Cruz, sc-29473) or PRK-2 (Ambion, AM51333) were transfected for two rounds into HEK293 cells at 75 pmol per well in a 6-well dish using lipofectamine. ARN127(Q65)CFP/YFP and Htt exon 1(Q72)CFP/YFP were co-transfected in the second round.

2.3. FRET measurements and calculations

FRET intensity in fixed HEK293 cells was measured using a SAFIRE Fluorescence Plate Reader (Tecan Inc.) and calculated as described previously [3,12]. Relative FRET/donor = [(FRET/donor)a − (FRET/donor)b]/(FRET/donor)b, where a = cells co-transfected or treated with aggregation modulators, and b = cells co-transfected with control vector (pcDNA3), or untreated. For dose–response of ROCK inhibitors, relative aggregation inhibition was calculated for each compound by arbitrarily setting the minimum aggregation inhibition to 0 (untreated cells) and maximum to 1 (at highest tolerated drug concentrations).

2.4. Western blot

Rabbit polyclonal anti-ROCK 1 (sc-5560) or ROCK 2 (sc-5561) antibodies were purchased from Santa Cruz, and used at 1:5000. Mouse anti-PRK-2 antibody (catalog number: 610794) was purchased from BD transduction laboratories, and used at 1:5000.

3. Results

3.1. Multiple ROCK inhibitors reduce polyglutamine aggregation

Off-target effects of chemically different kinase inhibitors are usually distinct. Thus, if multiple inhibitors of a candidate kinase produce a similar effect, it is highly likely that this results from inhibition of a specific target, rather than overlapping off-target effects. HA-1077 and H-1152P are ROCK inhibitors

