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New insights into Dok-4 PTB domain structure and function

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ABSTRACT

The seven members of the Dok adapter protein family share a highly conserved phosphotyrosine-binding (PTB) domain. In the case of Dok-1, 2 and 3, the PTB domain binds to the lipid phosphatase Ship1, a key component of their inhibitory signaling mechanisms in immune cells. In contrast to most other Dok family members, Dok-4 is expressed widely but is poorly understood, largely because of limited knowledge of its partner molecules. We previously showed that, in contrast to the Dok-1 PTB domain (defined as aa 107–260), the homologous sequence in Dok-4 (aa 100–233) bound very poorly to Ret, a known Dok-4 partner. In the current study, we show that binding of Dok-4 to Ret requires residues C-terminal to the previously defined PTB domain boundaries (up to aa 246). These residues are predicted to form an extension in a critical C-terminal α-helix. We show that the Dok-4 PTB domain also binds the phosphorylated NPXY motifs in Ship1 but not Ship2. Finally, we found that a rare human single nucleotide polymorphism causing a R186H substitution in the PTB domain abolishes tyrosine phosphorylation of Dok-4 by Ret. In addition to providing a clearer understanding of Dok-4 PTB domain structure and function, our findings point to a potential mechanism for Dok-4 inhibitory signaling in T-cells and to the possibility of a rare Dok-4-related phenotype in humans.

1. Introduction

Like the related IRS (insulin receptor substrate) family, the seven members of the Dok family of adapter proteins are characterized structurally by a tandem of N-terminal pleckstrin homology (PH) and phosphotyrosine-binding (PTB) domains followed by more divergent C-terminal sequences [1]. The first three members of the Dok family, Dok-1, 2 and 3, form a separate subfamily (Dok-A) that displays extensive homology and possess expression patterns mostly restricted to immune cells including monocyte/macrophages [1,2] where they inhibit antigen receptor signaling through multiple mechanisms, including PTB domain-mediated binding of the lipid phosphatase Ship-1 [2]. In contrast, Dok-4, 5 and 6 form a distinct sub-family (Dok-B) whose function is poorly understood. While expression of Dok-5 and 6 is essentially restricted to neural tissues [3,4], Dok-4 is expressed widely with a preference for neural, endothelial and epithelial tissues [5]. Dok-4 is also expressed in circulating T-cells where it participates in inhibitory signaling through a poorly defined mechanism [6]. Dok-7 is a more distant member of the Dok family with expression restricted to muscles, where it serves a critical role in neuromuscular junction formation [7]. In summary, Dok-4 – and to a lesser extent Dok-1 – is the only ubiquitous member of the Dok family but it remains one of the least understood.

Although its exact biological function remains unclear, Dok-4 has been reported to *enhance* Ret-mediated neurite outgrowth through enhanced activation of Erk [3]. Conversely, Dok-4 has also been shown to *inhibit* activation of the Erk target transcription factor Elk-1 downstream of multiple tyrosine kinases. These seemingly contradictory roles highlight how adapter protein function is dependent on an array of partner molecules to accomplish their tasks, a feature that renders them highly dependent on cellular context. Unfortunately, only two partner proteins to have so far been rigorously identified for Dok-4. The best studied of these is the receptor tyrosine kinase (RTK) Ret [3]. This interaction involves phosphorylated tyrosine residue 1062 of Ret presumably binding to the Dok-4 PTB domain since Y1062 represents a known site of Dok-1 and Shc PTB binding [8–10]. Another RTK, Tie2, also presumably binds Dok-4 through a PTB/phospho-Tyr dependent mechanism, though this has yet to be directly studied [3].

Despite often limited primary amino acid (aa) sequence homology, PTB domains share a highly conserved 3D structure. This consists of seven anti-parallel β-strands forming a sandwich of two orthogonal β-sheets capped on one side by a C-terminal α-helix of varying length. In most cases, peptide binding is mediated by residues from the β5 strand and the C-terminal α-helix of the PTB domain [11]. Although many PTB domains can bind their target proteins independently of a phosphotyrosine residue, the most conserved consensus target sequence for PTB domains is a phosphorylated NPXY motif.

To date, the boundaries of Dok family PTB domains have been deduced from their sequence homology to IRS-1. The validity of

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this approach has been confirmed by crystallographic analysis of the Dok-1 PTB domain bound to a phosphorylated Ret peptide [11]. However, we have previously shown that a similarly defined Dok-4 PTB domain displayed poor interaction with a known Dok-4 partner, Ret [5]. Unfortunately, the 3D structure of the Dok-4 PTB domain has not yet been described and crystallographic analysis of the highly homologous Dok-5 PTB domain using a peptide corresponding to aa 127–233 has been attempted but not completed [12].

In the current study, we have explored in more detail the structure and function of the Dok-4 PTB domain.

