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PICK-1: A scaffold protein that interacts with Nectins and JAMs at cell junctions

Nicolas Reymond, Sarah Garrido-Urbani, Jean-Paul Borg, Patrice Dubreuil, Marc Lopez*

INSERM UMR.599, Institut de Cancérologie de Marseille, IFR 137, 27 Bvd. Leï-roure, 13009 Marseille, France

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Abstract Nectin adhesion molecules are involved in the early steps of cell junction formation. Later during the polarisation process, Nectins are components of epithelial adherens junctions where they are indirectly associated with the E-cadherin/Catenins complex via the adaptator AF-6. To have a better understanding of Nectin-based cell junctions, we looked for some new Nectins' partners. We demonstrate that the scaffold molecule PICK-1, involved in the clustering of junctional receptors in synaptic junctions, interacts directly with Nectins in a PSD-95/Dlg/ZO-1 domain-dependent manner and is localised at adherens junctions in epithelial cells. Finally, we observed that protein interacting with C-kinase-1 (PICK-1) also interacts directly with the junctional adhesion molecules, and we suggest that PICK-1 could be involved in the regulation of both adherens and tight junctions in epithelial cells.

Keywords: Nectins; Junctional adhesion molecule; AF-6; Protein interacting with C-kinase-1; PDZ domain

1. Introduction

Nectin adhesion molecules are key players for the establishment of cell junctions in epithelial cells [1]. They are involved in the building of a protein network required for the formation of both adherens and tight junctions [1]. Nectins may potentially recruit, regulate and interact with E-cadherin at adherens junctions via their cytoplasmic associated molecules, i.e., AF-6/afadin and Catenins, respectively [1]. Nectins share common cytoplasmic interactors such as AF-6 and ASIP/PAR-3 with JAM-A, and are involved in JAM-A proper localisation at tight junctions [1–4]. Thus, E-cadherin, Nectins and JAM-A constitute a complex of cell adhesion molecules, communicating with each other's to maintain cell cohesion, cell polarity and regulate their physiological properties.

Five Nectins have been described in human: PVR/CD155, and Nectin-1, -2, -3 and -4 [5–9]. They display both calcium independent *trans*-homophilic and *trans*-heterophilic adhesion properties following *cis*-dimerisation at the cell surface, characterised by specific combinations of Nectin ectodomains: Nectin-3 *trans*-interacts with poliovirus receptor (PVR), Nectin-2 and Nectin-1, the latter is in turn able to *trans*-interact with Nectin-4 [1,9]. Some of these interactions have been described in vivo at the pre- and post-synaptic junctions of neurons (Nectin-1/Nectin-3), as well as at the junctions between Sertoli cells and spermatids during spermiogenesis (Nectin-2/Nectin-3) [1]. Moreover, Nectin-2 and PVR have been described to interact with the NK triggering molecule, DNAM-1 (CD226), to induce the NK-mediated lysis of tumour cells [10] and we recently described that PVR interacts with DNAM-1 to regulate the *trans*-endothelial process of monocytes [11]. CD96 has also been described to *trans*-interact with PVR [12].

To characterise the molecular organisation of Nectin-based adherens junctions, we looked for cytoplasmic proteins interacting with the intracellular region of Nectins. Nectins do not show any sequence similarity within this region except, for most of them, a common carboxy-terminal consensus sequence that enable them to bind to PSD-95/Dlg/ZO-1 (PDZ) domains [1,8,9]. We thus tested several PDZ domain-containing proteins for their ability to interact with Nectins. Among them, we found that protein interacting with C-kinase-1 (PICK-1) is a new Nectin partner. PICK-1 was initially described as a PKCα interacting protein [13,14]. It interacts with tyrosine kinase receptors including EphA7 and EphB2 as does AF-6 [15,16]. PICK-1 is required for the control of synaptic transmission by metabotropic glutamate receptors [17–19]. We found that PICK-1 interacts with Nectins in vivo and, as Nectins, localises at adherens junctions in epithelial cells. Moreover, we found that PICK-1 also interacts with the junctional adhesion molecules (JAMs). From our results, we show that PICK-1 is a new component of epithelial cell adherens junctions and likely plays a role in epithelial physiology through its interactions with Nectins and JAMs.

2. Materials and methods

2.1. Construction of expression vectors

Mammalian expression vectors encoding the full-length (FL) human PVRα (pSV2PVRα), Nectin-1δ (pCF18), Nectin-1α (pLX1.12), Nectin-2α (LX2Sb1), Nectin-2δ (LX2Lc12) and Nectin-3α (pFLR3V.1) have been already described [5–9]. Mammalian expression vectors encoding the FL mouse JAM-A (pRC/106) and JAM-C (pDel5′3′ JAM-2#6) were provided by Dr. M. Aurrand-Lions (Geneva, Switzerland).