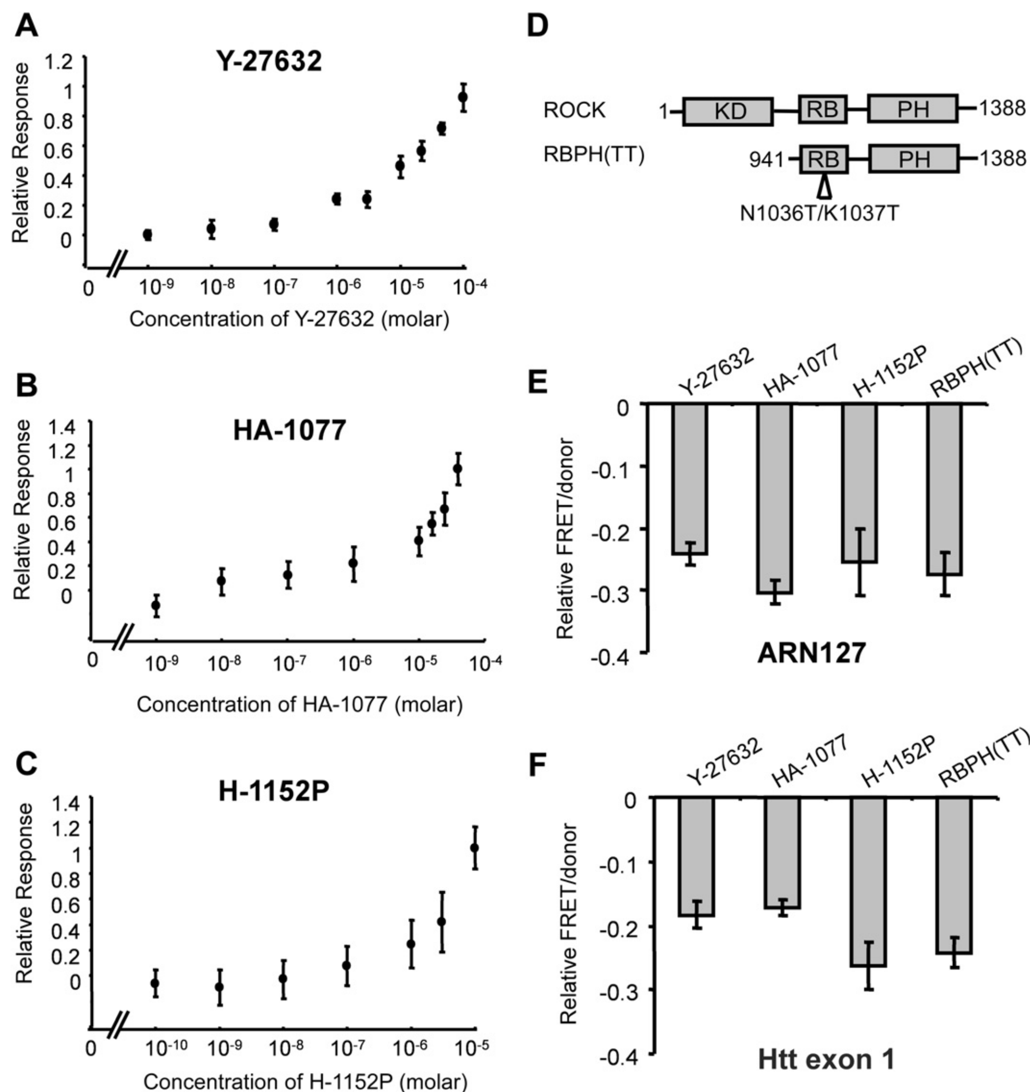

Fig. 1. Inhibition of ROCK by multiple inhibitors suppresses polyglutamine aggregation. (A–C) Dose–response of pharmacologic inhibitors of ROCK. HEK293 cells were transfected with ARN127(Q65)CFP/YFP and treated with (A) Y-27632, (B) HA-1077, or (C) H-1152P at the indicated concentrations. FRET values representing aggregation were determined on the fluorescence plate reader. The relative drug responses were calculated, with one indicating maximal aggregation inhibition. All drugs dose-dependently inhibited polyglutamine aggregation. (D) A C-terminal auto-inhibitory fragment of ROCK 2 containing the RBPH domain was mutated at two sites (N1036T, K1037T) to abolish Rho-binding. (E, F) Pharmacologic and peptide-mediated inhibition of ROCK reduces polyglutamine aggregation to similar extents. HEK293 cells were transfected with ARN127(Q65)CFP/YFP (E) or Htt Exon 1(Q72)CFP/YFP (F) alone or with RBPH(TT), or were treated for 24 h with Y-27632 (50 μM), HA-1077 (40 μM) or H-1152P (10 μM). Effects were expressed as relative FRET/donor ratios vs. untreated cells or cells transfected with an empty vector. All inhibitors reduced aggregation by 25–30%. Error bars represent the S.E.M.

chemically distinct from Y-27632. Compared to Y-27632 and HA-1077, H-1152P is more potent and selective for ROCK [13,14]. We tested each compound as an aggregation inhibitor using the FRET-based aggregation assay. HEK293 cells were transfected with ARN127(65)CFP/YFP, and relative aggregation was measured by FRET using a fluorescence plate reader. All three compounds dose-dependently inhibited polyglutamine aggregation (Fig. 1A–C). IC_{50}s were approximately 5 μM for Y-27632 and HA-1077 and 0.5 μM for H-1152P, consistent with the relative potencies they exhibit against ROCK in vitro and in cells [6,14]. At maximal concentrations tolerated by the cells, each compound inhibited ARN127(Q65) aggregation by 25–30% (Fig. 1E). Each also inhibited Htt exon 1(Q72) aggregation dose-dependently (data not shown) and to a comparable extent at the highest concentrations (Fig. 1F).

As an additional test for the role of ROCK, we exploited its auto-inhibitory mechanism to achieve more specific inhibition. In the absence of activating stimuli (e.g. RhoA), the carboxy-terminal RBPH (rho-binding and pleckstrin-homology) domain of ROCK binds and inhibits its own amino-terminal kinase domain [5,6]. A mutated form of this domain (RBPH(TT)), cannot bind RhoA [11], and specifically blocks ROCK activity, while not affecting closely related kinases that are sensitive to inhibitors such as Y-27632 [15] (Fig. 1D). Expression of RBPH(TT) reduced aggregation of ARN127(Q65)CFP/YFP by up to 30% in HEK293 cells (Fig. 1E). It also inhibited Htt exon 1(Q72) aggregation to a similar extent (Fig. 1F). Taken together, these results suggest that ROCK regulates polyglutamine aggregation, and inhibition of its kinase activity contributes to the anti-aggregation effect of Y-27632.

