Introduction

Schistosomiasis, caused by members of the genus Schistosoma, afflicts more than 200 million people living in the endemic areas of 77 countries worldwide, representing a major health and economic burden in tropical and developing nations [1]. The treatment of schistosomiasis is largely based on the long-term application of the single available drug, praziquantel (PZQ). However, concerns were raised about the problem of drug resistance [2,3]. The advances of schistosome genomics have provided a valuable basis for dissecting the parasite biology and identifying novel drug targets against the parasite [4–6].

Protein-protein interactions (PPIs) mediate multiple biological processes and physiological functions in organisms, and offer a variety of opportunities for exploring potential drug targets [7]. The PSD-95/Dlg/ZO-1 (PDZ) domain is one of the most crucial modules for PPI, and is emergently recognized as a novel target of drug discovery [8–11]. PDZ domains consist of approximately 80–90 amino acids, that typically form a packed structure composed of six β-strands (β1-β6) and two α-helices (α1, α2) [12]. A
confirmed the ligand binding specificities, including the canonical peptide library and introducing point mutations, we revealed and prefer typical I C-terminal PDZ binding motifs (PBMs) [43].

including human Scribble (hScrib), have been investigated by specificity profiles for a number of PDZ domains of LAP proteins, important role in clustering synaptic vesicles at developing [21,34,35], regulating cell migration [36,37] and act as a tumor neurons, and T cells [30–33], mediating PCP signalling [25], and ZO-2 [26], as well as proteins from pathogenic viruses b of Scribble mediate interactions with a number of partners, such as to negatively regulate Akt signalling [19], while the PDZ domains membrane of epithelial cells [17,18], and can interact with the LRR domain is necessary for targeting to the basolateral internal motifs of their partner proteins [15,16].

Scribble is a member of the evolutionarily conserved LAP (LRR (leucine-rich repeats) and PDZ) protein family. The N-terminal LRR domain is necessary for targeting to the basolateral membrane of epithelial cells [17,18], and can interact with the PH domain leucine-rich-repe t protein phosphatase 1 (PHLPP1) to negatively regulate Akt signalling [19], while the PDZ domains of Scribble mediate interactions with a number of partners, such as β-PIX (PAK-interacting exchange factor β) [20], Vangl2, a core planar cell polarity (PCP) protein [21–23], NOS1AP [24], zyxin [25], and ZO-2 [26], as well as proteins from pathogenic viruses [27–29]. Scribble has been implicated to be involved in establishing and maintaining membrane polarity in epithelia, neurons, and T cells [30–33], mediating PCP signalling [21,34,35], regulating cell migration [36,37] and act as a tumor suppressor [38–41]. Also, it has been shown that Scribble plays an important role in clustering synaptic vesicles at developing synapses via interaction with β-catenin [42]. The binding specificity profiles for a number of PDZ domains of LAP proteins, including human Scribble (hScrib), have been investigated by screening phage display library, most of which were found to prefer typical I C-terminal PDZ binding motifs (PBMs) [43].

In this study, by yeast two-hybrid (Y2H) screening of a random peptide library and introducing point mutations, we revealed and confirmed the ligand binding specificities, including the canonical C-terminal and non-canonical internal binding specificities for all four PDZ domains of the S. japonicum cell polarity protein Scribble (SjScrib). The binding specificity profiles were compared with those of human Scrib ortholog. The potential ligands of SjScrib were further predicted based on the ligand binding specificities of the PDZ domains, which were validated in the Y2H system. This work will facilitate the identification of novel drug targets against schistosomal infection.

Methods

Ethical statement

All procedures carried out on animals within this study were conducted following animal husbandry guidelines of the Chinese Academy of Medical Sciences and with permission from the Experimental Animal Committee of Chinese Academy of Medical Sciences with the Ethical Clearance Number IPB-2011-6.

Parasites and animals

Schistosoma japonicum-infected Oncomelania hupensis were purchased from Jiangxi Institute of Parasitic Diseases, Nanchang, China. Cercariae were freshly shed from the infected snails. New Zealand White rabbits were percutaneously infected with ~1,000 S. japonicum cercariae. Hepatic schistosomula and adult worms were recovered from the rabbits by hepatic-portal perfusion at 2 and 6 weeks post-infection (p.i.), respectively. Male and female adult worms were manually separated with the aid of a light microscope. Eggs were isolated from the liver tissues of the infected rabbits at 6 weeks p.i. by the sieving and enzymatic digestion method [44].

Cloning of the S. japonicum Scribble gene

Two EST sequences (GenBank accession number AY809835 and AY815909), respectively encoding the N-terminal and C-terminal fragment of SjScrib, were identified and retrieved directly from NCBI using the S. mansoni Scribble cDNA sequence (GenBank accession number: XM_002581163) as a bait. Forward (5’-GGCTCTAC-TAATGTTCAAGTGTTTGCCAATTATAGG-3’) and reverse (5’-CCAGAAGTGCTCCCATATTTGCGTCATCG-3’) primers were designed based on these EST sequences and used to amplify the full-length ORF of SjScrib from adult worm pair cDNA templates with high fidelity Phusion DNA polymerase (New England Biolabs, NEB, UK). The resulting PCR fragment was cloned into T-vector and sequenced. The amino acid sequence of SjScrib (GenBank accession number: AHC92618) was deduced from the obtained cDNA sequence (GenBank accession number: KF730248) for further bioinformatic analysis.

Bioinformatical analysis of SjScrib

The amino acid sequences of the Scrib orthologs of S. mansoni (GenBank accession number: CCD76510), Drosophila melanogaster (GenBank accession number: NP_733154) and Homo sapiens (GenBank accession number: AAL38976) were retrieved from GenBank. The domain boundaries of SjScrib and hScrib were analyzed using the following public domain tools: the NCBI CDD (http://www.ncbi.nlm.nih.gov/cdd) [45], PFAM [46], and SMART [47]. Peptide sequences of all PDZ domains from SjScrib, SmScrib, and hScrib were aligned by ClustalX 2.0. The structure-based alignment of these PDZ domains was produced by ESPript [48]. The three-dimensional (3D) structures of SjScrib-PDZ1, SjScrib-PDZ2 and SjScrib-PDZ3 were predicted by the online service Phyre 2 [http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index] [49]. The 3D structure of SjScrib-PDZ4 was predicted based on the NMR structures of hScrib-PDZ1 (PDB code 1X5Q), hScrib-PDZ2 (PDB code 1WHA), and

