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Review

Epithelial cell polarity and tumorigenesis: new perspectives for cancer detection and treatment

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Loss of cell-cell adhesion and cell polarity is commonly observed in tumors of epithelial origin and correlates with their invasion into adjacent tissues and formation of metastases. Growing evidence indicates that loss of cell polarity and cell-cell adhesion may also be important in early stage of cancer. In first part of this review, we delineate the current understanding of the mechanisms that establish and maintain the polarity of epithelial tissues and discuss the involvement of cell polarity and apical junctional complex components in tumor pathogenesis. In the second part we address the clinical significance of cell polarity and junctional complex components in cancer diagnosis and prognosis. Finally, we explore their potential use as therapeutic targets in the treatment of cancer.

Keywords: cell polarity; epithelial tumors; differential diagnosis

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Introduction

In humans, more than 80% of all tumors are carcinomas and originate from epithelial tissues[1]. A characteristic hallmark for almost carcinomas is the loss of epithelial morphology and the acquisition of a mesenchymal-like phenotype, through a process called epithelial-to-mesenchymal transition (EMT)[2, 3]. Activated during embryogenesis and adult tissue remodelling[4, 5], EMT is a complex molecular program by which epithelial cells shed their differentiated characteristics and acquire mesenchymal features, including motility, invasiveness and a heightened resistance to apoptosis[6, 7].

A strong correlation between malignancy and loss of epithelial organization has been histologically documented for almost types of tumor deriving from epithelial cells[8–10], and disruption of cell-cell adhesion *per se* has been found to promote the development of some cancers[11, 12]. Thus, understanding the molecular mechanisms that regulate tissue organization and how such mechanisms are disrupted during neoplastic transformation, could provide important and useful insights to be exploited for diagnostic and therapeutic purposes.

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Epithelial cells and the apical junctional complex

Generally located at the interface between the organism and the outside world or at the free surface of tubes or cavities as in the case of digestive, respiratory, urinary and reproductive tract, epithelial cells are organized in mono- or multi-layered sheets in which any single cell is strictly in contact with neighboring cells by specific cell-cell adhesion structures. To allow a correct sheet alignment, each epithelial cell presents a well-defined orientation with an apical pole directly in contact with the luminal space and a basal pole in contact with basement membrane (Figure 1A). In contrast, mesenchymal cells display a fusiform or spindle-like morphology, do not form organized cell layers, are not polarized, contact the neighboring cells only focally, are not associated with the basement membrane and tend to be highly mobile (Figure 1B).

The characteristic polarization of epithelial cells is obtained by an asymmetric distribution of cellular components along the internal apicobasal axis. This property, known as apicobasolateral polarity, is part of a crucial differentiation process, termed epithelial polarity program that governs the spatial asymmetry required for a correct epithelial cell morphology and tissue homeostasis[13]. Schematically, the epithelial polarity program utilizes three cellular machineries dynamically interplaying: the polarized trafficking machinery, the domain-identity machinery and the 3D-organization machinery[14]. The polarized trafficking machinery is an adaptation of secretory and endocytic systems to sort and deliver proteins and

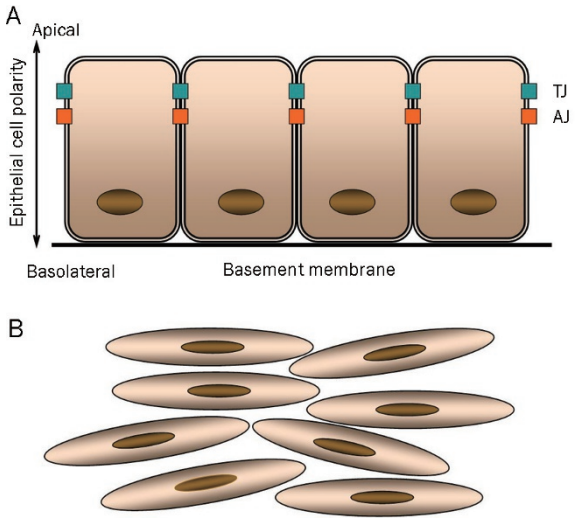

Figure 1. Epithelial cells (A) are organized in mono- or multi-layered sheets in which any single cell is strictly in contact with neighboring cells by specific cell-cell adhesion structures: tight junction (TJ) and adherens junction (AJ). Conversely, mesenchymal cells (B) display a fusiform or spindle-like morphology, do not form organized cell layers, are not polarized, contact the neighboring cells only focally, are not associated with the basement membrane and tend to be highly mobile.

lipids to apical and basolateral plasma membrane domains; the domain-identity machinery governs a highly conserved set of proteins and lipids to the task of generating and maintaining the 'identity' of the apical and basolateral domains; the 3D-organization machinery controls cytoskeleton organization and coordinates extracellular signals with the polarized trafficking and domain-identity machineries.