PICK-1 expression constructs were generated by subcloning the FL mouse PICK-1 cDNA and its mutants into pRK5-myc (Stratagene) and the FL mouse PICK-1 cDNA into pGFP-C1 (Clontech). Mouse

*Corresponding author. Fax: +33 4 91 26 03 64.
E-mail address: lopez@marseille.inserm.fr (M. Lopez).

Abbreviations: JAM, junctional adhesion molecule; PDZ, PSD-95/Dlg/ZO-1; PICK-1, protein interacting with C-kinase-1; PVR, poliovirus receptor

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PICK-1 and its mutants cloned in pRK5-myc were named pRKPICK-1 (PICK-1 FL), pRKPICK-1-KD (PICK-1 PDZ domain mutant: K27D28 amino acids mutated in A27A28), pRKPICK-1.1-305 (residues 1–305) as described in previous studies [14,35]. Mouse PICK-1 was also cloned in pGFP-C1 and named pGFPC-PICK-1 [35].

2.2. Yeast two-hybrid system

Oligonucleotide adaptators encoding the last seven amino acids of the COOH termini of the various human Nectins, and their mutants, were cloned into the LexA DNA-binding domain bait expression vector pBTM116B.Kana. Mouse cDNA encoding the FL PICK-1 was cloned into the Gal4 DNA-activation domain prey vector pGAD (pGADPICK-1). PICK-1 mutants were constructed as described previously [14] and cloned into pGAD: pGADPICK-1-KD (K27D28 amino acids mutated in A27A28) and into pACT2 Gal4 DNA-activation domain prey vector: pACPICK-1.1-305 (residues 1–305). cDNAs encoding the PDZ domain of various proteins were cloned into pACT2: pACPDZ-AF6g (PDZ domain of AF-6), pACERBIN (PDZ domain of ERBIN) and pACYE15.126 (PDZ domain of *Caenorhabditis elegans* LET 413). The integrity of all inserts was confirmed by DNA sequencing (Genome Express, Grenoble, France).

Yeast assays were performed as described previously using lithium acetate-based method. Interaction between bait and prey were first monitored by a LacZ reporter assay: β-galactosidase activity was tested with X-gal as a substrate using the filter method. Interactions were also evaluated by HIS3 reporter assay.

2.3. Cells and culture conditions

Madin Darby Canine kidney cells (MDCK cells) and COS cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), 1% penicillin, 1% streptomycin and 1% glutamine. $CaCO_2$ cells were cultivated in DMEM medium supplemented with 20% FCS, 1% penicillin, 1% streptomycin, 1% glutamine and 1% non-essential acids (Life Technologies, France). Cells were cultivated in an air–5% CO_2 atmosphere at constant humidity.

2.4. Antibodies

Murine monoclonal anti-myc 9E10 (Oncogene Research Products, Cambridge, MA) and rabbit polyclonal anti-β-Catenin (Zymed) were purchased from commercial sources. Murine monoclonal antibodies anti-Nectin-1 (R1.302), anti-PVR (PV.404) and rabbit immune sera directed against the intracellular domain of Nectin-1, Nectin-2α and PVR were obtained in the laboratory. Rat monoclonal antibodies anti-JAM-A (H202.106) and anti-JAM-C (XIXH36) were provided by Dr. M. Aurrand-Lions (Geneva, Switzerland). Rabbit polyclonal anti-PICK-1 antibody JPB-9 was raised against a GST-PICK-1 fusion protein.

2.5. DNA transfection, cell lysis, immunoprecipitation immunoblot analysis and immunofluorescence

Cells were grown to 50–80% confluency then were transfected with plasmids using FuGENE6 reagent method according to manufacturer recommendations (Boehringer–Mannheim). The cells were cultivated for 1 day and the medium was replaced. Cells were analysed either after transient transfection or after selection in the presence of 0.5 mg/ml of G418 in the case of stable transfectants establishment. Cell lysis, immunoprecipitation and immunoblot were performed as previously described [20].

MDCK and COS cells were grown on 13-mm-round glass coverslips as a confluent monolayer. Immunofluorescence was performed as previously described [9].

