Cell polarity and cancer – cell and tissue polarity as a non-canonical tumor suppressor

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Accepted 19 February 2008
Journal of Cell Science 121, 1141-1150 Published by The Company of Biologists 2008
doi:10.1242/jcs.016634

Summary

Correct establishment and maintenance of cell polarity is required for the development and homeostasis of all metazoans. Cell-polarity mechanisms are responsible not only for the diversification of cell shapes but also for regulation of the asymmetric cell divisions of stem cells that are crucial for their correct self-renewal and differentiation. Disruption of cell polarity is a hallmark of cancer. Furthermore, recent evidence indicates that loss of cell polarity is intimately involved in cancer: several crucial cell-polarity proteins are known proto-oncogenes or tumor suppressors, basic mechanisms of cell polarity are often targeted by oncogenic signaling pathways, and deregulation of asymmetric cell divisions of stem or progenitor cells may be responsible for abnormal self-renewal and differentiation of cancer stem cells. Data from in vivo and three-dimensional (3D) cell-culture models demonstrate that tissue organization attenuates the phenotypic outcome of oncogenic signaling. We suggest that polarized 3D tissue organization uses cell-cell and cell-substratum adhesion structures to reinforce and maintain the cell polarity of pre-cancerous cells. In this model, polarized 3D tissue organization functions as a non-canonical tumor suppressor that prevents the manifestation of neoplastic features in mutant cells and, ultimately, suppresses tumor development and progression.

Key words: Cancer, Cell polarity, Stem cells

Introduction

The human body consists of billions of cells that exist together as an intricately organized and mutually supportive community. This cell community is a dynamic system that is maintained by a well-regulated balance between cell proliferation and death. However, when this balance is skewed in favor of cell accumulation, the result is tumor development and, potentially, the death of the entire cell community. Fortunately, cells have evolved many mechanisms to prevent such an unfavorable development. Loss of cell polarity and subsequent tissue disorganization is a hallmark of cancer (Fig. 1). Although loss of cell polarity was previously considered a by-product of abnormal cell accumulation, recent evidence supports the idea that disruption of cell-polarity mechanisms plays a causal role in tumor initiation. This Commentary concentrates on the role of cell and tissue polarity mechanisms as potential non-canonical tumor suppressors. Unlike canonical tumor suppressors that function autonomously within the cell, higher-order tissue polarity may function as a non-cell-autonomous tumor suppressor that relies on the symbiotic relationship of a community of cells to suppress the malignant phenotype of individual mutant cells in order to secure the further survival of the entire community. Here, we will discuss the evidence implicating cell-polarity mechanisms in cancer development and progression, and discuss the potential mechanisms responsible for a hypothetical tumor-suppressor function of three-dimensional (3D) tissue organization.

Core mechanisms of cellular polarity and their connection to the apical junctional complex

Histological analyses of mammalian organisms reveal an extraordinarily complex organization of cells in normal organs and tissues. Focal disruption or complete loss of this high-order structural organization usually accompanies neoplastic transformation. To understand why cells become disorganized in solid tumors, it is important to understand the mechanisms that are responsible for normal tissue organization. Two components are required for this function: (1) the establishment and maintenance of cell polarity and, (2) spatially organized intercellular adhesion. Much of what is known about the mechanisms of apical-basal cell polarity (Fig. 2) comes from studies in *Drosophila melanogaster* (Bilder, 2004; Wodarz, 2005). Whereas mechanisms of cell polarity are quite complex, three groups of proteins play a central role in the establishment and maintenance of apical-basal cell polarity. The Crumbs-Pals1(Stardust)-Patj and the Par3(Bazooka)-Par6-aPKC protein complexes localize to the apical membrane domain and promote apical-membrane-domain identity (Margolis and Borg, 2005; Suzuki and Ohno, 2006). Their function is antagonized by the basolaterally localized Lethal giant larvae (Lgl), Scribble (Scrib) and Discs large (Dlg) proteins, which together promote basolateral membrane identity (Bilder, 2004) (Fig. 2). Epistatic genetic experiments in *Drosophila* revealed that the apical aPKC-Par3-Par6 protein complex and the basolateral Lgl, Scrib and Dlg proteins are involved in a tug-of-war-type interaction in which a fine balance between their activities defines the boundary position and the sizes of the apical and basolateral membrane domains (Suzuki and Ohno, 2006). Although the molecular mechanisms of mutual inhibition between the apical Par3-Par6-aPKC complex and the basolateral Lgl, Scrib and Dlg proteins are only beginning to be understood, an important discovery revealed that aPKC phosphorylates and ‘inactivates’ Lgl at the apical membrane domain (Betschinger et al., 2003; Plant et al., 2003). The molecular basis for the reverse inhibitory interaction by Lgl, Scrib and Dlg is unknown, although the basolateral cell-polarity-protein Par1

Journal of Cell Science

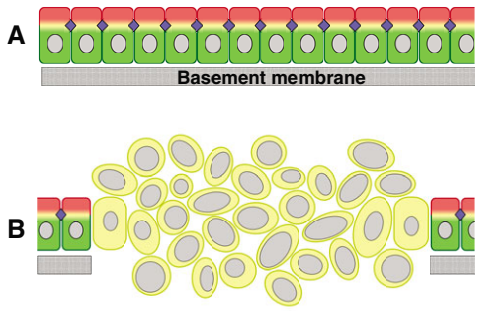

Fig. 1. Disruption of cell polarity and tissue disorganization is a hallmark of advanced epithelial tumors. (A) Normal simple epithelium comprises a monolayer of individual cells that display distinct apical-basal polarity. Cells are tightly packed and connected to each other by the apical junctional complexes (blue), which separate apical (red) and basolateral (green) membrane domains. (B) Cells in high-grade epithelial tumors display loss of apical-basal polarity and overall tissue disorganization.