Among the cellular processes under the domain-identity machinery control there is the establishment of the apical junctional complex, which is formed by two specific cell-cell membrane structures: adherens (AJs) and tight junctions (TJs). Also known as zonula adherens (ZA) and zonula occludens (ZO), these structures are located in the upper portion of a polarized epithelial cell and are composed of transmembrane proteins that interact outside with homotypically molecules in the adjacent cells, and several intracellular scaffolding proteins and signaling molecules connected with the cytoskeletal network[15, 16]. Whereas the main function of AJs is to provide a strong cell-cell adhesion, TJs form a continuous, circumferential, belt-like, selective barrier to solutes leakage across the cellular sheet, and serve, at the same time, as a boundary between apical and basolateral membrane domains to prevent the diffusion of integral proteins and lipids from one to the other domain[17]. As well, TJs are also critical for the polarized location of ion channels, receptors, and enzymes to the membrane domains necessary for structurally and functionally developed epithelia, a function referred to as the "fence" function. In addition, to these barrier and fence functions, TJs are an important site for regulation of epithelial cell differentiation and proliferation due to the interactions of some scaffolding proteins with a large number of signaling molecules[18, 19].

Initially described as membrane "kissing points" (the external leaflets of the lateral plasma membrane of opposing cells appear fused), TJs are located at the apical end of the basolateral domain just above AJs that form a continuous adhesion belt with a sealing function apparently less stronger than that of TJs because the opposing cell membranes are 15−20 nm distant (Figure 2). However, electron microscopy has revealed that the intercellular space of the AJs is not empty but stuffed by the extracellular portion of several transmembrane proteins, and that at the cytoplasmic side, they are characterized by a "plaque" into which actin microfilaments are particularly condensed and connected with the actin filaments forming the cytoskeleton[20].

Both TJs and AJs are composed of a variety of membrane-localized cell adhesion molecules (CAMs) and cytoplasm-localized adaptor proteins that are responsible, respectively, for the mutual recognition and adhesion of adjacent cells in an epithelial layer and for the anchorage of TJ and AJ structure to the cytoskeleton (Figure 2).

The molecular composition of TJs

To date four types of CAMs or transmembrane proteins have been found in TJs: claudin protein family (mammals express about 24 claudins)[21], occludin[22], tricellulin[23] and junctional adhesion molecules (JAMs)[24] (Table 1).

Claudins, occludin and tricellulin are tetraspan proteins that exhibit two extracellular loops, through which they establish contact with homotypic molecules located in the corresponding TJ region of adjacent cells (Figure 2). Experimental evidence from knock-out animals, fibroblasts and epithelial cell lines transfected with different claudin constructs, as well as clinical evidence from human pathological conditions[25, 26]

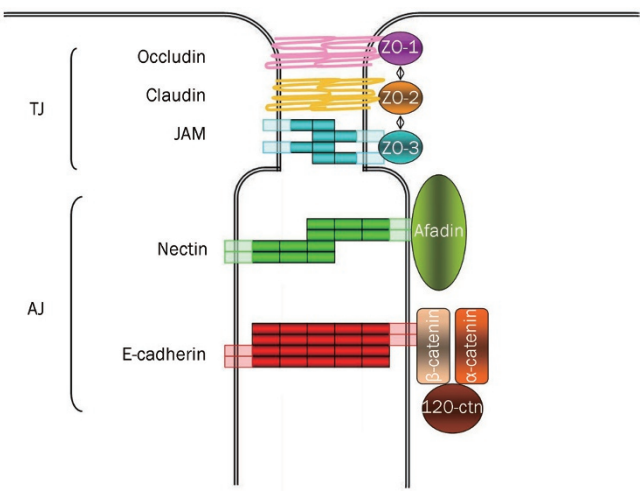

Figure 2. Tight and adherens junctions components. Tight junctions (TJs) are the most apical intercellular junctions in epithelial cells. Their major transmembrane components are the claudins, occludin and adhesion molecules (JAMs) whereas their cytoplasmic components are zonula occludens (ZOs) proteins. Adherens junction (AJs) are localized just below TJs; their transmembrane components are cadherins and nectins that complex respectively the cytoplasmic components β-catenin and afadin.

Table 1. Apical junctional complex components.

	Cell adhesion molecules (transmembrane proteins)	Adaptor proteins (cytoplasmic proteins)
Tight junction	Claudins (about 24 members)	Proteins without PDZ domains
		• Cingulin
	Occludin	• Symplekin
		• Junction-enriched and junction-associated protein (JEAP)
	Tricellulin	
		Proteins with PDZ domains
	Junctional adhesion molecules	*MAGUK protein family*
	• JAM-1	• ZO-1
	• JAM-2	• ZO-2
	• JAM-3	• ZO-3
		• Pals1
		MAGUK inverted proteins
		• MAGI-1
		• MAGI-2
		• MAGI-3
		multi-PDZ domain proteins
		• MUPP-1
		• PATJ
Adherens junction	Cadherins (over 80 members)	Catenins
	Nectins	• α-catenin
	• Nectin-1	• β-catenin
	• Nectin-2	• p120 catenin
	• Nectin-3	Afadin
	• Nectin-4	

indicate that claudin proteins constitute the molecular backbone of TJs and are responsible for the tissue-specific barrier properties of such structures according to the different claudins combination[27]. On the contrary, the role of occludin on TJ structure and function remains rather controversial since occludin knock-out mice are viable and exhibit TJs with an apparent normal morphology[22]. Finally, tricellulin, the latest TJ integral protein to be identified, is concentrated at contact points of three epithelial cells and it appears to play a crucial role in the paracellular barrier mechanism[23].

