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#### COMMENTARY 8 OPEN ACCESS

## P-cadherin-mediated Rho GTPase regulation during collective cell migration

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#### **ABSTRACT**

This commentary addresses the role of P-cadherin in collective cell migration (CCM), a cooperative and coordinated migration mode, used by cells during normal and pathological migration processes. We discuss how cadherin-mediated cell-cell junctions (CCJs) play a critical role in CCM through their ability to regulate Rho GTPase-dependent pathways and how this leads to the generation and orientation of mechanical forces. We will also highlight the key function of P-cadherin (a poor prognostic marker in several tumors) in promoting collective cell movement in epithelial and mesenchymal cells.

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β-PIX; Cdc42; collective cell migration; mechanical forces; P-cadherin; polarity; Rac1

#### Introduction

Tissue modeling, repair, wound healing, cell invasion and metastasis formation require CCM.<sup>14</sup> This process, whereby each single cell within a group communicates with its neighbors, allows the orchestrated movement of cells toward specific locations. To achieve this, cells need to form a cohesive tissue through physical interactions and force transmission between cells and the surrounding substrates. Adhesion and force transmission between cells is mediated by proteins of the cadherin superfamily, whereas adhesion and force transmission to the extracellular matrix components is mediated via integrin receptors.

Cancers of epithelial (carcinomas) and mesenchymal (sarcomas) origin use CCM to invade surrounding tissues. Indeed, rhabdomyosarcoma (a tumor of muscle origin), breast cancer, colorectal carcinoma and melanoma show collective cell behavior. Most of the mortality associated with solid tumors is the final result of CCM (*i.e.*, tumor cell invasion and metastasis formation). Therefore, targeting the biomechanical pathways leading to CCM might be an efficient therapeutic strategy to block cancer progression.

During the last decade, efforts have been made to understand the molecular and physical mechanisms involved in CCM. However, to fully capture CCM biomechanical mechanisms is challenging due to the complexity of the biological systems. Indeed, collectively migrating cells during embryo development or tumor invasion are heterogeneous and complex structures that are the result of the crosstalk between different cell types and their surrounding environment. To efficiently tackle all the complex biological questions concerning CCM, robust and reproducible in vivo and in vitro experimental models have been developed. For instance, lateral line migration during zebrafish development, neural crest migration during chicken neurogenesis and border cell migration in the fly egg chamber are good in vivo models for understanding CCM mechanism. On the other hand, in vitro models (i.e., organotypic culture systems, wound healing-like and 3-dimensional invasion assays) allow the fast study of specific aspects of the underlying molecular processes and the high resolution imaging of specific cellular events. We took advantage of a reproducible in vitro directional migration assay in which cells are allowed to migrate in the direction perpendicular to the free edges after removal of a physical barrier to study the biomechanical pathways leading to CCM upon P-cadherin expression. We performed quantitative analysis of cell movement, cell organization and mechanical parameters using time-lapse, confocal and Föster resonance energy transfer imaging and traction force microscopy. Our study shows that P-cadherin

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specifically induces CCM when expressed in myoblasts. We then demonstrated that P-cadherin recruits the guanine exchange factor (GEF)  $\beta$ -PIX that allows Cdc42 activation. This signaling cascade leads to massive reorganizations, from the polarization of cells, membrane protrusions and focal adhesions (FA) to the global collective movement of the entire cell monolayer. Mechanically, the P-cadherin/β-PIX/Cdc42 axis drives CCM by increasing the intercellular stress through a physical process called plithotaxis and promotes the strength and orientation of traction forces in the migration direction<sup>39,44</sup> (Fig. 1).

# Cadherins in CCM of epithelial and mesenchymal

Cadherins are a central CCJ component and major CCM drivers. 16 There are 5 main type-1 classical cadherins in mammals: E, M, N, P and R-cadherin. E, N and P-cadherin have been involved in CCM in different models, whereas R-cadherin and M-cadherin do not seem to contribute to CCM. However, we can easily imagine that depending on the cell system, the cadherin type involved in CCM could be different. The tissue anatomy and peripheral microenvironment geometry also could influence the cadherin type involved in CCM. For instance, N-cadherin regulates CCM of MDCK cells in 3D, but not in 2D environments.<sup>37</sup> CCM is observed in both epithelial and mesenchymal cells, but the involved cadherins are different. Specifically, E-cadherin plays a role exclusively in CCM of epithelial cells, while N-cadherin regulates CCM of mesenchymal cells. On the other hand, P-cadherin is involved in CCM of both cell types.<sup>2,25,33</sup>