3.2. Over-expression of ROCK and PRK-2 increase polyglutamine aggregation

ROCK has two main isoforms, ROCK 1 and ROCK 2, which are highly conserved in sequence and function, but differ in tissue distribution. ROCK 1 is expressed in many tissues including brain, while ROCK 2 is restricted primarily to the brain [6,11,16]. Our preceding experiments indicated that ROCK inhibition reduces polyglutamine aggregation. Thus, we tested the effects of over-expression of the two isoforms in cultured HEK293 cells and measured aggregation using FRET. Over-expression of ROCK 1 and ROCK 2 by transient transfection (Fig. 2A) increased the aggregation of ARN127(Q65)CFP/YFP by 40–60% and Htt exon 1(Q72)CFP/YFP by 35% (Fig. 2B) without changing the size of the inclusions. Thus, ROCK activity increases polyglutamine aggregation, whereas its inhibition decreases aggregation.

Because Y-27632 inhibits ROCK and PRK-2 with similar IC_{50}s in vitro, we tested whether PRK-2 also plays a role in polyglutamine aggregation. Over-expression of PRK-2 in cultured HEK293 cells increased aggregation of ARN127(Q65)CFP/YFP by 60% and Htt exon 1(Q72)CFP/YFP by 40% (Fig. 2B). These data suggested that PRK-2 inhibition could be an additional contributor to the effect of Y-27632 on polyglutamine aggregation.

3.3. ROCK and PRK-2 are both required for the anti-aggregation activity of Y-27632

To test whether ROCK and PRK-2 mediate the aggregation-inhibitory effect of Y-27632, we used RNA interference to knock down the endogenous proteins. We reasoned that if both proteins are responsible for the effect of Y-27632, knocking them down should eliminate the target(s) of Y-27632 and diminish its effect. We transfected HEK293 cells with a control siRNA, or siRNAs specifically targeting ROCK 1, PRK-2, or both. ARN127(Q65)CFP/YFP or Htt exon 1(Q72)CFP/YFP were co-transfected with the siRNAs, followed by treatment of cells with the vehicle control or 50 μM Y-27632. Western blot for ROCK 1 and PRK-2 confirmed the specific knockdown of target proteins (Fig. 3A). We measured aggregation by FRET.

We first calculated the direct effects of siRNAs on aggregation. ROCK 1 knockdown inhibited aggregation of AR by 5% and Htt by 15%. Though modest (in particular for AR), this is qualitatively similar to the effects of pharmacologic inhibition of ROCK. PRK-2 knockdown inhibited aggregation of AR by 24% and Htt by 34% (Fig. 3B). Simultaneous knockdown of

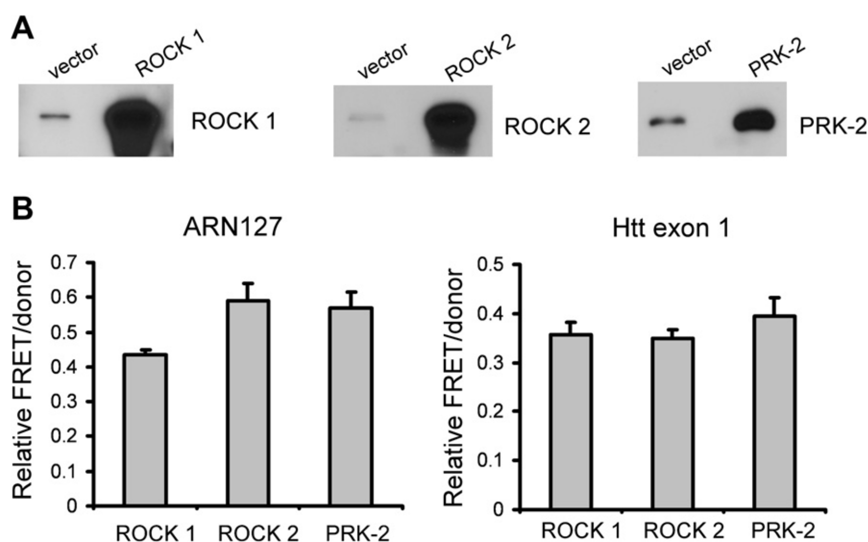

Fig. 2. Over-expression of ROCK and PRK-2 increases polyglutamine aggregation. HEK293 cells were co-transfected with ARN127(Q65)CFP/YFP or Htt exon 1(Q72)CFP/YFP with an empty vector (pcDNA3), ROCK 1, ROCK 2, or PRK-2. (A) Western blot against each protein confirmed their over-expression. (B) Effects of over-expression of each protein on aggregation were expressed as relative FRET/donor ratios vs. cells co-transfected with the empty vector. Over-expression of ROCK 1, ROCK 2, and PRK-2 each increased aggregation. Error bars represent the S.E.M.

