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Polarity proteins regulate mammalian cell–cell junctions and cancer pathogenesis

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The epithelial cells of multicellular organisms form highly organized tissues specialized for the tasks of protection, secretion, and absorption, all of which require tight regulation of the core processes of cell polarity and tissue architecture. Disruption of these core processes is a critical feature of epithelial tumors. Cell polarity and tissue architecture are intimately linked, as proteins controlling cell shape are also responsible for proper localization and assembly of cell–cell junctions and three-dimensional tissue organization. The extracellular matrix underlying epithelial tissues supports tissue architecture and suppresses malignant growth through regulation of cell adhesion and activation of protective signaling cascades. Emerging evidence is uncovering the mechanisms by which polarity pathways alter the way epithelial cells organize and interact with the tissue microenvironment to promote aberrant growth and invasion during tumorigenesis. We discuss how cell polarity pathways regulate cell–cell junctions and highlight the new insights gained by investigating the role played by polarity pathways during the transformation of epithelial cells.

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Introduction

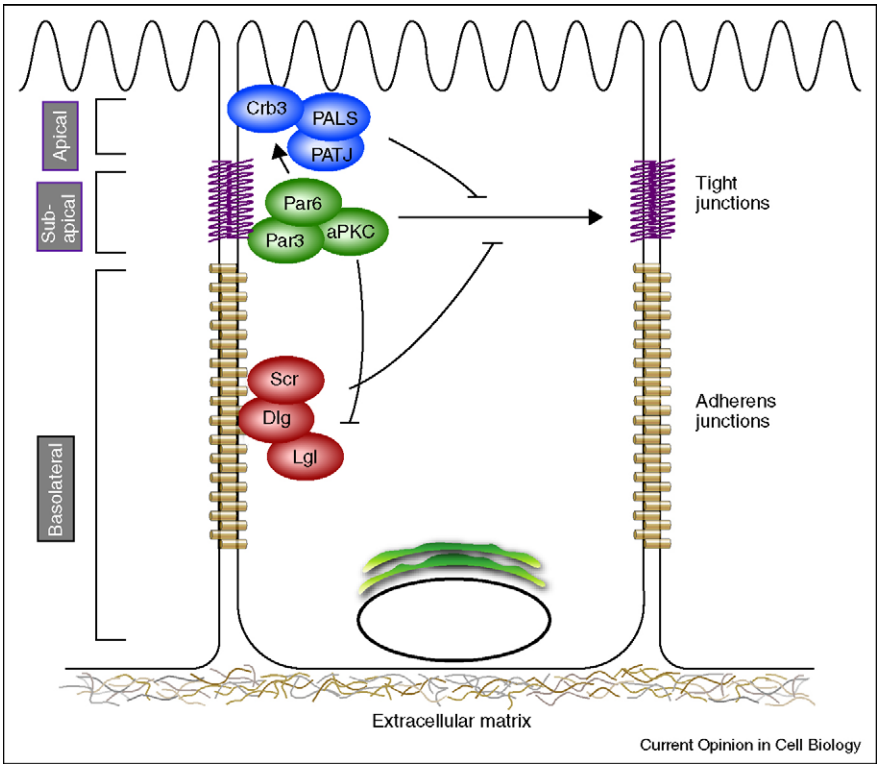
Normal epithelial cell structure and organization is lost early during tumorigenesis. We are far from developing an understanding of the molecular mechanisms by which cell and tissue structure is lost during carcinogenesis; however, we are beginning to understand how epithelial cells establish structure and undergo morphogenesis during development. Cell–cell adhesions play critical roles during establishment and maintenance of cell structure and tissue organization and hence understanding how they are regulated is likely to provide novel insights into the mechanisms by which cell and tissue structure is lost in carcinoma.