Author Summary

Schistosomiasis japonica remains a major public health problem in China and Southeast Asia. The long-term of treatments with the only available drug, praziquantel, has raised the concerns about drug resistance. Protein-protein interactions (PPIs), for highly discriminating specificities, are thought to be the innovative targets for a generation of new drugs. The PDZ domain is one of the most important modules for PPIs. A number of compounds screened based on binding specificities of PDZ domains have shown their potential therapeutic power in several disease models with less side effects. Although domain loss events are widespread in S. japonicum, a panel of PDZ domains is conserved in this species. So far, however, little is known about ligand binding specificities and the molecular functions of parasite-derived PDZ domain-containing proteins. In this study, by yeast two-hybrid screening of a random library, we confirmed the ligand binding properties of a multiple PDZ domain-containing protein Scribble of S. japonicum for the first time. Divergent ligand specificities between the homologous PDZ domains of S. japonicum and human Scribble orthologs were revealed. Internal motif recognition and irregular ligand interaction models for the SjScrib-PDZ domains were identified. These results provide an important basis for the rational discovery of anti-schistosomal drugs.
hScrib-PDZ4 (PDB code 1UJU) using the program EasyModeller 4.0 [50]. The structural alignments of PDZ domains between SjScrib and hScrib were performed by using the Swiss PDB Viewer (SPDBV) [51]. The solution NMR structures of hScrib-PDZ1 (PDB code 1X5Q), hScrib-PDZ2 (PDB code 1WHA), and hScrib-PDZ4 (PDB code 1UJU) were used as templates for superposition of the predicted structures of SjScrib-PDZ1, SjScrib-PDZ2, and SjScrib-PDZ4, respectively. The superimposed structures were further refined with PyMOL Viewer program (DeLano Scientific, San Carlos, CA, http://www.pymol.org/). Free, open source Java software for visualizing ligand binding specificity profiles is available from http://badelab.org/Software/LOLA.

Transcriptional characterization of the SjScrib gene by quantitative RT-PCR

Total RNAs of S. japonicum at different developmental stages (cercariae, hepatic schistosomula, separated male and female adult worms, and eggs) were extracted using Trizol reagent (Invitrogen, CA, USA). The possible contaminating genomic DNA was completely removed from RNA samples with TURBO DNA-free kit (Ambion, CA, USA). RNA quantification and quality control was conducted by 1% agarose gel electrophoresis and the Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). For each sample, 1 μg total RNA was reverse transcribed into first-strand cDNA using SuperScript III Reverse Transcriptase Kit (Invitrogen). The synthesis procedure was performed as follows: 25°C for 5 min, 50°C for 1 h, 70°C for 15 min. Each PCR reaction contained 12.5 μl of 2×Brilliant II SYBR Green QPCR Master Mix (Agilent, USA), 1 μl diluted cDNA (20×), 1 μl of the forward and reverse primer pair (Table S1), and 10.5 μl of sterile water. The PCR program included 40 cycles with denaturation at 95°C for 30 s, followed by annealing and extension at 60°C for 1 min. Quantification of the transcriptional level of the SjScrib gene was performed by normalizing against the PSMD4 transcript (26S proteasome non-ATPase regulatory subunit 4, GenBank Accession Number: FN320595) [52,53] and applying the comparative 2–ΔΔCt method, according to the SDS 1.4 software.

Construction of bait plasmids and Y2H screening of a random peptide library

Primer pairs were designed based on the boundaries of PDZ domains (shown in Table S1). The DNA fragments encoding PDZ domains of SjScrib were respectively amplified from the adult worm cDNA templates using Phusion DNA polymerase. The PCR was performed with an initial denaturation at 1 min for 98°C, followed by thirty cycles: 98°C for 5 s, 50°C for 30 s and 72°C for 15 s. The final extension was 5 min at 72°C. The PCR products were digested with EcoRI and HindIII sites of the GAL4 BD vector, pBridge (Clontech). Each GAL4 BD-fusion bait plasmid was transformed into the yeast strain CG1945 using the lithium acetate procedure. The transformants were grown on SD/-Trp plates and lacZ assays were performed to make sure that the mutation was correctly introduced. Each of the mutated plasmids was co-transformed with the corresponding PDZ domain bait plasmids into yeast strain CG1945, respectively. The interactions between the mutants and the corresponding PDZ domain were validated by Y2H assays as mentioned above.

Confirmation of candidate SjScrib-ligand interactions

The GAL4 AD plasmids expressing the carboxyl-terminus of the candidate ligands of SjScrib-PDZ1 and SjScrib-PDZ4 were first constructed. The self primer template PCR reactions were performed to produce DNA templates encoding the 15 amino acids of extreme carboxyl-terminus of each ligand candidate (The primer pairs were listed in Table S1). The PCR procedure included thirty cycles as follows: 98°C for 5 s; 50°C for 25 s, and 72°C for 1 s, with a final extension at 72°C for 2 min. The resulting DNA fragments were recovered, and further digested with EcoR I and BamH I endonucleases, and cloned into the EcoR I/BamH I sites of the GAL4 AD vector, pBridge (Clontech). Each GAL4 BD-fusion bait plasmid was transformed into the yeast strain CG1945 using the lithium acetate procedure. The transformants were grown on SD/-Trp plates and lacZ assays were performed to examine self-activation. The transformants were further spread on SD/-His/-Trp plates with different concentration of 3-amino-1,2,4-triazole for leakage test. A random peptide library (constructed with Tsg509l-digested human genomic DNA fragments) [54] was screened following the MATCHMAKER Two-Hybrid System protocol (Clontech). The transformants were screened on SD/-Trp/-His/-Leu plates with different concentration of 3-amino-1,2,4-triazole (2.5 mM for the SjScrib-PDZ1, SjScrib-PDZ2, and SjScrib-PDZ4 bait transformants, and 7.5 mM for the SjScrib-PDZ3 bait transformant) and further verified by the improved lacZ assays. After rescue, the potential positive plasmids were isolated and retransformed into the yeast strain CG1945 containing corresponding bait plasmid. Only the clones that were positive for all the reporter assays and confirmed by at least two independent tests were selected for specific interactions and sequenced [53].

Prediction of native ligand candidates

The consensus C-terminal binding sequences for each SjScrib-PDZ domain were deduced from sequence alignments of positive clones from Y2H screening. The S. japonicum predicted peptide sequences were downloaded from SDSPB (http://lifecenter.sgst.cn/schistosoma/cn/schdownload.do). The Tailfit software [56] was used to search against the S. japonicum peptide dataset to retrieve the potential ligands whose carboxyl termini matched the consensus-binding sequences. These peptide sequences were then manually BLAST in NCBI to filter the truncated fragments lacking of the C-terminus. Further, the most promising candidates were selected based on biological information such as subcellular localization and potential molecular function, as well as the C-terminal homology between S. japonicum and S. mansoni orthologs [57].