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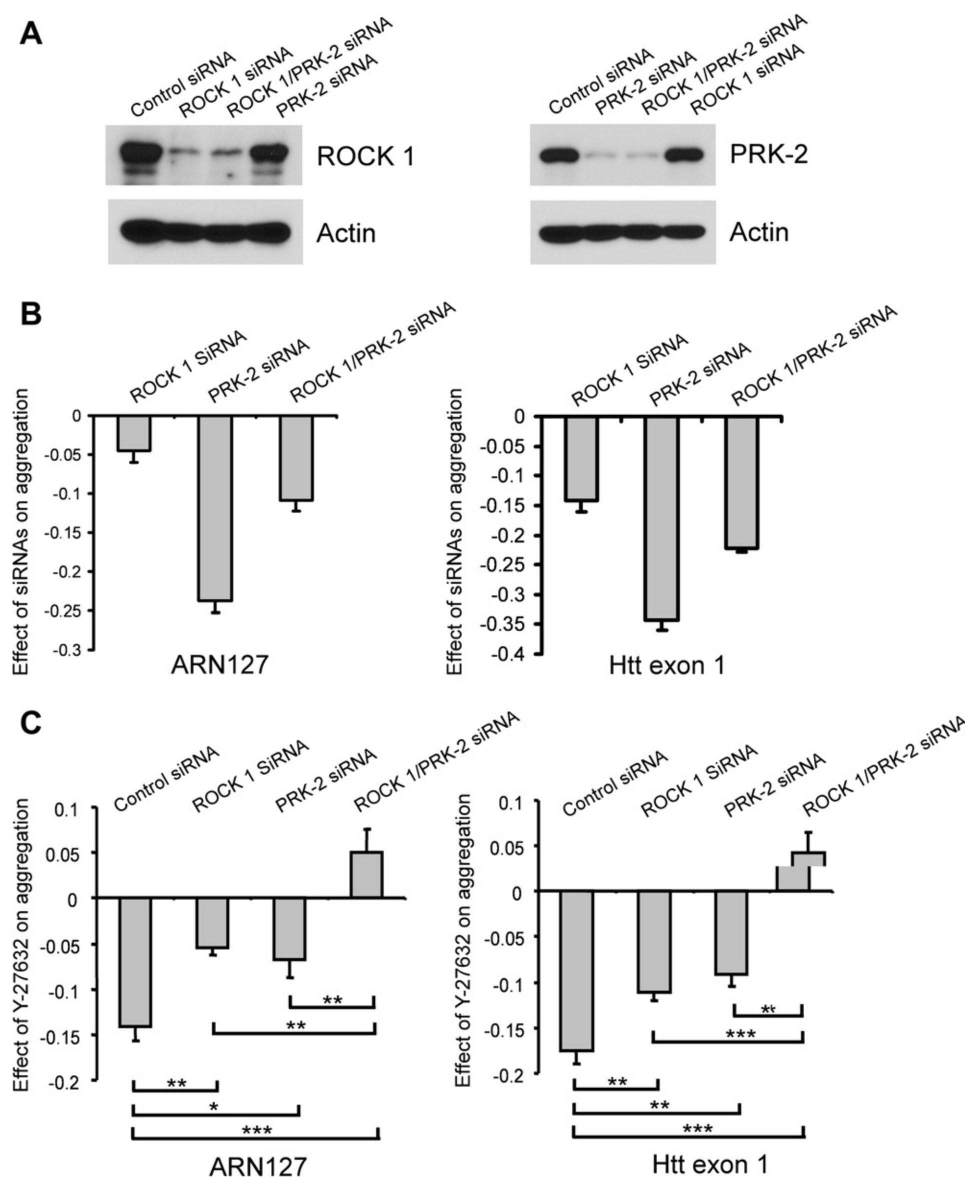