2. Materials and methods

2.1. Plasmids and cDNAs

The expression vectors pME18S-Fyn Y528F, pCDNA3.1-Ret C634R, pCDNA3.1-Dok-4-Myc/His were described previously [5]. Additional Dok-4 constructs (point mutants G207A and R186H; deletion mutant Δ130–233) were generated by PCR, cloned in frame in pCDNA3.1-Myc/His as previously described [5] and fully sequenced. The pEGFP-N1-Ship1 wild type and Y918/1021F mutant as well as pCDNA-Ship1 constructs were obtained from Dr. Gerry Krystal. The c-Abl cDNA was obtained from Addgene (pCX-EGFP-Abl) and subcloned into the pcDNA3.1 vector. The HA-Ship2 expression construct was obtained from Dr. Christina Mitchell.

2.2. Cells

293HEK and COS-1 cells were cultured in DMEM-high glucose containing pyridoxine–HCl and sodium pyruvate with 10% FBS.

2.3. Antibodies, immunoprecipitation (IP) and immunoblotting

Antibodies for Ret (C-19), Ship1 (P1C1), Myc (9E10), Fyn (Fyn3), GST (B-14), Abl (K-12) were from Santa Cruz. Anti-phosphotyrosine (4G10) was from Millipore. Dok-4 antisera were previously described [5]. Horseradish peroxidase-coupled secondary antibodies were from Jackson Immunoresearch. Cell lysis, IP and immunoblotting were performed according to standard protocol as detailed previously [5].

2.4. Yeast two-hybrid (Y2H) screening

Y2H screening was performed by Hybrigenics (Paris, France). A LexA-Dok-4 chimera was constructed by cloning full-length mouse Dok-4 sequence in frame with the LexA DNA-binding domain in vector pB27. To allow detection of phosphotyrosine-dependent interactions, a yeast strain expressing mammalian Lyn tyrosine kinase was used. After yeast transformation with pB27-Dok-4, titration with 3-aminotriazole (3-AT) was performed to minimize background transactivation. Yeast were then transformed with a mouse kidney library (cloned in the vector pP6) and screened for growth on His- plates with 20 mM of 3-AT.

2.5. Glutathione S-transferase (GST) pulldown assays

GST and GST-Dok-4 fusion proteins were created in the pGEX-2T vector (GE Healthcare) using the BamH1 and EcoRI cloning sites as described previously [5].GST-Dok-4(100–233) was described previously [5]. GST-Dok-4 (100–325) and GST-Dok-4(130–246) were generated by PCR and fully verified by sequencing. Recombinant proteins expression and pull downs assays were performed according to standard protocol as previously described [5]. Bead volume was identical for all pull-down conditions and comparable loading of GST fusion proteins was verified on blotted membranes by either Ponceau stain or anti-GST immunoblotting.

3. Results

3.1. Redefining the boundaries of the Dok-4 PTB domain

Ret, the receptor for GDNF, is one of only two confirmed partners of Dok-4, the other being Tie2, the receptor for angiopoietin [3]. Surprisingly, we had previously found that, compared to the Dok-1 PTB domain, the Dok-4 PTB domain bound Ret very poorly in GST pull-down assays [5]. We wondered if delineation of the Dok-4 PTB domain according to homology with Dok-1 and IRS-1 (i.e. aa 100–233) might have unknowingly excluded key structural elements. Indeed, a recombinant Dok-4 construct containing aa 100–325, which comprises the entire C-terminal region of Dok-4, was able to bind Ret much more efficiently in GST pull-down assays, whereas a construct limited to aa 100–233 did so only very weakly (Fig. 1A). This suggested that residues located C-terminally to aa 233 were required for proper PTB function. To clarify this, we analyzed the sequences of Dok-1 and Dok-4 with the 2D prediction algorithm PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) [13]. PSIPRED accurately predicted all of the secondary structures of the Dok-1 PTB domain defined crystallography [14] including the C-terminal α helix (α2) (Fig. 1B and data not shown) with the exception of the β5 strand. PSIPRED also predicted a similar arrangement of β-strands and α-helices for the Dok-4 PTB domain with boundaries matching those predicted by alignment with the resolved Dok-1 PTB domain [14]. However, it predicted that the α2 helix of the Dok-4 PTB domain extended to aa 246, 13 aa beyond the boundary predicted by homology to Dok-1 (Fig. 1C). Interestingly, while these 13 aa (i.e. aa 234–246) are not conserved in Dok-1, they are conserved in Dok-6 and to a lesser extent in Dok-5, where PSIPRED also predicted them to form an extended α-helix (data not shown). This suggested that, unlike Dok-1 and IRS-1, the PTB domain of Dok-4 (and perhaps other Dok-B family members) possesses an extended C-terminal α-helix, similar to that found in more distant PTB domains such as Shc and Dab1 [11,15]. Given the typical involvement of β5-strands and C-terminal α-helices of PTB domains in peptide binding [15], the integrity of this extended α-helix might be critical for binding of Dok-4 to its target proteins. To explore this possibility more directly, we created a new GST-Dok-4 PTB construct that comprised aa 131–246 of Dok-4 (including the putative extended α-helix) instead of our previously used PTB construct comprising aa 100–233 [5]. Amino acid 131 was used as the N-terminal boundary of this new construct based on homology to the resolved crystallographic structure of the Dok-1 PTB domain [14] as well as on PSIPRED analysis of Dok-4 PTB, which both pointed to aa 138 as the start of the first β-strand (data not shown). The ability of the GST-Dok-4 constructs to bind activated Ret (C634R) was then compared in pull-down assays. As shown in Fig. 1D, whereas the GST-Dok-4(100–233) fusion protein bound barely more Ret than did GST alone, the GST-Dok-4(131–246) chimera could bind Ret more strongly, similar to the larger GST-D4(100–325) chimera. These results confirmed that the Dok-4 PTB domain is contained within aa 131–246 and that the C-terminal extension of its boundary is necessary for binding to one of its target proteins.