#### **CCM** of epithelial cells

A specific feature of epithelial cells is that they maintain stable CCI during CCM, as observed during carcinoma ductal invasion through E- and P-cadherin, 12,39 or during angiogenesis and tubular ramification through VEcadherin (a type-2 cadherin).<sup>32</sup> In most of these CCM models, epithelial cells show highly directional movement and E-cadherin inhibition increases randomness. For instance, in vivo studies on D. melanogaster border cell migration in the ovary have demonstrated that collective movement, cell cohesion, directionality and mechanical sensing are controlled through E-cadherin engagement.<sup>5,34</sup> For years, E-cadherin was considered to be the main cadherin involved in CCM of epithelial cells. However, recently P-cadherin emerged as an additional key player. P-cadherin depletion in epithelial cells

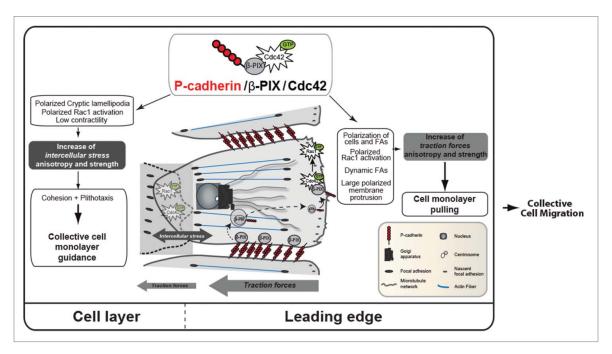


Figure 1. P-cadherin expression induces CCM. P-cadherin expression promotes a mechanical tug-of-war. Indeed, P-cadherin expression is associated with increased intercellular stress anisotropy and strength that promote collective cell guidance, called plithotaxis. P-cadherin expression also increases traction-force anisotropy (by increasing the Tx/Ty ratio that is the ratio between the traction forces parallel to the direction of migration (Tx) and the traction forces perpendicular to the direction of migration (Ty)) and strength that pull the cell layer. P-cadherin expression activates CDC42 through the GEF  $\beta$ -PIX. This generates biological responses, such as polarization of the cell layer, of RAC1 activity, cryptic lamellipodia and FAs in the migration direction, polarized membrane protrusions and FA dynamics, thereby controlling mechanical force anisotropy and strength.



impairs drastically CCM in both 2D and 3D in vitro culture systems. 25,26 Our collaborators Bazellières and colleagues showed that during CCM of epithelial cells, "while P-cadherin predict the level of intercellular force, E-cadherin predicts the rate at which intercellular force builds up," suggesting, for the first time, a real mechanical function of P-cadherin in force transmission within the cell monolayer.<sup>2</sup>

#### **CCM** of mesenchymal cells

CCM of mesenchymal cells also requires cadherinmediated CCJs. 41 Mesenchymal cells that originate from epithelial to mesenchymal transitions during development or tumor progression undergo cadherin switching from E- to N-cadherin or to other cadherin types. 42,48 This cadherin switch is associated with increased cell migration and invasion. P-cadherin is up-regulated in invasive alveolar rhabdomyosarcoma (a tumor of mesenchymal origin). When expressed in myoblasts, P-cadherin promotes a cadherin switch, inducing their transformation, migration and invasion.<sup>43</sup> By examining in details the migration parameters of P-cadherin-expressing myoblasts we found that P-cadherin promotes CCM very efficiently.<sup>33</sup>