JAMs belong to a family of type I proteins characterized by two extracellular immunoglobulin-like domains, a single transmembrane region and a cytoplasmic domain that ends with a canonical PDZ domain. The latter is a module of about 80−90 amino acid residues, that derived its name from the first three proteins in which it was found: postsynaptic density protein PSD-95, the *Drosophila* lethal disc large tumor suppressor protein Dlg and the TJ scaffold protein zonula occludens 1, ZO-1. The length of the cytoplasmic tail differs among JAMs, and this difference affects their association to different sets of molecules containing one or more PDZ domains. JAMs appear essential for TJs assembly[28] and growing evidence indicates that they contribute to the regulation of paracellular permeability.

At the cytoplasmic side of TJs, a wide spectrum of scaffold proteins is found. Named peripheral proteins or cytoplasmic adaptor proteins, they can be classified according to the presence or absence of PDZ domains in their sequences, a feature that gives them the possibility of establishing specific protein-protein interactions (Table 1).

Among the cytoplasmic adaptor proteins without PDZ domains there are cingulin[29], symplekin[30] and the junction-enriched and junction-associated protein (JEAP), a component of TJs specifically expressed in exocrine cells but not in the epithelial cells of the small intestine[31].

Among the cytoplasmic adaptor proteins with one or more PDZ domains, at least three subgroups can be distinguished. The first one corresponds to the membrane-associated guanylate kinase (MAGUK) protein family and it includes ZO-1, ZO-2, ZO-3, and Pals1[32, 33]. The establishment of cultured epithelial cells in which the expression of ZO-1 and ZO-2 is suppressed by RNA interference, has given crucial clues for understanding the role of these proteins in TJ strand formation: ZO-1 and ZO-2 are essential for the polymerization of claudins and to determine their correct localization at the uppermost portion of the lateral membrane[34].

The second subgroup corresponds to the MAGUK inverted proteins MAGI-1, MAGI-2, and MAGI-3 in which, differently from ZO proteins, the guanylate kinase domain is located at the amino portion of the molecule and PDZ domains are at the carboxyl region[35]. The experimental evidence that MAGI proteins can bind and counteract viral oncoproteins and may

interact with PTEN (phosphatase and tensin homologue) tumor suppressor suggests possible role of MAGIs in the recruitment of growth suppressors[36–38].

The third subgroup includes the multi-PDZ domain protein 1 (MUPP-1) and Pals1-associated TJ protein (PATJ) that, respectively, contain 13 and 10 PDZ domains[39, 40]. MUPP-1 is exclusively localized to the TJ of polarized epithelial cells, where it binds to claudin-1 via PDZ10 domain and to claudin-8 via PDZ9 domain. Like ZO-1, MUPP-1 also links to the membrane-associated JAM-1 protein suggesting a pivotal role of MUPP-1 as multivalent scaffolding protein to recruit several other proteins and participate in the regulation of epithelial cell growth and differentiation.

The molecular composition of AJs

AJs consist of two basic adhesive units: the cadherin/catenin and nectin/afadin complexes (Figure 2)[16]. Classic cadherins are type I, single-pass transmembrane glycoproteins that mediate Ca^{2+}-dependent intercellular adhesion[41]. They consist of over 80 members, among which, E-cadherin is primarily expressed in epithelia. Specific adhesive binding is conferred by the extracellular portion that serves as a template and engages an identical molecule on the surface of neighboring cells. Conversely, the cytoplasmic domain, highly conserved in length, sequence and interaction partners, mediates key structural and signaling activities by the interaction with some cytoplasmic adaptor proteins belonging to the class of catenins (specifically, α-catenin, β-catenin or the highly homologous γ-catenin/plakoglobin and p120 catenin)[42–44]. In particular, an E-cadherin-β-catenin complex is required for the transport of newly synthesized E-cadherin molecules to the plasma membrane where they contribute to the formation of structured AJs[45]. Once at the plasma membrane, the E-cadherin-β-catenin complex rapidly recruits α-catenin, which is essential to reinforce the association of E-cadherin to filamentous actin (F-actin) by linking other actin-binding proteins, including α-actinin, zyxin and vinculin[42, 46]. Cadherins also associate with p120 catenin that specifically prevents cadherin degradation enhancing its surface clustering and helping the formation of strong and more "compacted" structures[47].

In addition to the association with F-actin, α-catenin binds to afadin, the main cytoplasmic binding partner of nectins, recruiting in this way, the second basic adhesive unit (nectin/afadin) that cooperatively organizes AJs[48].

The four nectins till now identified, are members of the immunoglobulin (Ig) superfamily of calcium-independent cell adhesion molecules and are characterized by an extracellular domain with three IgG-like loops, a single transmembrane region, and a cytoplasmic tail that binds to afadin through a C-terminal PDZ binding domain[49]. Nectin-1, nectin-2, and nectin-3 are ubiquitously expressed in a variety of cells including epithelial cells, neurons, and fibroblasts whereas nectin-4 is mainly expressed in the placenta[50]. Experimental evidence indicates that similar to cadherins, nectins are thought to form cis-homodimers and trans-homodimers with similar molecules of adjacent cells[51] (Figure 2).