Accession numbers

PDB: 1X5Q, PDB: 1WHA, PDB: 1UJU, GenBank: AY809835, GenBank: AY815909, GenBank: XM_002581163, GenBank: AHC92618, GenBank: KF730248, GenBank: CDD76510, GenBank: NP_733154, GenBank: AAL38976, GenBank: XP_002581546, GenBank: FN320595.
Results and Discussion

Molecular characteristics of *S. japonicum* Scribble

The *S. japonicum* Scribble gene encodes a protein of 1,486 amino acids, with a theoretical molecular weight of 168 kDa. Homology blast showed that *SjScrib* shares a relatively high degree of sequence homology with the orthologous *S. mansoni* scribble, SmScrib (GenBank accession number: CDD76510) (Figure 1A). A leucine-rich repeat (LRR) domain within the N-terminus of *SjScrib* is relatively conserved with orthology of *Drosophila* and *H. sapiens*, indicating that the function mediated by this domain may be evolutionarily conserved. Four PDZ domains are located in the relatively extreme C-terminal region of schistosome Scribs (Figure 1A). It is obvious that the carboxyl-terminal (CT) domain, which has previously been suggested not being essential for any aspect of Scrib localization or function [17], is missing in *SjScrib* and SmScrib when compared with DmScrib and hScrib (Figure 1A). Among the four PDZ domains of *SjScrib*, the third PDZ domain shares the highest degree of homology (60%) with hScrib-PDZ3 (Figure 1A). The peptide sequences of the *SjScrib* PDZ domains were aligned with homologous sequences of SmScrib and hScrib (Figure 1B). The secondary structures of the PDZ domains were determined using the NMRs structure of hScrib-PDZ1 (PDB codes 1X5Q) as a template. It is notable that the loop between β2 and β3 in the fourth PDZ domain of schistosome Scribs is longer than that of hScrib-PDZ4, which may increase the flexibility of this domain (Figure 1B). Totally, *SjScrib* and SmScrib possess the general primary characteristics as that of the homologous proteins of other organisms, indicating that they may play analogous roles in schistosome parasites. The Scribbles polarity module is composed of Scribble, lethal giant larva (Lgl) and discs large (Dlg), which are well conserved across species from worms and flies to mammals [41,58]. Recently, it has been shown that the *S. japonicum* Lgl protein is localized in the worm tegument and knockdown of this gene can significantly deform the surface structure of adult worm and impair egg hatching [59]. To some extent, these data may reveal the potential function of the Scribble polarity module in *S. japonicum*.

Homology modeling reveals a different degree of structural divergence between *SjScrib*-PDZs and *hScrib*-PDZs

To assess the structural divergence between *SjScrib* and hScrib PDZ domains, we further superimposed the structures of *SjScrib*-PDZs with three available NMR structures of hScrib PDZ domains. Although the primary sequence identity of the second PDZ domain between *SjScrib* and hScrib is higher than that of the first and fourth PDZ domains (Figure 1A), the structural similarity of the second PDZ domain between *SjScrib* and hScrib is the lowest among the three PDZ domains (Figure 2). The root mean square deviation (RMSD) value for the first, second, and fourth PDZ domain is 0.41 Å, 1.36 Å, and 0.64 Å, respectively, indicating that the ligand binding specificity for *SjScrib*-PDZs may be more likely to diverge from that of hScrib-PDZ2 than those of *SjScrib*-PDZ1 and *SjScrib*-PDZ4 from the corresponding hScrib PDZ domains, and the ligand binding specificity may be relatively conserved between *SjScrib*-PDZ1 and hScrib-PDZ1.

Transcriptional analysis of the *SjScrib* gene at different developmental stages of *S. japonicum*

QRT-PCR was performed to determine the transcriptional profiles of *SjScrib* at different developmental stages and between sexes of the parasite. As a result, we observed that the *SjScrib* gene was ubiquitously expressed during different developmental stages, but in a stage-biased pattern. The transcription levels of the *SjScrib* gene were similar in cercariae and schistosomula at a medium level. In adult worms, the transcriptional level was significantly higher in male than that in female worms. In the egg stage, the expression of *SjScrib* was relatively higher than that in any other stages detected (Figure 3). The data suggest that *SjScrib* is a necessity for all developmental stages of the parasite, particularly during the development of egg embryo. The reason that *SjScrib* was less transcribed in the female parasites is puzzling, but it could be postulated that in female adult worms, a relatively low number of cells need to maintain membrane polarity when compared to other developmental stages of the parasite.

Y2H screening against each of *SjScrib*-PDZ domain

To determine the binding properties of *SjScrib*-PDZs, an arbitrary peptide library was screened by Y2H assays with a similar approach for the determination of the binding specificity of *SjGIPC3*-PDZ [57]. For *SjScrib*-PDZ1, a total of 35 positive clones were obtained, which encodes 25 unique carboxy-termini. Among them, 16 belong to Class I PBMs, 6 belong to irregular Class I PBM (which can be viewed as a projection of an amino acid at C-terminus of regular PBMs), and 3 cannot be classified (Figure 4A). For *SjScrib*-PDZ2, only 2 positive clones were probed. One is a Class II PBM, and the other is an irregular Class II PBM (Figure 4B). For *SjScrib*-PDZ3, 37 positive clones which encode 30 unique carboxy-termini were obtained. Among them, 16 belong to Class I PBM, 2 belong to irregular Class I PBM, and 12 are irregular (Figure 4C). For *SjScrib*-PDZ4, 58 positive clones encoding 56 unique carboxy-termini were obtained. Among them, 53 belong to Class II PBM, 1 belongs to Class I PBM, and 2 are irregular (Figure 4D).

Determination of the specific binding sites within irregular ligands

For each PDZ domain of *SjScrib*, ligands with irregular C-termini were subjected to further analysis. Consequently, putative binding sites were inferred within the internal sequences of these ligands according to the pattern of regular C-terminal PBMs (Table 1, bold and underline characters). To confirm these putative binding motifs, point mutations were introduced into the key amino acid residues and those mutants were further validated in the Y2H system (Table 1).