Fig. 3. RNAi knockdown of ROCK 1 and PRK-2 abolishes the inhibitory effect of Y-27632 on polyglutamine aggregation. Control siRNA or siRNAs targeting ROCK 1 or PRK-2 were transfected into HEK293 cells alone, before co-transfection with ARN127(Q65)CFP/YFP or Htt exon 1(Q72)CFP/YFP. Aggregation was measured by FRET. (A) Western blot confirmed the successful knockdowns of ROCK 1 and PRK-2. (B) RNAi knockdown of ROCK 1 and PRK-2 reduces polyglutamine aggregation. Effects of ROCK 1 or PRK-2 knockdown were expressed as relative FRET/donor ratios vs. cells transfected with the control siRNA. (C) RNAi knockdown of ROCK 1 and PRK-2 abolishes the inhibitory effect of Y-27632 on polyglutamine aggregation. Effects of 50 μM Y-27632 were calculated as relative FRET/donor ratios vs. untreated cells in the presence of control, ROCK 1, or PRK-2 siRNAs. ROCK1 or PRK-2 knockdowns each partially reduced the inhibitory effect of Y-27632, and their simultaneous knockdown abolished it, slightly increasing aggregation in response to treatment. Error bars represent the S.E.M. Statistical significance was calculated using the unpaired t-test ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$).

ROCK 1 and PRK-2 inhibited aggregation of AR by 11% and Htt by 22% (Fig. 3B). The non-additive nature of these phenomena likely indicate complex homeostatic mechanisms induced by RNAi [17].

We next compared the anti-aggregation effect of Y-27632 in the presence or absence of RNAi knockdowns. Y-27632 inhibited AR and Htt aggregation in the presence of the control siRNA, however, ROCK 1 and PRK-2 knockdown each significantly reduced its effect (Fig. 3C). This suggests that each protein is partially, but not entirely, responsible for the effect of Y-27632 on polyglutamine aggregation. Simultaneous knockdown of ROCK 1 and PRK-2 abolished the Y-27632 inhibition of AR and Htt aggregation, and engendered a slight (<5%) increase in aggregation in the presence of the inhibitor. This may represent unmasking of a weak, off-target effect of the compound. Taken together, these data suggest that both ROCK and PRK-2 are targets of Y-27632, which together mediate the full anti-aggregation effect of Y-27632.

4. Discussion

Our initial discovery of Y-27632 as a polyglutamine aggregation inhibitor implicated, but did not confirm, the role of

ROCK in this process [3]. It also left uncertain whether other cellular proteins might be targeted by Y-27632 to suppress aggregation. We now observe that inhibition of ROCK by chemically distinct inhibitors, an auto-inhibitory peptide, and RNAi knockdown reduces polyglutamine aggregation. Conversely, ROCK over-expression in cultured cells increases polyglutamine aggregation. These results directly implicate ROCK in the regulation of polyglutamine aggregation. However, knockdown of ROCK 1 in cultured cells only partially diminishes the effect of Y-27632 on polyglutamine aggregation, suggesting additional Y-27632 targets. Over-expression of PRK-2, another Rho-associated kinase inhibited by Y-27632 [7], increases aggregation. Likewise, knockdown of PRK-2 suppresses polyglutamine aggregation, and partially diminishes the Y-27632 effect. Simultaneous knockdown of ROCK and PRK-2 eliminates the inhibitory effect of Y-27632. Taken together, our data imply that Y-27632 inhibits both kinases to suppress polyglutamine aggregation.

It remains a challenge to identify and validate cellular targets that underlie the effects of bioactive compounds. Yet this could hold the key to the development of more effective therapies. Exploiting the fact that ROCK and PRK-2 are known targets of Y-27632, we validated their importance in regulating polyglutamine aggregation. Effects of siRNA vs. each kinase were not additive. This could be due to complex compensatory effects that occur in the context of chronic depletion of each protein. Likewise, the quantitative difference between pharmacologic inhibition vs. genetic knockdown could be due to the distinct mechanisms of these two approaches. Indeed, this is consistent with the well-described discrepancies between genetic knockdown and pharmacologic kinase inhibition, which can even be paradoxical within a complex intracellular regulatory milieu [17]. Our data overall strongly suggest that simultaneous ROCK and PRK-2 inhibition mediates the intracellular anti-aggregation effect of Y-27632. More potent inhibitors of these kinases together might provide effective treatment for HD and SMBA.