Cell polarity complexes and formation of the junctional complex

A key event in the establishment of cell-cell junctional complex is the polarization of epithelial cells. In fact, upon establishing a cell-cell contact and before forming AJs and TJs, epithelial cells must reorganize their cytoskeleton and polarize trafficking routes[52]. However, to correctly assemble and localize the junctional complex components, a set of evolutionarily conserved polarity proteins that cross-regulate one each other and interplay with cytoskeleton structures is required (Figure 3).

Originally identified by genetic experiments in *Drosophila melanogaster* and *Caenorhabditis elegans*, in mammals, core polarity regulators are organized in three distinct polarity complexes: (1) the PARtition (PAR) (Par3/Par6/atypical protein kinase (aPKC)) complex; (2) the CRB (Crb/Pals1/Patj) complex; and (3) the SCRIB (Scrib/Dlg/Lgl) complex[53–55].

The PAR complex was initially identified in *Caenorhabditis elegans* mutants (partitioning-defective) as involved in the regulation of the anterior-posterior cell polarity of the one-cell embryo[56]. In mammals, it is composed of two scaffold proteins (Par3 and Par6) and aPKC that form a ternary complex able to bind JAMs and nectins through the PDZ-domain of Par3. PAR complex is localized to the apical junction domain and significant evidence indicates that it has a critical role in TJ formation and epithelial polarization[55, 57].

The CRB and SCRIB complexes were initially identified in the fruit fly *Drosophila melanogaster*, as responsible for epithelial defects: fly embryos the lack Crumbs and stardust proteins fail to form a zonula adherens (the *Drosophila* homolog of TJ) and display a disruption of the apico-basal polarity[54]. In mammals, Crumbs3, one of the three mammalian homologs of Crumbs protein, is localized to the apical membrane where it forms a complex with Pals1 (the mammalian homolog of *Drosophila* stardust protein), and Patj (Pals-associated tight junction protein) the mammalian homolog of *Drosophila* Dlt (Disc lost)[58]. By the binding of the amino terminus of Pals1 and the PDZ domain of Par6, the CRB complex interacts with PAR complex and together regulate TJ assembly[59].

The mammalian SCRIB complex is composed of three proteins, Scribble (Scrib), Disc large (Dlg) and Lethal giant larvae (Lgl)[60]. While Scrib colocalizes with Dlg and overlaps with the AJs, the localization of Lgl depends on its phosphorylation status, which is mediated by aPKC. In fact, phosphorylated Lgl is unable to localize to the membrane and is inactive[61].

In addition to these three core complexes, further components and protein kinases, including Rac1/Cdc42 GTPase, are increasingly being implicated in the organization of cell polarity[54].

Based on the work mainly performed in *Drosophila* and *Caenorhabditis elegans*, the formation of apical polarity seems to be a hierarchical and interactive process in which PAR and CRB complexes cooperate in establishing the apical domain and in the assembly of TJs, whereas SCRIB complex should define the basolateral plasma membrane domain.

Experimental evidence indicates that, in mammals, the earliest stage of junction biogenesis consists of the trans-interaction

of nectins that recruit E-cadherin and JAMs to the apical side of AJs[51]. JAMs and nectins then recruit Par3 protein, which in turn recruits Par6, the second element of the PAR complex characterized by a binding site for aPKC[62]. Experimental evidence indicates that the recruitment of aPKC by Par6 and the following phosphorylation of Par3, that allows the activation of the ternary complex, is a critical step for the maturation of the junctional complex into distinct TJ and AJs. In fact, the localization of active PAR complex to the apical domain is stabilized by the CRB complex whose distribution is reciprocally dependent on the PAR complex. As a consequence, CRB complex forces TJs to remain lateroapical by maintaining the PAR complex in this plasma membrane region[63, 64]. At the same time, the basolaterally located SCRIB complex antagonizes the apical localization of the active PAR complex. In fact, Lgl protein, one of the SCRIB complex components, competes with Par3 for binding to the PAR complex, thereby sequestering the PAR complex away from the apical junctional domain. Such an antagonist function of SCRIB complex is controlled by aPKC as the aPKC-dependent phosphorylation of Lgl protein inactivates the SCRIB complex[54] (Figure 3).

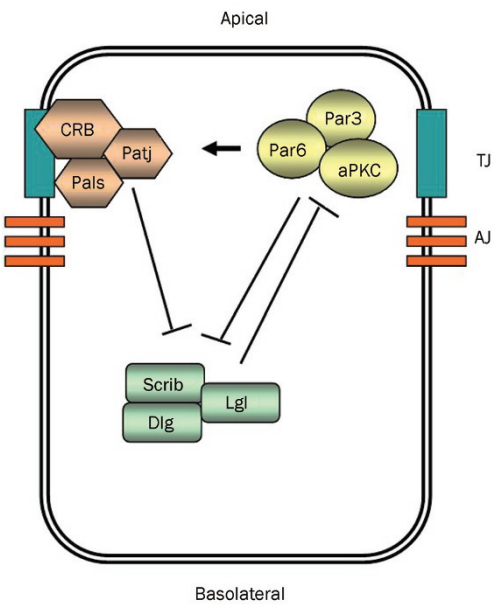

Figure 3. Epithelial cells are polarized along their apicobasal axis, due to the action of three polarity complexes. PAR (Par3/Par6/aPKC) and CRB (Crumbs/Pals1/Patj) complexes are localized to the apical region and promote apical membrane identity. SCRIB complex is localized to the basolateral region, antagonizes the function of the PAR complex and promotes basal membrane identity. SCRIB complex is reciprocally inactivated by aPKC-dependent phosphorylation of Lgl protein.