For *SjScrib*-PDZ1, a core binding sequence was deduced from its canonical C-terminal PBMs, -E[T/S][S][L][I][F]*- (Figure 4A). A putative binding motif, [E][T/S][x][IF], was inferred for each irregular ligand. Substitution of the hydrophobic residue Ala for the polar residue Thr/Ser totally interrupted the interactions between *SjScrib*-PDZ1 and the irregular ligands. For *SjScrib*-PDZ2, only two unique sequences were obtained, probably due to the stringent recognition at particular binding sites. We hypothesized that the typical II C-terminal PBMs, -WELSI*- and the atypical II C-terminal PBMs, -FELMLE*- with one amino acid Glu projected at the C-terminus of the binding site, are likely to be the core binding motifs for *SjScrib*-PDZ2. This hypothesis was confirmed by a series of point mutations introduced into each amino acid of the putative PBMs (Table 1). For *SjScrib*-PDZ3, a putative core binding sequence, [-E][T][Φ][Φ] was deduced based on its canonical typical I C-terminal PBMs (Figure 4C). Potential PBMs was postulated for each irregular ligand of *SjScrib*-PDZ3. Substitutions the conserved polar residue Thr with the hydrophobic residue Ala led to the failure of the interactions between the PDZ domain and these mutants (Table 1). For *SjScrib*-PDZ4, a consensus sequence -[FVY][x][Φ][x][Φ]- was inferred from most...
Figure 1. Schematic structures and multi-alignment of PDZ domains of different Scribble orthologs. (A). Diagram of the structures of SjScrib, SmScrib, hScrib, and DmScrib, which consist of an N-terminal leucine-rich repeat (LLR), and four PDZ domains. Sequence identities of full-length protein, as well as LRR and PDZ domains between SjScrib and the orthologs from S. mansoni, H. sapiens, and D. melanogaster are shown between schematic structures. (B). Structure-based sequence alignments of four PDZ domains of Scrib orthologs. Secondary structure elements are indicated above the alignment and refer to the structure of hScrib-PDZ1 domain (PDB code 1X5Q). Each fundamental residue that mediates ligand recognition is denoted and numbered according to its position in an element of secondary structure; and within loops, the residues are numbered according to the sequence of SjScrib-PDZ4. The black arrowheads under the sequence indicate residues that are potentially involved in ligand recognition.
of the typical C-terminal PBMs (Figure 4D). The binding sites within the five irregular ligands were located based on the consensus sequence. Two of them, P4-L32 and P4-L39 were found to be particularly unusual, as they potentially bear 7 and 6 amino acids, respectively, for interacting with SjScrib-PDZ4. However, as residues of small size are dominant within both PBMs ("PCGAS" in P4-L32 and "PSG" in P4-L39), it is reasonable to speculate that they can be well accommodated within the binding groove of SjScrib-PDZ4. To confirm our prediction, three point mutations were introduced in each of the five putative binding sites (Table 1), and as a result, these mutants were found to be unable to bind to SjScrib-PDZ4. Together, these data support our speculation that SjScrib-PDZ1, SjScrib-PDZ3, and SjScrib-PDZ4 have the ability to bind both C-terminal and internal PBMs, which will be further discussed below.

Determination of ligand binding specificities of the SjScrib-PDZ domains based on the statistical analysis of amino acid composition within C-terminal and internal PBMs

SjScrib-PDZ1 showed particular preference for hydrophobic residues at the carboxyl terminus of its ligand binding site (P^0, not the extreme carboxyl terminus of the ligand), especially Ile (44.0%), Leu (36.0%), or Phe (20.0%). At P^−1, no clear preference was observed. At P^−2, only the polar amino acids Thr (30.0%) and Ser (20.0%) were selected. At P^−3, Glu (100%) was absolutely preferred. At P^−4, the hydrophobic residues, such as Leu (28.0%), Phe (24.0%) and Tyr (16.0%), along with a rare occurrence of other hydrophobic residues were selected (Figure 5A). Based on the statistical analysis of its unique PBMs, a consensus-binding sequence was determined as -[Φ][x][E][TS][x][ILF] for SjScrib-PDZ1.

Hydrophobic residues, especially Leu (36.7%), Ile (26.7%), or Val (26.7%), were also predominantly preferred at P^0 site of SjScrib-PDZ3 ligands. At P^−1, the preference for Trp (36.7%) was observed, followed by other hydrophobic residues Val (13.3%), Phe (10.0%) and Tyr (10.0%). The P^−2 site demonstrated an absolute preference for the polar residue Thr (100%). At P^−3, Glu (60.0%) was predominantly selected, along with a rare selection of residues Thr (16.7%) and Ser (13.3%). While the positive charged residues, Arg (23.3%) and Lys (20.0%) were slightly prone to be at P^−4 site. At P^−5 site, no obvious preference was observed (Figure 5B). Based on these results, a consensus-binding sequence, -[x][RK][x][E][T][W][Φ][ILV], was deduced for SjScrib-PDZ3.

For the ligands of SjScrib-PDZ4, hydrophobic residues, especially, Ile (25.9%), Leu (25.9%), or Val (16.47%), were preferred at P^0, with a weak preference for the residues of Ala (11.1%) and Cys (9.3%). The P^−2 site was also occupied by the hydrophobic residues of Ile (44.4%) and Leu (27.8%). At P^−3, Phe (81.5%) was predominantly selected, with a less occurrence of Trp (14.8%). No clear preference was observed for the sites of P^−1, P^−4 and P^−6 (Figure 5C). Therefore, a consensus-binding sequence, -[x][FW][x][IL][x][ILV], was inferred for SjScrib-PDZ4 based the amino acid occurrence at particular positions.

Ligand specificity analysis by comparison of SjScrib-PDZs and other LAP family PDZ domains according to the key residues for ligand recognition

The comparative structural analysis of human LAP family members ZO-1-PDZ1 and Erbin-PDZ has provided a valuable insight for ligand recognition across the entire binding site [60]. To better understand the molecular basis for ligand recognition of SjScrib-PDZ domains, comparative analyses were conducted for ligand specificities of the SjScrib-PDZ domains according to the key residues for ligand recognition.
The pdz domains of LAP family that contain His and Val at P2-1 and P2-5, respectively, usually have a strong preference for Thr/Ser at P0 [60]. Strikingly, SjScrib-PDZs obey this rule quite well, i.e., SjScrib-PDZ1 and SjScrib-PDZ3 that bear His and Val at P2-1 and P2-5, respectively prefer Thr at P0 [Figure 6A and C], whereas SjScrib-PDZ2 that has an Ile at P2-5 and SjScrib-PDZ4 that has a Leu at P2-1 prefer Ile and Leu at P0, respectively (Figure 6B and D). Also, it has been suggested that the positively charged residue Arg or Lys at position β3-5 of the LAP family PDZ domains favors the electrostatic interactions with a negatively charged ligand side chain at P0 [60]. A similar situation was observed in regarding of the selectivity of P0 of SjScrib-PDZs. SjScrib-PDZ1, SjScrib-PDZ2, and SjScrib-PDZ3 that contain an Arg or a Lys at position β3-5, exhibit a strong preference for a Glu at P0 [Figure 6A, B, and C]. In contrast, SjScrib-PDZ4 that has a Thr at position β3-5 displays promiscuity for residues at P0 [Figure 6D].

In addition, SjScrib-PDZ2 and SjScrib-PDZ4 display a preference for ligands that contain an aromatic side chain at P2-1. Both recognitions are predominantly mediated by residues within the β2-β3 loop. In the case of SjScrib-PDZ2, the side chain of Phe/Trp at β4-5 may interact with residues in β2 (Ala β2-4) and the β2-β3 loop (Pro β2-3-9) (Figure 6B). While in the case of SjScrib-PDZ4, the aromatic side chain at site β4 of its PBMs may potentially interact with Thr β2-4 and Phe (β2-3-17), based on the structural analysis (Figure 6D). However, the interactions between the PDZ domains and the artificial ligands may not completely reflect the nature of the molecular recognitions in vivo, thus approaches such as co-immunoprecipitation with native SjScrib will be needed to further address the binding capacity of these domains.