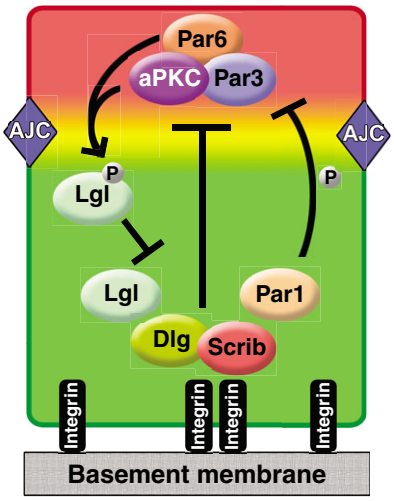

Fig. 2. The core mechanisms of apical-basal cell polarity. Cell-polarity complexes that contain the Par3-Par6-aPKC protein complex (and the Crumbs-Pals1-Patj complex; not shown) localize to the apical membrane domain and promote apical membrane identity. The function of Par3-Par6-aPKC complex is counteracted by the Dlg, Scrib, Lgl and Par1 proteins that localize to the basal membrane domain, and promote basolateral-membrane-domain identity. aPKC counteracts the activity of basal polarity proteins via direct phosphorylation and 'inactivation' of Lgl. Although the mechanisms that are responsible for negative regulation of Par3-Par6-aPKC activity by the Dlg, Scrib and Lgl proteins are not well understood, basal Par1 can negatively regulate the apical polarity complex by phosphorylation and inactivation of apical Par3.

phosphorylates and inhibits Par3 by promoting its dissociation from the Par6-aPKC complex (Benton and St Johnston, 2003).

In epithelial tissues, the apical and basolateral membrane domains are separated by a physical barrier called the apical junctional complex (AJC) (Fig. 2), which is also the most significant epithelial cell-cell adhesion structure and comprises tight junctions (TJs) and adherens junctions (AJs) (Hartsock and Nelson, 2007). Whereas TJs are crucial for epithelial barrier function by providing a tight seal between the membranes of the neighboring cells, AJs use the forces that are generated by the actin cytoskeleton to keep the cellular membranes of neighboring cells together (Hartsock and Nelson, 2007; Perez-Moreno and Fuchs, 2006; Vasioukhin et al., 2000). Cell polarity mechanisms have an intimate relationship with the AJCs and the activities of the apical and basolateral polarity complexes are required for maintenance of AJCs (Bilder and Perrimon, 2000; Chen and Macara, 2005; Firestein and Rongo, 2001; Harris and Peifer, 2007; Hutterer et al., 2004; Imai et al., 2006; Rolls et al., 2003; Woods et al., 1997).

AJCs as a crucial physical link between internal cell polarity and 3D tissue organization

Segregation of the apical and basolateral membrane domains is a crucial function of AJCs in the maintenance of internal cell polarity. However, this is not the only function of AJCs. These structures are indispensable for intercellular adhesion, which is necessary for the integration of cells into 3D organized organs and tissues. Indeed, the formation of cell-cell junctions only at specific membrane locations determines how the building blocks (individual cells) are put together to form 3D organized structures (tissues and organs). As the intracellular position of AJCs is connected to, and partially controlled by, internal cell-polarity mechanisms, AJCs provide a physical link between intracellular cell polarity and polarity of the entire organ. When the cell is already a part of the organ, AJCs may provide external cues that help to orient and reinforce internal cell polarity to ensure correct positioning of the apical and basolateral membrane domains. In this scenario, AJCs may serve as a bi-directional communication link between internal cell-polarity mechanisms and external 3D tissue polarity, and this link may help them to strengthen and provide necessary redundancy to each other. This function makes AJCs one of the most crucial structures responsible for the formation and maintenance of metazoan organisms. Perhaps not surprisingly, AJCs are the major signaling centers that serve as biosensors of the external cellular environment and mediate communication between the neighboring cells in the normal organism (Lien et al., 2006). We will now discuss what happens to cell polarity and AJCs in cancer.

Core cell-polarity mechanisms and mammalian cancer

Changes in activity or expression of core cell-polarity proteins in cancer

The most convincing evidence that implicates cell-polarity pathways in cancer comes from studies in *Drosophila*. The *Drosophila* polarity proteins Lgl, Dlg and Scrib are potent tumor suppressors, and the loss of these proteins results in neoplastic transformation and tumor-like growth in larval imaginal discs and brains (Bilder, 2004). Concordantly, a number of studies found decreased expression or a complete loss of Lgl, Dlg and Scrib polarity proteins in primary tumors from human patients (Cavatorta et al., 2004; Gardiol et al., 2006; Kuphal et al., 2006; Nakagawa et al., 2004; Navarro et al., 2005; Schimanski et al., 2005). Interestingly, re-expression of Dlg and Lgl proteins in tumor-cell lines resulted in the attenuation of neoplastic phenotypes, suggesting that these also have a tumor-suppressor function in human cells (Kuphal et al., 2006; Massimi et al., 2004).

Epistasis experiments in *Drosophila* revealed that activation of aPKC, an Lgl antagonist, promotes tumorigenesis in *lgl* mutants (Lee et al., 2006b). Activation of aPKC has also been implicated in human cancer. Human aPKCι is amplified and overexpressed in ovarian and non-small-cell lung cancers (Eder et al., 2005; Regala et al., 2005; Zhang et al., 2006a), and loss-of-function experiments demonstrated that aPKC plays a causal role in the regulation of cell proliferation, anchorage-independent