Epithelia in glandular structures contain an apical membrane that faces the lumen and a basolateral surface that interacts with the neighboring cells and the basement membrane. This asymmetric organization is referred to as apical–basal cell polarity and is a characteristic trait of epithelial cells. Cell–cell adhesions are mediated by different types of junctional complexes, including tight junctions (TJ), adherens junctions (AJ), gap junctions, and desmosomes. These junctions comprise transmembrane proteins with extracellular domains that mediate interactions between neighboring cells and intracellular surfaces that facilitate interaction with signaling molecules and cytoskeletal proteins. In polarized epithelial cells, the junctional complexes are asymmetrically localized. For example, TJ are located at the apical–basal border and act to separate the apical and basolateral membrane domains, hold adjacent cells together and create an impermeant fluid barrier between cells [1]. AJ are located basal to the TJ and are considered as primary determinants of cell–cell adhesion [2]. The mechanisms by which cells develop cell–cell junctions and localize proteins to create the intracellular asymmetry are an active area of investigation. Most of our understanding of the molecular mechanisms by which cell polarity is established and maintained stems from genetic studies in model organisms and biochemical studies in cultured epithelial cells. This review will focus on how cell polarity pathways regulate the establishment and maintenance of cell–cell junctions and highlight the new insights gained on initiation and progression of carcinoma by investigating the role played by polarity pathways during transformation of epithelial cells.

Cell junctions and polarity pathways

The spatially asymmetric localization of these junctional complexes is mediated by an evolutionarily conserved class of proteins that are herein referred to as polarity proteins [3]. Functional analysis of the polarity determinants in a broad array of model organisms has resulted in their placement into three functional groups: the Crumbs complex, the Scribble complex, and the Par complex. The apical domain is specified by the Crumbs complex, which is made up of the transmembrane protein Crumbs (Crb) and intracellular signaling adaptors PALS1 (Protein associated with lin-7) and PATJ (PALS1-associated TJ protein) [4]. The basolateral domain is thought to be specified by the Scribble complex, consisting of signaling

Figure 1



A simplified view of polarity protein complexes. The figure depicts subcellular localization of polarity proteins along the apical–basal axis of polarized epithelia and positive (arrows) and negative (blunt head) interactions between the polarity complexes.

adaptors Scribble (Scr), Discs large (Dlg), and Lethal giant larvae (Lgl) [5]. The subapical domain that defines the apical–basal border is specified by the Par (Partitioning defective) complex, which consists of Par3, Par6, atypical protein kinase C (aPKC), and Cdc42 [6]. These protein complexes cross-regulate each other during epithelial polarization. For example, in addition to its role in regulating TJ formation, Par6 interacts with the Crumbs complex [7•] and aPKC negatively regulates Lgl [8•,9•,10•] (Figure 1).

Cell polarity proteins regulate tight junctions

TJ are composed of transmembrane proteins such as occludins and claudins and intracellular proteins such as Zonula occludens 1 (ZO-1) that coalesce apical to AJ and seal the spaces between neighboring epithelial cells, separate apical and basal membrane domains, and interact with the cytoskeletal network [1]. The Par3/Par6/aPKC complex localizes to mammalian TJs and is required for TJ assembly and maintenance [11,12]. Overexpression of Par6 in Madin-Darby canine kidney (MDCK) epithelial cells disrupts the localization of Par3 at cell–cell adhesion sites and alters TJ structure as measured by mislocalization of the TJ marker ZO-1 [12]. Par3 mediates its effects on TJ formation through a direct interaction with junctional adhesion molecule (JAM), a TJ component that associates with ZO-1 [13]. Par3 localizes to the apical region of TJs and overexpression of Par3 in MDCK cells leads to rapid onset of transepithelial electrical resistance (TER), a hallmark of TJ function [14]. In addition, loss of Par3 disrupts TJ formation, and rescue experiments provided evidence that Par3 coordinates TJ assembly through its C-terminus, independently of interaction with Par6, JAM and aPKC [15]. However, the inhibition of aPKC activity, through the expression of a dominant negative aPKC mutant, leads to aberrant redistribution of Par3 and ZO-1 during TJ assembly [16], suggesting that aPKC is required for TJ formation.

The nature and identity of targets of the Par complex that are required for TJ assembly are only beginning to be understood. The C-terminal region of Par3 binds directly to the Rac guanine nucleotide exchange factor (GEF), Tiam1. Loss of Tiam1 alone disrupts TJ formation in epidermal keratinocytes [17]. While Tiam1 is not required for the development of primordial junction complexes, it is required for the maturation of TJ in a Rac1 activation dependent manner [17]. It is likely that Par3 localizes Tiam1 to sites of developing cell–cell adhesions and activates Rac to promote TJ maturation [15]. Par3 also regulates actin dynamics at the TJ through inhibition of LIM kinase 2 (LIMK2) activity, which leads to the activation of cofilin and promotion of TJ formation [18].