3.2. Dok-4 interacts with Ship1 but not Ship2

In order to identify novel phosphotyrosine-dependent interactions involving Dok-4, we performed yeast two-hybrid (Y2H) screen using full-length Dok-4 as bait with co-expression of Lyn tyrosine kinase in a mouse kidney library (see Section 2). A total

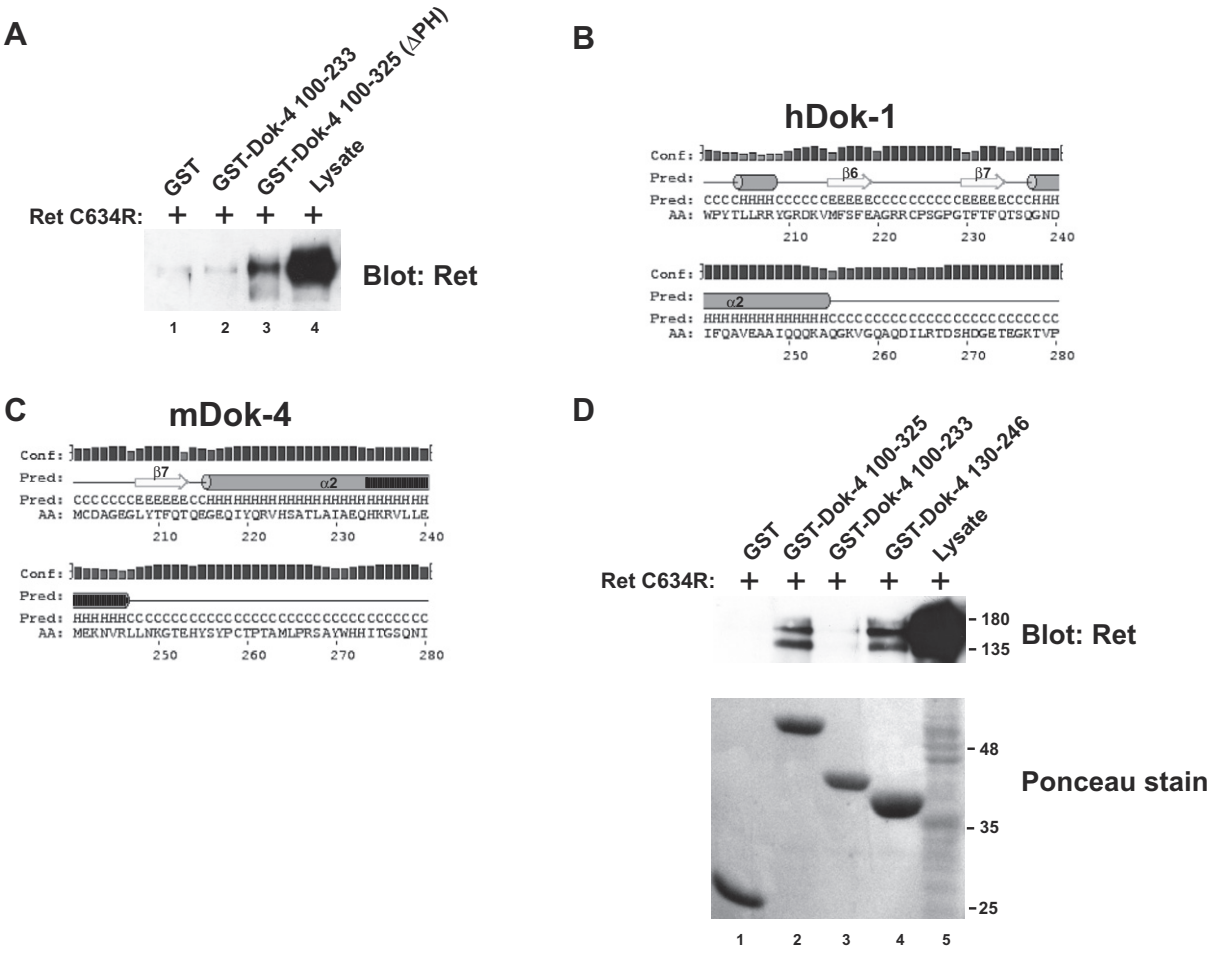

Fig. 1. Redefining the boundaries of the Dok-4 PTB domain. A: The constitutively activated Ret C634R mutant was transiently expressed in COS-1 cells and lysates were subjected to pull-down assay with recombinant GST, GST-Dok-4(100–233), and GST-Dok-4(100–325), followed by anti-Ret immunoblotting. A sample of lysate was also loaded as positive control (lane 4). B–C: The aa sequence of Dok-1 (B) and Dok-4 (C) was analyzed for secondary structure prediction using the PSIPRED algorithm. Open arrows indicate β-strands whereas gray cylinders indicate α-helices and are numbered according to their position within the PTB domain. The shaded area within the Dok-4 α2 helix indicates aa not previously assigned to the PTB domain. D: GST pull-down assay performed as in A but comparing GST-Dok-4(100–233) (lane 3) with GST-Dok-4(130–246) (lane 4) and GST-Dok-4(100–325) (lane 2) for their ability to bind activated Ret.