3. Results

3.1. Identification of PICK-1 as a new Nectin partner by the yeast two-hybrid system

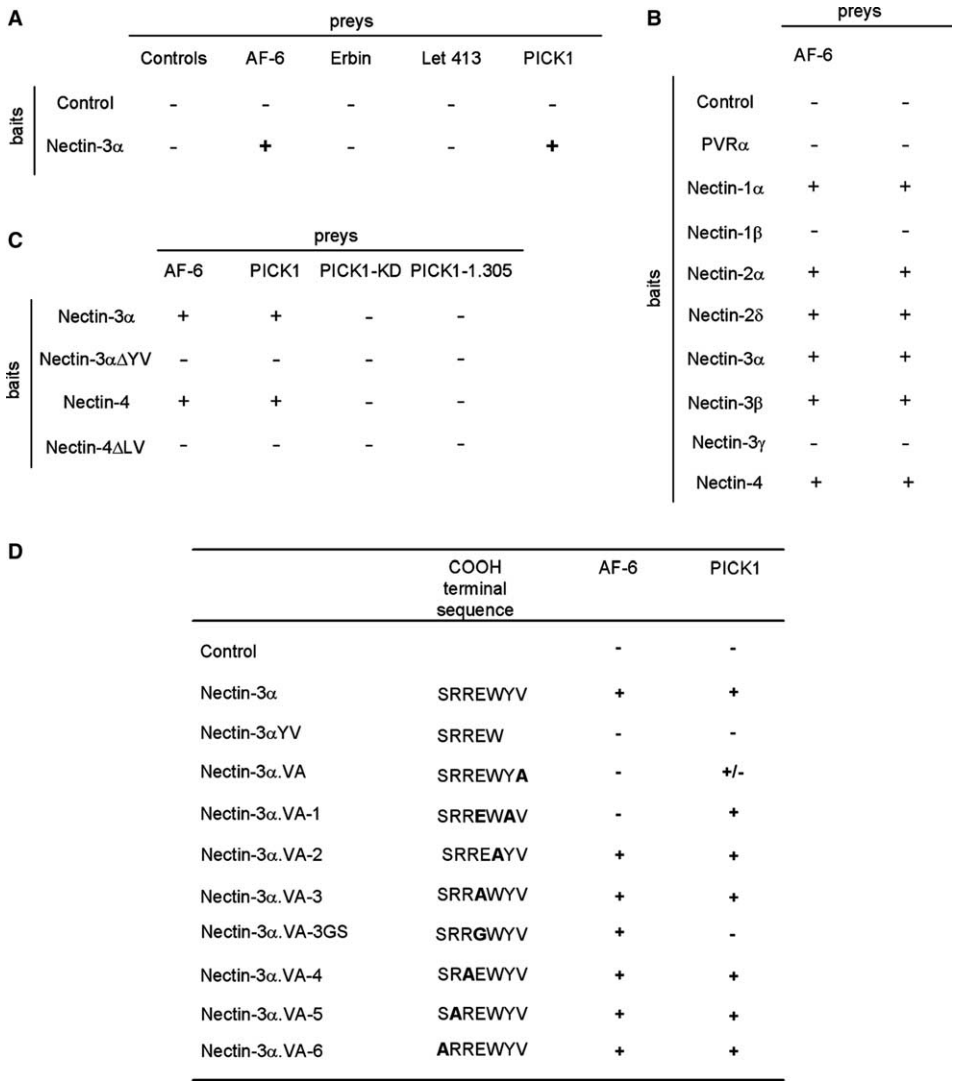
Nectins constitute a family of five structurally related cell adhesion molecules [5–9]. Different isoforms have been described for each Nectin, sharing identical extra-cellular domains but different carboxy-terminal regions. Two subgroups have been determined: a first group carries a carboxy-terminal PDZ domain-binding sequence, identified in Nectin-1α, Nectin-2 (α and δ), Nectin-3 (α and β) and Nectin-4; a second group (PVRα, PVRδ, Nectin-1β and Nectin-3γ) does not carry this PDZ domain-binding sequence.

Looking for new Nectin cytoplasmic partners by the yeast two-hybrid system, we selected Nectin-3α as a bait representative of Nectins: we tested its ability to bind to various known PDZ domain-containing proteins. We found that Nectin-3α interacts with PICK-1 and AF-6 (taken as a positive control) but not with Erbin or Let413 (Fig. 1A). We tested the ability of other Nectins to bind to PICK-1 and we found that AF-6 and PICK-1 have the same specificity for Nectins: we show that all the AF-6 interacting Nectins (i.e., Nectin-1α, Nectin-2 and -2δ, Nectin-3α, -3β and Nectin-4) also bind to PICK-1, whereas PVRα, Nectin-1β and Nectin-3γ neither interact with AF-6 nor with PICK-1 (Fig. 1B).

We challenged PICK-1 with Nectin mutants, unable to interact with AF-6, as they are deleted of the last two C-terminal amino acids [9]. In a similar way, Nectin-3α and Nectin-4 mutants were not able to interact with PICK-1 (Fig. 1C). These results confirm that the carboxy-terminal sequence of Nectins is involved in the interaction with PICK-1, and, as for AF-6, that the last two residues of Nectins are critical in this interaction. The carboxy-terminal tails of Nectins were also challenged with two PICK-1 mutants described to abolish the interaction between PKCα and the AMPA receptor GluR2 [14]. PICK-1-KD has two point mutations within its PDZ domain and PICK-1-1.305 is deleted from its acidic region but still possesses its PDZ domain. Interactions with Nectins are also abrogated with both PICK-1 mutants, suggesting that the PDZ domain of PICK-1 is involved and that the acidic region of PICK-1 is also necessary for the interaction probably by stabilising the conformation of its PDZ domain, as previously suggested [14]. In conclusion, we describe PICK-1 as a new partner for Nectins. As described for AF-6, PICK-1 interacts with the carboxy-terminal region of Nectins via its PDZ domain. Also, the carboxy-terminal sequence of PICK-1 is necessary to ensure a strong binding affinity.