Phosphorylation of Par complex components plays critical roles in the regulation of TJ maintenance. Activation of epidermal growth factor receptor (EGFR) signaling leads to the phosphorylation of Par3 by c-Src and c-Yes and subsequent dissociation of the Par3/LIMK2 interaction [19]. The protein phosphatase PP2A accumulates at TJs, binds to aPKC, and inhibits aPKC activity, leading to impaired TJ assembly [20]. Similarly, the protein phosphatase PP1 associates with Par3 and regulates the Par3/aPKC interaction [21]. These results underscore the possibility that signaling pathways that alter TJ dynamics interact with and post-transcriptionally modify the Par polarity proteins.

The Par complex also interacts with other polarity complexes. Expression of Crb3 in a human mammary epithelial cell line, MCF-10A, which lacks TJs, induces *de novo* TJ formation, highlighting the importance of this complex in TJ assembly [22]. Crb3 can interact either directly with Par6 or indirectly through PALS1 [7•,23]. Expression of a dominant negative PATJ in MDCK cells led to redistribution of PALS1 and aPKC away from TJs [7•]. Par complex proteins also interact with the basolateral polarity proteins. For example, aPKC phosphorylates and inactivates Lgl [8•,9•,10•] and Lgl is required for the disassembly of Par3–Par6–aPKC complex in remodeling epithelia [24]. Thus, polarity proteins form an intricate signaling system within epithelial cells to assemble and maintain TJ integrity.

Cell polarity proteins regulate adherens junctions

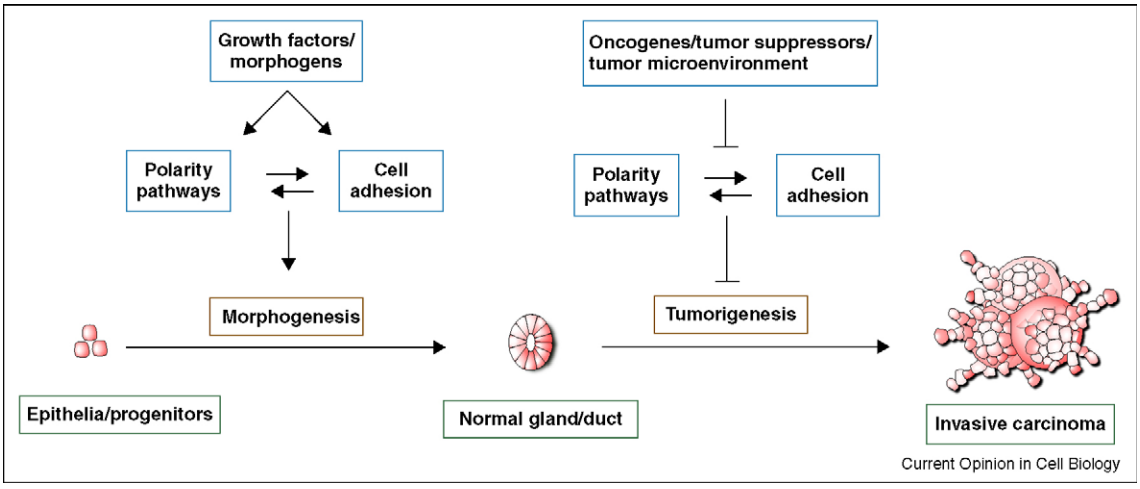
The role polarity proteins play during AJ biogenesis and function is only beginning to be understood. Several polarity proteins have been implicated in AJ formation and maintenance through regulation of E-cadherin. The basolateral polarity protein Scr is recruited to AJs in an E-cadherin-mediated manner, and loss of E-cadherin in human colorectal adenocarcinoma Caco-2 cells results in Scr mislocalization [25]. Loss of Scr decreased cell adhesion in a cell aggregation assay, although no changes were observed in levels of cellular E-cadherin. However, cells lacking Scr were deficient in binding to tissue culture plates coated with the extracellular domain of E-cadherin, suggesting a role for Scr in E-cadherin-mediated cell–cell adhesion. There has been no evidence of a direct interaction between Scribble and the E-cadherin complex, so it is likely that Scribble regulates E-cadherin in an indirect manner. Similarly, Dlg colocalizes with E-cadherin and knockdown of Dlg in Caco-2 cells inhibited the localization of E-cadherin and F-actin at cell junctions [26]. At the AJ, Dlg binds directly to PI3K (phosphatidylinositol 3-kinase) and is required for E-cadherin-mediated signaling. Finally, loss of PALS1 in MDCK cells disrupts AJ formation and E-cadherin localization [27]. In PALS1-deficient cells E-cadherin is retained in intracellular puncta suggesting a role for PALS1 in E-cadherin exocytosis to the plasma membrane. Thus, analyses of cell polarity proteins are providing novel insights into the mechanisms by which AJ are formed and maintained.