Apical junctional complex and gene expression

In addition to their role in establishing and maintaining a correct epithelial phenotype, several components of the apical junctional complex have also been found able to actively influence gene expression. In fact, accumulating evidence indicated that some nontransmembrane proteins involved in the formation of the apical junctional complex can shuttle between plasma membrane and nucleus where they participate in the formation of chromatin-associated complexes and hence in the control of gene transcription.

Initially reported for β-catenin, concerning to its role in Wingless (Wnt) signaling pathway[65], this shuttling activity has then been broadened to other TJs proteins (principally ZO-1 and ZO-2)[66, 67] and some cell polarity complex components including Par3, Par6 and Dlg1[68–70] suggesting for these apical polarity proteins a powerful role as epigenetic factors[71, 72].

As recently proposed by Lelièvre[73], the impact of the nuclear localization of apical polarity proteins on gene transcription should depend on the integrity of cell-cell junctions. In fact, if the apical junctional complex is intact, some apical proteins move into the nucleus and participate in the transcription repression of the genes that have to be silenced in order to maintain a differentiated phenotype. Conversely, when apical junctional complex is disrupted certain transcription regulators, normally compartmentalized at cell-cell junctions by apical polarity proteins, are free to move into the nucleus where they induce apical proteins to switch from nuclear repressors to nuclear promoters. Such an hypothesis has recently been supported by the finding that nuclear overexpression of symplekin, a transcriptional regulation normally localized at TJs, promotes tumorigenesis in the human colon and that the regulation of claudin-2 expression is instrumental in this effect[74].

Disassembly of junctional complex and cancer

Disruption of intercellular junctions and alterations in cell polarity are specific hallmarks of epithelial cancer cells. In fact, most human tumors arising in epithelial tissues, gradually lose their polarized morphology and acquire a more peculiar mesenchymal phenotype according to a process termed epithelial-mesenchymal transition (EMT)[75].

Physiologically associated with embryogenesis and wound healing in the adult, EMT is a process characterized by the loss of cell-cell adhesion and apicobasal cell polarity and the concomitant acquisition of a fibroblastoid motile phenotype. When pathologically activated during tumor development, EMT provides cells with ability to escape from the primary tumor mass and to migrate to distant region where they metastasize[76].

Disruption of cell-cell adhesion is principally due to the disassembly of the junctional complex that may be induced by a direct modification of the any junctional components (transmembrane and cytoplasmic proteins) or by indirect effects through the actin cytoskeleton that controls the integrity of TJs and AJs. In particular, alterations in the structure and function of TJs have been reported in adenocarcinomas of various organs, including colon and breast[77, 78], whereas absent or defective TJs have also been associated with the development of the neoplastic phenotype in endometrial cells[79]. TJs dysfunction has also been described in inflamma-

tory processes that end up in cancerous transformation. For example, Crohn's disease and ulcerative colitis are inflammatory illnesses involving the gastrointestinal tract in which an abnormal paracellular permeability defect, probably induced by cytokines and growth factors associated to inflammation including interleukin-1, interleukin-4, interleukin-13, transforming growth factor-α, insulin-like growth factor-I and II, interferon-γ, tumor necrosis factor-α and hepatocyte growth factor, appears to precede the development of both syndromes[80]. Interestingly, it has also been shown that the transforming potential of some oncoproteins, encoded by human adenovirus, papillomavirus (HPV), T-cell leukaemia virus type, depends in part on their ability to interact with TJ-associated proteins of epithelial cells[81].

As previously illustrated, alterations in some apical proteins and transcription regulators compartmentalization, due to TJs and AJs disassembly, may have a dramatic impact on gene transcription with the switch in the apical proteins activity from nuclear repressors to nuclear promoters as supported by the observation that ZO-1 protein is found at the plasma membrane in noninvasive cells whereas it is located in the nucleus of invasive cells[82]. Similarly, the dissociation of E-cadherin-catenin complex results in an increase of the cytosolic pool of β-catenin, the key regulator of Wnt signaling pathway, that drives cell proliferation once migrated to the nucleus[83]. It is important to note that, in addition to transforming growth factor-β (TGF-β), Wnt is one of the extracellular growth factors that have been shown to trigger epithelial dedifferentiation and EMT by the downregulation of epithelial genes and the upregulation of genes coding for fibroblastoid-associated features as N-cadherin, the typical mesenchymal marker[84–86]. That, according to a process known as "cadherin switch" during which cells shift to express different isoforms of this transmembrane component[87].