Divergence of ligand specificities of SjScrib-PDZs and hScrib-PDZs

Zhang et al. have previously investigated the binding specificity profiles of a panel of PDZ domains of LAP proteins by phage display screening [43]. They found that all four C-terminal residues of the ligands constituted a core recognition motif for interactions, while the more upstream residues also supported the core binding sites. Here, we performed a comparative analysis to figure out the convergent and divergent ligand specificities between SjScrib-PDZs and hScrib-PDZs. The ligand binding profile for SjScrib-PDZ1 ([Φ][Φ][EDQ][TS][x][ILF]) was found to be similar with that of hScrib-PDZ1 ([Φ][Φ][EDQ][TS][x][ILV][*]), but not exactly the same. For example, hScrib-PDZ1 prefers a hydrophobic residue at P0, whereas this is not the case for SjScrib-PDZ1. Except that both PDZ domains prefer the hydrophobic residues Leu and Ile at P0, SjScrib-PDZ1 exhibits a preference of the aromatic residue Phe; whereas hScrib-PDZ1 prefers an aliphatic residue Val at this site. The binding specificity pattern for SjScrib-PDZ3 ([Φ][Φ][EDQ][TS][x][ILV][*]) resembles that of hScrib-PDZ3 ([Φ][Φ][EDQ][TS][x][ILV][*]) [43], but with certain distinctions for SjScrib-PDZ3. For instance, SjScrib-PDZ3 shows no clear preference of hydrophobic residues at P0-5. At P0-4, unlike hScrib-PDZ3 that prefers hydrophobic residues, SjScrib-PDZ3 exhibits a preference for the positively charged residues, Arg and Lys, which may form electrostatic interactions with the negatively charged residue Asp at P2-2 or P2-2-3 (Figure 6C). Further, there is a strong preference for hydrophobic residues, particularly the aromatic residue Tyr, at P0-1 for SjScrib-PDZ3, which was not the case for hScrib-PDZ3.

The most significant divergence was observed when comparing the binding preferences of SjScrib-PDZ2 with that of hScrib-PDZ2. In contrast to hScrib-PDZ2, which prefers typical I C-terminal PBMs, -[Φ][Φ][EDQ][TS][W][V][*] [43], SjScrib-PDZ2 displays a preference for a typical II PBM, -WELS[I*], and an irregular typical II PBM, -FELMLE[*]. Also, SjScrib-PDZ2 exhibits no preference of hydrophobic residues and Tyr at P3-4 and P3-1, respectively, in contrast to that observed in those of hScrib-PDZ2. These results are consistent with that of homology modeling, i.e. the structural similarity between SjScrib-PDZ2 and hScrib-PDZ2 is poor (Figure 2B). As no binding preference of hScrib-PDZ4 is available so far, we cannot directly compare the binding specificity profile of SjScrib-PDZ4 with that of hScrib-PDZ4. However, given the relatively low homology between SjScrib-PDZ4 and hScrib-PDZ4 (Figure 1A), as well as the difference in several key residues involved in ligand recognition, such as β3-5, β2-1, β1-β2-7, β2-β3-7, β2-4, and β2-6 between the two domains (Figure 1B) [60], we postulate that the binding specificity of SjScrib-PDZ4 may be highly divergent from that of hScrib-PDZ4. Totally, these different ligand binding characteristics between the homologous PDZ domains of SjScrib and hScrib can be potentially utilized for the drug design against SjScrib.
The internal ligand binding ability of SjScrib-PDZs

For the first time, the internal motifs binding ability of the three SjScrib-PDZ domains, SjScrib-PDZ1, SjScrib-PDZ3, and SjScrib-PDZ4 was confirmed in this study. Based on the ratio of internal PBMs to C-terminal PBMs obtained from each SjScrib-PDZ, we can infer that SjScrib-PDZ3 (12:18) displays a relatively strong preference for internal motif binding, followed by SjScrib-PDZ1 (3:22); while SjScrib-PDZ4 (1:55) shows a weak ability of internal motif recognition. Interestingly, preferences at P2 between the C-terminal and internal PBMs of SjScrib-PDZ3 are different. The C-terminal PBMs of SjScrib-PDZ3 display a strong preference for Glu2; whereas its internal PBMs show a preference for Thr/Ser2, which may interact with the residue Cys at b2-4 (Figure 6C) as previously suggested [60]. Also, the negatively charged residues (Glu and Asp) frequently appear at the position +1 (P+1) in the irregular and internal PBMs of SjScrib-PDZ1. In contrast, the hydrophobic residues (Leu, Ile, and Val) or the aromatic amino acids (Phe and Tyr) are preferred at this position in the irregular and internal PBMs of SjScrib-PDZ3. The structural analysis indicated that the P+1 residue may interact with the residues of the carboxylate-binding loop or a2-9 (Figure 6C). Similar to the case of SjScrib-PDZ1, the Glu+1 of the atypical PBM of SjScrib-PDZ2 (P2-L2) and the Asp+1 in the internal ligand of SjScrib-PDZ4 (P4-L4) can potentially interact with Arg (b1-b2-4) (Figure 6B) and Arg (a2-9) (Figure 6D), respectively. These results indicate that SjScrib-PDZs may favor the unconventional interactions with a mode of Par6-Pals internal recognition [61].

In Drosophila and zebrafish, it has been shown that some PDZ domains of Scribble can interact with ligands via internal recognition [24,62]. These data together with ours suggest that the internal interaction pattern of the Scrib-PDZ domains may be more common than currently appreciated. The internal binding model of PDZ domains can dramatically increase the diversity of its native ligands. Thus, it is possible that SjScrib may mediate multiple biological pathways through a manner of internal recognition [61]. The finding of the internal binding preferences of SjScrib-PDZs has raised an opportunity for designing small molecular drugs against this target.

Potential native ligand prediction and validation for the SjScrib-PDZ domains

To identify the native ligand candidates of SjScrib-PDZs, the Tailift program was employed to search potential ligands in the
Table 1. Validation the interaction between the mutants and the SjScrib-PDZ domains by Y2H assays.

| SjScrib-PDZ4 | Ligand sequence and point mutation | Y2H² |
|--------------|-----------------------------------|------|
| P1-L8M       | CMESI[A]HFY*                      | ×    |
| P1-L10M      | SLOET[A]DIE*                      | ×    |
| P1-L23M      | FHSYRTTET[A]HIK*                  | ×    |
| P1-L34M      | YPHSAEMET[A]SIE*                  | ×    |
| P1-L35M      | SFTET[A]IFIE*                     | ×    |
| P1-L44M      | LFLTVNTMVAEKPAES[A]EI[S]EMGFPEA S LK* | ×    |
| P1-L25M      | CDEKYMVARET[A]AEIMGFPEASHLK*      | ×    |