of 79.5 million independent transformants were screened. DNA was extracted and sequenced from 137 positive clones. Two of those clones corresponded to the lipid phosphatase Ship1 and encoded for its C-terminal portion (aa 723–1053) (Fig. 2A). This sequence contained the extreme C-terminal end of the phosphatase domain, most of the proline rich region as well as the two NPXY motifs centered on Y918 and Y1021, both known to bind Dok-1, 2, and 3 [16]. This interaction was validated in 293HEK cells by co-IP of full-length Dok-4 with Ship1 in the presence of a constitutively active form of the Src family kinase, FynY528F (Fig. 2B). This was further validated by pull-down assays with glutathione S-transferase (GST) fusion proteins. As shown in Fig. 2C, GST-Dok-4(130–246) was able to pull down wild-type Ship1 and this interaction was increased in the presence of co-expressed Fyn. Moreover, mutation of the two NPXY tyrosine residues in Ship1 to phenylalanine (Y918/1021F) abolished binding of GST-Dok-4 even in the presence of co-expressed Fyn (Fig. 2D). These data suggest that, like Dok-A family members, Dok-4 can interact with Ship1 through a prototypical PTB-mediated interaction involving NPXpY motifs.

Although Dok-4 is expressed widely, including in a subset of T-cells [6,17], Ship1 is expressed in a more restricted pattern, mostly in immune cells [16]. Therefore, we wondered if Dok-4 might also interact with the more ubiquitous Ship family member, Ship2, which also contains a single C-terminal NPXY motif. However, in contrast to Ship1, Ship2 was not pulled down by GST-Dok-4(131–246) (Fig. 2E).

3.3. Ship1 increases Abl-mediated phosphorylation of Dok-4

Dok-4 contains multiple tyrosine residues that can be phosphorylated by upstream kinases. We and others have previously shown that Dok-4 can be phosphorylated downstream of both receptor tyrosine kinases, such as Ret, Tie2 [3,5] as well as several non-receptor tyrosine kinases such as, Src, Fyn and Jak2 [5]. The ubiquitously expressed c-Abl tyrosine kinase is uses Dok-1 an upstream kinase for Dok-A family members and it has been shown to exist in Dok-1/Ship1 complexes [18]. We therefore wondered if Dok-4 could be phosphorylated by Abl and if Ship1 could facilitate this process. Following transient transfection in 293HEK cells, immunoprecipitated Dok-4 was phosphorylated in the presence of c-Abl (Fig. 3, lane 3), but this was indeed markedly increased in the presence of co-expressed Ship1 (lane 5), suggesting that Ship1 might facilitate coupling of Abl to its substrate Dok-4.

3.4. A rare human SNP of Dok-4 is associated with altered PTB function

Single nucleotide polymorphisms (SNPs) are present within the human genome and have been estimated to account for large