Also transmembrane and cytoplasmic proteins with at least a PDZ domain have been found target of some oncoproteins including HPV E6 protein, one of the major oncogenic determinants of the sexually transmitted HPV, the primary etiologic agent of cervical cancers. HPV E6 protein has a functional PDZ binding motif that interacts with the corresponding PDZ domain of the TJ proteins MUPP-1, MAGI-1, MAGI-2, MAGI-3, and PATJ[36, 88]. Through such a binding E6 targets these proteins as well as the Dlg and Scrib molecules to ubiquitin-mediated degradation in the proteosome[89–91]. In this way, expression of HPV E6 protein in epithelial cells destabilizes TJs, generates ZO-1 mislocalization, disrupts AJs and promotes carcinogenesis.

Cell polarity complexes dysregulation and cancer

The first hint that polarity proteins might contribute to cancer was provided by experiments in *Drosophila* which showed that knockout of any member of the Scribble complex results in the loss of apicobasal cell polarity, mislocalization of apical junctions and failure to form the zonula adherens, and neoplastic overgrowth of imaginal discs, the ectodermal structure from which originate epithelia, that lead to death at the larval stage suggesting for these proteins an oncosuppressive key function[92, 93].

Also in mammals, several reports describe a strong correlation between decreased expression of mammalian homologues of Scrib, Lgl and Dlg and tumor progression. Mice lacking Lgl1, one of the two Lgl homologues, show severe brain dysplasia, accompanied by loss of cell polarity in neuroepithelial cells[94], whereas loss or aberrant localization of Lgl2 is associated with human gastric epithelial dysplasia and adenocarcinomas[95]. In addition, as already described, ubiquitin-mediated degradation of Dlg and Scrib proteins may contribute to the development of the HPV-induced cervical carcinoma[91, 96]. It should be noted that cancer promoting viruses, such as HPV also destabilize the CRB/PAR complexes interaction required for TJs formation and induce epithelial depolarization[97] suggesting for CBR components a role as tumor suppressors[98].

Recent findings also implicate the PAR complex in human carcinogenesis. Gene amplification and elevated activity of aPKC have been detected in ovarian, lung and colon cancer and correlated with a poor prognosis[99–101]. In addition, as SCRIB complex components are known to cooperate with those of PAR complex in the assembly of TJs, dysregulation of Scrib, Lgl and Dlg proteins affects also PAR complex activity. As the members of PAR complex play a pivotal role in signaling pathways that control polarity and proliferation, PAR complex implication in human carcinogenesis is not surprising[102]. An example of the link between growth factors signaling, cell polarity and cancer is provided by the ErbB2 receptor tyrosine kinase signaling pathway in breast cancer, in which an overexpression or amplification of the gene is found in 25%-30% of cases. It has recently been demonstrated that after activation, the ErbB2 receptor binds directly to Par6 protein, leading to the competitive recruitment of aPKC and the disruption of the apicobasal polarity[103]. Further recent insights into the regulation of cell polarity during EMT have converged on PAR complex. In fact, in rat intestinal epithelial cells, upon exposure to TGF-β, Par3 protein level has been found decreased, concomitantly to E-cadherin suppression and the induction of mesenchymal markers. The decrease in Par3 mediated by TGF-β resulted in a redistribution of Par3-aPKC complex from the membrane to the cytoplasm leading to the disorganization of PAR complex and loss of polarity[104].

Also mutations in tumor suppressor proteins, such as von Hippel-Lindau protein (VHL), may affect PAR complex efficiency and junctional complex assembly. In fact, as demonstrated by Okuda *et al*[105], VHL, a tumor suppressor involved in the von Hippel-Lindau syndrome which leads to the development of hemagioblastoma, clear-cell renal carcinoma and pheochromocytomas, mediates ubiquitination of a variety of proteins, including activated aPKC, and it regulates the assembly of intercellular junctions. Because VHL tumor suppressor simultaneously affects cell polarity and growth, inactivating mutations result in an abnormal AJs and TJs organization and cell proliferation as evident in VHL-negative cancer cells.

Cell polarity and cell-cell adhesion proteins in cancer diagnosis and prognosis

Due to their multiple links to cancer, many structural and regulatory components of the junctional complex may represent valuable tumor biomarkers. Even though the acquisition of new insights is increasing, at present the largest body of clinical evidence derived from studies on E-cadherin expression. Changes in the normal expression pattern of the E-cadherin/catenin complex have been found in various human cancers from epithelial origin where a partial or total loss of E-cadherin expression correlates with loss of differentiation characteristics, increased tumor grade and metastatic behavior, and poor prognosis. Such a downregulation of E-cadherin may occur as a consequence of gene mutation or epigenetic silencing. Thus, for example, mutations in CDH1, the gene encoding human E-cadherin, are found in more than 50% of stomach cancers[106] whereas, in lobular breast cancer, E-cadherin expression is irreversibly lost due to loss of heterozygosis (LOH) in more than 85% of cases; an LOH which is frequently associated with epigenetic silencing of the remaining CDH1 allele via promoter hypermethylation or transcriptional repression[12]. The prevalence of E-cadherin loss in non-invasive precursor lesions as lobular carcinomas *in situ* of the breast or pancreas adenomas[107] highlights the importance of E-cadherin as a tumor and invasion suppressor and it suggests E-cadherin loss as a critical step in the transition from adenoma to carcinoma.