| SjScrib-PDZ2 |
|--------------|
| P2-L1M1      | SNNVNKMKVMLTKVTEEEVSINRGLTPKFEDV PRKNTVTEASVTYAFQRGFWELSI(T)* | ×    |
| P2-L1M2      | SNNVNKMKVMLTKVTEEEVSINRGLTPKFEDVPRKNTVTEASVTYAFQRGFWELSI(A)*  | ×    |
| P2-L1M3      | SNNVNKMKVMLTKVTEEEVSINRGLTPKFEDVPRKNTVTEASVTYAFQRGFWELSI(T)I* | ×    |
| P2-L1M4      | SNNVNKMKVMLTKVTEEEVSINRGLTPKFEDVPRKNTVTEASVTYAFQRGFWELAI[S]*  | ×    |
| P2-L1M5      | SNNVNKMKVMLTKVTEEEVSINRGLTPKFEDVPRKNTVTEASVTYAFQRGFWTI[E]LSI* | ×    |
| P2-L1M6      | SNNVNKMKVMLTKVTEEEVSINRGLTPKFEDVPRKNTVTEASVTYAFQRGFWELSI*     | ×    |
| P2-L2M1      | CLVTDELWTWFELML*                  | ×    |
| P2-L2M2      | CLVTDELWTWFELMI[L]E*              | ×    |
| P2-L2M3      | CLVTDELWTWFELMI[L]E*              | ×    |
| P2-L2M4      | CLVTDELWTWFELMI[L]E*              | ×    |
| P2-L2M5      | CLVTDELWTWFELMI[L]E*              | ×    |
| P2-L2M6      | CLVTDELWTWFELMI[L]E*              | ×    |

| SjScrib-PDZ3 |
|--------------|
| P3-L2M       | SRKKYIKRWA KDMNHRHLSEKNIHMPTIRKET[A]IFLE* | ×    |
| P3-L59M      | FHFYPISRET[A]WFL*                   | ×    |
| P3-L3M       | SFGSSXCRCVTRFKEINAELYTT[A]AVCVYCLMSEKEPIDSKRPEFPLPPGWASI RDPS SSCR* | ×    |
| P3-L34M      | GNTT[A]SIFTPFKKKENPKVTI*            | ×    |
| P3-L35M      | SSAAGTKHNVS[T]AIFHHPPQKM*           | ×    |
| P3-L41M      | NFTT[A]VILLLHCQHPDQETEH*            | ×    |
| P3-L50M      | SPGFCLPSPSRCCPGSS[T]AVILWLGNP*      | ×    |
| P3-L51M      | STTLNLKVPKFHFTET[A]WIWF*            | ×    |
| P3-L56M      | WLNPSRLGSGPTMQNNTT[A]ATVYFRGFTNSV NKSLSSWDDYHRHAPPOQAOA*       | ×    |
| P3-L70M      | LRQEPHYWAGLPSY ELEYECPLKDRQXQATVTR[T]AI[L]FHIHKPS*              | ×    |
| P3-L79M      | NFKVPSFLPREDKTVFST[A]CILFRSSYEYASAVN KPIKLNSTRVGIDTGESIELEQMNR RY* | ×    |
| P3-L104M     | FIRE[T]AVW[Q]SKRASVYSGFLIST*       | ×    |
| P3-L117M     | NSST[A]FYLFIFA*                    | ×    |
| P3-L134M     | FPLKHFVLHTQST[A]AVCLFLSSHKR[T]*    | ×    |

| SjScrib-PDZ4 |
|--------------|
| P4-L4M1      | LRKTRPHAINNHRPEKEKTNYQYKELWKS G RGWESSG[WQLCA(T)DCLSLIYL*]     | ×    |
| P4-L4M2      | LRKTRPHAINNHRPEKEKTNYQYKELWKS G RGWESSG[WQL(T)CADSLIYL*]      | ×    |
| P4-L4M3      | LRKTRPHAINNHRPEKEKTNYQYKELWKS G RGWESSG[WITQLCA]DCLSLIYL*     | ×    |
| P4-L32M1     | ASALIRYGRKILNCGCFLRSSLP SHGCRGV DIHLWEGKVRNARLFYALPFSVTTF P C GAS W(T)I* | ×    |
| P4-L32M2     | ASALIRYGRKILNCGCFLRSSLP SHGCRGV DIHLWEGKVRNARLFYALPFSVTTF P C GAS W(T)SW* | ×    |
| P4-L32M3     | ASALIRYGRKILNCGCFLRSSLP SHGCRGV DIHLWEGKVRNARLFYALPFSVTTF P C GAS W(T)PC GAS W* | ×    |
| P4-L39M1     | YSPLLMARRYSYS[V]LPSGFT(T)*        | ×    |
| P4-L39M2     | YSPLLMARRYSYS[V]LPSR(G)*          | ×    |
| P4-L39M3     | YSPLLMARRYSYS[V]LPSGF*            | ×    |
| P4-L8M1      | SII[F]LLET(R)*                    | ×    |
| P4-L8M2      | SII[F]SNIET(T)*                   | ×    |
Table 1. Cont.

| SjScrib-PDZ4 | Ligand sequence and point mutation¹ | Y2H² |
|--------------|------------------------------------|------|
| P4-L8M3      | SITLET*                            | X    |
| P4-L42M1     | CFIMCFVIRNGVSFVIAT(B)*             | X    |
| P4-L42M2     | CFIMCFVIRNGVSFVIAT*                | X    |
| P4-L42M3     | CFIMCFVIRNGVSFVIAT*                |      |

¹: The potential PBMs within the irregular ligands of each PDZ domain of SjScrib are shown in bold and underline. The substituted residues are indicated in brackets.
²: !: positive in the Y2H system; X: negative in the Y2H system.
doi:10.1371/journal.pntd.0002837.t001

Figure 5. Ligand binding specificities of SjScrib-PDZ1, SjScrib-PDZ3, and SjScrib-PDZ4. Each aligned C-terminal and internal PBMs set was used to create a position weight matrix (PWM) for SjScrib-PDZ1 (A), SjScrib-PDZ3 (B), SjScrib-PDZ4 (C), respectively. Two irregular PBMs (-TFPCGASW* and -KSYLPSGF*) were excluded from the analysis of the ligand binding specificity of SjScrib-PDZ4. The percentages of each type of the amino acids at a particular position (from P₅ to P₀) are presented in the tables on the right panel.
doi:10.1371/journal.pntd.0002837.g005
S. japonicum predicted proteomic database (sjr2_protein.fasta) with less stringent core binding motifs as baits (Table 2). A panel of protein sequences was retrieved. After filtering, 2 and 9 proteins were selected as potential ligand candidates for SjScrib-PDZ1 and SjScrib-PDZ4, respectively (Table 2). However, only one protein, Prepro-cathepsin C, was confirmed to be potential ligand of SjScrib-PDZ4 in the Y2H system. Although the C-terminal tails of most of the candidates fit the ligand consensus sequence quite well, they are not native partners of SjScrib. One possibility is that the interaction between PDZ domain and ligand partner was not simply determined by the consensus sequence. For example, the consensus sequence ([x][FW][x][IL]](ILV) of SjScrib-PDZ4 is

![Figure 6. Modeling of ligand binding specificities for the SjScrib-PDZ domains.](image)

*The Scribble Protein of Schistosoma japonicum*
just a “primary”, but not “tertiary” determinant of specificity. The characteristics, such as the charge and size of the residues at position -1 and -3, may also affect the ligand recognition. These residues must coordinate with the ones at position 0, -2 and -4 and function as a whole determinant for recognition. Vice versa, this fact may explain why some unusual PBMs (e.g. P4-L32, -FPCGASW* and P4-L39, -YLPSGF*) could interact with SjScrib-PDZ4, although they do not fit the typical consensus sequence well. As it has been suggested that each PDZ domain is capable of interacting with 17 partners on average [63], it is obvious that more potential native ligands of SjScrib are still under discovery, which probably due to the poor quality of the proteomic database, or in the other case, these PDZ domains may interact with the native ligands via internal recognition. Also, it is worth noting that this positive ligand confirmed in Y2H assay is merely a "primary", but not "tertiary" determinant of specificity. The characteristics, such as the charge and size of the residues at position -1 and -3, may also affect the ligand recognition.

