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Research Article

Polycystins and intercellular mechanotransduction: A precise dosage of polycystin 2 is necessary for alpha-actinin reinforcement of junctions upon mechanical stimulation

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ABSTRACT

Polycystins 1 and 2, which are mutated in Autosomal Polycystic Kidney Disease, are involved in mechanotransduction through various mechanisms. In renal cells, polycystins not only have an important mechanotransductive role in primary cilia but are also present in intercellular contacts but their role there remains unclear. Here, we address the hypothesis that polycystins are involved in mechanotransduction *via* intercellular junctions, which would be expected to have consequences on tissue organization. We focused on the role of polycystin 2, which could be involved in mechanical organization at junctions either by its channel activity or by the direct recruitment of cytoskeleton components such as the F-actin cross-linker α-actinin. After mechanical stimulation of intercellular junctions in MDCK renal epithelial cells, α-actinin is rapidly recruited but this is inhibited upon overexpression of PC2 or the D509V mutant that lacks channel activity, and is also decreased upon PC2 silencing. This suggests that a precise dosage of PC2 is necessary for an adequate mechanosensitive α-actinin recruitment at junctions. At the multicellular level, a change in PC2 expression was associated with changes in velocity in confluent epithelia and during wound healing together with a loss of orientation. This study suggests that the mechanosensitive regulation of cytoskeleton by polycystins in intercellular contacts may be important in the context of ADPKD.

1. Introduction

Epithelia form highly cohesive structures with intercellular contacts, such as adherens junctions, tight junctions and desmosomes, that need to be tightly regulated so that cell-cell adhesion is maintained despite fluctuations in the forces that originate from each cell. At a higher time-scale, this regulation allows the morphological and functional adaptation of the epithelia to mechanical stress arising from the environment or morphogenetic movements. In adherens junctions, homophilic contacts between cells are ensured by E-cadherins, which are indirectly coupled to the F-actin cytoskeleton through β-catenin/α-catenin links [1,2] with F-actin bundles cross-linked primarily by vinculin and α-actinin. Adherens junctions experience forces originating from neighboring cells due to either the contraction of actomyosin at steady-state or the reorganization (*e.g.* division) of these adjacent cells [3]. Mechanotransduction in junctions relies on a strong interconnection between the cadherin/catenin system and the actomyosin system, with α-catenin–vinculin as a central functional module. The application of a force causes α-catenin to stretch, which leads to the exposure of a cryptic site and its binding to actin-bundling proteins, in particular vinculin [4–9] or α-actinin as more recently described [9]. The recruitment of these complexes reinforces cell-cell junctions, due to either the bundling activity of vinculin/α-actinin or the subsequent recruitment of other factors that remodel the actin cytoskeleton such as zyxin, vasodilator-stimulated phosphoprotein (VASP) or Arp2/3 [6].

The aim of this study is to address whether putative mechanosensitive channels are also important for mechanotransduction in intercellular contacts. Interestingly, cation transient receptor

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potential (TRP) channels [10] are involved in mechanotransduction in focal contacts: the ultra-fast activation of mechanosensitive TRPV4 channels by integrins in endothelial cells leads in a retroactive loop to additional integrin recruitment, and mediates cyclic strain-induced endothelial reorientation [11]. Some TRP channels have been reported to be both mechanosensitive and present at intercellular contacts: in particular, complexes comprising polycystin 1 (PC1) and polycystin 2 (PC2). The mechanosensitivity of polycystins in the primary cilia strongly suggests a similar role in adherens junctions, where they are found within a complex with cadherins and catenins [12].

PC1 and PC2 are mutated in the most common renal genetic disease Autosomal Dominant Polycystic Kidney Disease (ADPKD), which leads not only to progressive renal insufficiency in adulthood as a result of the development of numerous renal cysts, but also in the disorganization of other tissues (*e.g.* hepatic cysts, cerebral aneurysms). PC2 is a cation non-selective TRP channel with a complex gating mechanism that is dependent on phosphatidylinositol 4,5-bisphosphate (PIP_2) binding and interactions with protein partners, and has been reported to be mechanically gated in some cellular systems. PC2 can form a complex with PC1, a transmembrane protein comprising a very large extracellular loop with multiple functional domains [13]. PC1 and PC2 are present in a variety of subcellular localizations (in particular, primary cilia, intercellular and basal contacts and, for PC2, the endoplasmic reticulum) where they establish links with numerous cytoskeleton and adhesion proteins. For example, PC1 forms complexes with α2β1 integrin, tropomyosin, troponin 1, talin, vinculin and α-actinin at focal contacts [14]. PC2 binds to mDia, a Rho GTPase effector protein [15], and to the F-actin cross-linker α-actinin [16]. Polycystins are also coupled to several signaling pathways (mTOR, JAK/STAT, PI3K/Akt, G proteins). *In vitro* loss-of-function or the overexpression of polycystins have major effects on tissue organization including proliferation, apoptosis, tubulogenesis, planar polarity and the control of cell size [17–21].