Also the expression of occludin, one of the major transmembrane components of TJs, has been found decreased with disease progression. For example, moderately and poorly differentiated gastric carcinomas show a reduced occludin mRNA and corresponding protein level when compared to well differentiated ones[108]. Similarly, loss of occludin has been found in human breast cancer[109], in unpolarized prostate cancer cells of Gleason grades 4 and 5[110], and in grades 2 and 3 endometrioid carcinoma with respect to grade 1[79].

More debated is the diagnostic/prognostic role of claudins. In fact, because of their tissue-specific pattern of expression, a causal relationship between claudins expression/localization and cancer has not been established. In some cancers, including prostate, breast, melanocytic neoplasia, esophageal squamous cell carcinoma and lung[111–115], a decreased expression of some claudins, especially claudin-1, has been associated with cancer progression, recurrence and a shorter disease-free survival, suggesting a cancer invasion/metastasis suppressive role. Conversely, in other cancers such as papillary thyroid, oral squamous cell carcinoma, ovarian, colon, melanoma and gastric[116–121], overexpression of claudins has been associated with a more aggressive phenotype.

With regards to the clinical relevance of cytoplasmic proteins, such as ZOs and JAMs proteins, several studies have reported that their reduced expression correlates with dedifferentiation in tumors arising from various compartments of the biliary tract and progression of clear cell renal carcinoma or endometrial cancer[122–124]. However, ZO-1, which is generally considered as a tumor suppressor, has unexpectedly been found overexpressed and associated with N-cadherin in melanoma[125], overexpressed and aberrantly localized in primary and metastatic pancreatic cancer[126], whereas JAM-A has been found overexpressed and positively correlated with poor prognosis in breast cancer patients[127].

About cell polarity complex components recent data provide evidence that aPKC is overexpressed in gastric cancer and associated with unfavorable prognosis[128]. In addition, in a very recent study, aPKC overexpression has been found associated with the loss of Lgl2, a component of the SCRIB polarity complex, in foveolar-type gastric dysplasia suggesting their combined evaluation useful to refine dysplasia diagnosis[129]. The immunohistochemical determination of Lgl2 expression has also provided to be useful in separating pancreatic intraepithelial neoplasia-3 and pancreatic ductal adenocarcinoma from lower-grade pancreatic intraepithelial neoplasias; in fact, while loss or abnormal Lgl2 expression is seen in pancreatic intraepithelial neoplasia-3 and adenocarcinoma, pancreatic intraepithelial neoplasia-1 and pancreatic intraepithelial neoplasia-2 express the protein in a normal-like basolateral fashion[130].

Similarly, the loss of Lgl1 has been associated with several solid tumors including melanoma, prostate, breast, colon, liver and lung cancer[131–134]. In these cancers Lgl1 loss is generally associated with higher tumor grade, and is more pronounced in lymph node or distant metastases.

Also Scrib and Dlg, the other two members of the SCRIB complex, have been found of clinical relevance. In fact, an emerging finding is the association of Dlg1 with HPV-induced gynecological malignancies: in later-stage and poorly differentiated tumors, cytoplasmic Dlg1 protein level increases while it decreases at the membrane sites of cell-cell adhesion[135, 136]. A similar trend of early-stage overexpression and mislocalization has been described in colon carcinogenesis: in adenomatous polyps and well- and moderately differentiated adenocarcinomas, Dlg1 protein level increased but become more diffuse with an increase cytoplasmic staining[137] suggesting its potential use as selective biomarker. Similarly even though preliminary, the evidence that alterations in Scrib protein expression and localization are associated with some cancer of epithelial origin including endometrial, cervical and colorectal neoplasias[138, 139]. In particular in the former, Scrib protein alterations have been found associated with clinical stage, histopathological differentiation, and lymph node metastasis[140] whereas in cervical carcinoma the histochemical analysis showed a dramatic decrease in the expression of Scrib with the progression of disease from normal uterine cervical tissues to invasive cervical cancers through the precursor lesions[139]. Finally, in colorectal carcinoma the overexpression and distribution of Scrib were observed to extensively overlap with the cytoplasmic accumulation of β-catenin and an intense immunoreactivity of Scrib was often observed in small adenomas suggesting that Scrib could be involved in an early step of colon carcinogenesis and supporting the experimental finding of a link between cell polarity disruption and EMT[140].

While no clear evidence of a direct link between CRB complex components and tumor features and clinical outcome, it

has now emerged, Par6 protein, a PAR complex component, has recently been found overexpressed in breast cancer-derived cell lines and in both precancerous breast lesions and advanced primary human breast cancers, especially in estrogen receptor-positive ones[141].