CCM of mesenchymal cells is associated with highly dynamic CCJs, as recently shown for N-cadherin during astrocyte collective migration.<sup>31</sup> In P-cadherinexpressing myoblasts, cells within the monolayer maintain low cell-cell interaction by forming cryptic lamellipodia, membrane extensions seen in front of cells that migrate collectively and extending underneath the cell behind. Similarly, some studies showed that cryptic lamellipodia formation is essential for collective movement of epithelial cells.<sup>8,11</sup> This means that in epithelial and mesenchymal cells, dynamic CCJs are required for efficient CCM. Indeed, cells migrating as a collective tissue move more continuously and persistently. 49 Moreover, CCM is associated with increased polarity, which is a key process in CCM.<sup>7,30</sup> Cadherins are considered to be important regulators of polarity. In astrocytes, N-cadherin-mediated CCJs directly regulate the polarity pathways, leading to FA organization at the leading edge of migrating cells and strengthening the persistence and orientation of the migrating cell monolayer.<sup>6,10</sup> We found that P-cadherin (but not E- or R-cadherin) expression in myoblasts induces a strong polarization of cells and their trajectories, of their membrane protrusions and cryptic lamellipodia at the front of migrating cells across the layer, and of their FAs at the leading edge and in the monolayer.<sup>33</sup>

#### Cadherin-dependent mechanical coupling

CCM occurs through the generation and transmission of physical forces that drive cellular movement. Two different force types are important in CCM: traction forces that are exerted on the cell substrate through FA modules<sup>9,45</sup> and intercellular forces transmitted between neighboring cells through CCJs.  $^{21,22,36,39,45,46}$ In the last years, several groups have been working on the identification of the molecular mechanisms that govern force transmission between cells, together with the activation of contractility and the crosstalk to cell substrate adhesion molecules.<sup>2,5,25,47</sup> These studies highlight the key function of different proteins, particularly cadherins and integrins, in force transmission.

#### Cadherin role in inter-cellular force generation and transmission at CCJs

Cadherins, particularly E- and N-cadherin, regulate the physical properties of cell monolayers by transducing mechanical forces between the actomyosin cytoskeleton and the plasma membrane of neighboring cells.3,5,20 Bazellières et al have shown that during CCM of epithelial cells, P-cadherin allows force transmission through the entire cell monolayer.<sup>2</sup> By using magnetic tweezers, they could confirm that E-cadherin and P-cadherin, when co-expressed, play different mechanical roles. E-cadherin strengthens cell adhesion and P-cadherin regulates the amount of tension that a FA can transmit. Furthermore, they demonstrated that when E-cadherin is knocked down, P-cadherin can take over its role as a tension regulator. In myoblasts, only P-cadherin expression increases the intercellular stresses within the cell monolayer, while E- and Rcadherin co-expression does not.33 All these data clearly indicate that E-cadherin is not the only cadherin involved in the regulation of intercellular tension, and that P-cadherin also should be taken into account to understand how forces are generated and transmitted within a cell monolayer.

Polarization of the cell cytoskeleton, organelles and forces is a crucial step for directed and efficient CCM. CCJs play a crucial role in inducing coordinated migration by allowing the alignment of intercellular forces with their velocity, a phenomenon referred to as plithotaxis. 8,36,39,44 However, the identity of the proteins that lead to this mechanical polarized behavior is still under investigation. As explained above, P-cadherin expression in myoblasts strongly affects cell polarity. P-cadherin also increases the anisotropy of intercellular forces across cell-cell junctions as well as

plithotaxis, which allows the efficient translocation of the entire cell layer.<sup>33</sup> Thus, P-cadherin expression not only polarizes cells toward the migration direction, but also extensively polarizes intercellular stresses.

#### Cross-talk between intercellular and cell matrix forces and generation of traction forces

The architecture and polarity of a cell monolayer are maintained thanks to the crosstalk and mutual inhibition of CCJs and FAs. Interestingly, Mertz el al., using an in vitro model, demonstrated that E-cadherin has a key role in the reorganization and relocalization of traction forces around the cell cluster, and highlighted the existence of a bi-directional feedback loop between intercellular and cell-matrix forces.23

Theoretical studies suggest that single cells within the monolayer tend to align their traction forces and that cell-substrate traction may play a role in polarizing neighboring cells in the same direction.<sup>1,15</sup> Furthermore, Zaritsky et al. demonstrated that a subpopulation of cells within the monolayer transmit mechanical cues by inducing normal stress (i.e., traction/compression forces) on follower rear cells and shear stress (i.e., parallel stress) on neighboring cells on their side.<sup>51</sup> Propagation of this cell-cell mechanical communication over time and space results in group of cells that migrate and exert forces in a coordinated and polarized manner. Taken together these theoretical studies strongly support the notion that cellcell crosstalk can feedback with cell-substrate signaling to promote polarized CCM.