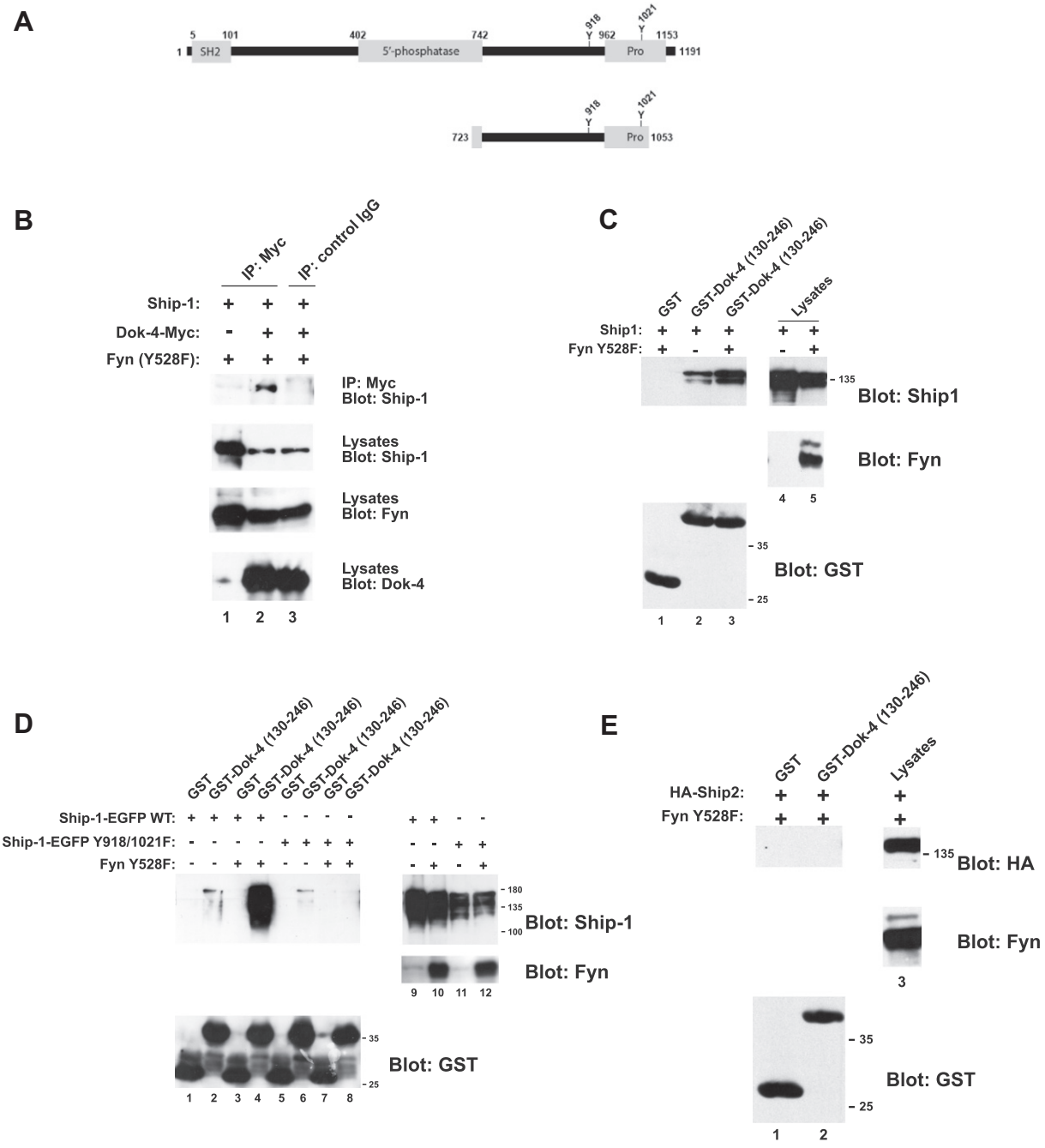

Fig. 2. Dok-4 interacts with Ship1 but not Ship2. A: Schematic representation of mouse Ship1 and C-terminal Ship1 sequence (aa 723–1053) obtained as Dok-4 partner in a yeast two-hybrid screen. B: Ship1 and Fyn Y528F were transiently transfected in 293HEK cells with (lanes 2–3) or without (lane 1) Dok-4-Myc. IP with anti-Myc antibody (lanes 1–2) or control mouse IgG (lane 3) was performed, followed by anti Ship1 immunoblotting. C: GST-Dok-4 pull-down assay was performed as in Fig. 1 but using Ship1 as the target for binding. D: GST pull-down experiment was performed as above, this time comparing the ability of GST-Dok-4 chimeras to pull-down EGFP-tagged constructs of either wild type (WT) Ship1 or Y918/1021F mutant Ship1. E: HA-tagged Ship2 was transiently overexpressed in COS-1 cells and lysates were subjected to pull-down assay with GST or GST-Dok-4(130–246) followed by anti-HA immunoblotting. Eluates (lanes 1–2) and a lysate sample (lane 3) were probed for expression of HA-Ship2 (top panel).

percentage of genetic variability within the population. While the vast majority of SNPs involve non coding sequences and are functionally silent, a small number of non-synonymous SNPs result single aa changes. Several rare non-synonymous Dok-4 SNPs appear to exist in humans based on information contained in the dbSNP, 1000 Genomes and Exome Sequencing Project databases (data not shown). One of these SNPs (accession number rs150421010) encodes for substitution of arginine 186 by histidine (R186H), which according to sequence alignment corresponds to a critical phosphotyrosine binding residue of the PTB domain (corresponding to Arg 207 of Dok-1) [14]. To clarify this, we generated a mouse Dok-4 R186H mutant, and examined its ability to undergo tyrosine phosphorylation by Ret, a PTB-mediated event (Fig. 4). As a control for inactivation of PTB function, we also generated a G207A Dok-4 mutant, which mimics a missense mutation in the Dok-7 PTB domain (G180A) responsible for cases of congenital myasthenic syndrome [19,20], and which represents a universally conserved glycine residue in Dok/IRS PTB domains [14]. Whereas Dok-4 WT was highly phosphorylated in the presence of activated Ret (lane 2), both the G207A and R186H mutants (lanes 3 and 4) were severely impaired in their phosphorylation levels despite comparable expression levels. The Δ130–233 deletion mutant of Dok-4, which