In summary, for the first time, we presented the molecular characterization of the PDZ domain-containing protein Scribble from S. japonicum. We defined the ligand binding specificities of the SjScrib-PDZ domains by screening a random peptide library in the Y2H system. The convergent and divergent ligand specificities between the SjScrib-PDZ domains and those of its human ortholog were denoted. The confirmation of internal ligand specificities and the identification of irregular ligands for the SjScrib-PDZ domains will assist in the rational design of novel drugs against the parasite. The strategy used here can also be generically applied to the determination of the ligand binding specificities of other parasite-derived PDZ domains.

Supporting Information

Table S1 Primer sequences used in this study.

Author Contributions

Conceived and designed the experiments: PC YM QC. Performed the experiments: PC YM XP NH SL. Analyzed the data: PC YM QC. Contributed reagents/materials/analysis tools: YG HW. Wrote the paper: PC QC.

Reference

1. Gray DJ, McManus DP, Li Y, Williams GM, Bergquist R, et al. (2010) Schistosomiasis elimination: lessons from the past guide the future. Lancet Infect Dis 10: 733-736.
2. Fenwick A, Rollinson D, Southgate V (2006) Implementation of human schistosomiasis control: Challenges and prospects. Adv Parasitol 61: 567-622.
3. McManus DP, Gray DJ, Li Y, Feng Z, Williams GM, et al. (2010) Schistosomiasis in the People’s Republic of China: the era of the Three Gorges Dam. Clin Microbiol Rev 23: 442-466.
4. Zhou Y, Zheng HJ, Chen YV, Zhang L, Wang K, et al. (2009) The Schistosoma japonicum genome reveals features of host-parasite interplay. Nature 460: 345-351.
5. Beirman M, Haas BJ, LoVerde PT, Wilson RA, Dillon GP, et al. (2009) The genome of the blood fluke Schistosoma mansoni. Nature 460: 352-359.
6. Young ND, Jex AR, Li B, Liu S, Yang L, et al. (2012) Whole-genome sequence of Schistosoma haematobium. Nat Genet 44: 221-225.
7. Archakov AI, Govorun VM, Dubanov AV, Ivanov YD, Veselovsky AV, et al. (2003) Protein-protein interactions as a target for drugs in proteomics. Proteomics 3: 380-391.
8. Dev KK (2004) Making protein interactions druggable: targeting PDZ domains. Nat Rev Drug Discov 3: 1047-1056.
9. Patra CR, Rupasinghe CN, Dutta SK, Bhattacharya S, Wang E, et al. (2012) Chemically modified peptides targeting the PDZ domain of GIPC as a therapeutic approach for cancer. ACS Chem Biol 7: 770-779.
10. Chittenden TW, Pak J, Rubio R, Cheng H, Holton K, et al. (2010) Therapeutic implications of GIPC silencing in cancer. PLoS One 5: e115381.
11. Zhou L, Li F, Xu HB, Luo CX, Wu HY, et al. (2010) Treatment of cerebral ischemia by disrupting ischemia-induced interaction of nNOS with PSD-95. Nat Med 16: 1439-1443.
12. Lee HJ, Zheng JJ (2010) PDZ domains and their binding partners: structure, specificity, and modification. Cell Commun Signal 8: 8.
13. Kalyoncu S, Keskin O, Gursoy A (2010) Interaction prediction and classification of PDZ domains. BMC Bioinformatics 11: 357.
14. Schluecker C, Mogk A, Bukau B (2004) A PDZ switch for a cellular stress response. Cell 117: 417-419.
15. Hillier BJ, Christopherson KS, Prehoda KE, Brod DS, Lim WA (1999) Unexpected modes of PDZ domain scaffolding revealed by structure of nNOS-syntrophin complex. Science 284: 812-815.
16. Mu Y, Cai P, Hu S, Ma X, Gao Y (2014) Characterization of diverse internal binding specificities of PDZ domains by yeast two-hybrid screening of a special peptide library. PLoS One 9: e88286.
34. Montcouquiol M, Rachel RA, Lanford PJ, Copeland NG, Jenkins NA, et al. (2003) Functional recruitment of mammalian Scribble relies on E-cadherin engagement. Oncogene 24: 4310–4319.

31. Kim E, Sheng M (2004) PDZ domain proteins of synapses. Nat Rev Neurosci 5: 277–289.

27. Nakagawa S, Huibregtse JM (2000) Human scribble (Vartul) is targeted for the Plk1 kinase by the ubiquitin ligase complex SCF. Mol Cell 5: 647–664.

26. Metais JY, Navarro C, Santoni MJ, Audebert S, Borg JP (2005) hScrib interacts with the basolateral PDZ protein Scribble. Nature 434: 676–680.

23. Belotti E, Polanowska J, Daulat AM, Audebert S, Thome V, et al. (2013) The human PDZome: a Gateway to PSD95-Disc Large-Zonula Occludens (PDZ)-mediated Functions. Mol Cell Proteomics 12: 2587–2603.

21. Montcouquiol M, Sans N, Hass D, Kach J, Dickman JD, et al. (2006) Mammalian Scribble forms a tight complex with the betaPIX exchange factor. Curr Biol 16: 987–995.

20. Montcouquiol M, Sans N, Hass D, Kach J, Dickman JD, et al. (2006) Asymmetric localization of Vangl2 and Fzd3 indicate novel mechanisms for planar cell polarity in mammals. J Neurosci 26: 5265–5273.

19. Li X, Yang H, Liu J, Schmidt MD, Gao T (2011) Scribble-mediated membrane protrusion. Nature 474: 173–177.

18. Navarro C, Nola S, Santoni MJ, Audrèbert S, Santoni MJ, et al. (2005) A network of PDZ-containing proteins regulates T cell polarity and asymmetric lymphocyte division.Curr Biol 15: 737–748.

17. Albertson R, Chabu C, Sheehan A, Doe CQ (2004) Scribble protein domain mapping reveals a multistep localization mechanism and domains necessary for establishing cortical polarity. J Cell Sci 117: 6001–6007.