In contrast to PRK-2, for which there is little pre-clinical data, ROCK is a promising therapeutic target. It has been implicated in multiple disease models, including hypertension, vasospasm, renal and pulmonary fibrosis (reviewed in [18]). In the CNS, ROCK is involved in neurite retraction [19–22], and its inhibition by Y-27632 and HA-1077 promotes axon regrowth after spinal cord injury in experimental animals [23–26]. Additionally, inhibition of RhoA/ROCK signaling by Y-27632 reduces the expression of amyloid-β peptide in mouse models of Alzheimer disease [27]. Despite its lack of selectivity for ROCK [7], HA-1077 is currently approved for clinical use in Japan in the treatment of primary pulmonary hypertension, and has shown efficacy in preventing vasospasm after subarachnoid hemorrhage in animal models and humans [28,29]. Its IC_{50} in vitro against PRK-2 is only two-fold higher than that against ROCK [7], and its efficacy in vivo could result in part from inhibition of PRK-2. However, due to reportedly poor CNS penetration [6], in its current form it is not optimal for treating neurodegenerative diseases such as HD.

The downstream mechanism by which ROCK and PRK-2 inhibition affects polyglutamine aggregation is unknown, and is the subject of active investigation. Both kinases are effectors of small Rho GTPases, and regulate actin cytoskeleton rearrangement [4,5,9,30]. Thus, the actin cytoskeleton might somehow play a role in the regulation of polyglutamine aggregation. Indeed, recent work by others has implicated actin regulatory factors such as Arp2 and N-WASP as modulators of polyglutamine aggregation in yeast and mammalian cells [31]. Thus, further study of this pathway will yield important insights about cellular mechanisms that inhibit polyglutamine protein misfolding and toxicity.

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References

[1] The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72, 971–983.

[2] La Spada, A.R., Wilson, E.M., Lubahn, D.B., Harding, A.E. and Fischbeck, K.H. (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352, 77–79.

[3] Pollitt, S.K., Pallos, J., Shao, J., Desai, U.A., Ma, A.A., Thompson, L.M., Marsh, J.L. and Diamond, M.I. (2003) A rapid cellular FRET assay of polyglutamine aggregation identifies a novel inhibitor. Neuron 40, 685–694.

[4] Riento, K. and Ridley, A.J. (2003) Rocks: multifunctional kinases in cell behaviour. Nat. Rev. Mol. Cell Biol. 4, 446–456.

[5] Amano, M., Fukata, Y. and Kaibuchi, K. (2000) Regulation and functions of Rho-associated kinase. Exp. Cell Res. 261, 44–51.

[6] Mueller, B.K., Mack, H. and Teusch, N. (2005) Rho kinase, a promising drug target for neurological disorders. Nat. Rev. Drug Discov. 4, 387–398.

[7] Davies, S.P., Reddy, H., Caivano, M. and Cohen, P. (2000) Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem. J. 351, 95–105.

[8] Vincent, S. and Settleman, J. (1997) The PRK2 kinase is a potential effector target of both Rho and Rac GTPases and regulates actin cytoskeletal organization. Mol. Cell Biol. 17, 2247–2256.

[9] Mukai, H. (2003) The structure and function of PKN, a protein kinase having a catalytic domain homologous to that of PKC. J. Biochem. 133, 17–27.

[10] Ishizaki, T., Naito, M., Fujisawa, K., Maekawa, M., Watanabe, N., Saito, Y. and Narumiya, S. (1997) p160ROCK, a Rho-associated coiled-coil forming protein kinase, works downstream of Rho and induces focal adhesions. FEBS Lett. 404, 118–124.

[11] Leung, T., Chen, X.Q., Manser, E. and Lim, L. (1996) The p160 RhoA-binding kinase ROK alpha is a member of a kinase family and is involved in the reorganization of the cytoskeleton. Mol. Cell Biol. 16, 5313–5327.

[12] Desai, U.A., Pallos, J., Ma, A.A., Stockwell, B.R., Thompson, L.M., Marsh, J.L. and Diamond, M.I. (2006) Biologically active molecules that reduce polyglutamine aggregation and toxicity. Hum. Mol. Genet. 15, 2114–2124.

[13] Sasaki, Y., Suzuki, M. and Hidaka, H. (2002) The novel and specific Rho-kinase inhibitor (*S*)-(+)-2-methyl-1-[(4-methyl-5-isoquinoline)sulfonyl]-homopiperazine as a probing molecule for Rho-kinase-involved pathway. Pharmacol. Ther. 93, 225–232.

[14] Ikenoya, M., Hidaka, H., Hosoya, T., Suzuki, M., Yamamoto, N. and Sasaki, Y. (2002) Inhibition of rho-kinase-induced myristoylated alanine-rich C kinase substrate (MARCKS) phosphorylation in human neuronal cells by H-1152, a novel and specific Rho-kinase inhibitor. J. Neurochem. 81, 9–16.