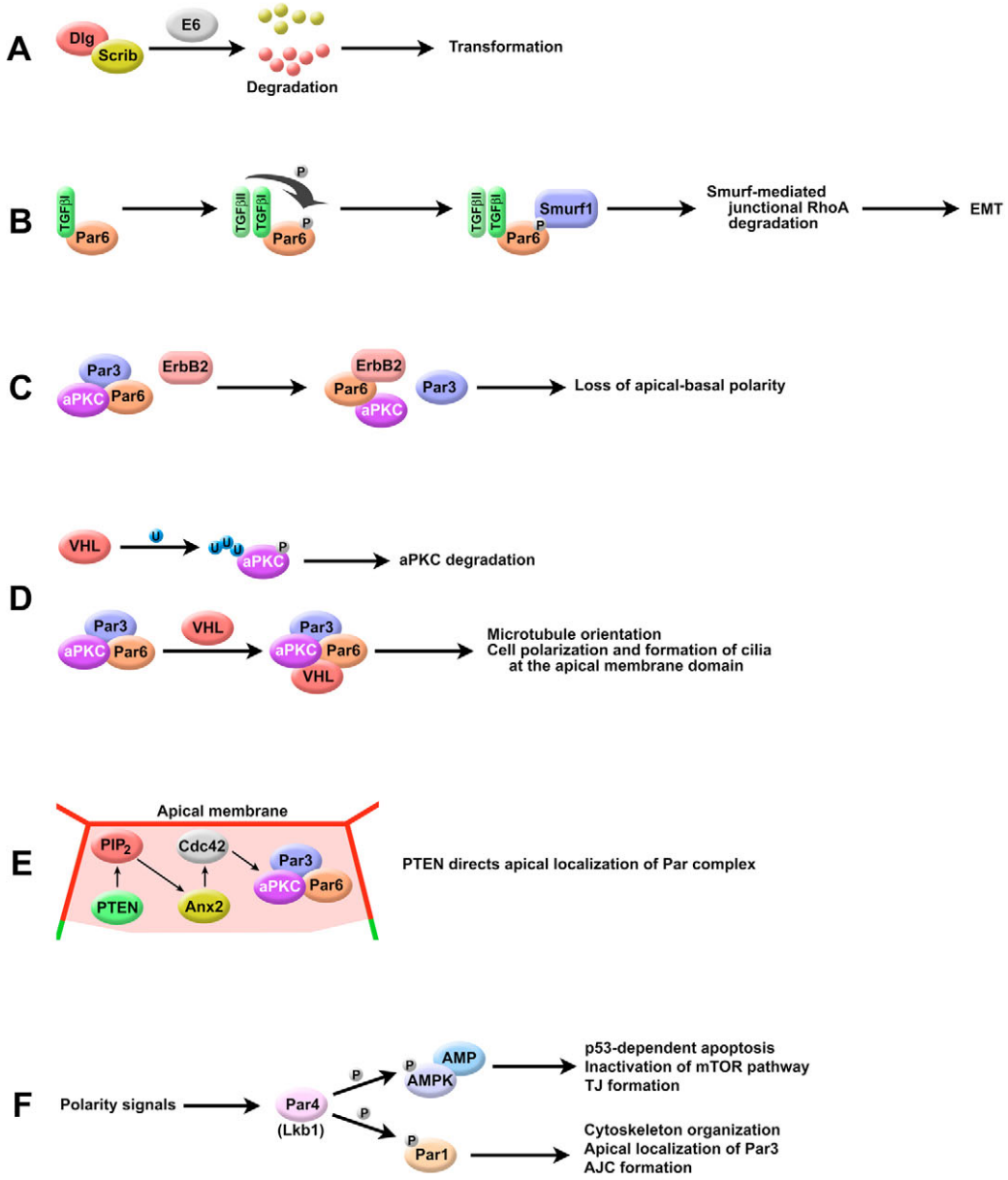

Fig. 3. Oncogenic and tumor-suppressor signaling pathways target cell-polarity mechanisms. (A) Viral E6 oncogene from human HPV targets Dlg and Scrib proteins for ubiquitin-mediated degradation. (B) Activation of TGFβ signaling results in phosphorylation of Par6, and targeting of the E3 ubiquitin ligase Smurf1 to the AJCs, where Smurf1 destroys RhoA, disrupts the integrity of AJCs and causes EMT. (C) Activation of ErbB2 signaling results in disruption of the apical Par6-Par3-aPKC polarity complex by promoting dissociation between Par3 and Par6-aPKC. This function is crucial for ErbB2-mediated disruption of cell polarity and tissue disorganization. (D) The VHL tumor suppressor interacts with core cell-polarity proteins in two ways. First, VHL ubiquitylates active aPKC and targets it for proteasome-mediated degradation. In addition, interaction between VHL and the Par3-Par6-aPKC polarity complex is necessary for correct orientation of the microtubules and for primary cilia formation. (E) The tumor suppressor PTEN is crucial for apical-basal polarization of epithelial cells. During polarization of epithelial cells PTEN is targeted to the future apical membrane domain, where it generates PtdIns(4,5)P_2 (PIP2), which facilitates recruitment of annexin 2 (Anx2), Cdc42 and the apical Par6-Par3-aPKC complex. (F) The tumor suppressor LKB1 controls cell polarity, growth and proliferation by regulating the activities of AMPK and Par1 protein kinases.

growth and xenograft tumor development in cell lines derived from these tumors (Regala et al., 2005; Stallings-Mann et al., 2006; Zhang et al., 2006a). Furthermore, experiments in mice revealed that aPKCι has an important role in Ras-induced colon carcinogenesis, in which aPKC is necessary for Ras-mediated activation of Rac1 (Murray et al., 2004). In addition to aPKCι, aPKCζ has also been implicated in human cancer. aPKCζ is hyperactivated in the squamous-cell carcinoma of the head and neck, where it is required for EGF-induced proliferation by mediating the activation of MAPK (Cohen et al., 2006). aPKCζ is also crucial for the proliferation of human glioblastoma cell lines (Donson et al., 2000) and the regulation of cancer-cell chemotaxis and motility, which may be important for tumor-cell invasion and metastasis (Kuribayashi et al., 2007; Sun et al., 2005). These experiments demonstrated that the crucial apical cell-polarity-protein aPKC is also a potent proto-oncogene in mammalian cells. Taken together, these studies directly implicate cell-polarity pathways in the development of human cancer.