Although, as reported above, several polarity and junctional complex components have been found singly associated with initiation or progression in many cancers of epithelial origin, an integrated picture of the mechanisms controlling their expression and activity during cancer development is lacking. To this aim, novel approaches such as gene expression profile analysis are needed to investigate the associations between the expression of genes coding for cell polarity and cell-cell adhesion determinants and the biologic features of preneoplastic and neoplastic lesions. In such a perspective, for example, we have investigated whether, with respect to normal tissue, epithelial or mixed malignant pleural mesothelioma (MPM) were associated with specific patterns of expression of a selected set of genes related to EMT and cell polarity and adhesion[142]. Using active and passive Principal Components Analysis-based biplots, to visualize specific patterns of expression for epithelial and mixed MPMs, we found that epithelial MPMs, mixed MPMs and normal tissues were characterized by different patterns of expression of genes contributing to a better characterization of MPM histologies, thus suggesting the possibility of using gene expression profile analysis in refining the morphological diagnosis also in other controversial pathological situations as for example preneoplastic lesions.

Cell polarity and cell-cell adhesion proteins as therapeutic targets

Blocking cell proliferation in neoplasias and preventing EMT in preneoplastic lesions eventually restoring normal epithelial morphology represent the main goal of differentiation therapy. Several attempts, targeting cell polarity and adhesion components, have been made at experimental level with interesting and promising results. For example, it has been observed that, in suspension 3D breast tumor spheroids, the retrovirally-induced reexpression of claudin-1, downregulated or completely lost in breast cancer cells *in vitro*, resulted in plasma membrane homing of the protein and reconstitution of paracellular flux[143]. Similarly, in several cancer cell lines, the treatment with demethylators and histone deacetylase inhibitors or retinoids was able to reexpress occludin (silenced by CpG island hypermethylation on its promoter region) with a decreased cellular invasiveness and motility, thereby abrogating the metastatic potency of cancer cells[144].

Conversely, as regard to claudins which appear overexpressed in tumors, the use of siRNA to silence gene expression has provided a significant reduction in cell proliferation, reduction in tumor growth and a significant increase in the number of apoptotic cells[145]. A negative control of claudins overexpression has also been achieved using monoclonal antibody against specific members of the family and promising results have been obtained in pancreatic and ovarian cancers[146, 147]. The use of monoclonal antibodies has also been applied to inhibit the activity of JAM proteins and in particular to block the proangiogenic activity of JAM-3, another component of TJs that in tumor cells mediates the homophilic interaction with endothelial cells. In a murine model of lung carcinoma, monoclonal antibody against JAM-3 was able to inhibit the growth of lung pulmonary tumor grafts by reducing tumor vasculature[148].

In addition to the immunotherapeutic strategy, the potential of claudins as targets for cancer therapy has been highlighted by the observation that treatment with *Clostridium perfringens* enterotoxin (CPE) elicits a rapid and specific cytolysis of breast, ovarian, pancreatic and prostate carcinoma cells. This toxin, responsible for the gastrointestinal symptoms associated with *Clostridium perfringens* food poisoning, is released into the intestinal lumen where it binds specifically to claudin-3 and -4 present in the intestinal epithelial. This triggers the formation of a large multiprotein membrane pore that ultimately results in cell lysis. Since claudin-3 and -4 are overexpressed in numerous carcinomas, this condition represents a unique opportunity for innovative therapy using CPE[149, 150].

As regard polarity complex components, preliminary data on the ternary Par6/Par3/aPKC complex as a novel target for therapeutic intervention are emerging. In particular, Par6 protein appears a promising target both in early and late stage of breast cancers[141] whereas aPKC, as well as being a new prognostic indicator, has been proposed as a novel therapeutic target for the treatment of gastric cancer[128].

Conclusion and future perspectives

Increasing evidence clearly shows that the loss of apico-basal polarity and changes in cellular junctions can be initiating events in tumor formation and that they are hallmark of malignant transformation. Although in recent years there has been a significant advance regarding our understanding of the physiologic mechanisms governing cell polarity, the exact sequence of events leading to cell polarity dysregulation are still being uncovered whereas it is clear that the expression of genes coding for core polarity and junctional adhesion components can be directly or indirectly transcriptionally regulated by several factors including growth factors, oncogene products, tumor suppressors and EMT inducers.

Of particular interest, the emerging relationship among EMT, cell polarity and stem cell phenotype based on the observation that the proteins regulating cell polarity also have an important function in asymmetric cell division, a critical event that guarantees the two essential properties of stem cells: self-renewal and differentiation[151]. In fact, studies in *Drosophila* have shown that the asymmetric localization of cell-fate determinants is controlled by PAR and SCRIB complexes and that mutations in their components provoke defects in asymmetric stem cell division which finally results in tumor development[93, 94].

Also in adult somatic stem cells, which play a central role in epithelial homeostasis, it is thought that polarity is particularly important with respect to fate decisions on stem cell division and that the failure in establishing or regulating stem

cell polarity might result in neoplastic transformation[152]. The recent demonstration that Par3 influences the progenitor cell compartment during mammary gland morphogenesis[153] supports this hypothesis and strengthens the urgency to thoroughly investigate the relationship between apico-basal polarity and progenitor/stem cells. That taking into consideration also the recently proposed link between EMT activation and the acquisition by tumor cells of a stem-like phenotype[154].

Interfering with the signaling pathways that promote the loss of epithelial integrity may be one of the most promising avenues for the development of new therapeutic strategies to impair tumor progression and to revert preneoplastic lesion toward a more differentiated phenotype.

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