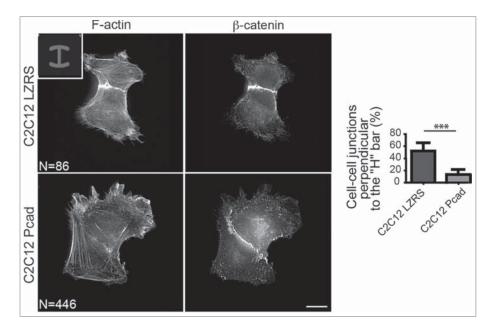
In our study, we used an in vitro system to study P-cadherin contribution to CCM physical properties. We demonstrated that P-cadherin expression in myoblasts induces a highly oriented and directed CCM, a phenotype that was never observed upon expression of other cadherins, such as E- and R-cadherin. The reorientation of FAs and cell protrusions in the first row of Pcadherin-expressing cells in the monolayer led us to investigate the mechanical properties of these cell monolayers using traction force microscopy and monolayer stress microscopy, a technique based on the principle that, according to Newton's laws, traction forces applied at the cell-gel interface must be balanced by intra- and intercellular forces.<sup>22,45</sup> We found that traction forces and intercellular stress anisotropy were increased in Pcadherin-expressing cells. Specifically, cells at the leading edge could generate more traction thanks to FA remodeling, leading to an increase in the pulling forces on the follower cells. P-cadherin, which is localized at CCJs, then propagates the tension toward the entire monolayer, leading to an increase of intercellular tension. In our model, progression of P-cadherin expressing cells is achieved through the development of traction forces that counterbalance the intercellular stresses and drive the CCM of the cell layer (Fig. 1). Our data confirm the predictive theory of soft active matter according to which high polarization and high viscosity/friction (i.e., traction exerted on the cell substrate) would lead to rapid and cohesive migration in the presence of high intercellular stresses. Our study demonstrates that P-cadherin has a major role in intercellular force generation and polarization to promote cell guidance, and also in traction force generation at the extracellular matrix to drag the cell layer.

#### Cadherins signal to Rho GTPases during CCM

Cell-cell adhesion molecules of the cadherin family are important for CCM, but insights into how they regulate multicellular migration and into the involved signaling pathways are only beginning to emerge. GTPases of the Rho family, which are well-known regulators of adhesion and migration dynamics, appear to be key actors of cadherin-mediated CCM. In mammals there are 21 Rho family members, but so far, only RhoA, Rac1, Cdc42 and RhoE have been identified as key regulators of CCM, as reviewed in ref. 52. However, it is not precisely known how cadherin-mediated cell-cell adhesion affects Rho GTPase activity. Our recent work brings some clues.

We found that in myoblasts (cells of mesenchymal origin), P-cadherin (but not E-cadherin or R-cadherin) expression specifically activates Cdc42 during CCM.<sup>33</sup> Pcadherin-mediated Cdc42 activation requires cadherin homodimer formation and promotes the polarized cell organization (i.e., polarization of cells, membrane protrusions and FA in the migration direction) and the polarization of mechanical forces to allow CCM. Our work in myoblasts not only confirmed CDC42 main role in CCM, which was first identified by Alan Hall and Sandrine Etienne-Maneville and collaborators using several cell systems, but also identified a new P-cadherin/Cdc42 connection. Using a scratch-induced fibroblast migration assay, Cau et al reported that Cdc42 controls the polarization of both membrane protrusions and the Golgi/centrosome.<sup>7</sup> In astrocytes, N-cadherin-mediated cell-cell adhesion controls cell polarization. Reduced N-cadherin expression in tumor glial cells is associated with loss of Cdc42 and cell polarity and increased cell velocity.<sup>6,10</sup> Finally, our work shows that P-cadherin-mediated Cdc42 activation coordinately controls at least 2 major pathways during CCM: cell polarity and the actin cytoskeleton dynamics.