Proto-oncogenes and oncogenes directly target basic cell-polarity mechanisms

Recent evidence indicates that loss of cell polarity is not merely a by-product of abnormal proliferation, but rather is caused by the direct disruption of cell-polarity mechanisms by oncogenic signaling (Fig. 3). Examples can be found in the function of several viral oncogenes. E6 oncogenes found in human papilloma virus (HPV) target cell-polarity proteins Dlg and Scrib for proteolytic degradation (Fig. 3A) (Gardiol et al., 1999; Handa et al., 2007; Massimi et al., 2004; Massimi et al., 2007; Nakagawa and Huibregtse, 2000; Pim et al., 2000; Thomas et al., 2001; Zhang et al., 2007). The ability to degrade these cell-polarity proteins

correlates with the malignant potential of E6 oncogenes. The interaction between an oncoprotein and Dlg is not unique to E6. Dlg also binds to the Tax1 oncoprotein from human T-lymphotropic virus type 1 (HTLV-1) and the adenovirus oncoprotein 9ORF1 (Hirata et al., 2004; Kanamori et al., 2003; Lee et al., 1997). As viral proteins are under selective evolutionary pressure to keep only the most essential functions, these findings suggest that degradation of Dlg and Scrib is necessary for malignant transformation.

In addition to viral oncogenes, malignant transformation can be induced by the abnormal activation of various growth-factor signaling pathways, which not only stimulates cell proliferation, but also results in disruption of apical-basal polarity, cell-cell adhesion and sometimes a complete epithelial-mesenchymal transition (EMT). Transforming growth factor β (TGFβ) signaling, for example, promotes EMT during normal development and tumor progression (Huber et al., 2005; Massague and Gomis, 2006; Thiery, 2003). An interesting insight into the mechanisms responsible for this phenotype was recently provided by the laboratory of Jeffrey Wrana, who found that AJC-localized Par6 directly interacts with TGFβ receptors and is a substrate for TGFβRII-mediated phosphorylation (Ozdamar et al., 2005). Activation of TGFβ signaling results in phosphorylation of Par6, which promotes its interaction with the E3 ubiquitin ligase Smurf1. Localized to the AJCs, Smurf1, in turn, targets junctional RhoA for degradation. As RhoA is crucial for the maintenance of the actin cytoskeleton and stabilization of AJCs, activation of TGFβ signaling ultimately results in the destabilization and loss of AJCs and the initiation of EMT (Fig. 3B).

Abnormal activation of the receptor tyrosine kinase ErbB2 (also known as HER2) is implicated in human breast cancer, and ovarian, gastric, esophageal and endometrial cancers (Hynes and Lane, 2005; Linggi and Carpenter, 2006; Moasser, 2007). Whereas ErbB2 stimulates cell proliferation by activating the Ras-PI3K-PLC pathway, it can also directly disrupt cell polarity and provide protection from apoptosis through its interaction with the Par6-aPKC protein complex (Aranda et al., 2006). Activation of ErbB2 results in the dissociation of Par3 from the Par6-aPKC complex (Fig. 3C). Inhibition of this dissociation restores correct cell polarity and abrogates the anti-apoptotic effects of ErbB2, but does not affect its role in the stimulation of cell proliferation. These findings indicate that growth-factor receptors can use independent mechanisms to regulate proliferation and polarity, and that it is possible to activate cell proliferation without inducing cell-polarity defects.

TGFβRII and ErbB2 are probably not the only growth-factor receptors that can directly target cell-polarity mechanisms. In addition to TGFβ and ErbB2 signaling, loss of polarized cell architecture and EMT can be induced by a variety of signal-transduction pathways, including receptor tyrosine kinases, Ras, Wnt, Notch, Hedgehog and nuclear factor κB (NFκB) (Huber et al., 2005). Future research will help to reveal the molecular mechanisms that connect these relevant cancer signaling pathways to the disruption of cell polarity and EMT.

Connections between tumor suppressors and cell-polarity pathways

In addition to oncogenes and proto-oncogenes, tumor suppressors are also involved in the regulation of apical-basal cell polarity. Mutations in the von Hippel-Lindau (VHL) tumor suppressor are responsible for von Hippel-Lindau disease, which is characterized by the development of hemangioblastoma, clear-cell renal carcinoma and pheochromocytomas (Kaelin, Jr, 2005; Kaelin, Jr, 2007). VHL polyubiquitinates and targets the transcription factor hypoxia-inducible factor 1 for degradation. VHL can also directly impact cell-polarity pathways by ubiquitin-mediated degradation of activated aPKC (Fig. 3D) (Okuda et al., 2001). Furthermore, interaction between VHL and the Par3-Par6-aPKC complex is involved in VHL-mediated regulation of polarized microtubule growth and formation of primary cilia (Schermer et al., 2006). This function makes VHL an important regulator of cell polarity because polarized growth of microtubules is crucial for cell polarization. Moreover, because primary cilia play an important role in cancer-relevant Hedgehog signaling and plateled-derived growth factor (PDGF) signaling pathways, the cell-polarity function of VHL might be directly involved in its function as a regulator of cell proliferation and tumor suppressor.

Phosphatase and tensin homolog (PTEN) is another tumor suppressor protein that is implicated in the regulation of cell polarity. PTEN negatively regulates the phosphatidylinositol 3-kinase (PI3K) pathway by dephosphorylating the PI3K product, phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3], and producing phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] (Rossi and Weissman, 2006). Spatial membrane segregation of PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 is crucial for apical-basal cell polarity (Fig. 3E) (Martin-Belmonte et al., 2007). PtdIns(4,5)P_2 is localized to the apical membrane domain and it maintains its identity by promoting apical localization of annexin2, Cdc42 and aPKC proteins. Apical accumulation of PtdIns(4,5)P_2 depends on apical membrane targeting of PTEN. Thus, PTEN is pivotal for the establishment and maintenance of apical-basal cell polarity.

