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Rapid Communication

Mutant huntingtin replaces Gab1 and interacts with C-terminal SH3 domain of growth factor receptor binding protein 2 (Grb2)

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ABSTRACT

Huntington's disease (HD) is caused due to expansion of CAG repeats in the gene huntingtin (Htt). Adaptor protein Grb2, involved in Ras-MAP kinase pathway, is a known interactor of Htt. Mutant Htt–Grb2 interaction reduces Ras-MAPK signaling in HD models. In normal cells Grb2 forms Grb2–Sos1–Gab1 complex through its N-SH3 and C-SH3 domains respectively, essential for sustained activation of Ras. We found that C-SH3 of Grb2 mediates the interaction with mutant Htt and this interaction being stronger could replace Gab1, with mutant Htt becoming the preferred partner. This would have immense effect on downstream signaling events.

Huntington's disease (HD), an autosomal dominant neurodegenerative disorder, is caused due to expansion of CAG repeats (coding for glutamine) in the gene huntingtin (Htt) (The Huntington's Disease Collaborative Research Group, 1993). Over the years, various cellular processes like excitotoxicity, oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, axonal transport, ubiquitin proteasome pathway, autophagy, transcriptional deregulation and apoptosis have been implicated in HD (Ross and Tabrizi, 2011; Seredenina and Luthi-Carter, 2012). Disease causing mutations in Htt often recruit many of its interacting partners of the mutant protein leading to the biological consequences of the disease (Basu et al., 2013).

Growth factor receptor binding protein 2 (Grb2) is a well-known adaptor protein involved in Ras-MAP kinase pathway. Grb2 signaling has been implicated in many human malignancies (Cheng et al., 1998; Dharmawardana et al., 2006; Lowenstein et al., 1992). Apart from its several signaling role, Grb2 is also known to act as a scavenger molecule in Alzheimer's disease like condition (Raychaudhuri and Mukhopadhyay, 2010).

The adaptor protein Grb2 is made up of a SH2 domain flanked by two SH3 domains. Through the SH2 domain Grb2 interacts with the receptor tyrosine kinases (RTKs) and non-RTKs having phospho tyrosine motifs (Dharmawardana et al., 2006; Lowenstein et al., 1992; Rozakis-Adcock et al., 1992, 1993). The N-terminal SH3 (N-SH3) domain of Grb2 binds to SOS, Cbl, HYK1 and other proteins having the consensus proline rich motif (P-X-X-P-X-R) (Chardin et al., 1993; Giubellino and Arany, 2010; Lewitzky et al., 2001; Li et al., 1993; Odai et al., 1995; Schaeper et al., 2000; Seedorf et al., 1994; Vidal et al., 1998). However, a lack of consensus motif for the C-terminal SH3 (C-SH3) domain compromises its binding specificity and it has been reported that Grb2 C-SH3 can bind to peptides which even lack the core P-X-X-P sequence (Lewitzky et al., 2001), typically required by most SH3 domains. While Grb2 binds to the RTK's via its SH2 domain, the SH3 domains remain available for binding a plethora of proline rich motif containing proteins to trigger different signaling (McDonald et al., 2010). The most common and well studied of these partners is Sos1 (Chardin et al., 1993; Li et al., 1993; McDonald et al., 2010; Schaeper et al., 2000), which upon recruitment to the inner membrane surface, facilitates GDP–GTP exchange within the membrane bound Ras GTPase and thereby turns on MAPK signaling cascade, crucial for cellular growth and proliferation. Further, the recruitment of Gab1 provides docking platforms for the Shp2 tyrosine phosphatase and the PI3K lipid kinase, which account for further amplification of Ras activity. For sustained activation of Ras, both Sos1 dependent and Gab1 dependent pathways (Araki et al., 2003; Cunnick et al., 2002; Gu and Neel, 2003; McDonald et al., 2010) have been implicated.

Abbreviations: HD, Huntington's disease; Htt, huntingtin; Grb2, growth factor receptor binding protein 2; Sos1, Son of Sevenless homolog 1; Gab1, Grb2 associated binding protein 1; SH2, Src homology 2; SH3, Src homology 3; N-SH3, N-terminal SH3 domain; C-SH3, C-terminal SH3 domain; RTK, receptor tyrosine kinase; PRD, proline rich domain.