3.2. PICK-1 and AF-6 differentially interact with Nectins

PICK-1 is described to interact via its PDZ domain with PKCα, AMPA and Eph receptors [13–15,21,22]. PDZ domains bind to their ligands with a high selectivity driven by each amino acid of the carboxy-terminal domain of their ligands [23]. We performed a comparative analysis to identify critical residues important for Nectin/PICK-1 and Nectin/AF-6 interactions. As shown in Fig. 1A, the seven last residues of Nectin-3α are sufficient to interact with both proteins. Each amino acid within the Nectin-3α peptide was mutated to alanine (A) from position 0 (the carboxy-terminal valine) to position −6 and challenged with either PICK-1 or the PDZ domain of AF-6 in the yeast system (Fig. 1D). Nectin-3α/AF-6 interaction is abolished when the V in position 0 is mutated, while Nectin-3α/PICK-1 interaction is diminished but not completely disrupted. Nectin-3α/AF-6 but not Nectin-3α/PICK-1 interaction is altered when the Y in position −1 is mutated. Concerning the mutation in position −3, the Nectin-3α/PICK-1 but not Nectin-3α/AF-6 interaction is altered when E in position −3 is mutated in G, whereas no change was observed when the E in position −3 is mutated in A. The latter



A

baits	preys: Controls	AF-6	Erbin	Let 413	PICK1
Control	-	-	-	-	-
Nectin-3α	-	+	-	-	+

B

baits	preys: AF-6	
Control	-	-
PVRα	-	-
Nectin-1α	+	+
Nectin-1β	-	-
Nectin-2α	+	+
Nectin-2δ	+	+
Nectin-3α	+	+
Nectin-3β	+	+
Nectin-3γ	-	-
Nectin-4	+	+

C

baits	preys: AF-6	PICK1	PICK1-KD	PICK1-1.305
Nectin-3α	+	+	-	-
Nectin-3αΔYV	-	-	-	-
Nectin-4	+	+	-	-
Nectin-4ΔLV	-	-	-	-

D

	COOH terminal sequence	AF-6	PICK1
Control		-	-
Nectin-3α	SRREWYV	+	+
Nectin-3αYV	SRREW	-	-
Nectin-3α.VA	SRREWY**A**	-	+/-
Nectin-3α.VA-1	SRRE**W**A**V**	-	+
Nectin-3α.VA-2	SRRE**A**YV	+	+
Nectin-3α.VA-3	SRR**A**WYV	+	+
Nectin-3α.VA-3GS	SRR**G**WYV	+	-
Nectin-3α.VA-4	SR**A**EWYV	+	+
Nectin-3α.VA-5	S**A**REWYV	+	+
Nectin-3α.VA-6	**A**RREWYV	+	+

Fig. 1. PICK-1 interaction with Nectins in the yeast two-hybrid system. (A) The carboxy-terminal region of Nectin-3α was tested for interaction with different PDZ domain-containing proteins using the yeast two-hybrid system. The carboxy-terminal residues of Nectin-3α interact strongly with AF-6 (positive control) and with PICK-1, whereas no interaction was detected with Erbin and Let413. (B) The carboxy-terminal region of Nectins was tested for interaction with AF-6 and PICK-1. All the AF-6 interacting Nectins interact with PICK-1. (C) The carboxy-terminal regions of Nectins-3α and -4 were deleted of their last two residues and the resulting mutants were tested for their abilities to interact with AF-6 and PICK-1. Neither of the mutants was able to interact with the wild-type AF-6 or PICK-1. No interaction was detected with wild-type Nectin-3α or -4 and the carboxylate-binding loop mutant (PICK-1-KD) or the acidic region deleted mutant (PICK-1-1.305). (D) Alanine scan of Nectin-3α carboxy-terminal region. Each residue of Nectin-3α carboxy-terminal region was mutated into alanine. Nectin-3 point mutants were tested for interaction with AF-6 or PICK-1. Different Nectin-3α residues seem to be important for these two interactions.

observation could be explained by the fact that the E/A substitution preserves the consensus motif of Nectins (A/E)XYV. None or only a slight change in both interactions was noted when the W in −2, the R in −4, the R in −5, the S in −6 positions were replaced by A. Our results demonstrate that amino acids important for the interactions between Nectin-3α/AF-6 and Nectin-3α/PICK-1 are in part different. Amino acids that form βB strand and αB helix of the PICK-1 and AF-6 PDZ domains probably bind with different C-terminal amino acids of Nectins.