In addition to PTEN, serine/threonine kinase 11 (STK11, herafter referred to as LKB1) is another dual-function protein that is directly involved in both cell polarity and tumor suppression. Mutations in LKB1 are responsible for Peutz-Jeghers syndrome, which is characterized by the development of benign hamartomatous polyps throughout the gastrointestinal tract and a 18-fold increased risk of cancer (Giardiello et al., 1987; Katajisto et al., 2007). LKB1 is a homologue of the *Drosophila* and *Caenorhabditis elegans* cell-polarity protein Par4, and its function is also crucial for the polarization of mammalian cells (Baas et al., 2004). LKB1 phosphorylates and activates several other serine/threonine kinases including AMP-activated protein kinase (AMPK) and Par1 (Fig. 3F) (Lizcano et al., 2004). Although it was thought that LKB1 signaling regulates cell polarity via Par1 and proliferation via AMPK, recent evidence revealed that *Drosophila* and mammalian AMPK are also crucial for LKB1-mediated regulation of cell polarity (Lee et al., 2007; Mirouse et al., 2007; Zhang et al., 2006b). AMPK controls cell polarity by regulating the acto-myosin cytoskeleton via direct phosphorylation of the non-muscle myosin regulatory light chain (MRLC) protein (Lee et al., 2007). The discovery of AMPK as a crucial regulator of cell polarity indicates that LKB1-mediated control of cell growth and polarity are, ultimately, connected to each other.

Together, these studies demonstrate an intimate connection between cell-polarity pathways and tumor suppression. This relationship is either direct, because many known tumor suppressor proteins and proto-oncogenes are also crucial cell-polarity proteins, or indirect and mediated by specific signaling pathways that connect cancer-relevant proteins with core polarity mechanisms. Knowledge of the mechanisms that are used by pro-tumorigenic pathways to disrupt cell polarity opens the

possibility of targeted inactivation of these mechanisms to reveal their specific contribution to cancer initiation and progression. This approach will help to determine the role of disruption of cell polarity in mammalian cancer. We will now discuss emerging data that directly implicate cell-polarity mechanisms in cancer through their role in the self-renewal and differentiation of stem cells.

Cell-polarity mechanisms in self-renewal and differentiation of stem cells and the emergence of cancer stem cells

Core cell-polarity mechanisms regulate asymmetric cell division of *Drosophila* stem cells

Mammalian tissues are constantly renewed through a well-controlled process that involves the removal of old cells and the generation of new cells that originate from the stem-cell population. To ensure correct self-renewal and differentiation, a stem cell divides asymmetrically to generate two daughter cells that can have the same or different cell fates (Fig. 4A). One daughter cell acquires the mother-cell position near the stem-cell niche and retains the stem-cell fate (self-renewal), whereas the other acquires a more differentiated fate (differentiation) (Fig. 4B).

Studies on *Drosophila* neuroblasts, stem-cell-like progenitors in the central nervous system, revealed that cell-polarity mechanisms are pivotal for the regulation of asymmetric cell division (Wodarz, 2005; Wodarz and Nathke, 2007). Asymmetric cell division is regulated by the polarized localization of cell fate determinants (Numb, Pros, Brat, Pon and Mira) and the correct orientation of the mitotic spindle, and both are necessary to ensure that cell fate determinants are inherited by only one daughter cell (Fig. 4B). Asymmetric localization of cell fate determinants is controlled by the function of the apical Par3-Par6-aPKC polarity-protein complex and basolateral Lgl, Dlg and Scrib polarity proteins. Remarkably, *lgl*, *dlg* or *scrib* mutant neuroblasts fail to correctly localize cell fate determinants and generate daughter cells that maintain characteristics of dividing neuroblasts. Dividing cells accumulate quickly, leading to the development of a tumor (Albertson and Doe, 2003). Animals that have mutations in cell-fate determinants themselves (such as *mira*, *pros*, *numb* and *brat*) also develop tumors (Betschinger et al., 2006; Caussinus and Gonzalez, 2005; Lee et al., 2006c). Thus, abnormal asymmetric cell division is the primary defect responsible for tumor formation in *lgl*, *dlg* and *scrib* mutants.

In addition to correct segregation of cell fate determinants, correct spindle orientation is also required for the successful execution of asymmetric cell division of *Drosophila* neuroblasts. The mitotic spindle is positioned by dynamic interactions between astral microtubules and the cell cortex. Partner of Inscuteable (Pins), Gαi and Mushroom body defect (Mud) proteins link astral microtubules to the apical Par3-Par6-aPKC polarity complex to regulate correct spindle orientation. Neuroblasts with *mud* mutations show correct localization of the apical polarity proteins and basal-cell fate determinants before cell division; the mitotic spindle, however, is not aligned correctly in these mutants, and this eventually leads to missegregation of cell-fate determinants between the daughter cells and the accumulation of excessive neuroblasts (Bowman et al., 2006; Izumi et al., 2006; Lee et al., 2006a; Siller et al., 2006). Finally, the mitotic kinases Aurora-A and Polo are necessary for both asymmetric segregation of cell fate determinants and correct orientation of the mitotic spindle, and *Drosophila aurA* and *polo* mutants also show accumulation of neuroblasts and tumor development (Lee et al., 2006a; Wang et al., 2007; Wang et al., 2006).

Abnormal asymmetric cell division as a potential cause of mammalian cancer stem cells

Overall, studies in *Drosophila* demonstrated that cell-polarity mechanisms regulate asymmetric cell division of stem cells, and perturbation of correct asymmetric cell division results in the abnormal accumulation of dividing cells and cancer. These findings became especially intriguing recently, after it was discovered that human cancers are driven by a small population of cancer stem cells (CSCs) (Al-Hajj et al., 2003; Huntly and Gilliland, 2005; Lapidot et al., 1994; O'Brien et al., 2007; Singh et al., 2004). Isolated from tumors, CSCs are similar to undifferentiated progenitors, which are committed to a specific cell lineage. Unlike normal progenitors, for which the proliferative life span is limited, CSCs are immortal and unable to withdraw from the cell cycle. Instead, they self-renew and produce cells of various degrees of differentiation that proliferate but have only a limited life span and form the bulk of the tumor (Fig. 5). CSCs in human tumors have distinct similarities to mutant *Drosophila* neuroblasts, which are unable to divide asymmetrically and instead keep dividing and form a tumor. One of the most exciting questions in modern cancer biology is whether the core cell-polarity mechanisms that govern the asymmetric cell division of stem cells are disrupted in human cancer, and whether these abnormalities are causally involved in the emergence of CSCs. Indeed, many apical-basal cell-polarity mechanisms that have been discovered in *Drosophila* are also conserved in mammalian cells.