[15] Amano, M., Chihara, K., Nakamura, N., Kaneko, T., Matsuura, Y. and Kaibuchi, K. (1999) The COOH terminus of Rho-kinase negatively regulates rho-kinase activity. J. Biol. Chem. 274, 32418–32424.

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[16] Nakagawa, O., Fujisawa, K., Ishizaki, T., Saito, Y., Nakao, K. and Narumiya, S. (1996) ROCK-I and ROCK-II, two isoforms of Rho-associated coiled-coil forming protein serine/threonine kinase in mice. FEBS Lett. 392, 189–193.
[17] Weiss, W.A., Taylor, S.S. and Shokat, K.M. (2007) Recognizing and exploiting differences between RNAi and small-molecule inhibitors. Nat. Chem. Biol. 3, 739–744.
[18] Wettschureck, N. and Offermanns, S. (2002) Rho/Rho-kinase mediated signaling in physiology and pathophysiology. J. Mol. Med. 80, 629–638.
[19] Bito, H. et al. (2000) A critical role for a Rho-associated kinase, p160ROCK, in determining axon outgrowth in mammalian CNS neurons. Neuron 26, 431–441.
[20] Katoh, H., Aoki, J., Ichikawa, A. and Negishi, M. (1998) p160 RhoA-binding kinase ROKalpha induces neurite retraction. J. Biol. Chem. 273, 2489–2492.
[21] Hirose, M. et al. (1998) Molecular dissection of the Rho-associated protein kinase (p160ROCK)-regulated neurite remodeling in neuroblastoma N1E-115 cells. J. Cell Biol. 141, 1625–1636.
[22] Arimura, N. et al. (2000) Phosphorylation of collapsin response mediator protein-2 by Rho-kinase. Evidence for two separate signaling pathways for growth cone collapse. J. Biol. Chem. 275, 23973–23980.
[23] Hara, M., Takayasu, M., Watanabe, K., Noda, A., Takagi, T., Suzuki, Y. and Yoshida, J. (2000) Protein kinase inhibition by fasudil hydrochloride promotes neurological recovery after spinal cord injury in rats. J. Neurosurg. 93, 94–101.
[24] Dergham, P., Ellezam, B., Essagian, C., Avedissian, H., Lubell, W.D. and McKerracher, L. (2002) Rho signaling pathway targeted to promote spinal cord repair. J. Neurosci. 22, 6570–6577.
[25] Fournier, A.E., Takizawa, B.T. and Strittmatter, S.M. (2003) Rho kinase inhibition enhances axonal regeneration in the injured CNS. J. Neurosci. 23, 1416–1423.
[26] Tanaka, H., Yamashita, T., Yachi, K., Fujiwara, T., Yoshikawa, H. and Tohyama, M. (2004) Cytoplasmic p21(Cip1/WAF1) enhances axonal regeneration and functional recovery after spinal cord injury in rats. Neuroscience 127, 155–164.
[27] Zhou, Y. et al. (2003) Nonsteroidal anti-inflammatory drugs can lower amyloidogenic Abeta42 by inhibiting Rho. Science 302, 1215–1217.
[28] Takanashi, Y., Ishida, T., Meguro, T., Kiwada, H., Zhang, J.H. and Yamamoto, I. (2001) Efficacy of intrathecal liposomal fasudil for experimental cerebral vasospasm after subarachnoid hemorrhage. Neurosurgery 48, 894–900 (discussion 900-1).
[29] Tachibana, E., Harada, T., Shibuya, M., Saito, K., Takayasu, M., Suzuki, Y. and Yoshida, J. (1999) Intra-arterial infusion of fasudil hydrochloride for treating vasospasm following subarachnoid haemorrhage. Acta Neurochir. (Wien) 141, 13–19.
[30] Fukata, Y., Amano, M. and Kaibuchi, K. (2001) Rho–Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. Trends Pharmacol. Sci. 22, 32–39.
[31] Meriin, A.B., Zhang, X., Alexandrov, I.M., Salnikova, A.B., Ter-Avanesian, M.D., Chernoff, Y.O. and Sherman, M.Y. (2007) Endocytosis machinery is involved in aggregation of proteins with expanded polyglutamine domains. Faseb J. 21, 1915–1925.

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