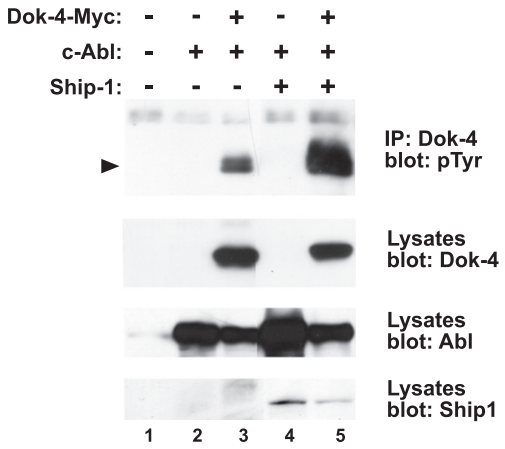

Fig. 3. Ship1 increases Abl-mediated phoshorylation of Dok-4. 293HEK cells were transiently co-transfected with Dok-4-Myc, c-Abl tyrosine kinase and Ship1, either alone or in combination. After anti-Dok-4 IP, anti-phosphotyrosine immunoblotting was performed (top panel). Dok-4 phosphorylation (arrowhead) was increased in the presence of co-expressed Ship1 (compare lanes 3 and 5).

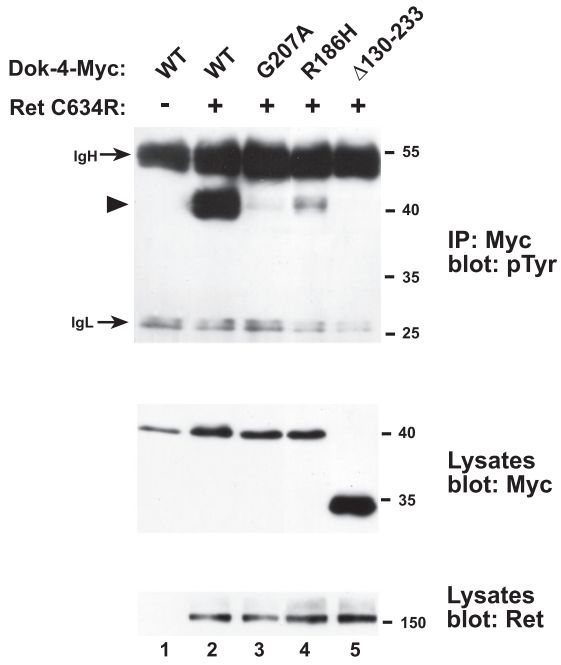

Fig. 4. Mutations disrupting Dok-4 PTB function. Myc-tagged Dok-4 constructs were co-transfected with activated Ret in 293HEK cells. G207A and R186H correspond to PTB point mutants. The Δ130–233 constructs represents an almost complete PTB domain deletion. Anti-Myc IP was followed by anti-phosphotyrosine immunoblotting (top panel). The position of phosphorylated Dok-4 is indicated by an arrowhead.

lacks most of the PTB domain, lacked any detectable tyrosine phosphorylation (lane 5). These data suggest that both Dok-4 point mutants result in severely impaired PTB function, thus preventing the necessary binding to Ret.

4. Discussion

Whether Dok-4 is primarily a positive or negative regulator of cell signaling likely depends on context-specific interactions and requires a detailed understanding of Dok-4 structure and interaction repertoire. In the current study we identified Ship1 as a novel partner of Dok-4 adapter protein (Fig. 2A and B). This association involves both the PTB domain of Dok-4 and the phosphorylated NPXY motifs of Ship1 (Fig. 2C and D). However, we found that proper Dok-4 PTB function required aa residues downstream of what homology alignments had previously identified as its C-terminal boundary. This explains the puzzlingly weak binding of the Dok-4 PTB domain to Ret observed in our previous studies. Based on the PSIPRED secondary structure prediction algorithm, these additional residues (aa 234–246) are likely to form an extension of the C-terminal α-helix of the Dok-4 PTB domain (Fig. 1C), which may render them critical for overall PTB domain integrity and/or peptide binding. Moreover, since these residues are partially conserved in Dok-5 and Dok-6, our findings imply that an extended C-terminal α-helix may be a shared feature of all three Dok-B family PTB domains. This could explain why a previous attempt at crystallization of the Dok-5 PTB with Ret peptide could not be brought to completion ([12] and Ning Shi, personal communication).

To date, PTB domains have been divided into three main categories according to structural features and preference for binding to phosphotyrosine or non-phosphorylated peptides: IRS-like, Shc-like, and Dab-like [11]. The presence of a truncated C-terminal α-helix is what distinguishes IRS-like PTB domains from the other two categories [11]. Therefore our current report highlights the unique place of Dok-4 within the IRS-like family of PTB domains.

Finally, the current study shows that a putative human SNP involving Dok-4 (R186H) results in disrupted PTB domain function, similar to a G207A substitution, which mimics a known pathogenic Dok-7 mutation (G180A) (Fig. 4).