If the failure of core cell-polarity mechanisms can result in tumor development in *Drosophila*, why not in mammals? Presently, there is no definitive evidence that this happens in mammalian tumors. Whereas mutations in the cell-polarity genes *dlg*, *lgl* or *scib* in *Drosophila* result in cancer, mutations in their respective mammalian orthologues either do not show overt problems with asymmetric cell division (*Dlg1*, *Dlg2*, *Dlg3*, *Dlg4*, *Scrib*) or show defects in asymmetric cell divisions but do not develop cancer (*Lgl1*) (Cuthbert et al., 2007; Klezovitch et al., 2004; Mahoney et al., 2006; McGee et al., 2001; Migaud et al., 1998; Murdoch et al., 2003). One of the reasons for the differences between *Drosophila* and mammalian systems might be the significant redundancy of core cell-polarity components in mammalian cells. Indeed, whereas the *Drosophila* genome has only one of each of the *Dlg*, *Lgl*, *Par6*, *Par3* and *aPKC* genes, mammalian genomes have four *Dlgs* (*Dlg1-4*), two *Lgls* (*Llgl1* and *2*), three Par6 (*PARD6A*, *PARD6B*, *PARD6G*), two *Par3* (*PARD3* and *PARD3B*) and two *aPKC* (*PRKCI* and *PRKCZ*) genes. Owing to this redundancy, loss of any one of the mammalian genes might not be sufficient for the manifestation of abnormal asymmetric cell division and cancer. Future double- or triple-knockout experiments in mice will help to determine whether this is indeed the case.

In contrast to core cell-polarity genes, a number of genes that play an important role in the correct positioning of the mitotic spindle during asymmetric cell division in *Drosophila* have been shown to be involved in mammalian cancer. For example, Aurora-A and Polo kinases are implicated in human cancer (Malumbres and Barbacid, 2007). It is believed that genomic instability resulting from abnormal mitotic checkpoints and missegregation of chromosomes is responsible for the cancer-related roles of mammalian *Aurora-A* and *Polo*. However, it is conceivable that abnormal asymmetric cell division is also

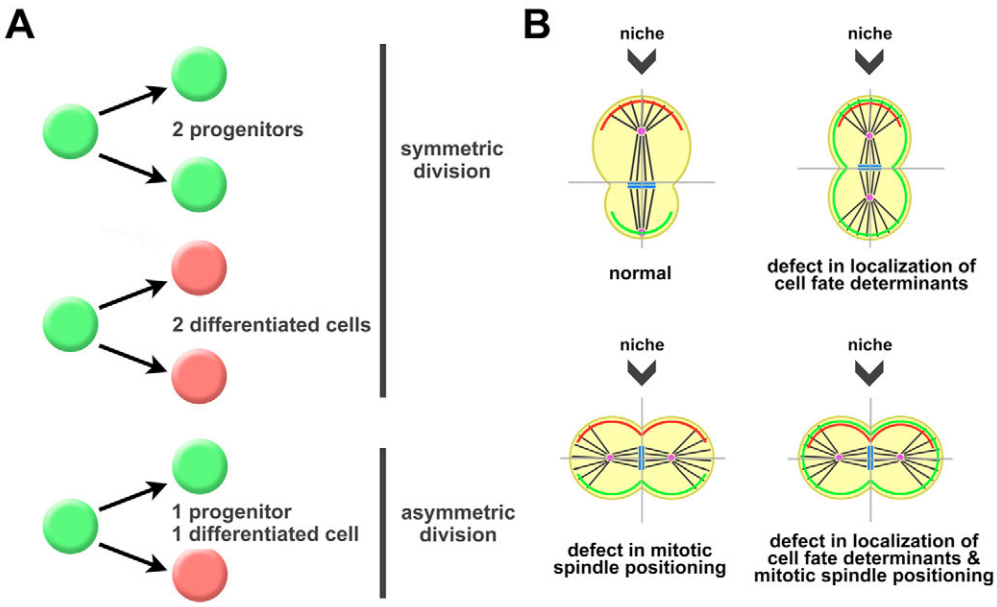

Fig. 4. Symmetric and asymmetric divisions of stem or progenitor cells. (A) Stem or progenitor cells can divide symmetrically and generate either two stem or progenitor cells (expansion), or two differentiated cells (depletion). Alternatively, a stem or progenitor cell can divide asymmetrically and generate one stem or progenitor cell (self-renewal) and one differentiated cell (differentiation). Asymmetric cell division is the predominant type of stem or progenitor cell division during adult mammalian tissue homeostasis. (B) Mechanisms of normal asymmetric cell division and its potential perturbation in cancer. The stem or progenitor cells occupy specific positions within tissues that are called niches and are defined by the presence of the particular neighboring cells and cell-cell and/or cell-substratum adhesion structures. The mother cell asymmetrically localizes the Par3-Par6-aPKC complex (red) and cell fate determinants (green), and orients the mitotic spindle in such a way that basal cell fate determinants are inherited by only one daughter. Segregation of cell-fate determinants to one daughter assures that this cell acquires a fate different to that of the mother cell. Asymmetric cell division may fail because of an inability to asymmetrically localize cell fate determinants (*Drosophila lgl*, *dlg*, *scribl*, *pros*, *brat* and *mira* mutants), an inability to correctly orient the mitotic spindle (*Drosophila pins*, *mud* mutants) or both of these defects (*Drosophila polo*, *aurA* mutants).

involved in Aurora-A and Polo-mediated tumors, and future studies will probably examine this possibility. In addition to the regulation of asymmetric cell division, internal cell-polarity mechanisms are important for the assembly of individual cells into complex 3D structures of tissues and organs. In the following section we discuss the available information concerning a potent regulatory function of 3D tissue organization and introduce a hypothetical model of tissue polarity as a non-canonical tumor-suppressor.