3.3. PICK-1 and Nectins interact in vivo and co-localise at cell junctions in COS cells

We analysed the interaction of PVRα, Nectin-1β, Nectin-2α and -2δ, Nectin-3α and Nectin-4 with PICK-1 within COS cells. Cells were transiently transfected with each Nectin cDNA and myc-tagged PICK-1. Nectin-1α (Fig. 2A), Nectin-2α and -2δ, Nectin-3α and Nectin-4 (data not shown) were co-immunoprecipitated with PICK-1 within COS cells in contrast to PVRα (Fig. 2B) and Nectin-1β (data not shown) that were not. These data confirm our two-hybrid analyses and demonstrate that PICK-1 shares the same Nectin partners as AF-6 in vivo within COS cells.

On the basis of previous experiments, we next examined the localisation of Nectins and PICK-1 within COS cells. Subconfluent cells were transiently transfected with EGFP-PICK-1 and Nectin-1α. As seen in Fig. 2C, PICK-1 is present in the cytoplasm and the perinuclear regions, as already described [13,14,24]. PICK-1 also co-localised with Nectin-1α at cell contacts (see arrow) but neither PICK-1 nor Nectin-1α are present

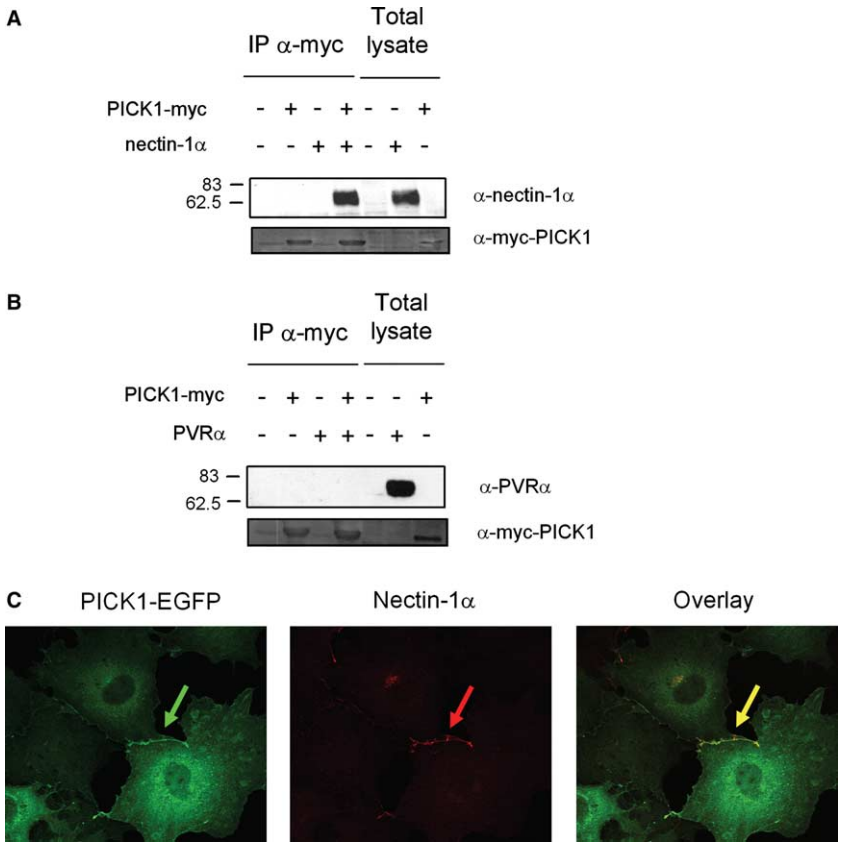


Fig. 2. Detection of PICK-1 interactions with Nectins in COS cells. COS cells were transfected with expression vectors encoding tagged myc-PICK-1 and Nectin-1α (A) or PVRα (B). Extracts were prepared from transfected cells and were immunoprecipitated with anti-myc antibody. Following separation of immunoprecipitates by SDS–PAGE, Western-blotting was performed using an anti-Nectin-1α (A) or an anti-PVRα (B) polyclonal antibody. The Nectin-1α protein is co-immunoprecipitated with PICK-1 (A) whereas PVRα is not (B). Controls were obtained using the anti-myc antibody. (C) Immunolocalisation of PICK-1 in Nectin-1α transfected COS cells. COS cells were transiently transfected with EGFP tagged PICK-1 and pLX1.12 (encoding Nectin-1α) expression vectors. Two days after transfection, COS cells were stained with the anti-Nectin-1 monoclonal antibody (R1.302). EGFP-PICK-1 is mostly localised to the perinuclear region as described [13] and slightly observed at cell junctions where it co-localises with Nectin-1α.

at the plasma membrane not engaged in a cell-to-cell contact. Our results suggest that Nectins could recruit PICK-1 at cell junctions.