Ship1 is only the third known partner of Dok-4. Dok1, Dok2 and Dok3 have previously been reported to interact with Ship1 [2,21,22]. Ship1 is a lipid phosphatase primarily expressed in cells of hematopoietic lineages [16] and its genetic ablation results in a lethal hematopoietic deregulation [23] and, in the case of B-cell specific targeting, autoimmunity [24]. In contrast, Dok-4 has been shown to exist primarily in neuronal, epithelial and endothelial cells, but significant expression has also been detected in the spleen and in circulating T lymphocytes [17] where its inhibitory role in T-cell receptor signaling has recently been shown [6]. Interestingly, the closely related Dok-5 has been shown to exist in activated but not resting T-cells [17]. Although we were able to identify the Dok-4/Ship1 interaction using a mouse kidney cDNA library, Ship1 protein expression appears to be low in renal epithelial cells. Indeed, we were unable to detect Ship1 protein in either kidney lysates or in cultured renal tubular MDCK II cells (data not shown) despite previous reports of its expression in this cell line [25,26]. Moreover, we found no evidence that the more ubiquitous lipid phosphatase Ship2 interacted with Dok-4 (Fig. 2E). Therefore, Dok-4/Ship1 interaction likely takes place mostly in subsets of immune cells including T lymphocytes where Dok-4 has been shown to regulate MAP kinase activation [6]. How Ship1 might negatively regulate MAP kinase signaling remains unclear, but the Dok-3/Ship1 interaction has previously been shown to inhibit c-Jun kinase activation in B lymphocytes through an as yet undefined mechanism [27]. While Dok-4 inhibitory signaling might be mediated by the phosphatase activity of Ship1, our results hint at a possible alternate mechanism involving enhanced tyrosine phosphorylation of Dok-4 by upstream kinases, presumably through a Ship1 scaffolding function (Fig. 3). Validation of this hypothesis will require further studies.

By redefining the boundaries of the Dok-4 PTB domain, the results presented herein set a more solid foundation on which to pursue the identification and characterization of a broader Dok-4 interaction repertoire and its possible pathophysiological relevance.