Cell-polarity-mediated 3D tissue organization as a potential non-canonical non-cell-autonomous tumor suppressor

Correct functioning of cell-polarity mechanisms is crucial for the integration of individual cells into tissues and organs. Indeed, as discussed earlier, internal cell-polarity pathways regulate localization and function of AJCs, and this, in turn, controls the pattern of assembly of individual cells into 3D organized tissues. Evidence is emerging that the 3D structure of the organ and the overall tissue microenvironment plays an important role in the regulation of proliferation, survival and apoptosis of individual epithelial cells (Bissell et al., 2003; Kenny and Bissell, 2003; Nelson and Bissell, 2006; Potter, 2007; Zahir and Weaver, 2004). The significance of tissue organization was underestimated for decades because the field of cancer biology concentrated on using tumor-derived cell lines, either growing them in vitro or as a disorganized cellular mass in mouse xenograft tumors. The, perhaps first, evidence that the tissue microenvironment may be of importance in tumor development can be traced to early experiments with teratoma tumor cells injected into normal mouse blastocysts. Despite high malignancy and euploidy, tumor cells integrated into the wild-type host tissue and displayed normal behavior (Astigiano et al., 2005; Brinster, 1974; Illmensee and Mintz, 1976; Mintz and Illmensee, 1975).

The most obvious example of the non-cell-autonomous tumor suppressor effect of normal tissue organization comes from studies using genetically modified mice. It is well known that significant differences are often seen between the outcomes of experiments performed in vivo using an animal model, and in vitro using unorganized populations of cells in culture. In general, cancer-relevant phenotypes are much more readily apparent in vitro than in vivo. For example, expression of activated Ras results in a potent transformation in cultured cells; yet, expression of the same gene in vivo results in normal tissue that shows some clonal tumor development in cells that had presumably acquired additional oncogenic modifications (Frame and Balmain, 2000). Similar results were obtained after infection of organisms with oncogenic viruses. For example, cells in chicken embryos infected with v-Src-containing virus do not show a malignant phenotype, but when the same cells are dissociated and placed in culture, they show massive transformation (Dolberg and Bissell, 1984).

In fact, it is extremely rare that one or even several genetic modifications can turn an entire tissue into a tumor, suggesting that it is much more difficult to transform cells that grow as part of a tissue than single cells that grow in a culture dish. Furthermore, in addition to mutant cells, wild-type cells also show differences in cancer-relevant cell behavior depending on whether they grow as a part of the tissue or an unorganized cell population in culture. For example, it was thought that normal cells have only a limited

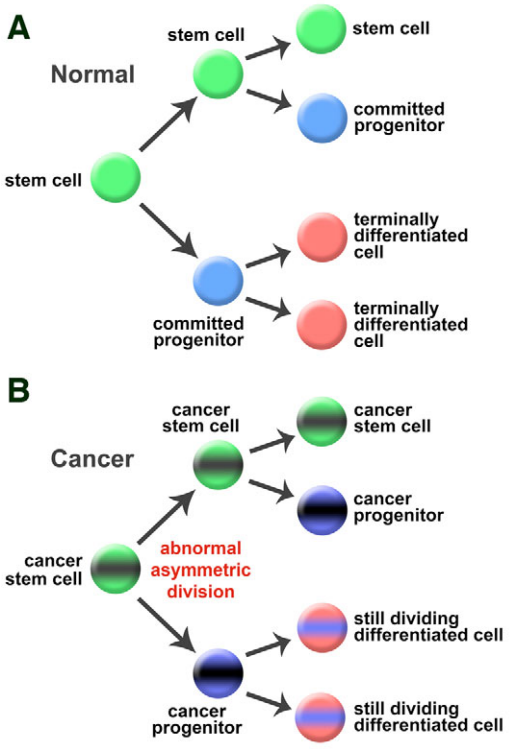

Fig. 5. Maintenance of normal tissue homeostasis and a hypothetical model of the cancer-stem-cell-based origin of tumors. (A) Asymmetric cell divisions of stem and progenitor cells result in the generation of differentiated non-proliferating cells, which often constitute the bulk of the normal tissue. (B) Abnormal asymmetric cell division of stem and progenitor cells might be responsible for the accumulation of cells that fail to withdraw from the cell cycle and continue to divide. In this scenario, both progenitor- and stem-cell populations are collectively known as cancer stem cells.