3.4. PICK-1 is expressed at adherens junctions in epithelial cells

PICK-1 is expressed in many tissues but it has been mainly studied in the neuronal system where it binds and clusters several junctional synaptic components including AMPA and Eph receptors [15,21,22]. Nectins are expressed in many tissues and, among them, in epithelial derived tissues. We thus investigated the expression of PICK-1 in epithelial cells: we detected the 52 kDa specific band of PICK-1 in $CaCO_2$ and MDCK epithelial cells as well as in mouse brain (Fig. 3A, left). No band was detected with the corresponding pre-immune serum (Fig. 3A, right).

Nectins are specifically localised at E-cadherin-based adherens junctions in polarised epithelial cells. In fully polarised MDCK cells, we observed that PICK-1 is present in the cytoplasm and the perinuclear regions, but also co-localises with β-catenin and AF-6 at the basolateral membranes (Fig. 3B and C). This co-localisation with β-catenin could be attributable to the presence of endogenous Nectins expressed in MDCK cells as previously described [1]. We failed to detect an endogenous interaction between PICK-1 and Nectins in $CaCO_2$ human epithelial cells, but this could be due to a weak or a transient interaction as described for the interaction between Nectins and ASIP/PAR-3 or JAM-A and ASIP/PAR3 and a low expression level of PICK-1 at cell junctions [1,3,4].

3.5. PICK-1 interacts with the JAM family members

Investigating PICK-1 distribution at epithelial cell basolateral membranes with polarised epithelial cells, we found that PICK-1 also co-localises with AF-6 at cell junctions (Fig. 3B) [25,26]. PICK-1 and AF-6 share some common transmembrane partners (Nectins and Eph) and we thus hypothesise that some other AF-6 transmembrane partners like JAM-A could also interact with PICK-1. To test this hypothesis, JAM-A was challenged with PICK-1 in a two-hybrid assay in yeast, and we found that PICK-1 interacts with JAM-A in a way similar to Nectins. This interaction is abrogated with both KD27/28 and 1–305 PICK-1 mutants, suggesting that the carboxy-terminal region of JAM-A interacts directly with the PDZ domain of PICK-1 (Fig. 4A). We tested PICK-1 ability to interact with the other members of the JAM family (JAM-B and JAM-C) and found that PICK-1 is also able to interact with them (Fig. 4A). No interaction was detected between PICK-1 with Claudins (-2, -10 and -15) that also have PDZ domain-binding motifs and localise at tight junctions in epithelial cells (data not shown).