Acknowledgments

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References

[1] R. Mashima, Y. Hishida, T. Tezuka, Y. Yamanashi, The roles of Dok family adapters in immunoreceptor signaling, Immunol. Rev. 232 (2009) 273–285.
[2] S. Lemay, D. Davidson, S. Latour, A. Veillette, Dok-3, a novel adapter molecule involved in the negative regulation of immunoreceptor signaling, Mol. Cell. Biol. 20 (2000) 2743–2754.
[3] J. Grimm, M. Sachs, S. Britsch, S. Di Cesare, T. Schwarz-Romond, K. Alitalo, W. Birchmeier, Novel p62dok family members, dok-4 and dok-5, are substrates of the c-Ret receptor tyrosine kinase and mediate neuronal differentiation, J. Cell Biol. 154 (2001) 345–354.
[4] R.J. Crowder, H. Enomoto, M. Yang, E.M.J. Johnson, J. Milbrandt, Dok-6, a novel p62 Dok family member, promotes ret-mediated neurite outgrowth, J. Biol. Chem. 40 (2004) 42072–42081.
[5] A. Bedirian, C. Baldwin, J.-i. Abe, T. Takano, S. Lemay, Pleckstrin homology and phosphotyrosine-binding domain-dependent membrane association and tyrosine phosphorylation of Dok-4, an inhibitory adapter molecule expressed in epithelial cells, J. Biol. Chem. 279 (2004) 19335–19349.
[6] A. Gerard, M. Ghiotto, C. Fos, G. Guittard, D. Compagno, A. Galy, S. Lemay, D. Olive, J.A. Nunes, Dok-4 is a novel negative regulator of T cell activation, J. Immunol. 182 (2009) 7681–7689.
[7] K. Okada, A. Inoue, M. Okada, Y. Murata, S. Kakuta, T. Jigami, S. Kubo, H. Shiraishi, K. Eguchi, M. Motomura, T. Akiyama, Y. Iwakura, O. Higuchi, Y. Yamanashi, The muscle protein Dok-7 Is essential for neuromuscular synaptogenesis, Science 12 (2007) 1802–1805.
[8] N. Asai, H. Murakami, T. Iwashita, M. Takahashi, A mutation at tyrosine 1062 in MEN2A-Ret and MEN2B-Ret impairs their transforming activity and association with shc adaptor proteins, J. Biol. Chem. 271 (1996) 17644–17649.
[9] M.J. Lorenzo, G.D. Gish, C. Houghton, T.J. Stonehouse, T. Pawson, B.A. Ponder, D.P. Smith, RET alternate splicing influences the interaction of activated RET with the SH2 and PTB domains of Shc, and the SH2 domain of Grb2, Oncogene 14 (1997) 763–771.
[10] H. Murakami, Y. Yamamura, Y. Shimono, K. Kawai, K. Kurokawa, M. Takahashi, Role of Dok1 in cell signaling mediated by RET tyrosine kinase, J. Biol. Chem. 277 (2002) 32781–32790.
[11] M.T. Uhlik, B. Temple, S. Bencharit, A.J. Kimple, D.P. Siderovski, G.L. Johnson, Structural and evolutionary division of phosphotyrosine binding (PTB) domains, J. Mol. Biol. 345 (2005) 1–20.
[12] N. Shi, W. Zhou, K. Tang, Y. Gao, J. Jin, F. Gao, X. Peng, M. Bartlam, B. Qiang, J. Yuan, Z. Rao, Expression, crystallization and preliminary X-ray studies of the recombinant PTB domain of human dok-5 protein, Acta Crystallogr. D. Biol. Crystallogr. 58 (2002) 2170–2172.
[13] D.W.A. Buchan, S.M. Ward, A.E. Lobley, T.C.O. Nugent, K. Bryson, D.T. Jones, Protein annotation and modelling servers at University College London, Nucl. Acids Res. 38 (2010) W563–W568.
[14] N. Shi, S. Ye, M. Bartlam, M. Yang, J. Wu, Y. Liu, F. Sun, X. Han, X. Peng, B. Qiang, J. Yuan, Z. Rao, Structural basis for the specific recognition of RET by the Dok1 phosphotyrosine binding domain, J. Biol. Chem. 279 (2004) 4962–4969.
[15] J.D. Forman-Kay, T. Pawson, Diversity in protein recognition by PTB domains, Curr. Opin. Struct. Biol. 9 (1999) 690–695.
[16] L.R. Rohrschneider, J.F. Fuller, I. Wolf, Y. Liu, D.M. Lucas, Structure, function, and biology of SHIP proteins, Genes Dev. 14 (2000) 505–520.
[17] C. Favre, A. Gerard, E. Clauzier, P. Pontarotti, D. Olive, J.A. Nunes, DOK4 and DOK5: new Dok-related genes expressed in human T cells, Genes Immun. 4 (2003) 40–45.
[18] M. Sattler, S. Verma, Y.B. Pride, R. Salgia, L.R. Rohrschneider, J.D. Griffin, SHIP1, an SH2 domain containing polyinositol-5-phosphatase, regulates migration through two critical tyrosine residues and forms a novel signaling complex with DOK1 and CRKL, J. Biol. Chem. 276 (2001) 2451–2458.
[19] J.S. Muller, A. Herczegfalvi, J.J. Vilchez, J. Colomer, L.L. Bachinski, V. Mihaylova, M. Santos, U. Schara, M. Deschauer, M. Shevell, C. Poulin, A. Dias, A. Soudo, M. Hietala, T. Aarimaa, R. Krahe, V. Karcagi, A. Huebner, D. Beeson, A. Abicht, H. Lochmuller, Phenotypical spectrum of DOK7 mutations in congenital myasthenic syndromes, Brain 130 (2007) 1497–1506.
[20] D. Beeson, O. Higuchi, J. Palace, J. Cossins, H. Spearman, S. Maxwell, J. Newsom-Davis, G. Burke, P. Fawcett, M. Motomura, J.S. Muller, H. Lochmuller, C. Slater, A. Vincent, Y. Yamanashi, Dok-7 mutations underlie a neuromuscular junction synaptopathy, Science 313 (2006) 1975–1978.
[21] N.M. Dunant, D. Wisniewski, A. Strife, B. Clarkson, M.D. Resh, The phosphatidylinositol polyphosphate 5-phosphatase SHIP1 associates with the dok1 phosphoprotein in bcr-Abl transformed cells, Cell. Signaling 12 (2000) 317–326.
[22] I. Tamir, J.C. Stolpa, C.D. Helgason, K. Nakamura, P. Bruhns, M. Daeron, J.C. Cambier, The RasGAP-binding protein p62dok is a mediator of inhibitory FcgammaRIIB signals in B cells, Immunity 12 (2000) 347–358.
[23] C.D. Helgason, J.E. Damen, P. Rosten, R. Grewal, P. Sorensen, S.M. Chappel, A. Borowski, F. Jirik, G. Krystal, R.K. Humphries, Targeted disruption of SHIP leads to hemopoietic perturbations, lung pathology, and a shortened life span, Genes Dev. 12 (1998) 1610–1620.
[24] S.K. O'Neill, A. Getahun, S.B. Gauld, K.T. Merrell, I. Tamir, M.J. Smith, J.M. Dal Porto, Q.Z. Li, J.C. Cambier, Monophosphorylation of CD79a and CD79b ITAM motifs initiates a SHIP-1 phosphatase-mediated inhibitory signaling cascade required for B-Cell anergy, Immunity 35 (2011) 746–756.
[25] M. Stefan, A. Koch, A. Mancini, A. Mohr, K.M. Weidner, H. Niemann, T. Tamura, Src homology 2-containing inositol 5-phosphatase 1 binds to the multifunctional docking site of c-Met and potentiates hepatocyte growth factor-induced branching tubulogenesis, J. Biol. Chem. 276 (2001) 3017–3023.
[26] A. Mancini, A. Koch, R. Wilms, T. Tamura, The SH2-containing inositol 5-phosphatase (SHIP)-1 is implicated in the control of cell-cell junction and induces dissociation and dispersion of MDCK cells, Oncogene 21 (2002) 1477–1484.
[27] J.D. Robson, D. Davidson, A. Veillette, Inhibition of the Jun N-terminal protein kinase pathway by SHIP-1, a lipid phosphatase that interacts with the adaptor molecule Dok-3, Mol. Cell. Biol. 24 (2004) 2332–2343.