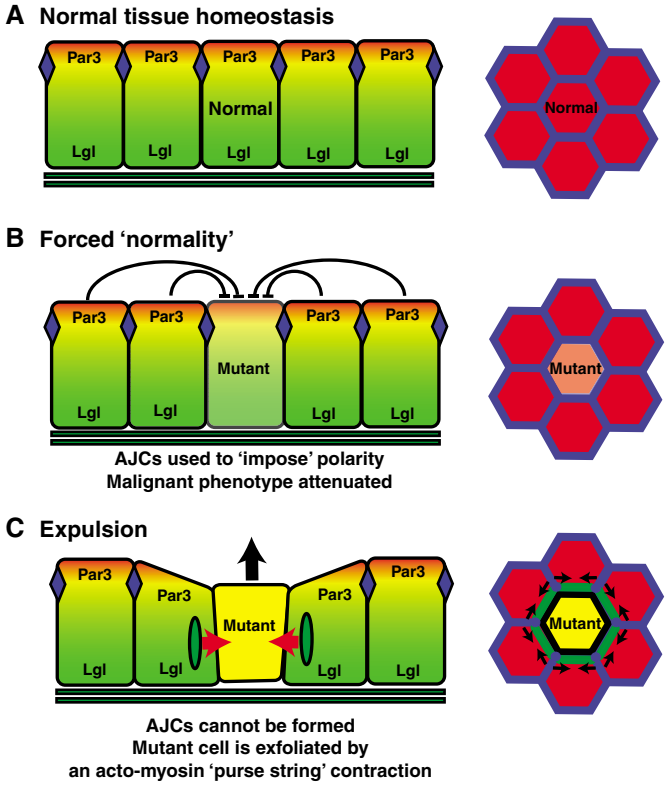

Fig. 6. Hypothetical mechanisms of tissue polarity as a non-cell-autonomous tumor suppressor. (A) In normal tissues, cells use their AJCs to link internal cell-polarity mechanisms (asymmetrically localized cell-polarity proteins) with polarized 3D tissue organization. (B) Pro-tumorigenic changes in the mutant cells might disrupt internal cell-polarity mechanisms (see Fig. 3). If mutant cells express AJC proteins, the normal cell neighbors could use AJCs to maintain polarity and attenuate the malignant phenotype of the mutant cells. In this context, both 3D tissue polarity and AJC proteins have an important tumor-suppression function. (C) When the mutant cell is unable to form AJCs with its neighbors, an alternative 3D-mediated tumor-suppression mechanism may be engaged. Epithelial cells use AJCs as a biosensor of the external cellular microenvironment (Lien et al., 2006). Normal cells will treat the AJC-negative mutant as an empty space and may exfoliate it by an actin-myosin-mediated 'purse-string' wound-healing mechanism (Brock et al., 1996; Danjo and Gipson, 1998). Only when the mechanisms describend in B and C both fail will the mutant cells progress and form the tumor.

life span in culture. However, recent studies demonstrated that this might be due to inferior culture conditions (Papini et al., 2003; Ramirez et al., 2001). When primary mouse and human keratinocytes grow as an unorganized cell population in the presence of fibroblasts, the keratinocytes display almost limitless proliferative capacity, although this is not due to transformation because the same cells behave normally after being grafted back onto human or mouse skin (Rheinwald and Green, 1977; Ronfard et al., 2000). These results indicate that completely normal cells can exhibit tumor-like behavior when they are grown outside of their normal tissue microenvironment.

3D cultures of epithelial cells, pioneered in Mina Bissell's laboratory, have provided substantial information concerning the role of tissue organization in the regulation of cell proliferation and cancer. Epithelial cells within polarized 3D structures are vastly different from the same cells that grow two-dimensionally (Wang et al., 1998). Polarization has a profound effect on the outcome of oncogenic signaling. For example, activation of Myc results in increased cell proliferation in cells that grow as an unorganized cell population, but has no effect in the same cells when they can form polarized 3D structures (Partanen et al., 2007).

Both intercellular communication and signals from the basement membrane are crucial for the manifestation of the transformed phenotype of epithelial cells. Disruption of the interaction between β1-integrin and the basement membrane suppresses the malignant phenotype of breast carcinoma cell lines (Liu et al., 2004; Wang et al., 1998; Weaver et al., 1997). Basement-membrane interaction of polarized epithelial cells not only provides them with integrin-mediated proliferation signals, but can also protect epithelial cells from apoptotic cell death (Weaver et al., 2002). Accordingly, elimination of signaling from the crucial basement-membrane-receptor β4-integrin in epithelial cells in vivo results in the attenuation of ErbB2-mediated mammary tumorigenesis (Guo et al., 2006).

Taken together, these results demonstrate that the 3D integration of epithelial cells into a tissue, and the establishment and maintenance of a polarized cell phenotype exercise a potent non-cell-autonomous control over cellular phenotype and may potentially serve as a higher-order tumor suppressor. What might be the mechanism responsible for such regulation? It has recently been proposed that normal tissue produces secreted factors called morphostats, and that these factors are responsible for the tumor-suppressor function of 3D tissue organization (Potter, 2007). We

propose that the internal cell-polarity mechanisms of the normal cells function as a non-cell-autonomous tumor suppressor by using 3D tissue organization to reinforce cell polarity, and attenuate proliferation and invasion of the mutant cells (Fig. 6). In this model, normal cells use their internal cell-polarity mechanisms to establish a polarized 3D tissue organization (Fig. 6A), which, in turn, uses the AJCs and cell-substratum adhesions to reinforce and maintain polarity of the mutant cells with disrupted internal cell-polarity pathways (Fig. 6B). If tumor cells are unable to form AJCs with their wild-type neighbors, AJ biosensors on wild-type cells will not recognize the mutant as a neighboring cell and will treat it as a gap in the epithelial layer that needs to be repaired. Subsequent activation of the actin-myosin-based 'purse-string' epithelial wound-repair mechanism may result in expulsion and loss of the mutant cell (Brock et al., 1996; Danjo and Gipson, 1998) (Fig. 6C).

Overall, in cases of both the 'imposed' cell polarity and physical removal of the mutant cells, 3D tissue polarity may function to inhibit primary tumor development. This is a non-canonical mechanism of tumor suppression because, unlike the canonical tumor suppressors that function within the cells, 3D tissue polarity functions non-cell-autonomously and probably depends on the activities of multiple cell-polarity and cell-adhesion genes. Although such a mechanism of tumor suppression currently represents only a hypothetical model, specific experiments can be designed to test its validity in the future.

Conclusion and future outlook

A significant body of evidence demonstrates the intimate relationships between cell-polarity mechanisms, 3D tissue polarity and tumor formation. Recent breakthrough studies in *Drosophila* revealed that cell-polarity mechanisms are causally involved in cancer through their role in asymmetric cell division that governs self-renewal and differentiation of stem and progenitor cells. These findings might reveal the mechanisms responsible for the origin of human CSCs, and this possibility will have to be carefully examined in the future. Here, we have discussed the idea that cell polarity and 3D tissue organization function as a non-canonical tumor suppressors that inhibits neoplastic phenotypes in mutant pre-cancerous cells. Significant new research will be necessary to evaluate this idea and to clarify the molecular mechanisms that link cell and tissue polarity with the regulation of normal tissue homeostasis and cancer.

We thank all members of the Vasioukhin laboratory for critical comments on this manuscript. We apologize to those authors whose work is not described in this review owing to space limitations. The studies on cell polarity and cancer in our laboratory are supported by the NIH grants R01 CA098161 and R01 CA102365 and Developmental Biology Training Grant 5T32HDO7183.

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