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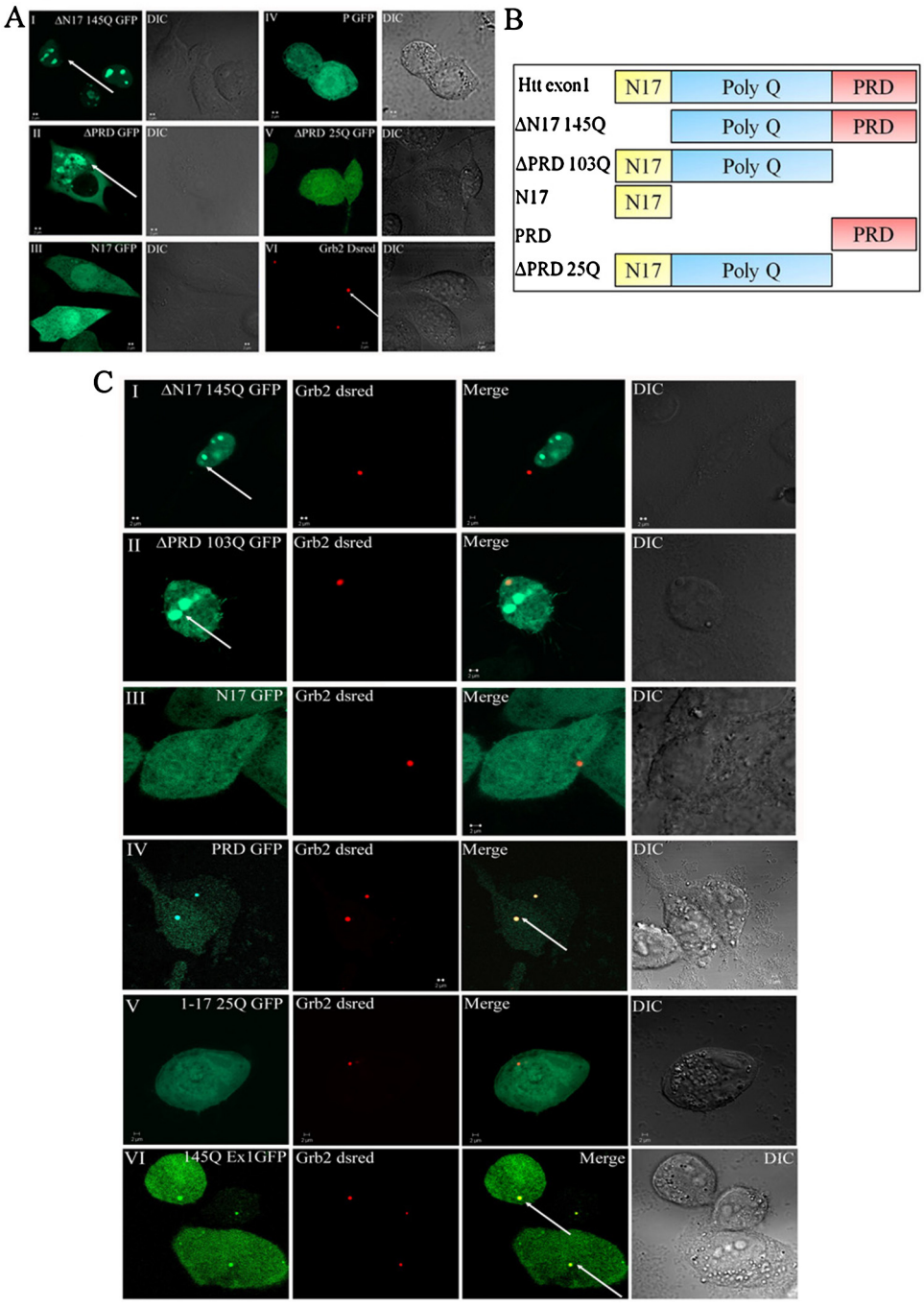

Fig. 1. (A) Representative confocal microscopy images of Htt deletion mutants cloned in GFP. Different clones are (I) ΔN17-145Q, (II) ΔPRD-145Q, (III) 1–17, (IV) P, (V) 1–17 25Q GFP, (VI) Grb2 Dsred in Neuro2A cell. Arrows show the aggregates of Htt GFP clones and vesicular structures of Grb2 Dsred. (B) Domain organization of Htt exon1 and the construction of deletion mutant clones. (C) Confocal images of co-transfected cells with Htt clones in A (I–V) and Grb2 Dsred. VI representative confocal image of neuro2A cell co-transfected with Htt 145Q exon1 GFP and Grb2 Dsred. Arrows show the colocalized structures

Grb2 is a known interactor of Htt and this interaction is known to be regulated by epidermal growth factor receptor (EGFR) activation (Liu et al., 1997). It is also reported that polyQ expansion leads to alteration in EGFR related trafficking (Lievens et al., 2005). Previously, we showed that Grb2 can interact with mutant Htt without any EGFR activation and that the interaction leads to downregulation of ERK signaling in striatal HD cell (Baksi et al., 2013). In this study, using biophysical tools, we tried to find out the mechanism of mutant Htt–Grb2 interaction leading to inhibition of ERK signaling in HD cell model.

Htt exon1 145Q and 23Q clones used in our study, were kindly gifted by Patrick Lajoie, Albert Einstein College of Medicine, USA (Lajoie and Snapp, 2010). ΔN17 GFP i.e. 145Q-PRD GFP: was cloned into GFPC1 vector sing Htt exon1 145Q as template and 5′-ACGCGTCGACGTATGCAGCAGCAGCAGCAGC-3′ forward, 5′-TGGGATCCGGTC GGTGCAGCGGCTCCTCAGC-3′ reverse primer Rest of the deletion mutant clones of Htt exon1 were kindly provided by Leslie Michels Thompson of University of California, Irvine (Rockabrand et al., 2007). The full length Grb2, N-SH3 and C-SH3 domains of Grb2 were cloned into pET28A,