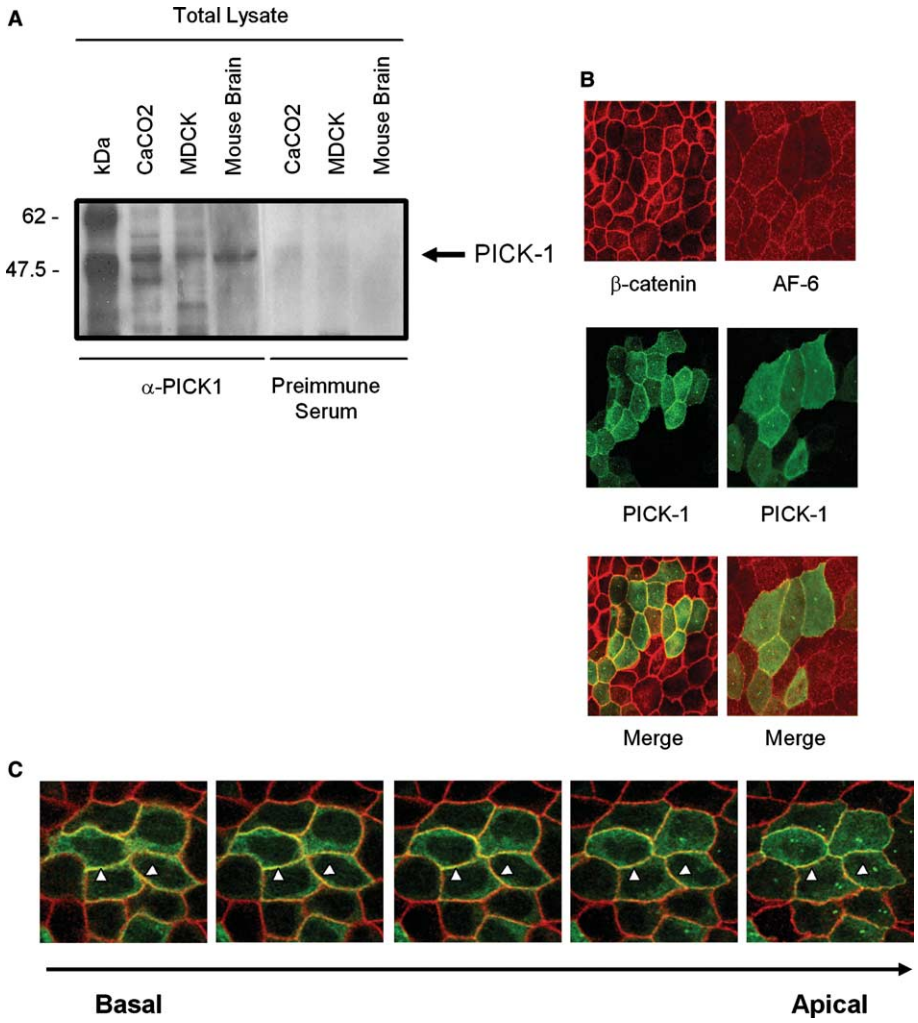


Fig. 3. Expression and localisation of PICK-1 in epithelial cells. (A) Cell extracts from MDCK and $CaCO_2$ epithelial cells were tested for the expression of PICK-1. The PICK-1 protein (52 kDa) is detected from both epithelial cell line lysates as well as in rat brain lysate with a polyclonal anti-PICK-1 antibody. Other bands were detected but not identified. No protein was detected in the same lysates using the pre-immune serum of this antibody. (B) Immunolocalisation of PICK-1 in MDCK cells. MDCK cells were stably transfected with EGFP tagged PICK-1 expression vector. Cells were cultivated at confluency during several days for an optimal polarisation. PICK-1 localisation mostly overlaps with β-catenin and AF-6, suggesting that PICK-1 is localised at adherens junctions of epithelial cells. (C) Gallery of PICK-1 and β-catenin localisation in MDCK cells. PICK-1 and β-catenin co-localise at the basolateral domain of epithelial cells within adherens junctions.

We then analysed the interaction between PICK-1 and the JAMs within COS cells. We thus performed transfection experiments with JAM-A, JAM-C and PICK-1 cDNAs. JAM-A and JAM-C are co-immunoprecipitated with myc-PICK-1 (Fig. 4B and C, lane 4). JAM-B/PICK-1 interaction was not evaluated as no specific reagent was available. JAM-A, JAM-C and Nectin-2 are endogenously expressed within COS cells and we evaluated the possible simultaneous association of PICK1 with endogenous JAMs and Nectins. As shown in Fig. 4B–D (lane 2), PICK-1 may simultaneously interact with endogenous JAM-A, JAM-C and Nectin-2α expressed by COS cells. Whether these simultaneous interactions are conserved in polarised epithelial cells will need further investigations.

Previous studies reported that JAM-A shares some cytoplasmic partners with Nectins including AF-6 and ASIP/PAR-3, both of them via PDZ domains interactions [2–4]. Using two different approaches, we show for the first time that PICK-1 interacts specifically with Nectins and JAMs. The specificity of these interactions is strengthened by a positive interaction between PICK-1 and the different members of two distinct families of cell adhesion molecules and the lack of interaction with Claudins.

4. Discussion

We describe PICK-1 as a scaffold protein that may potentially link two components of the apical junctional complex in epithelial cells. PICK-1, previously described in neuronal cells, is indeed expressed in epithelial cells, localised mostly at adherens junctions and interacts with transmembrane components of both adherens and tight junctions (Fig. 4). Indeed, PICK-1 interacts directly with Nectins (Figs. 1 and 2), localised at adherens junctions [1,9], and with JAMs (Fig. 4), described to be localised at tight junctions [27]. We showed that these interactions are ensured by the carboxy-terminal tail of Nectins or JAMs and the PDZ domain of PICK-1 (Figs. 1