The response of the polycystin complex to mechanical stimuli involves very different mechanisms depending on the cellular system [22]. In primary cilia of renal epithelial cells, the bending of primary cilia induced by extracellular flow was classically proposed to lead to direct calcium entry, requiring both PC1 and PC2 possibly acting as a "primary mechanosensor" [23]. However, this role for calcium-responsive mechanosensing by primary cilia has recently been debated [24]: a detailed imaging analysis with high temporal resolution suggested that physiological or supraphysiological mechanical stimuli (bending of cilia with flow) did not elicit a fast calcium response in cilia, which is in contrast with the prevailing view. Polycystins might thus have another mechanosensitive role in cilia that is not linked to PC2 channel function; alternatively, they could have a mechanosensory role in other cell substructures, related (or not) to their channel activity. (Indeed in cilia, a slow increase of calcium influx from the cytoplasm was observed after 10–20 s of mechanical stimulation [24]). On the other hand, an entirely different mechanism for the mechanosensitive properties of the polycystin complex was reported in vascular smooth muscle, in which the PC1/2 ratio was shown to determine pressure sensing: stretch-activated channels are inhibited by PC2 *via* its effects on cytoskeletal organization (filamin), and PC1 releases this inhibition [25]. Polycystins have also been implicated in bone development and mechanosensing [26–28]. It is thought that the tissue defects observed in ADPKD kidneys might result from aberrant mechanosensing. In healthy tissue, some signaling pathways (such as mTOR, STAT6) are activated only in situations such as wound healing, which generate abnormal flow and would be detected by bending of the primary cilia through the mechanosensory role of polycystins. However in ADPKD, the defective mechanosensitive behavior of polycystins would lead to constitutive activation of these pathways [29].

We hypothesized that the mechanosensor activity of polycystins is not only important in primary cilia, but also in intercellular contacts. PC1 is present in tight junctions but is even more abundant in adherens junctions and in desmosomes where it associates with intermediate filaments. In renal tissue, PC1 is present in a complex containing E-cadherin and α-, β- and γ-catenins [12], and it can bind to cytoskeleton proteins such as mDia, vinculin and α-actinin. This link with cadherin is physiologically important because in ADPKD renal epithelium, E-cadherin is improperly sequestered in intracellular pools [30] and shows an increased shedding [31]. PC1 accelerates the recruitment of E-cadherin to reforming cell-cell contacts in Ca^{2+} switch experiments in MDCK cells [32]. The overexpression of PC1 in MDCK cells leads to a scattered morphology with actin reorganization and an increased migration, altered behavior in wound-healing experiments, and a decrease in cell-cell adhesion in confluent monolayers [33]. Therefore, PC1 seems to have a key role in the adhesive properties of epithelia. The role of PC2 is less well documented; interestingly, in MDCK cells it is localized at lateral junctions only in confluent or near-confluent cells, and its localization between the membrane and the cytoplasm is reorganized during wound-healing experiments [34].

PC2 could be important in intercellular contacts because of its ability to either form a mechanosensitive channel or recruit cytoskeleton molecules. In this study, we specifically focused on the link between PC2 and α-actinin, which is present in adherens junctions and has been shown to bind to PC2 and modulate its activity [16]. α-actinins belong to the spectrin family and are abundant in muscle (isoforms 2, 3) and non-muscle cells (isoforms 1, 4). They comprise an N-terminal actin-binding domain, a central domain with spectrin-like repeats (which can bind to α-catenin and vinculin, and is sufficient for junctional targeting [35]) and a C-terminal calmodulin-like domain with 2 EF-hands (that confers calcium and phosphoinositide regulation) [36]. Ubiquitous isoforms 1 and 4 are involved in calcium-dependent membrane attachment in adherens junctions and focal contacts: two identical molecules associate in a head-to-tail manner to bundle F-actin filaments, and calcium binding leads to the release of an actin monomer from their actin-binding domain. α-actinin also binds to some phospholipids, leading to a decrease in its F-actin bundling activity through competition [37]. Finally, α-actinin interacts with a number of cytoskeletal and membrane proteins, including Kv channels [38],zyxin [39-40], integrins [41], catenins [42], the NMDA receptor [43], as well as PC2 [16]. Both the N-terminal domain (residues 1–215) and the C-terminal domain (residues 821–878) of PC2 bind to α-actinin (isoforms 1 and 2) in a calcium-independent manner [16]. These domains directly contact the spectrin repeats and the C-terminal part of α-actinin but not its N-terminal actin-binding domain [16]. The direct interaction between these two proteins has a functional effect because in lipid bilayers with reconstituted PC2, α-actinin overexpression strongly increases single channel currents [16] in a calcium-dependent manner [44].

Similar to other cytoskeletal proteins such as vinculin and paxillin, α-actinin is recruited upon mechanical stimulation of focal contacts. This recruitment is dependent on zyxin targeting to stress fibers and is crucial for actin remodeling [45,46]. The kinetics of α-actinin binding was recently shown to be involved in cell dynamics and force generation [47]. At a multicellular level, changes in α-actinin levels are associated with changes in motility and invasive capacities, and have been linked to a variety of cancers [48–52].

The role of α-actinin mechanosensing during junction maturation has recently been studied [9]. A mechanical force induced actin- and α-actinin-4 accumulation at junctions in a time- and