Cell polarity, epithelial morphogenesis and cancer initiation

In addition to regulating cell junctions, cell polarity pathways also play important roles during morphogenesis of epithelial cells. Disruption of polarity, by the overexpression or loss of polarity proteins, induces defective morphogenesis. When plated on a bed of extracellular matrix, the MDCK and MCF-10A cells undergo a programmed morphogenetic process that results in the formation of three-dimensionally organized glandular structures composed of polarized epithelial cells surrounding a central lumen. These model systems have been used extensively to study the role played by polarity proteins during three-dimensional morphogenesis. Overexpression of Crumbs or downregulation of Junctional adhesion molecule-A (JAM-A) in MDCK cells significantly delays the establishment of apical polarity in monolayer cultures and blocks lumen formation in 3D cysts [28,29]. Par6 and aPKC are required for lumen formation in MDCK cysts, regulating both polarization and cell death through a pathway involving glycogen synthase kinase 3β [30]. We have shown that deregulation of Scribble in MCF-10A cells, while only producing moderate effects on establishment of apical–basal polarity, significantly blocks morphogenesis by inhibiting cell death during lumen formation in 3D structures [31•]. Loss of Lgl reorients the apical membrane to the basal surface and blocks lumen formation in MDCK cells [24] demonstrating that disruption of polarity proteins can significantly affect morphogenesis. Furthermore, components of the polarity complex such as Rac1 and Cdc42 are critical regulators of MDCK and Caco2 3D morphogenesis where they play critical roles during polarization of MDCK cells and establishment of normal lumen in 3D cysts [32–34].

Consistent with the role for polarity proteins in tissue morphogenesis, loss of polarity proteins can initiate tumorigenesis in animal models of cancer. For example, downregulation of Scribble is sufficient to induce initiation of mammary tumors in an immortalized pluripotent mouse mammary epithelial cell line that harbors a mutant allele of the tumor suppressor gene p53 [31•]. Loss of Crb3 is required for the loss of contact inhibition of mouse kidney epithelial cells [28,29] and loss of Lgl1 results in severe dysplasia in the mouse brain [35•]. Loss of the polarity proteins can directly deregulate cell adhesion processes, which in turn will disrupt morphogenesis and promote tumorigenesis. Consistent with this notion, loss of E-cadherin cooperates with p53 to induce invasive mouse mammary tumors [36]. Thus, cell polarity pathways are likely to constitute a new class of tumor

Figure 2



A model that attempts to summarize the relationships between cell polarity, cell adhesion, morphogenesis, and tumorigenesis pathways.

suppressors, disruption of which can initiate tumorigenesis (Figure 2).

Cell polarity pathways function downstream of oncogenes

Oncogenes transform cells by deregulating multiple processes including cell proliferation and apoptosis. In addition, oncogenes have been known to disrupt cell polarity and architecture of epithelial cells, although the mechanisms by which this process is accomplished are just beginning to be uncovered. While high levels of expression of v-Src are sufficient to transform MDCK cells, low levels of v-Src in MDCK cells are unable to induce anchorage independent growth. However, cells expressing low levels of v-Src have defective cell–cell junctions and are unable to undergo normal 3D morphogenesis, suggesting that the ability of epithelial cells to form proper cell–cell junctions and undergo normal morphogenesis is exquisitely sensitive to the presence of aberrant oncogenic signals [37•]. In addition, aberrant expression of genes associated with cell transformation such as v-K-ras, RhoA, Rac1, Raf-1, and β-catenin affects cell polarity and morphogenesis [38–41]. Several other oncogenes and soluble factors have been shown to disrupt cell polarity, including hepatocyte growth factor (HGF) [42] and the insulin-like growth factor receptor (IGFR) [43]. Interestingly, not all oncogenes have the ability to disrupt polarity. For example, activation of c-Myc or expression of Cyclin D1 does not induce the disruption of polarity in mammary epithelial cells [44–46].