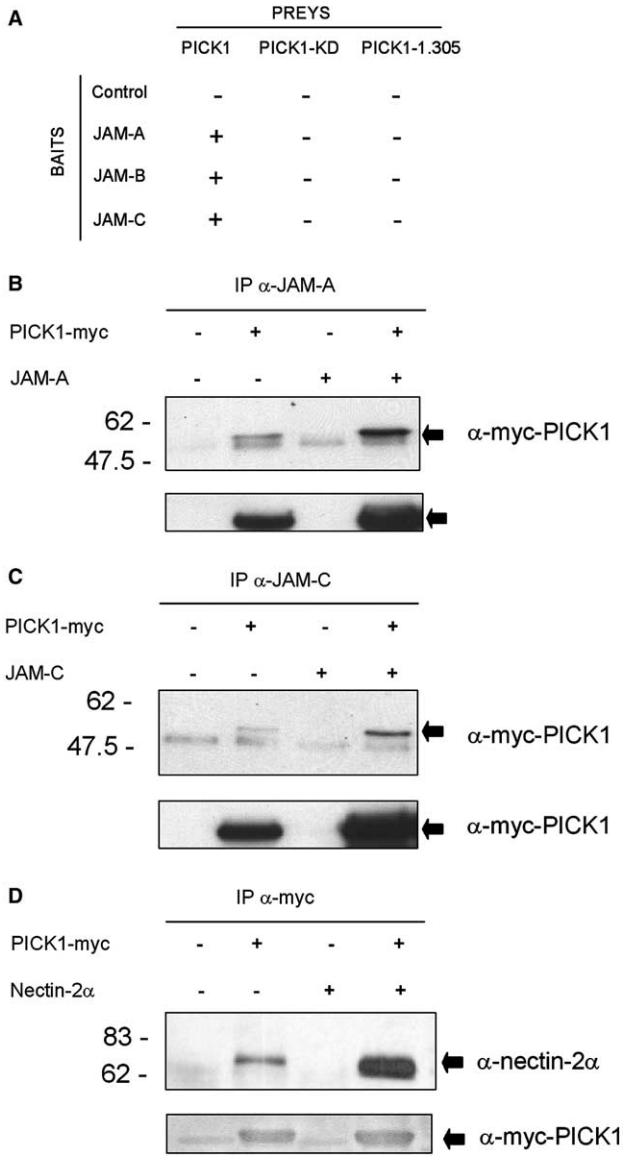


Fig. 4. PICK-1 interaction with JAMs. (A) The carboxy-terminal region of JAM-A, -B and -C were tested for interaction with PICK-1 or point mutants of PICK-1 using the yeast two-hybrid system. The carboxy-terminal regions of JAM-A, -B and -C strongly interact with PICK-1 probably via its PDZ domain. (B–D) (top) COS cells were transfected with expression vectors encoding tagged myc-PICK-1 and/or JAM-A, JAM-C, Nectin-2α. Extracts were prepared from transfected cells and were immunoprecipitated with anti-JAM-A (B), anti-JAM-C (C) and anti-myc (D) antibodies. Western-blotting was performed using an anti-myc (B, C) or an anti-Nectin-2 (D) antibodies (black arrow: PICK-1, white arrow: Nectin-2α). (bottom) For each conditions, total lysates were analysed using the anti-c-myc antibody.

and 4). Interestingly, a recent report showed that PICK-1 also interacts with CAR, another cell-to-cell junction protein described to be localised at tight junctions in epithelial cells [28,29]. Altogether, these studies suggest that PICK-1 is an important scaffold molecule in epithelial cells. They also raise the question of an eventual competition between all these proteins to bind Nectins, JAMs and CAR or a potential spatio-temporal regulation of these interactions. Interestingly, we found that the residues involved in AF-6 and PICK-1 binding to Nectins are probably different. Moreover, Nectins, JAMs or CAR interactions with PICK-1 could be simultaneous which could be linked to the fact that PICK-1 can form homo-dimers [14].

The characterisation of new adaptator proteins that interact with Nectins, JAMs or CAR within their carboxy-terminal region provides new insights into the molecular composition of epithelial cell-to-cell contacts. PICK-1 associated interactions may indeed be important for cell junction formation. PICK-1 has been first characterised to interact with the carboxy-terminal domain of PKCα [13,14]. Interestingly, PKC signalling has been reported to be crucial to organise adherens junctions. The engagement of E-cadherin seems to induce intracellular signalling pathways that lead to the activation of PKC [30]. However, less is known concerning the isotype of the PKC involved and its recruitment to cell junctions. Nectins and E-cadherin seem to be physically and functionally linked at adherens junctions [1]. They are indeed associated by their cytoskeletal partners, i.e., AF-6 and α-catenin [1] and are both needed to recruit the Sec6/Sec8 complex of exocyst at cell junctions [31]. Nectins are recruited early during cell contact formation and could thus recruit PKCα to cell junctions to participate in the recruitment of additional E-cadherin and Nectin molecules. Alternatively, it seems that E-cadherin can activate but also responds to PKC signalling. Interestingly, we observed a consensus sequence for PKCα phosphorylation within Nectin-1α intracellular domain (TKK: amino acids 391–394 and SSK: amino acids 511–513), suggesting that Nectins could be phosphorylated by PKCα.

Beyond its interaction with Nectins, an association between PICK-1 and CAR has been described [28] and we also found that PICK-1 interacts with the JAMs (Fig. 4). PICK-1 could thus also take part in the regulation of tight junctions as they are regulated by PKC signalling: inhibition of PKCs blocks tight junctions assembly and disassembly, implying PKC transient activation. Several PKC isoforms have been localised to tight junctions, but little is known about the molecular mechanisms by which they regulate junctional dynamics [32]. Interestingly, JAM-A has been described to be phosphorylated by a classical PKC (PKCα, β or γ) upon platelet activation [33]. JAM-A and other JAMs or CAR could recruit PKCα at tight junctions and could potentially be targets of PKCα. Interestingly, Nectins are involved in the correct targeting of JAM-A to tight junctions. It would be important to study the role of PICK-1 in JAM-A and CAR localisation and stability at tight junctions.

E-cadherin has been shown to mediate the coordinate organisation of adherens junctions [34]. The implication of Nectins during this step remains to be elucidated. Signalling downstream of Nectins and JAMs is unclear. Nectin engagement seems to activate Rho GTPases such as Cdc42 and Rac in a PI3K independent manner [1]. Investigating the potential connection between Nectins, JAMs and the PKC pathways could open new ways to understand the regulation of cell junction formation.

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