16. Navarro C, Nola S, Audrèbert S, Santoni MJ, Arasnoe JP, et al. (2003) Functional recruitment of mammalian Scribble relies on E-cadherin engagement. Oncogene 24: 4310–4319.

15. Li X, Yang H, Liu J, Schmidt MD, Gao T (2011) Scribble-mediated membrane targeting of PHLP1 is required for its negative regulation of Akt. EMBO Rep 12: 818–824.

14. Audrèbert S, Navarro C, Nourry C, Chasseur-Golas S, Lecine P, et al. (2004) Mammalian Scribble forms a tight complex with the betaPIX exchange factor. Curr Biol 14: 987–995.

13. Montcouquiol M, Sans N, Hass D, Kach J, Dickman JD, et al. (2006) Asymmetric localization of Vangl2 and Fzd3 indicate novel mechanisms for planar cell polarity in mammals. J Neurosci 26: 5265–5273.

12. Kalay LM, McNickle A, Brennessel PJ, Hubbard AL, Braiterman LT (2006) Scribble associates with two polarity proteins, Lgl2 and Vangl2, via distinct molecular domains. J Cell Biol 99: 647–664.

11. Beloti E, Polanowska J, Daulat AM, Audrèbert S, Thome V, et al. (2013) The Human PDZome: a Gateway to PSD95-Disc Large-Zonula Occludens (PDZ)-mediated Functions. Mol Cell Proteomics 12: 2587–2603.

10. Richler I, Willton K, Clattenburg L, Colwell K, O’Brien M, et al. (2010) NOSIAP associates with Scribble and regulates dendritic spine development. J Neurosci 30: 4796–4805.

9. Petit MM, Crenshaw KR, Verheugen HB, Weyns N, Van de Ven WJ (2005) The tumor suppressor Scribble selectively interacts with specific members of the axin family of proteins. FEBS Lett 579: 5061–5068.

8. Menta JY, Navarro C, Santoni MJ, Audrèbert S, Borg JP (2005) hScrib interacts with ZO-2 at the cell junctions of epithelial cells. FEBS Lett 579: 3725–3730.

7. Nakagawa S, Huijbregts JM (2006) Human scribble (Vartul) is targeted for ubiquitin-mediated degradation by the high-risk papillomavirus E6 proteins and the E6AP ubiquitin-protein ligase. Mol Cell Biol 20: 8244–8253.

6. Lazic D, Huibbauer M, Zigrino P, Buchholz S, Kazem S, et al. (2012) Human papillomavirus type II E6 oncoprotein inhibits transcription of the PDZ protein synemin-2. J Virol 86: 7943–7952.

5. Javier RT, Rice AP (2011) Emerging theme: cellular PDZ proteins as common targets of pathogenic viruses. J Virol 85: 11544–11556.

4. Bilder D, Perrimon N (2000) Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. Nature 403: 676–680.

3. Kim E, Sheng M (2004) PDZ domain proteins of synapses. Nat Rev Neurosci 5: 771–781.

2. Ludford-Menting MJ, Oliaro J, Sacrariove F, Cheah ET, Pedersen N, et al. (2005) A network of PDZ-containing proteins regulates T cell polarity and morphology during migration and immunological synapse formation. Immunity 22: 737–748.

1. Yeh JH, Sodha SS, Chan AC (2008) Regulation of a late phase of T cell polarity and effector functions by Cxcl3. Cell 132: 846–859.

33. Montcouquiol M, Rachel RA, Lanford PJ, Copeland NG, Jenkins NA, et al. (2003) Identification of Vangl2 and Scribl as planar polarity genes in mammals. Nature 423: 173–177.

32. Yates LL, Schwartzwender C, Hazlewood L, Chesum L, Paushal A, et al. (2013) Scribble is required for normal epithelial cell-cell contacts and lumen morphogenesis in the mammalian lung. Dev Biol 373: 267–280.

31. Dow LE, Kauffmann JS, Caddy J, Zarbalis K, Peterson AS, et al. (2007) The tumor-suppressor Scribble regulates cell polarity during directed epithelial migration: regulation of Rho GTPase recruitment to the leading edge. Oncogene 26: 2272–2282.

30. Nola S, Sebbagh M, Marchetto S, Osman N, Nourry C, et al. (2006) Scrib regulates Pak activity during the cell migration process. Hum Mol Genet 17: 3535–3546.

29. Bilder D, Li M, Perrimon N (2000) Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. Science 289: 113–116.

28. Wodarz A, Nathke I (2007) Cell polarity in development and cancer. Nat Cell Biol 9: 1016–1024.

27. Frank SR, Bell JH, Frodin M, Hansen SH (2012) A betaPIX-PAK2 complex confers protection against Scrib-dependent and cadherin-mediated apoptosis. Curr Biol 22: 1747–1754.

26. Humbert PO, Greschik NA, Brumby AM, Galea R, Elsoum I, et al. (2008) Control of tumourigenesis by the Scribble/Dlg/Lgl polarity module. Oncogene 27: 6818–6890.

25. Sun Y, Aiga M, Yoshida E, Humbert PO, Banjii SX (2009) Scribble interacts with beta-catenin to localize synaptic vesicles to synapses. Mol Biol Cell 20: 3390–3400.

24. Zhang Y, Yeh S, Appleton BA, Held HA, Kausalya PJ, et al. (2006) Convergent and divergent ligand specificity among PDZ domains of the LAP and zonula occludens (ZO) families. J Biol Chem 281: 22299–22311.

23. Cai P, Piao X, Hao L, Liu S, Hou N, et al. (2013) A deep analysis of the small RNA population in Schistosoma japonicum. PLoS Negl Trop Dis 6: e1745.

22. Humbert PO, Richardson HE (2012) The Scribble-Dlg-Lgl polarity module in development and cancer: from flies to man. Essays Biochem 51: 339–350.

21. Humbert PO, Lindholt JS, Pedersen N, et al. (2005) Identification of Vangl2 and Scribl as planar polarity genes in mammals. Nature 423: 173–177.

20. Dow LE, Kauffmann JS, Caddy J, Zarbalis K, Peterson AS, et al. (2007) The tumor-suppressor Scribble regulates cell polarity during directed epithelial migration: regulation of Rho GTPase recruitment to the leading edge. Oncogene 26: 2272–2282.

19. Nola S, Sebbagh M, Marchetto S, Osman N, Nourry C, et al. (2006) Scrib regulates Pak activity during the cell migration process. Hum Mol Genet 17: 3535–3546.

18. Bilder D, Li M, Perrimon N (2000) Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. Science 289: 113–116.

17. Wodarz A, Nathke I (2007) Cell polarity in development and cancer. Nat Cell Biol 9: 1016–1024.

16. Frank SR, Bell JH, Frodin M, Hansen SH (2012) A betaPIX-PAK2 complex confers protection against Scrib-dependent and cadherin-mediated apoptosis. Curr Biol 22: 1747–1754.