The precise mechanisms by which oncogenes disrupt epithelial polarity are only beginning to be understood. We demonstrated that the oncogene ErbB2 disrupts apical polarity of epithelial cells and this property of ErbB2 requires an interaction with the Par6 polarity protein [47••]. Activation of ErbB2 causes mislocalization of ZO-1 and Par6 from the apical–lateral border, increases TJ permeability, and dissociates Par3 from the Par6/aPKC complex. ErbB2 associates with Par6 and this interaction is required for ErbB2 to disrupt polarity, 3D morphogenesis and inhibition of apoptosis. Interestingly, the ErbB2–Par6 pathway is not required for ErbB2 to induce cell proliferation, demonstrating that the ability of ErbB2 to disrupt polarity can be uncoupled from its ability to induce cell proliferation.

ErbB2 also cooperates with the β4 integrin to disrupt TJ organization through the activation of signal transducer and activator of transcription 3 (STAT3) [48]. Expression of a dominant negative β4 integrin blocked the ability of ErbB2 to disrupt TJ in a STAT3-dependent manner. However, inhibition of the ErbB2–β4 pathway had no effect on the ability of ErbB2 to activate Erk and also to induce cell proliferation, further demonstrating that ErbB2 uses separate mechanisms to induce cell proliferation and disrupt cell–cell adhesions. Together these observations demonstrate that disruption of cell–cell junctions and induction of uncontrolled proliferation are regulated by separate pathways during oncogene-induced transformation of mammalian epithelial cells.

Cell polarity pathways and tumor progression

Defects in cell and tissue polarity are recognized hallmarks of advanced epithelial tumors. The mechanisms by which oncogenes regulate polarity proteins during cancer progression are now beginning to emerge [49]. The first indication that loss of cell polarity genes cooperates with oncogene activation to induce tumor progression was demonstrated in *Drosophila*, where flies expressing an activated form of Ras were screened for secondary mutations that would lead to metastatic growth [50••].

This screen identified several polarity proteins, including Scr, Dlg, and Lgl, whose loss induced noninvasive Ras tumors to spread. Subsequently, cooperation between loss of Scr and Ras or Raf has been observed to cause invasive growth in mammalian cells in a MAPK-dependent manner [51].

Transforming growth factor β (TGFβ) cooperates with oncogenes to induce epithelial to mesenchymal transition (EMT) [52]. Loss of polarity and disruption cell–cell adhesion is associated with cells undergoing EMT and is thought to be a critical step during metastatic tumor progression. TGFβ-induced disruption of TJ and EMT in a mouse mammary epithelial cell line, NMuMG, requires an interaction between TGFβ receptor I and Par6 [53••]. Upon TGFβ stimulation, TGFβRII is recruited to this complex where it phosphorylates Par6 at Ser^{345}. This phosphorylation is required for the ability of TGFβ to disrupt TJs. In this context, Par6 functions as a scaffolding protein to facilitate an interaction between Smurf1, an E3 ubiquitin ligase, and RhoA to promote localized degradation of RhoA, a required step for TJ disruption [53••]. In rat proximal epithelial cells, TGFβ disrupts polarity through downregulation of Par3 and mislocalization of the Par6/aPKC complex [54], the precise mechanism for this process is unknown. Interestingly, Snail, a transcriptional repressor that induces EMT, can target polarity proteins upon TGFβ stimulation [55]. Overexpression of Snail in MDCK cells leads to dissociation of both the Par and Crumbs complexes from TJs. In addition, Snail binds to the promoter region of Crb3 and directly represses Crb3 promoter activation in response to TGFβ [55]. The transcriptional repressor ZEB1, another inducer of EMT, inhibits transcription of several polarity proteins including Crb3, PATJ, and Lgl2 [56,57]. Thus, multiple regulators of EMT require an interaction with polarity proteins demonstrating a role for polarity proteins during tumor progression.

Summary

Polarity pathways regulate important functions during the formation and maintenance of cell–cell junctions and during morphogenesis. In addition, cell polarity pathways are emerging as critical regulators of initiation and progression of carcinoma by functioning as tumor suppressors, downstream of oncogenes, or promoters of the metastatic process (Figure 2). It is highly likely that further analysis of cell polarity proteins and the pathways they control will identify novel biomarkers and potential drug targets for managing and treating patients with carcinoma.

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