Cdc42 not only activates Rac1 through a mechanism that remains to be identified, but it is also responsible for



**Figure 2.** P-cadherin expression decreases cell contractility as indicated by intercellular junction positioning. Immunostaining of  $\beta$ -catenin and F-actin in control (C2C12 LZRS: C2C12 cells expressing only the empty vector) and P-cadherin-expressing cell doublets plated on H-shaped micropatterns. Most of control C2C12 myoblasts (82%) have intercellular junctions that are perpendicular to the H bar. Conversely, the intercellular junction position and orientation are strongly perturbed upon P-cadherin expression. Indeed, junctions that are perpendicular to the H bar, like in control cells, are observed only in 11% of P-cadherin-expressing cells. For all panels, the mean  $\pm$ SEM is shown; \*\*\* P < 0.0005.

the spatial localization of Rac1 activity, a process observed also in vivo during CCM of anterior visceral endoderm cells.<sup>28</sup> Rac1 activation is required for CCM, as demonstrated both in vitro and in vivo (reviewed in ref. 52). However, its activity must be polarized to promote oriented protrusion activity and migration, because isotropic Rac1 activation is inefficient. Moreover, Arf6-dependent membrane trafficking is required for the polarized recruitment of Rac1 and of the PAR6-aPKC polarity complex at the front edge of migrating cells.<sup>29</sup> Recently, using epithelial MDCK cells undergoing CCM, Das et al demonstrated that leading cells pull the membrane of follower cells, leading to redistribution of the cytoskeletal protein Merlin from CCJs to the cytoplasm, thus allowing the activation of polarized Rac1.8 Using Föster resonance energy transfer measurement of Cdc42 and Rac1 activities, we demonstrated that their spatio-temporal activity is dynamic during CCM. Specifically, these 2 GTPases are strongly activated at the cell front of migrating cells in the whole monolayer and also at the lateral cell-cell contact sites. This polarized Rac1 activation is required for the formation of protrusions and cryptic lamellipodia, which most probably contribute to the increase in traction forces. 35,38,40

Activation of most Rho GTPases is controlled by guanine exchange factors (GEF) that promote GTPloading in response to external cues. We identified the GEF  $\beta$ -PIX as a P-cadherin partner required for P-cadherin-mediated Cdc42 activation

polarization, mechanical force generation and polarization and CCM.  $\beta$ -PIX also controls CCM of anterior visceral endoderm cells during early mouse embryogenesis. <sup>28</sup> During CCM of astrocytes,  $\beta$ -PIX is detected at CCJs, the cell front and in intracellular vesicles. 6,29,30 At the cell front,  $\beta$ -PIX negatively regulates FA maturation and promotes protrusion formation and FA turnover to allow cell migration.<sup>19</sup> We detected  $\beta$ -PIX at the cell front and in intracellular vesicles of P-, E- or R-cadherin-expressing cells. Conversely,  $\beta$ -PIX was localized at CCJs only in P-cadherin-positive cells and during CCM. Because tension generated at cadherin adhesion sites is an important process for protein recruitment at CCJs, 4,50 the higher intercellular stress generated by P-cadherin during CCM, compared with E- or R-cadherin, 2,33 might be responsible of  $\beta$ -PIX recruitment. Finally, in P-cadherin-expressing cells we did not detect any global change in RhoA or ROCK activities.<sup>33</sup> Conversely we could show using cell doublets on an H-shaped micropattern<sup>46</sup> that P-cadherin locally decreases cell contractility (Fig. 2). This is in agreement with previous studies showing that RhoA/ROCK-mediated contractility decreases to allow efficient CCM. 18,27

#### **Concluding remarks**

CCM provides an effective strategy for transporting group of cells to a new location for colonization, for



instance during embryo development, but also during tumor cell invasion, thus facilitating metastasis formation.<sup>12</sup> Elucidating CCM mechanisms, particularly the role of cell-cell adhesion molecules that play key roles in multicellular migration, will not only improve our understanding of normal development, but will also provide insights on how to target and slow down pathological cell migration, thereby improving current strategies for suppressing tumor cell invasion and metastasis formation.

By comparing the behavior of myoblasts that express different cadherin types, we found that P-cadherin is a very efficient pro-migratory molecule, compared with Eor R-cadherin. P-cadherin is expressed in rhabdomyosarcoma, an invasive sarcoma of skeletal muscle origin, and in some invasive carcinomas. Targeting P-cadherin is probably an interesting strategy to control metastasis formation in patients with cancers in which P-cadherin expression is upregulated. A human monoclonal antibody against P-cadherin showed anti-tumor and antimetastatic activity in different P-cadherin overexpressing tumor models.53

#### **Abbreviations**

CCJ cell-cell junction

CCM collective cell migration

FA focal adhesion

**GEF** guanine exchange factor

#### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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